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Original Article

STEREO SELECTIVE SYNTHESIS OF NOVEL LIGNAN INTERMEDIATES AS ANTIMICROBIAL AGENTS

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ABSTRACT

Objectives: The aim of the present study was to synthesize a series of new lignan intermediates as potential antimicrobial agents.

Methods: Substituted benzene and aromatic acids or acid chlorides were converted to benzophenones 1(a-f). The benzophenones 1(a-f) on Stobbe condensation with and diethyl succinate in the presence of potassium t-butoxide yielded 4-(4-aryl)-4-(4-aryl)-3-ethoxycarbonyl-but-3-enoic acids (2a, 2f) and a mixture of E and Z-isomers of 4,4-diaryl-3-ethoxycarbonyl-but-3-enoic acids 2(b-e) and 3(b-e) in relatively good yields. The synthesized compounds were tested for their antimicrobial susceptibility against different fungi and bacteria species.

Results: The Stobbe condensation of benzophenones 1(a-f) and diethyl succinate in the presence of potassium *t*-butoxide yielded 4-(4-aryl)-4-(4-aryl)-3-ethoxycarbonyl-but-3-enoic acids (2a, 2f) and a mixture of E and Z-isomers of 4,4-diaryl-3-ethoxycarbonyl-but-3-enoic acids 2(b-e) and 3(b-e) in good yields. The compounds 1a and 1f yielded only 2a and 2f but not 3a and 3f due to symmetrical substitution in the aromatic rings. The structures of the new lignan intermediates were confirmed by spectral studies and elemental analysis.

Conclusions: Results of the antimicrobial activity reveal that some of the compounds particularly 2c, 2d, 3c and 3d act as potential antimicrobial agents different fungal and bacterial organisms.

Keywords: Antibacterial, Antifungal, Diffusion, Inhibition, Stobbe condensation.

INTRODUCTION

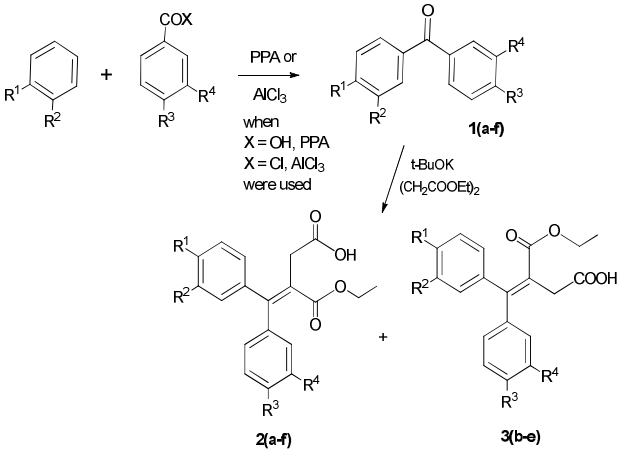
The lignans are a group of secondary metabolites found in plants, which are produced by oxidative dimerization of two phenyl propanoid units and show bioactive diversity as their chemical assembly. The range of their structures and biological activities is broad. Various lignans are known to have anti-tumour, antimitotic and antiviral activity and to specifically inhibit certain enzymes. Novel lignans continue to be described by natural products chemists at a steady rate and knowledge of their variety, as well as their range of occurrence in the plant kingdom, is continually expanding [1]. Thiophene-based lignan analogues were synthesized by a selective and high performance synthetic strategy based on the Negishi cross-coupling reaction [2]. An enantiomerically pure α-hydroxylated lactone lignans were synthesized starting from diisopropyl malate, which involve stereo selective alkylation with various benzyl bromides and saponification yielded the corresponding succinic acids. Acetalization afforded the dioxolanones, which were stereo selectively alkylated [3].

1-Phenyl-1H-benzo[b]azepine-2,5 dione was prepared by an intramolecular cyclisation of 4-N,N-diphenylamine-4-oxo-2-butenoic acid in good yield showed considerable activity against *F. Solani* and *A. Flavus* fungal strains [4]. Several reviews on lignan synthesis have been published, among them a recent reviews describe the synthetic strategies adopted for the synthesis of lignans and their scaffolds [5-6]. An asymmetric and regioselective total synthesis approach to 1,4-benzodioxane lignans in which (2R, 3R)-and (2S,3S)-3-(4-hydroxy-3-methoxyphenyl)-2-hydroxymethyl-1,4-benzodioxan-6-carbaldehyde were reported [7]. Intramolecular cyclization via nitrenium ion of 2-phenylpentanoic/2-phenylbutanoic acid esters with a terminal p-azidophenyl group gives direct access to tetrahydronaphthalene lignan esters, which showed good anti-tumor activity against cell lines MCF-7, NCI-H460, SF-268 and UACC-62 [8].

In view of enormous biological applications associated with lignan analogues, we herein report the stereoselective synthesis of lignan intermediates and their antimicrobial activity studies.

Experimental

Melting points were determined by open capillary method and are uncorrected. IR spectra were recorded on a Nujol mull on Shimadzu 8300 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Agilent-NMR 400 MHz and 100 MHz spectrophotometer respectively in $CDCl_3$ with TMS as an internal standard. The chemical shifts are expressed in δ ppm. Mass spectra were obtained on Mass Lynx SCN781 spectro photomer TOF mode. Elemental analysis was performed on a Thermo Finnigan Flash EA 1112 CHN analyzer. Chromatographic separations were carried out on silica gel (70-230 mesh, Merck) column using hexane: ethyl acetate (6:2 v/v) as eluent.



Scheme 1: Synthetic route for lignin intermediates

In a typical procedure, substituted benzene and aromatic acids or acid chlorides were converted to benzophenones 1(a-f) using PPA or $AlCl_3$. The Stobbe condensation of benzophenones 1(a-f) and diethyl succinate in the presence of a strong base potassium t-butoxide yielded 4-(4-aryl)-4-(4-aryl)-3-ethoxycarbonyl-but-3-enoic acids (2a, 2f) and a mixture of E and Z-isomers of 4,4-diaryl-3-ethoxycarbonyl-but-3-enoic acids 2(b-e) and 3(b-e) in relatively good yields (Scheme-1).

Antimicrobial activity of the synthesized compounds was done by paper disc diffusion method [9-11]. The bacteria species *Escherichia coli, Bacillus substilis*, *Staphylococcus aureus* and fungal species *Aspergillus niger, Aspergillus flavus, Fusarium oxysporium* were used as test stains. Briefly a lawn of the organism was prepared by spreading 50 ml of overnight cultures (conc. 10^6~10^7 CFU/ml) onto agar set in petri dishes. Sterile filter paper discs containing test compounds 2(a-f) and 3(b-e) at the concentration of 50 µg/ml in methanol/water are placed at equal distances on the agar plate. Control experiments with equivalent volumes of the only solvent were applied to the paper discs. The standard drugs ciprofloxacin (25 µg/disc) and nystatin (25 µg/disc) were used as the positive control against bacteria and fungi organisms. Plain dry discs were used as negative controls. The plates were incubated at 37 °C for 24 h to check clearing zones around the discs. Clearing zones around the disc (zone of inhibition) were measured as diameter in mm. The screening tests were carried out in triplicate and the results were expressed as a mean of three determinations±Standard Deviation (SD).

Preparation of poly phosphoric acid (PPA)

Initially reagent grade orhopolyphosphoric acid (85 ml) was heated for half an hour to remove traces of water present in it. Phosphorous pentoxide (100 g) was taken in a 250 ml three-necked flask and then the flask equipped with a mechanical stirrer, mercury seal tube and guard tube. The hot orhopolyphosphoric acid (75 ml) was added to phosphorous pentoxide and the mixture was vigorously stirred by maintaining the temperature between 170-200 °C. The whole mixture became a clear viscous liquid. The solid lump left over was removed by means of spatula and the liquid was stirred for again for 1 h and cooled to 90-100 °C.

Typical procedure for the synthesis of substituted benzophenone, 1a

Ethoxybenzene (0.15 mol) was slowly added to the freshly prepared PPA at 80 °C with swirling and the mixture was stirred for 15 min. 4-Ethoxybenzoic acid (0.15 mol) was added at a stretch and the mixture was vigorously stirred for 3 h on a oil bath at 90-100 °C. After the completion of the reaction, the mixture was cooled to room temperature and was poured into ice (250 g) with stirring. The dark grey colored precipitate formed was filtered, the residue is digested with 10% sodium hydroxide (100 ml) for half an hour using mechanical stirrer and filtered again. The solid obtained was repeatedly washed with water to free alkali and then recrystallized from alcohol to get bis (4-ethoxyphenyl) methanone in 74% yield.

Alternatively, the substituted benzophenone, 1a was prepared by alternative procedure as follows

A mixture of aluminium chloride (0.01 mmol) and ethoxybenzene (0.01 mmol) in dry carbon disulphide (50 ml) was stirred at 5 °C for 1 hour. 4-Ethoxybenzoyl chloride (0.01 mmol) was added to the reaction mixture slowly with swirling. Then the mixture was then stirred for 6h at room temperature. The reddish brown mixture obtained was acidified with 5N HCl and extracted into diethyl ether (3×25 ml). The organic layer washed successively with 10% sodium hydroxide (3×25 ml) and water (3×25 ml). The organic layer was evaporated to dryness. The crude solid was recrystallized from ethanol to get bis(4-ethoxyphenyl) methanone in 56% yield. The same procedure was used in all cases.

General procedure for the synthesis of 4-(4-ethoxyphenyl)-4-(4-ethoxyphenyl)-3-ethoxycarbonyl-but-3-enoic acid (2a)

To freshly prepared potassium t-butoxide [from potassium (0.09 mol) and t-butanol (120 ml)], 4,4'-diethoxy benzophenone 1a (0.09 mol) was added quickly under nitrogen atmosphere and refluxed for 1 h. Then freshly distilled diethyl succinate was added under nitrogen atmosphere and refluxed for 30 h. The progress of the reaction was monitored by TLC. After the completion of the reaction, the excess of *t*-butanol was removed by distillation under reduced pressure. The resulting residual mass was acidified with 5N HCl. The product was extracted into 10% $NaHCO_3$ (3×150 ml) solution and washed with diethyl ether (3×150 ml) and then acidified with 10% HCl. The resulting mass was recrystallized from methyl alcohol to get 2a in 78% yield. The same procedure was used for the synthesis of 2f from 1f.

General procedure for the synthesis of 4,4-diaryl-3-ethoxycarbonyl-but-3-enoic acids To freshly prepared potassium *t*-butoxide, 4,4'-diaryl benzophenone 1(b-e) (0.09 mol) was added quickly under nitrogen atmosphere and refluxed for 1 h. Then freshly distilled diethyl succinate was added under nitrogen atmosphere and refluxed for 30 h. The progress of the reaction was monitored by TLC. After the completion of the reaction, the excess of *t*-butanol was removed by distillation under reduced pressure. The resulting residual mass was acidified with 5N HCl. The product was extracted into 10% $NaHCO_3$ (3×150 ml) solution and washed with diethyl ether (3×150 ml) and then acidified with 10% HCl. The reaction furnished a mixture of E and Z-isomers of 4,4-diaryl-3-ethoxycarbonyl-but-3-enoic acids 2(b-e) and 3(b-e) in good yields. The isomers were separated by column chromatography. The E and Z-isomers are obtained due to the asymmetric substitution in the two aromatic rings.

RESULTS AND DISCUSSION

3-(Ethoxycarbonyl)-4,4-bis(4-ethoxyphenyl)but-3-enoic acid, 2a

Obtained from bis(4-ethoxyphenyl) methanone (1a) and diethyl succinate in 62% yield, m. p. 112-115 °C. IR (Nujol, γ cm^{-1}): 3030 (b) (OH str), 1748 (s) (ester C=O str), 1725 (s) (acid C=O str), 1667 (w) (C=C str), 1226 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.202 (t, 3H, CH_3), 1.310 (t, 6H, CH_3), 3.640 (s, 2H, CH_2), 4.080 (q, 4H, OCH_2), 4.236 (q, 2H, OCH_2), 6.984 (dd, 4H, Ar-H), 7.222 (dd, 4H, Ar-H), 10.556 (s, 1H, COOH).^{13}C NMR ($CDCl_3$): δ14.04 (1C, CH_3), 14.66 (2C, CH_3), 35.18 (1C, C-2), 61.26 (1C, OCH_2), 63.40 (2C, OCH_2), 114.20 (4C, Ar-C), 115.72 (1C, C-3), 128.94 (4C, Ar-C), ^{13}2.16 (2C, Ar-C), 157.38 (2C, Ar-C), 158.41 (1C, C-4), 168.30 (1C, COO), 174.14 (1C, COOH). MS (m/z): 398 (M^+, 22), 370 (24), 326 (36), 308 (100), 280 (42), 252 (54). Anal. Calcd. for $C_{23}H_{26}O_6$: C, 69.33; H, 6.58%; Found: C, 69.12; H, 6.51%.

(E)-3-(Ethoxycarbonyl)-4-(4-ethoxyphenyl)-4-(4-methoxyphenyl)but-3-enoic acid, 2b

Obtained from (4-ethoxyphenyl)(4-methoxyphenyl) methanone (1b) and diethyl succinate in 60% yield, m. p. 101-104 °C. IR (Nujol, γ cm^{-1}): 3022 (b) (OH str), 1745 (s) (ester C=O str), 1724 (s) (acid C=O str), 1665 (w) (C=C str), 1225 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.210 (t, 3H, CH_3), 1.324 (t, 3H, CH_3), 3.668 (s, 2H, CH_2), 3.853 (s, 3H, OCH_3), 4.123 (q, 2H, OCH_2), 4.254 (q, 2H, OCH_2), 6.978 (dd, 4H, Ar-H), 7.241 (dd, 4H, Ar-H), 10.598 (s, 1H, COOH). ^{13}C NMR ($CDCl_3$): δ14.10 (1C, CH_3), 14.68 (1C, CH_3), 35.18 (1C, C-2), 55.82 (1C, OCH_3), 61.20 (1C, OCH_2), 63.76 (1C, OCH_2), 114.28 (4C, Ar-C), 115.80 (1C, C-3), 128.90 (4C, Ar-C), ^{13}2.56 (2C, Ar-C), 157.40 (2C, Ar-C), 158.66 (1C, C-4), 169.04 (1C, COO), 174.40 (1C, COOH). MS (m/z): 384 (M^+, 25), 356 (30), 312 (28), 294 (100), 266 (51). Anal. Calcd. for $C_{22}H_{24}O_6$: C, 68.74; H, 6.29%; Found: C, 68.62; H, 6.10%.

(Z)-4-(3,4-Dimethoxyphenyl)-3-(ethoxycarbonyl)-4-(4-ethoxyphenyl)but-3-enoic acid, 2c

Obtained from (3,4-dimethoxyphenyl)(4-ethoxyphenyl) methanone (**1c**) and diethyl succinate in 52% yield, m. p. 96-97 °C. IR (Nujol, γ cm^{-1}): 3000 (b) (OH str), 1740 (s) (ester C=O str), 1721 (s) (acid C=O str), 1668 (w) (C=C str), 1220 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.208 (t, 3H, CH_3), 1.356 (t, 3H, CH_3), 3.555 (s, 2H, CH_2), 3.866 (s, 6H, OCH_3), 4.008 (q, 2H, OCH_2), 4.212 (q, 2H, OCH_2), 6.901-7.240 (m, 7H, Ar-H), 10.721 (s, 1H, COOH). ^{13}C NMR ($CDCl_3$): δ14.14 (1C, CH_3), 14.71 (1C, CH_3), 35.26 (1C, C-2), 55.90 (2C, OCH_3), 61.26 (1C, OCH_2), 63.67 (1C, OCH_2), 109.19 (1C, Ar-C), 112.04 (1C, Ar-C), 114.20 (2C,

Ar-C), 115.92 (1C, C-3), 122.30 (1C, Ar-C), 129.06 (2C, Ar-C), 133.14 (1C, Ar-C), 132.88 (1C, Ar-C), 148.70 (2C, Ar-C), 157.12 (1C, Ar-C), 159.34 (1C, C-4),168.54 (1C, COO), 174.88 (1C, COOH). MS (m/z): 414 (M^+, 24), 386 (33), 342 (28), 324 (100), 296 (47). Anal. Calcd. for $C_{23}H_{26}O_7$: C, 66.65; H, 6.32%; Found: C, 66.45; H, 6.22%.

(Z)-4-(3,4-Dimethoxyphenyl)-3-(ethoxycarbonyl)-4-(4-methoxyphenyl)but-3-enoic acid, **2d**

Obtained from (3,4-dimethoxyphenyl) (4-methoxyphenyl) methanone (1d) and diethyl succinate in 53% yield, m. p. 115-117 °C. IR (Nujol, γ cm^{-1}): 3015 (b) (OH str), 1742 (s) (ester C=O str), 1721 (s) (acid C=O str), 1667 (w) (C=C str), 1200 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.221 (t, 3H, CH_3), 3.604 (s, 2H, CH_2), 3.850 (s, 9H, OCH_3), 4.128 (q, 2H, OCH_2), 6.889-7.312 (m, 7H, Ar-H), 10.703 (s, 1H, COOH). ^{13}C NMR ($CDCl_3$): δ14.34 (1C, CH_3), 34.98 (1C, C-2), 55.85 (3C, OCH_3), 61.44 (1C, OCH_2), 109.12 (1C, Ar-C), 112.30 (1C, Ar-C), 114.28 (2C, Ar-C), 115.98 (1C, C-3), 121.96 (1C, Ar-C), 129.10 (2C, Ar-C), 133.20 (2C, Ar-C), 148.82 (2C, Ar-C),157.40 (1C, Ar-C), 158.56 (1C, C-4), 168.50 (1C, COO), 174.67 (1C, COOH). MS (m/z): 400 (M^+, 18), 372 (30), 328 (40), 310 (100). Anal. Calcd. for $C_{22}H_{24}O_7$: C, 65.99; H, 6.04%; Found: C, 65.78; H, 6.12%.

(Z)-3-(Ethoxycarbonyl)-4-(3-methoxyphenyl)-4-(4-methoxy phenyl) but-3-enoic acid, **2e**

Obtained from (3-methoxyphenyl)(4-methoxyphenyl) methanone (1e) and diethyl succinate in 55% yield, m. p. 122-124 °C. IR (Nujol, γ cm^{-1}): 2980 (b) (OH str), 1743 (s) (ester C=O str), 1714 (s) (acid C=O str), 1668 (w) (C=C str), 1188 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.218 (t, 3H, CH_3), 3.618 (s, 2H, CH_2), 3.862 (s, 6H, OCH_3), 4.131 (q, 2H, OCH_2), 6.894-7.402 (m, 8H, Ar-H), 10.733 (s, 1H, COOH). ^{13}C NMR ($CDCl_3$): δ14.20 (1C, CH_3), 34.52 (1C, C-2), 55.88 (2C, OCH_3), 61.40 (1C, OCH_2), 111.10 (1C, Ar-C), 112.76 (1C, Ar-C), 114.24 (2C, Ar-C), 115.12 (1C, C-3), 119.62 (1C, Ar-C), 129.14 (2C, Ar-C), 130.16 (1C, Ar-C), 133.21 (1C, Ar-C), 140.02 (1C, Ar-C), 157.66 (1C, Ar-C), 158.51 (1C, C-4), 160.62 (1C, Ar-C), 168.22 (1C, COO), 174.60 (1C, COOH). MS (m/z): 370 (M^+, 28), 342 (36), 298 (48), 280 (100). Anal. Calcd. for $C_{21}H_{22}O_6$: C, 68.10; H, 5.99%; Found: C, 68.01; H, 5.83%.

4,4-Bis(4-bromophenyl)-3-(ethoxycarbonyl)but-3-enoic acid, **2f**

Obtained from bis (4-bromophenyl) methanone (1f) and diethyl succinate in 58% yield, m. p. 133-135 °C. IR (Nujol, γ cm^{-1}): 2975 (b) (OH str), 1740 (s) (ester C=O str), 1718 (s) (acid C=O str), 1669 (w) (C=C str), 1176 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.266 (t, 3H, CH_3), 3.601 (s, 2H, CH_2), 4.122 (q, 2H, OCH_2), 7.118 (dd, 4H, Ar-H), 7.501 (dd, 4H, Ar-H), 10.796 (s, 1H, COOH). ^{13}C NMR ($CDCl_3$): δ14.26 (1C, CH_3), 34.20 (1C, C-2), 61.74 (1C, OCH_2), 115.34 (1C, C-3), 121.42 (2C, Ar-C), 128.64 (4C, Ar-C), 131.50 (4C, Ar-C), 140.14 (2C, Ar-C), 158.44 (1C, C-4), 167.69 (1C, COO), 174.14 (1C, COOH). Anal. Calcd. for $C_{19}H_{16}Br_2O_4$: C, 68.10; H, 5.99%; Found: C, 68.01; H, 5.83%.

(Z)-3-(ethoxycarbonyl)-4-(4-ethoxyphenyl)-4-(4-methoxy phenyl) but-3-enoic acid, **3b**

Obtained from (4-ethoxyphenyl)(4-methoxyphenyl)methanone (1b) and diethyl succinate in 38% yield, m. p. 87-89 °C. IR (Nujol, γ cm^{-1}): 2920 (b) (OH str), 1736 (s) (ester C=O str), 1700 (s) (acid C=O str), 1669 (w) (C=C str), 1112 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.212 (t, 3H, CH_3), 1.326 (t, 3H, CH_3), 3.670 (s, 2H, CH_2), 3.858 (s, 3H, OCH_3), 4.126 (q, 2H, OCH_2), 4.234 (q, 2H, OCH_2), 6.986 (dd, 4H, Ar-H), 7.278 (dd, 4H, Ar-H), 10.698 (s, 1H, COOH). ^{13}C NMR ($CDCl_3$): δ14.18 (1C, CH_3), 14.51 (1C, CH_3), 35.10 (1C, C-2), 55.88 (1C, OCH_3), 61.40 (1C, OCH_2), 63.70 (1C, OCH_2), 114.46 (4C, Ar-C), 115.65 (1C, C-3), 128.35 (4C, Ar-C), 132.58 (2C, Ar-C), 157.22 (2C, Ar-C), 158.34 (1C, C-4), 169.54 (1C, COO), 174.44 (1C, COOH). MS (m/z): 384 (M^+, 33), 356 (52), 338 (100), 310 (46). Anal. Calcd. for $C_{22}H_{24}O_6$: C, 68.74; H, 6.29%; Found: C, 68.46; H, 6.22%.

(E)-4-(3,4-Dimethoxyphenyl)-3-(ethoxycarbonyl)-4-(4-ethoxy phenyl)but-3-enoic acid, **3c**

Obtained from (3,4-dimethoxyphenyl)(4-ethoxyphenyl) methanone (**1c**) and diethyl succinate in 44% yield, m. p. 141-143 °C. IR (Nujol, γ cm^{-1}): 2935 (b) (OH str), 1739 (s) (ester C=O str), 1708 (s) (acid C=O str), 1668 (w) (C=C str), 1198 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.210 (t, 3H, CH_3), 1.342 (t, 3H, CH_3), 3.543 (s, 2H, CH_2), 3.812 (s, 6H, OCH_3), 4.108 (q, 2H, OCH_2), 4.234 (q, 2H, OCH_2), 6.944-7.222 (m, 7H, Ar-H), 10.739 (s, 1H, COOH). ^{13}C NMR ($CDCl_3$): δ14.09 (1C, CH_3), 14.86 (1C, CH_3), 34.66 (1C, C-2), 55.84 (2C, OCH_3), 61.31 (1C, OCH_2), 63.60 (1C, OCH_2), 110.10 (1C, Ar-C), 112.16 (1C, Ar-C), 114.45 (2C, Ar-C), 115.88 (1C, C-3), 122.78 (1C, Ar-C), 129.86 (2C, Ar-C), 132.84 (1C, Ar-C), 132.80 (1C, Ar-C), 148.90 (2C, Ar-C), 157.33 (1C, Ar-C), 159.39 (1C, C-4), 168.51 (1C, COO), 174.80 (1C, COOH). MS (m/z): 414 (M^+, 20), 386 (18), 368 (100), 340 (43). Anal. Calcd. for $C_{23}H_{26}O_7$: C, 66.65; H, 6.32%; Found: C, 66.42; H, 6.14%.

(E)-4-(3,4-Dimethoxyphenyl)-3-(ethoxycarbonyl)-4-(4-methoxyphenyl)but-3-enoic acid, **3d**

Obtained from (3,4-dimethoxyphenyl)(4-methoxyphenyl) methanone (1d) and diethyl succinate in 45% yield, m. p. 118-119 °C. IR (Nujol, γ cm^{-1}): 2943 (b) (OH str), 1737 (s) (ester C=O str), 1710 (s) (acid C=O str), 1669 (w) (C=C str), 1184 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.230 (t, 3H, CH_3), 3.624 (s, 2H, CH_2), 3.858 (s, 9H, OCH_3), 4.120 (q, 2H, OCH_2), 6.870-7.340 (m, 7H, Ar-H), 10.770 (s, 1H, COOH). ^{13}C NMR ($CDCl_3$): δ14.38 (1C, CH_3), 34.72 (1C, C-2), 55.86 (3C, OCH_3), 61.40 (1C, OCH_2), 109.66 (1C, Ar-C), 112.09 (1C, Ar-C), 114.55 (2C, Ar-C), 115.75 (1C, C-3), 121.44 (1C, Ar-C), 129.19 (2C, Ar-C), 133.23 (2C, Ar-C), 148.68 (2C, Ar-C), 157.57 (1C, Ar-C), 158.59 (1C, C-4), 168.70 (1C, COO), 174.61 (1C, COOH). MS (m/z): 400 (M^+, 41), 372 (24), 354 (100). Anal. Calcd. for $C_{22}H_{24}O_7$: C, 65.99; H, 6.04%; Found: C, 65.86; H, 6.10%.

(E)-3-(Ethoxycarbonyl)-4-(3-methoxyphenyl)-4-(4-methoxyphenyl) but-3-enoic acid, **3e**

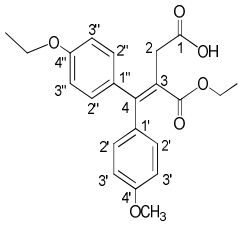
Obtained from (3-methoxyphenyl)(4-methoxyphenyl) methanone (**1e**) and diethyl succinate in 41% yield, m. p. 119-121 °C. IR (Nujol, γ cm^{-1}): 2934 (b) (OH str), 1738 (s) (ester C=O str), 1712 (s) (acid C=O str), 1668 (w) (C=C str), 1202 (s) (C-O str). ^{1}HNMR ($CDCl_3$): δ 1.220 (t, 3H, CH_3), 3.602 (s, 2H, CH_2), 3.865 (s, 6H, OCH_3), 4.140 (q, 2H, OCH_2), 6.887-7.433 (m, 8H, Ar-H), 10.754 (s, 1H, COOH). ^{13}C NMR ($CDCl_3$): δ14.22 (1C, CH_3), 34.56 (1C, C-2), 55.84 (2C, OCH_3), 61.46 (1C, OCH_2), 111.67 (1C, Ar-C), 112.70 (1C, Ar-C), 114.92 (2C, Ar-C), 115.86 (1C, C-3), 119.60 (1C, Ar-C), 129.74 (2C, Ar-C), 130.56 (1C, Ar-C), 132.26 (1C, Ar-C), 140.32 (1C, Ar-C), 157.61 (1C, Ar-C), 158.82 (1C, C-4), 160.93 (1C, Ar-C), 168.37 (1C, COO), 174.63 (1C, COOH). MS (m/z): 370 (M^+, 45), 342 (26), 324 (100). Anal. Calcd. for $C_{21}H_{22}O_6$: C, 68.10; H, 5.99%; Found: C, 68.16; H, 5.81%.

Structure proofs of the synthesized new compounds were provided by IR, ^{1}H NMR, ^{13}C NMR, MS studies and elemental analysis. The stretching frequencies in the IR spectrum of the compounds 2(a-f) and 3(b-e) showed a strong absorption bands in the region 1748-1736 $cm^{-1}$and 1725-1700 cm^{-1} for ester and acid C=O bonds respectively. A strong and intense absorption band absorbed in the region 1226-1112 $cm^{-1}$was assigned to C-O bonds. A broad absorption band in the region 3030-2920 cm^{-1} and weak band in the region 1667-1669 cm^{-1} were assigned to-OH and C=C stretching respectively.

In ^{1}H NMR spectra, the compounds 2(a-f) showed a consistent pattern signals due to COOH protons as singlets in the region δ 10.556-10.796 ppm., CH_2 protons of the side chain in the region δ 3.555-3.668 ppm. While the compounds 3(b-e) showed a consistent pattern signals due to COOH protons as singlets in the region δ 10.698-10.770 ppm., two CH_2 protons of side chain in the region δ 3.543-3.670 ppm, respectively. All these compounds showed signals due to aromatic and substituent protons in the expected region.

The structural assignments for the compounds 2(a-f) were made by ^{13}C NMR analysis by considering (E)-3-(ethoxycarbonyl)-4-(4-ethoxyphenyl)-4-(4-methoxyphenyl)but-3-enoic acid, 2b as the representative compound. In its ^{13}C NMR spectra, signals appeared at δ 174.40 ppm., δ 169.04 ppm were assigned to–COOH and–COO carbons respectively. The signals appeared at δ 35.18 ppm., δ 115.80 ppm. and δ 158.66 ppm., were assigned to C-2, C-3 and C-4 carbons. The signals observed at δ 14.10, 14.68, 55.82, 62.20 and 63.76 ppm were assigned to the two CH_3, OCH_3 and two OCH_3 carbons. Further, an array of signals observed at δ 114.28 ppm, 128.90 ppm, 132.56 ppm and 157.44 ppm were assigned to aromatic carbon atoms fig.1.

All the synthesized compounds 2(a-f) showed the similar ^{13}C NMR signals.



δ (ppm): 14.10 (CH_3), 14.68 (CH_3), 35.18 (C-2), 55.82 (OCH_3), 61.20 (OCH_2), 63.76 (OCH_2), 114.28 (4C, 3',3"-C), 115.80 (C-3), 128.90 (4C, 2',2"-C), 132.56 (2C, 1',1"-C), 157.40 (2C, 4',4"-C), 158.66 (C-4), 169.04 (COO), 174.40 (COOH).

Fig. 1: Carbon chemical shifts and couplings of 2b

In ^{13}C NMR, the compounds 3(a-e) showed a consistent pattern signals due to C-2, C-3 and C-4 carbons in the region δ 34.56-35.10 ppm., δ 115.65-115.88 ppm., and δ 158.34-159.39 ppm. While the signals due to COOH, COO of ester carbons in the region δ 174.44-174.80 ppm., δ 168.37-169.51 ppm respectively. Further they showed the signals due to the aromatic ring and in substituent carbons at the expected region.

Significantly stable molecular ion peaks were observed in mass spectra of synthesized compounds. The common possible fragmentation involves with the removal of CH_2=CH_2, CO_2, and H_2O etc. The compound 2(a-f) showed their base peaks at (M-90), while the compounds 3(b-e) showed the base peaks at (M-46). These spectral studies confirm the structures of the synthesized new products. Further their structures were supported by satisfactory elemental analysis.

The results of antifungal activity of the synthesized compounds 2(a-f) and 3(b-e) tested against different bacteria species were summarized in fig.2.

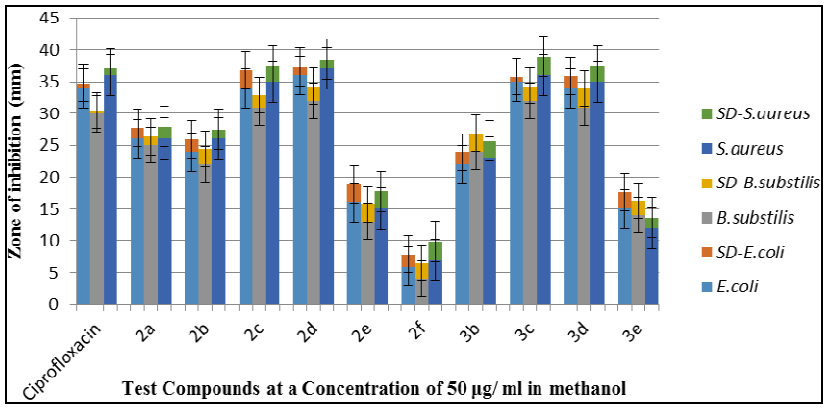


Fig. 2: Antibacterial activity of the synthesized compounds 2(a-f) and 3(b-e) at a concentration of 50 µg/ml in methanol/water tested against the bacterial strains. [Ciprofloxacin=25 µg/disc]

The antibacterial activity results of the synthesized compounds 2(a-f) and 3(b-e) revealed that all compounds exerted moderate to excellent activity against the tested organisms, except 4,4-bis(4-bromophenyl)-3-(ethoxycarbonyl)but-3-enoic acid 2f, that contain a bromo substitution on both the aromatic ring showed very poor inhibition against all the tested organisms. The compounds 2c, 2d, 3c and 3d which contain a strong electron donating OCH_3 substitution showed excellent activity in comparison with the standard. The compounds 2a, 2b and 3b showed good, while the compounds 2e and 3e moderate antibacterial activity against the tested organisms. The results of antifungal activity of the synthesized compounds 2(a-f) and 3(b-e) tested against different fungi species were summarized in fig.3.

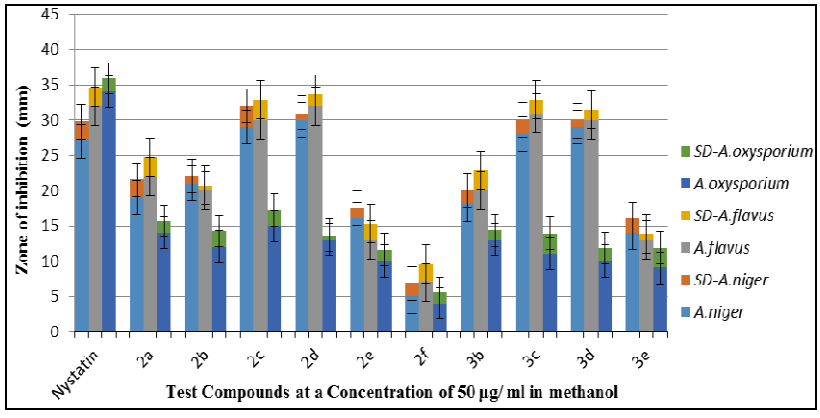


Fig. 3: Antifungal activity of the synthesized compounds 2(a-f) and 3(b-e) tested against the fungal strains [Nystatin=25 µg/disc]

The antibacterial activity results of the synthesized compounds 2(a-f) and 3(b-e) revealed that all compounds exerted moderate to excellent activity against *A. niger* and *A. flavus.* However, all showed poorer activity against *F. oxysporium* organism. The compound 4,4-bis(4-bromophenyl)-3-(ethoxycarbonyl)but-3-enoic acid 2f, that contain a bromo substitution showed very poor inhibition against all the tested organisms. The compounds 2c, 2d, 3c and 3d which contain a strong electron donating OCH_3 substitution showed excellent activity in comparison with the standard against *A. niger* and *A. flavus.* The compounds 2a, 2b and 3b showed good, while the compounds 2e and 3e moderate antibacterial activity against the *A. niger* and *A. flavus* organisms.

CONCLUSION

The accessible procedure for the synthesis of 4-(4-aryl)-4-(4-aryl)-3-ethoxycarbonyl-but-3-enoic acids (2a, 2f) and a mixture of E and Z-isomers of 4,4-diaryl-3-ethoxycarbonyl-but-3-enoic acids 2(b-e) and 3(b-e) in relatively good yields and efficacy of some of the molecules as antimicrobial agents validates the significance of this study. Among the series, the compounds 2c, 2d, 3c and 3d acts as potential antifungal and antibacterial agents.

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CONFLICT OF INTERESTS

Declare None

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