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Synthesis, Anti-Microbial Studies and Molecular Docking Studies of 2-Methyl-2-((2-Methyl-2-Phenoxypropanoyl)Oxy)Propanoic Acid Derivatives

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ABSTRACT

A simple and efficient protocol has been utilized to synthesis 2-methyl-2-((2-methyl-2-phenoxypropanoyl)oxy)propanoic acid derivatives using phenol and substituted phenol. Biological activities and molecular docking studies of these compounds were found to be effective against various microbial and docking pathogens of human and plants.

1. Introduction

In the past few years the synthesis and screening of small molecules based on natural products as templates have attracted many researchers all around the world [1]. Among the natural products, phenol or substituted phenol derivatives are well known for their anti-microbial activities and molecular docking studies 2-methyl-2-((2-methyl-2-phenoxypropanoyl)-oxy)propanoic acid moiety (**2**) [2], the structural core of phenol or substituted phenol is often found in more complex products which is frequently associated with biological activities and molecular docking studies , such as, obesity and cardiovascular drugs and anti-fungal activity [3]. The substituted phenol or phenol derivatives its potent anti HIV activity Fig. 1. Among phenol substituted compound and choline salt (**4**) is one of the most explored molecules [4]. Ramalakshmi et al have been reported that phenol derivatives can act as mGluR2 positive allosteric modulators (PMAMs) [5]. Recently novel phenol derivatives are known to act to a potent anti-fungal agent against Mtb H37Rv.Our research group is interested in the synthesis of alpha phenoxy isobutyric acid derivatives through simple and efficient methodologies [6]. Here, we present a facile methodology for synthesis of 2-methyl-2-((2-methyl-2-phenoxy propanoyl)oxy)propanoic acid frame work and its anti-microbial properties [7] and molecular docking studies against human and plant pathogens.

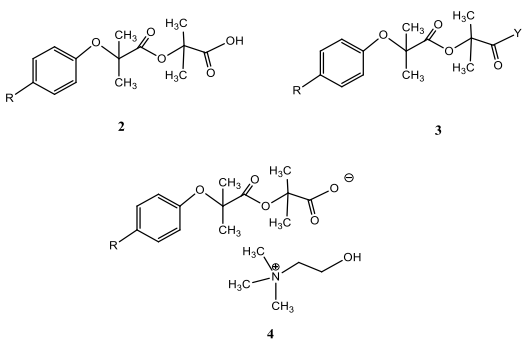

Fig. 1 α-phenoxy isobutyric acid, ester and choline salt derivative

2. Result and Discussion

The synthesis of 2-methyl-2-((2-methyl-2-phenoxypropanoyl)oxy) propanoic acid derivatives is well known in the literature and researchers and commercial users Bargellinc reaction have contributed significantly [8]. The synthesis of 2-methyl-2-((2-methyl-2-phenoxypropanoyl)-oxy) propanoic acid derivatives and choline salt (**4**) through the esterfication of phenyl derivatives did not afford the expected products [9]. Thereby adoptiding a modified literature protocol [10]. We have synthesized phenoxy derivatives from phenol (**1a-f**) or substituted phenol and acid derivatives **(2a-f)** [11]. The Bargellinic reaction phenol was condensed with acetone and chloroform in the presence of strong base to produce a sterically hindered α-phenoxy – isobutyric acid derivatives [12]. The synthesis of α-phenoxy – isobutyric acid derivatives strategy utilizes the Bargellinic reaction as the key bond forming step [13]. Several compounds were prepared by this route. The mechanism for this unusual condensation reaction was investigated [14]. Synthetic tool in organic synthesis and various nucleophiles and ketones have been employed in place of classic form (Fig. 2). In this manner it has potentially been a constructive lead for the organic chemists.

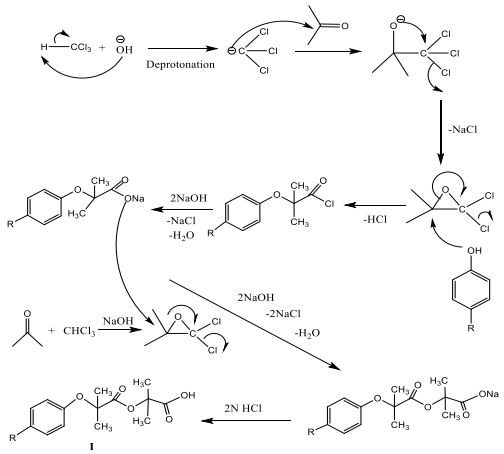

Fig. 2 Bargellinic type reaction dichloroepoxide mechanism

Intermediate are formed by the reaction of chloroform and ketones which are potentially active toward regioselective nucleophile attack

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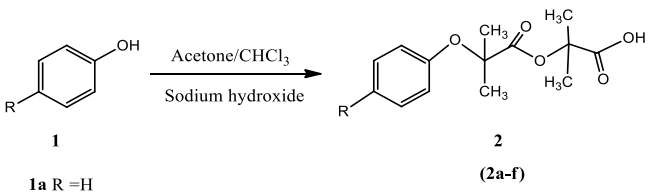
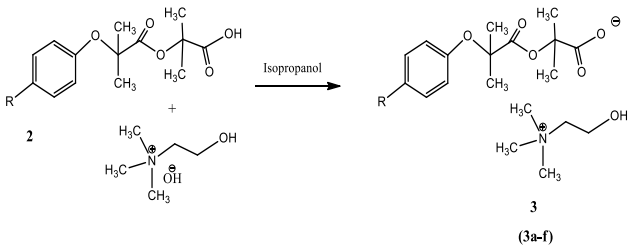
Scheme 1. The recent years have witnessed an increased activity in the area of α-phenoxy – isobutyric acid derivatives synthesis. α-phenoxy – isobutyric acid derivatives are persistent free radicals that exhibit remarkable stability primarily due to the absence of dimerization and disproportionation. Their unique properties have sparked considerable interest from theoretical, chemicals and biological standpoints [15].

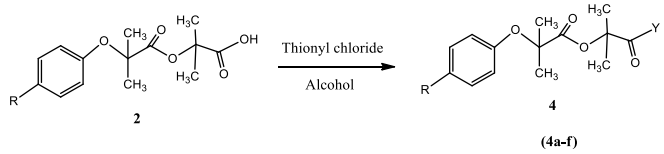


1a R =H
1b R =CH_3CH_2CO
1c R =C_6H_5CO
1d R =$CH_3(CH_2)_4CO$
1e R =$CH_3(CH_2)_6CO$
1f R =CH_3CO

Scheme 1 Regioselective nucleophilic attach reaction mechanism



Scheme 2 Synthesis choline salt of α-phenoxy isobutyric acid



Scheme 3 Synthesis ester of α-phenoxy isobutyric acid and its derivative

The ester derivatives are the most well-known phenoxy derivatives in the field of medicinal chemistry [16]. Alpha phenoxy isobutyric acid, thinoyl chloride was added then refluxes for 3 hours. The reaction mixture was distilled out at reflux temperature. The resulting suspension was quenched with benzene then the reaction mixture was distilled out at reflux temperature to remove the traces of thonlyl chloride. The resulting mixture was charged concern alcohol and pyridine as a base. The reaction mixture was heated to reflux for half an hour at reflux temperature [17]. The reacton mixture cooled to ambinent tempreatue extracted with ether washed aqueous bicarbonate solution and brine, dired over anhydrous mangnesium sulfate filtered and evaporated under reduced pressure to give the pure product Table 1 **(4a-f)**.

Table 1 Esters of α-phenoxy isobutyric acid and its derivatives

Compound	R	Y	Yield
4a	H	$OCH(CH_3)_2$	89%
4b	CH_3CH_2CO	OCH_3	55%
4c	C_6H_5CO	OC_2H_5	46%
4d	$CH_3(CH_2)_4CO$	$OCH(CH_3)_2$	45%
4e	$CH_3(CH_2)_6CO$	OC_2H_5	56%
4f	CH_3CO	OC_2H_5	41%

In connection with some work on the bactericidal properties of lecithins the author had occasion to prepare some of the salts of choline [18]. In looking through the literature it was found that the choline used in recent investigations was prepared and isolated by methods which it was thought might be simplified. In a second aspect, the present invention relates to novel salt of substituted alpha-phenoxy – isobutyric acids and derivatives preparation that are exhibit photo stability when compared to other salts of substituted alpha-phenoxy – isobutyric acid derivatives. These salts are useful for pharmaceutical formulations in a form of molecular dispersions that contain at least one of these novel salts. These novel salts can be used to treat hyperlipidemia or coronary heart diseases [19]. Their unique properties have sparked considerable interest from theoretical chemicals and biological stand points. The preparation of α-phenoxy isobutyric acid derivatives of choline salt, α-phenoxy isobutyric acid derivatives and choline hydroxide solution or choline chloride were added. The product crystallized out of solution and the slurry was mixed at reflux temperature for half an hour. Then reaction mixture stirred at ambient temperature for several hours [20]. The solution was filtered and the rinsed with isopropanol. The solid was dried in vacuum oven for several hours.

In our initial study choline salt preparation (**4a**) shows a better yield at reflux temperature but its ambient temperature [21]. Among several solvents studied isopropanol served as the prime solvent in (**4a-f**) choline salt mechanics. All the compounds synthesized were further analyzed without purification and exhibited nearly 98% pure [22].

On the basis of the results summarized synthesis of alpha phenoxy derivatives are feasible in a shot reaction time at ambient temperature with optimal purity [23]. As a result the identified synthetic strategy not only overcomes harsh and long reaction procedures but also overcomes time consuming purification techniques making them more attractive among the other procedures.

All synthesized compounds were characterized based on ^1H, ^{13}C NMR and elemental studies. Alpha phenoxy isobutyric acid derivatives (2a) resonates as a singlet broad in the region § 12.1-11.5 ppm corresponding to the –COOH proton. Similarly aromatic region §7.2-7.00 two proton doublet § 6.9-6.7 two proton triplet in region of §6.5 for 1 proton triplet. In the aliphatic region proton 2.7§ for 12 proton singlet. If choline salt present in the molecule the aliphatic region proton § 4.3 multiplet shows 2H §3.49 multiplets shows 2H, § 3.17 singlet shows 9H. Similarly the corresponding ^{13}C NMR spectra of the alpha phenoxy isobutyric acid of § 23.1- 54 ppm and § 69-92 ppm respectively. The aromatic region shows § 114.3-159.5 ppm. The ^{13}C spectra of the corresponding carbonyl group of alpha phenoxy isobutyric acid derivatives compounds resonates in the region § 175.7-202.4 ppm. The mass spectral data also were in accordance to the synthesized compounds

3.1 Antimicrobial Activity

3.1.1 Test Organisms Used

Staphylococcus aureus (96) *Micrococcus luteus (106)*	Gram positive bacteria
Enterobacter aerogenes (111) *Yersinia enterocolitica (840)*	Gram Negative bacteria
Staphylococcus aureus (841)	Gram positive bacteria
Micrococcus luteus (842) *Enterobacter aerogenes (843)* *Yersinia enterocolitica (844)*	Gram Negative bacteria
Staphylococcus aureus (854) *Micrococcus luteus (857)*	Gram positive bacteria
Aspergillus aureus (AA) *Candida albicans (CA)*	Fungal strains

Note: The number refers the culture code given by MTCC (Microbial Type Culture Collection), Chandigarh, India.

3.1.2 Concentration of the Sample Used

A weight of 120 mg of the given sample was dissolved in 300 µL of DMF. The 25 µL of the dissolved sample was loaded to the disc will contain 10 mg/disc concentration (Table 2, Figs. 3 and 4). DMF refers to Dimethyl Formamide.

Table 2 Anti-microbial activity of α-phenoxy isobutyric acid and its derivatives

S.No	Pathogen No.	1 (mm)	2 (mm)	3 (mm)	4 (mm)	5 (mm)	6 (mm)	CONTROL (mm)
3a	96	12	14	16	-	-	-	19
3b	106	-	-	-	-	-	-	22
3c	111	-	8	10	-	-	-	17
3d	840	-	-	-	-	-	-	19
4a	841	-	-	-	-	-	-	20
4b	842	-	-	-	-	-	-	18
4c	AA	-	-	-	-	-	-	15
4d	CA	-	-	-	-	-	-	13

Note: 1- 3a, 2- 3b, 3- 3c, 4- 3d, 5-4a, 6-4b, 7-4c, 8-4d Control-Streptomycin (for bacteria), Fluconazole (for Fungi).

Fig. 3 Spot 1, 2, 3, 4 relations with Compound **-3a, 3b, 3c, 3d**. (Yellow color spot (Active); white to off-white color spot (Inactive))

Fig. 4 Spot 1, 2, 3, 4 relations with Compound -4a, 4b, 4c, 4d. (Yellow color spot (Active); white to off-white color spot (Inactive))

3.2 Molecular Docking Study

The present invention will be described in two different aspects. Each of these two aspects of the present invention is treated separately under different headings for the convenience of the reader and should not be construed as limiting the present invention in any way. These headings are "Molecular studies of 2-methyl-2-((2-methyl-2-phenoxypropanoyl)oxy) propanoic acid derivatives [24], Physiologically in the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functions [25]. Docking of a small molecule (green) into the crystal structure (PDB: 3SN6) of the beta-2 adrenergic receptor. The associations between biologically relevant molecules such as proteins, nucleic acids, carbohydrates, and lipids play a central role in signal transduction. Furthermore, the relative orientation of the two interacting partners may affect the type of signal produced (e.g., agonism vs antagonism). Therefore, docking is useful for predicting both the strength and type of signal produced.

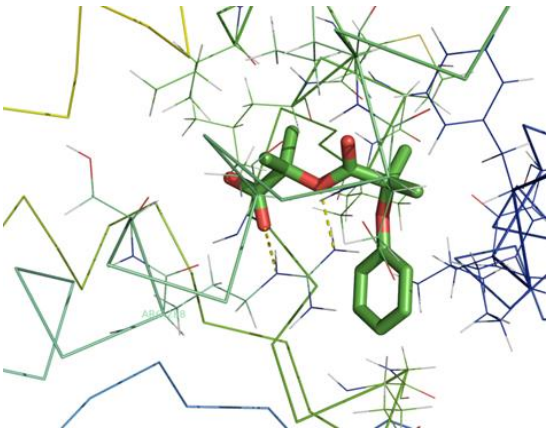


Fig. 5 methyl-2-((2-methyl-2-phenoxypropanoyl)oxy)-propanoic acid bonded with protein

3.2.1 Docking Analysis

The docking analysis was performed by Auto dock tools (Sanner 1999) (ADT) v 1.5.6 and Autodock v 4.2 programs; (Autodock, Autogrid, Autotors, Copyright-1991-2000) from the Scripps Research Institute http://www.scripps.edu/mb/olson/doc/autodock. The compound choline was docked with selected target protein; the protein molecule considered as a rigid body and the ligands being flexible. Affinity maps for all the atom types present, as well as an electrostatic map were computed with a grid spacing of 0.375 Å. The search was carried out with the Lamarckian Genetic Algorithm; populations of 150 individuals with a mutation rate of 0.02 were evolved for 10 generations. Evaluation of the results was done by sorting the different complexes with respect to the predicted binding energy. All docking simulation steps were followed by Balamurugan et al [26]. The ligand-protein interactions of all selected compound was analyzed by PyMol molecular viewer (The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC). The hydrophobic effect of ligands was developed by Pose View. This Applet provides interactive on-line prediction of protein-ligand interaction and environmental chemistry studies (Stierand and Rarey 2010).

The best possible binding modes of the Choline at the target protein's active sites are displayed in Fig. 5 and their corresponding energy values, inhibition constant values and ligand efficiency are listed in Table 3.

Table 3 Molecular docking studies of α-phenoxy isobutyric acid

Ligand	Protein PDB ID	Binding amino acid Residues	Binding Energy (kcal/mol)	Inhibition Constant uM	RMS D (Å)	Ligand efficiency
Choline	2PRG (PPAR gamma)	ARG`288/NE/ 1HH2 with 24 atoms	-5.92	45.73	48.37	0.31

Fig. 5 depicts the binding pattern of choline (ligand) with PPAR Gamma; it showed that ligand formed an extensive hydrogen bond network with ARG`288/NE/1HH2 with 24 atoms and its corresponding energy value is -5.92 kcal/mol.

3. Experimental Methods

2.1 Antibacterial Activity (Protocol)

Antibacterial activity was carried out using disc-diffusion method [28]. Petri plates were prepared with 20 mL of sterile Mueller Hinton Agar (MHA) (Hi-media, Mumbai). The 24 hrs grown cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. The tests were conducted at 10 mg concentrations of the given sample. The 25 µL of the sample was loaded to the disc and were placed on the surface of the medium and left for 30 min at room temperature for diffusion. Streptomycin (10 µg/disc) was used as positive control. The plates were incubated for 24 hr at 37 °C zone of inhibition was recorded in millimeters.

2.1.1 Antifungal Activity (Protocol)

Antifungal activity was performed according to the standard NCCLS method. Petri plates were prepared with 20 mL of sterile Sabouraud Dextrose Agar (SDA) (Hi-media, Mumbai). The 48 hrs grown cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. The tests were conducted at 10mg concentrations of the given sample. The 25 µL of the sample was loaded to the disc and were placed on the surface of the medium and left for 30min at room temperature for diffusion. Fluconazole was used as positive control. The plates were incubated for 48 hrs at 28 °C. Zone of inhibition was recorded in millimeters.

2.2 Ligand Preparation

Chemical structure of the ligand choline was taken from Pub Chem compound database http://www.ncbi.nlm.nih.gov/pccompound. Three dimensional structures for Choline Fig.6 were generated using Chem Draw Ultra, 11.0. The ligand molecules were structurally confirmed and energy minimized using PRODRG server.

2.2.1 Target Protein Selection

The crystallographic three dimensional structure of selected target protein PPAR Gamma (PDB ID: 2PRG) was retrieved from the Protein Data Bank (PDB) http://www.pdb.org. The Apo protein molecule was selected for docking analysis. The probable binding sites of preferred target receptors were searched using CastP server. It is important to keep the predicted ligand binding site as small as possible without compromising accuracy for a range of applications such as molecular docking, de novo drug design and structural identification and comparison of functional sites [27].

Liquid substances were distilled prior to use. Melting points were uncorrected. 1 HNMR spectra were measured on a Bruker Avance 400 (400 MHz) spectrometer using TMS as the internal standard. Elemental analysis were measured on a (HERAEUS CHNO, Rapid) analyzer. Sonication were performed in Shanghi Branson- CQX ultrasonic cleaner (with a frequency of 25 KHz and a nominal power 250 W) and SK 250 LH ultrasonic cleaner (with a frequency of 40 KHz, 59 KHz and nominal power

250 W Shanghai Kudos ultrasonic Instrument Co., Ltd.,) The reaction flask were located in the cleaner where the surface of reactants is slightly lower than the level of the water. The reaction temperature was controlled by addition or removal of water from ultrasonic bath.

2.3 Preparation-1

The preparation of α-phenoxy isobutyric acid derivatives (Scheme 1) phenol (21.2 mmol), acetone (20 mL), sodium hydroxide 215 mmol) were mixed in a 50 mL round bottomed flask. The reaction mixture was stirred at room temperature for a period of 0.5 hrs. A mixture chloroform 127 mmol and acetone 10 mL were added over a period of 3 hrs. Exothermic were observied controlled the reaction mixture below 55 °C. The reaction mixture was stirred at 53-55 °C for 40-52 hrs. After the completion of the reaction, distilled under reduced pressure. The resulting suspension was quenched with 40 mL water, the reaction mixture was extracted with diethylether (3x10 mL). The organic layer was discard. The obtained aqueous layer to added 2N hydrochloric acid adjusted the pH= 3 to 4. The solid was filtered and give the crude product solid which was separated by column chloromatography on silica (200-300mesh), eluted with ethyl acetate and n-hexane (incorporated in Table 4 (**2a-f**))

Table 4 Reaction time, yield comparison of α-phenoxy isobutyric acids

Compound	R	Yield	Time/ hours	Compound	R	Yield
2a	H	82%	48	**3a**	H	90%
2b	CH_3CH_2CO	65%	45	**3b**	CH_3CH_2CO	65%
2c	C_6H_5CO	75%	44	**3c**	C_6H_5CO	75%
2d	$CH_3(CH_2)_4CO$	55%	52	**3d**	$CH_3(CH_2)_4CO$	82%
2e	$CH_3(CH_2)_6CO$	68%	41	**3e**	$CH_3(CH_2)_6CO$	68%
2f	CH_3CO	73%	48	**3f**	CH_3CO	73%

2.4 Preparation -2

The preparation of α-phenoxy isobutyric acid ester derivatives Scheme 3, α-phenoxy isobutyric acid (preparation-1) (1.87 mmol), and thionyl chloride 5 mL were added then reflux for 3 hrs. The reaction mixture was distilled out at reflux temperature. The resulting suspension was quenched with benzene 5 mL the reaction mixture was distilled out at reflux temperature to remove the traces of thionyl chloride. The resulting mixture was charged isopropanol (2 mL) and pyridine (1.83 mmol) the reaction mixture was heated to reflux for 0.5 hrs at 65 °C. The reaction mixture was cooled to room temperature. The reaction mixture was extracted with diethylether (3x10 mL). The combined organic layers were washed aqueous bicarbonate (NaHCO3) solution and brine, dried over anhydrous magnesium sulfate for 2 hours and filtered; diethyl ether was evaporated under reduced pressure to give the pure product (**4a-f**).

2.5 Preparation-3

The preparation of α-phenoxy isobutyric acid derivatives Scheme 3 of choline salt, α-phenoxy isobutyric acid derivatives 3.75 mmol isopropanol 10 mL were mixed in a 50 mL round bottomed flask at 65 °C for 30-60 minutes. The reaction mixture to get clear solution. A solution of choline hydroxide in methanol (4.13 mmol, 45 Wt%) was diluted with 5mL isopropanol and approximately two thirds of the solution was added to the α-phenoxy isobutyric acid derivatives suspension. The reaction mixture was stirred at 30 minutes at 65 °C. The remaining one third of the choline hydroxide solution was added followed by a 4.5 mL rinse of the addition funnel with isopropanol. After 30 minutes solid was thrown out. The product crystallized out of solution and the slurry was mixed at 65 °C for half an hour. The reaction mixture cooled to 20-25 °C, over 5 hours. Then reaction mixture stirred at 20-25 °C for overnight. The product was filtered off and rinsed with 5mL isopropanol. The solid was dried in a vacuum oven at 35-40 °C for 24 hours. The dry weight of solid was 1.25 g or 90% yield (**3a-f**).

2.6 Preparation-4 Alternate Method for Making Choline Salt (Using Choline Chloride)

The preparation of α-phenoxy isobutyric acid derivatives of choline salt, α-phenoxy isobutyric acid derivatives 3.75 mmol and sodium bicarbonate 3.75 mmol were suspended in methanol 10 mL were mixed in a 50 mL round bottomed flask at 55 °C to dissolved the solids. A solution of choline chloride 4.13 mmol in 5 mL of methanol was added. The solution was filtered to remove the precipitated sodium chloride and the filter rinsed with 5 mL of methanol. The filtrate was diluted with 10 mL of isopropanol and concentrated to a volume of approximately 30 mL. The solution was filtered and the rinsed with 5 mL of isopropanol. The filtrate was concentrated to solid residue weighing 2.4 g. The residue was suspended in 10 mL of isopropanol and heated to 65 °C for 30-60 minutes cooled to 20-25 °C over 5 hours, and mixed at 20-25 °C for 24 hours. The product was filtered off and rinsed with 5 mL of isopropanol. The solid was dried in vaccum oven at 50 °C for 24 hours. The dry weight of solid was 1.1 g or 79.3% yield.

(**2a**): 2-methyl-2-((2-methyl-2-phenoxypropanoyl)oxy)propanoic acid. Melting point 95-97 °C. ^{1}H NMR (400 MHz, $CDCl_3$), §12.1-11.5 (broad, s, 1H), 7.2-7.00 (d, 11.2 Hz, 2H), 6.9-6.7 (t, 12 Hz, 2H) 6.5 (t, 9.2 Hz, 1H), 1.45 (s, 12H); ^{13}C NMR: 24, 25, 81, 91, 114, 120, 129, 155, 171, 175: m/z : 266, 265, 251, 248, 248, 238, 236, 221, 206, 179, 173, 134, 116, 93, 87, 65, 60, 45, 30, 28, 18; Anal. Calcd for $C_{14}H_{18}O_5$: C 63.16, H 6.77; found C 63.19, H 6.79;

(2b): 2-methyl-2-((2-methyl-2-(4-propionylphenoxy)propanoyl)oxy) propanoic acid. Melting point: 112 °C. ^{1}H NMR (400 MHz, $CDCl_3$), §11.8-11.2 (broad, s, 1H), 7.4-7.3(d, 9.5 Hz, 2H), 7.2-7.1(d, 10.1 Hz, 2H), 3.5(q, 2H), 1.52 (s, 12H), 1.2(t, 3H); ^{13}C NMR: 10, 24, 25, 31, 81, 91, 114, 128, 129, 159, 171, 175, 200: m/z : 322, 307, 293, 277, 262, 235, 219, 191, 190, 173, 149, 132, 131, 103, 87, 62, 60, 57, 45, 29, 15; Anal. Calcd for $C_{17}H_{22}O_6$: C 63.35, H 6.83; found C 63.39, H 6.92;

(**2c**): 2-((2-(4-benzoylphenoxy)-2-methylpropanoyl)oxy)-2-methyl propanoic acid. Melting point: 126-127 °C. ^{1}H NMR (400 MHz, $CDCl_3$), §12.0-11.5 (broad, s, 1H), 7.5-7.3 (d, 10.2Hz, 4H), 7.2(d, 9.5Hz, 2H), 7.1-7.00(t, 12.00Hz, 2H), 6.9(t, 1H), 1.45(s, 12H); ^{13}C NMR: 24, 25, 81, 91.8, 114, 128.4, 130, 130.9, 132.4, 138, 158.8, 170, 175, 194: m/z: 355, 325, 293, 267, 265, 238, 181, 173, 163, 153, 132, 105, 103, 77, 71, 66, 45, 28, 15; Anal. Calcd for $C_{21}H_{22}O_6$: C 68.11, H 5.94; found C 68.15, H 5.94;

(2d): 2-((2-(4-hexanoylphenoxy)-2-methylpropanoyl)oxy)-2-methyl propanoic acid. Melting point: 135-138 °C. ^{1}H NMR (400 MHz, $CDCl_3$), §11.9-11.4 (broad, s, 1H), 7.5-7.3(d, 9.3 Hz, 2H), 7.3-7.1(d, 10.5 Hz, 2H), 2.94(t, 2H), 1.53(s, 12H), 1.4(m, 2H), 1.28-1.29(s, 4H), 0.91(t, 3H); ^{13}C NMR : 14, 22, 24, 25, 31, 38.7, 81, 114.3, 129.4, 130.8, 159.8, 171, 175, 200: m/z: 364, 349, 319, 318, 304, 293, 277, 265, 232, 191, 173, 165, 132, 99, 87, 71, 60, 46, 45, 28, 15; Anal. Calcd for $C_{20}H_{28}O_6$: C 65.93, H 7.69; found C 65.94, H 7.65;

(**2e**): 2-methyl-2-((2-methyl-2-(4-octanoylphenoxy)propanoyl)oxy) propanoic acid. Melting point: 141-143 °C. ^{1}H NMR (400 MHz, $CDCl_3$), §12.1-11.5(broad, s, 1H), 7.5-7.3(d, 10.2 Hz, 2H), 7.2-7.00(d, 9.2 Hz, 2H), 2.94(t, 2H), 1.6(m, 2H), 1.55(s, 12H), 1.5-1.2(broad, s, 8H), 0.88(t, 3H); ^{13}C NMR : 14, 22.7, 24, 25, 29, 29.1, 29.2, 31.8, 38.7, 81, 91.8, 114, 129, 130.8, 159.5, 170.9, 175.7, 200: m/z: 392, 347, 346, 332, 305, 293, 265, 260, 219, 173, 132, 127, 99, 87, 60, 46, 45; Anal. Calcd for $C_{22}H_{32}O_6$: C 67.35, H 8.16; found C 67.40, H 8.19;

(**2f**): 2-((2-(4-acetylphenoxy)-2-methylpropanoyl)oxy)-2-methyl propanoic acid. Melting point: 105-106 °C. ^{1}H NMR (400 MHz, $CDCl_3$), §11.9-11.2 (broad, s 1H), 7.4-7.3 (d, 9.5 Hz, 2H), 7.2-7.1(d, 10.1 Hz, 2H), 2.3(s, 3H), 1.53(s, 12H),; ^{13}C NMR: 24, 25.5, 26.6, 81, 91.8, 114, 128.3, 129.4, 159.5, 170.9, 175.7, 197: m/z: 308, 293, 265, 263, 262, 248, 221, 200, 176, 173, 135, 108, 87, 60, 46, 45, 43, 15; Anal. Calcd for $C_{16}H_{20}O_6$:C 62.34, H 6.49; found C 62.32, H 6.48;

(3a): 2-hydroxy-N,N,N-trimethylethan-1-aminium 2-methyl-2-((2-methyl-2-phenoxy propanoyl)oxy)propanoate: Melting point 225-227 °C. ^{1}H NMR (400MHz, D_2O), §7.2-7.00 (d, 11.2Hz, 2H), 6.9-6.7 (t, 12Hz, 2H) 6.5 (t, 9.2 Hz, 1H), 4.03(t, 2H), 3.49(t, 2H) 3.17(s, 9H), 1.59(s, 12H); ^{13}C NMR: 23, 25, 54, 57, 69, 88, 92, 114, 120, 129, 155, 183: m/z : 369, 265, 250, 235, 220, 179, 134, 115, 104, 93, 86, 65, 49, 45, 30, 28, 18, 15; Anal. Calcd for $C_{19}H_{31}NO_6$: C 61.79, H 8.4, N 3.76; found C 61.71, H 8.3, N 3.79;

(3b): 2-hydroxy-N,N,N-trimethylethan-1-aminium 2-methyl-2-((2-methyl-2-(4-propionylphenoxy)propanoyl)oxy)propanoate; Melting point 232-235 °C. ^{1}H NMR (400 MHz, D_2O), § 7.4-7.3(d, 9.5Hz, 2H), 7.2-7.1(d, 10.1 Hz, 2H), 4.1(t, 2H), 3.5(q, 2H), 3.3(t, 2H) 3.17(s, 9H), 1.8(t, 3H), 1.59 (s, 12H); ^{13}C NMR : 18, 24, 25, 31, 54, 57, 69, 88, 92, 114, 128, 130, 159, 170, 183, 200: m/z : 425, 321, 292, 276, 264, 235, 219, 191, 190, 172, 149, 131, 130, 104, 102, 87, 73, 60, 57, 49, 45, 29, 17; Anal. Calcd for $C_{22}H_{35}NO_7$: C 62.09, H 8.23, N 3.27; found C 62.06, H 8.31, N 3.25;

(3c): 2-hydroxy-N,N,N-trimethylethan-1-aminium 2-((2-(4-benzoyl phenoxy)-2-methylpropanoyl)oxy)-2-methylpropanoate; Melting point 251-254 °C. ^{1}H NMR (400 MHz, D_2O), §7.5-7.3 (d, 10.2 Hz, 4H), 7.2(d, 9.5 Hz, 2H), 7.1-7.00(t, 12.00 Hz, 2H), 6.9(t, 1H), 4.4(t, 2H), 3.5(t, 2H) 3.2(s, 9H), 1.53(s, 12H); ^{13}C NMR : 23, 25, 54, 57, 69, 88, 91, 114, 128, 130, 131, 132, 138, 158, 163, 170: m/z: 473, 369, 324, 292, 283, 264, 238, 197, 173, 153, 131, 105, 104, 93, 86, 77, 73, 66, 49, 45, 17; Anal. Calcd for $C_{26}H_{35}NO_7$: C 65.94, H 7.34, N 2.96; found C 65.92, H 7.32, N 2.94;

(3d): 2-hydroxy-N,N,N-trimethylethan-1-aminium 2-((2-(4-hexanoyl phenoxy)-2-methylpropanoyl)oxy)-2-methylpropanoate; Melting point 243-246 °C. ^{1}H NMR (400 MHz, D_2O), § 7.5-7.3(d, 9.3 Hz, 2H), 7.3-7.1(d, 10.5Hz, 2H), 4.0(t, 2H), 3.2(t, 2H) 3.0(s, 9H), 2.4(t, 2H), 2.3(m, 2H), 2.1-2.00(s, 4H), 1.53(s, 12H)), 0.8(t, 3H); ^{13}C NMR : 14, 22, 23, 24, 25, 31, 38, 54, 57, 69, 88, 91, 114, 129, 130, 159, 170, 183, 200: m/z: 467, 363, 348, 318, 292, 277, 264, 233, 232, 191, 172, 131, 130, 104, 99, 93, 86, 79, 73,

71, 66, 49, 45, 17, 15; Anal. Calcd for $C_{25}H_{41}NO_7$: C 64.22, H 8.78 N 2.3; found C 64.21, H 8.76, N 2.98;

(**3e**): 2-hydroxy-N,N,N-trimethylethan-1-aminium 2-methyl-2-((2-methyl-2-(4-octanoylphenoxy)propanoyl)oxy)propanoate; Melting point 305-307 °C. ^{1}H NMR (400 MHz, D_2O), § 7.5-7.3(d, 10.2 Hz, 2H), 7.2-7.00(d, 9.2 Hz, 2H), 4.6(t, 2H), 3.4(t, 2H) 3.1(s, 9H), 2.9(t, 2H), 1.5(m, 2H), 1.42(s, 12H), 1.3-1.1(broad, s, 8H), 0.88(t, 3H); ^{13}C NMR : 14, 22, 23, 25, 31, 38, 54, 57, 69, 88,, 91, 114, 129, 130, 159, 170, 180, 200: m/z: 495, 391, 376, 346, 305, 292, 288, 261, 260, 219, 172, 127, 131, 130, 104, 99, 93, 86, 73, 66, 49, 45, 17, 15; Anal. Calcd for $C_{27}H_{45}NO_7$: C 65.43, H 9.09, N 2.83; found C 65.41, H 9.07, N 2.82;

(**3f**): 2-hydroxy-N,N,N-trimethylethan-1-aminium 2-((2-(4-acetyl phenoxy)-2-methylpropanoyl)oxy)-2-methylpropanoate; Melting point 235-237 °C. ^{1}H NMR (400 MHz, D_2O), § 7.4-7.3 (d, 9.5 Hz, 2H), 7.2-7.1(d, 10.1Hz, 2H), 4.1(t, 2H), 3.2(t, 2H) 3.1(s, 9H), 2.3(s, 3H), 1.53(s, 12H); ^{13}C NMR 23, 25, 26, 54, 57, 69, 88, 114, 128, 129, 159, 171, 197: m/z: 411, 307, 292, 264, 262, 221, 177, 176, 172, 135, 131, 130, 104, 93, 86, 73, 66, 49, 45, 43, 17, 15; Anal. Calcd for $C_{21}H_{33}NO_7$: C 61.29, H 8.03, N 3.40; found C 61.28, H 8.04, N 3.41;

(**4a**): Isopropyl-2-methyl-2-((2-methyl-2-phenoxy propanoyl)oxy) propanoate: Melting point 185 °C. ^{1}H NMR (400 MHz, $CDCl_3$); § 7.2-7.00(d, 11.2 Hz, 2H), 6.9-6.7(t, 12 Hz, 2H) 6.5(t, 9.2Hz, 1H), 5.2(m, 1H), 2.8(s, 12H); 1.9(d, 6H); m/z: 308, 265, 220, 215, 179, 174, 153, 134, 129, 88, 66, 43, 28; Anal. Calcd for $C_{17}H_{24}O_6$: C 66.23, H 7.79; found C 66.25, H 7.76;

(**4b**): Methyl-2-methyl-2-((2-methyl-2-(4-propionylphenoxy) propanoyl)oxy)-propanoate Melting point 206 °C. ^{1}H NMR (400 MHz, $CDCl_3$), § 7.4-7.3(d, 9.5 Hz, 2H), 7.2-7.1(d, 10.1 Hz, 2H), 4.1(s, 3H), 2.7(s, 12H) 2.3(q, 2H), 1.8(t, 3H); m/z: 336, 321, 305, 279, 277, 214, 202, 187, 149, 134, 122, 59, 57, 31, 15; Anal. Calcd for $C_{18}H_{24}O_6$: C 64.29, H 7.14; found C 64.32, H 7.16;

(**4c**): Ethyl 2-((2-(4-benzoylphenoxy)-2-methylpropanoyl)oxy)-2-methylpropanoate Melting point 191-193 °C. ^{1}H NMR (400 MHz, $CDCl_3$), §7.5-7.3(d, 10.2 Hz, 4H), 7.2(d, 9.5 Hz, 2H), 7.1-7.0(t, 12.00 Hz, 2H), 6.9(t, 1H), 3.9(q, 2H), 2.67(s, 12H), 1.9 (t, 3H); m/z: 398, 353, 325, 321, 293, 267, 239, 201, 197, 170, 159, 131, 105, 77, 73, 45; Anal. Calcd for $C_{23}H_{26}O_6$: C 69.35, H 6.53; found C 69.36, H 6.55;

(**4d**): Isopropyl -2-((2-(4-hexanoylphenoxy)-2-methylpropanoyl)oxy)-2-methyl-propanoate; Melting point 215 °C. ^{1}H NMR (400 MHz, $CDCl_3$); § 7.5-7.3(d, 9.3 Hz, 2H), 7.3-7.1(d, 10.5 Hz, 2H), 5.2(m, 1H), 2.7(s, 12H), 2.4(t, 2H), 2.3 (m, 2H), 2.1-2.00(s, 4H), 1.8(t, 3H), 1.5 (d, 6H); m/z: 406, 347, 308, 307, 273, 233, 215, 191, 173, 164, 145, 99, 88, 59; Anal. Calcd for $C_{23}H_{34}O_6$: C 67.98, H 8.37; found C 67.99, H 8.39;

(**4e**): Ethyl-2-methyl-2-((2-methyl-2-(4-octanoylphenoxy)propanoyl) oxy)propanoate.Melting point 221 °C. ^{1}H NMR (400 MHz, $CDCl_3$) §7.5-7.3(d, 10.2 Hz, 2H), 7.2-7.00(d, 9.2 Hz, 2H), 3.9(q, 2H), 2.7(s, 12H), 2.4(t, 2H), 2.2-2.00(m, 2H), 1.9(broad, s, 8H), 1.7(t, 3H), 1.6(t, 3H); m/z: 420, 375, 293, 289, 228, 260, 219, 201, 192, 160, 131, 127, 115, 74, 45; Anal. Calcd for $C_{24}H_{36}O_6$: C 68.57, H 8.57; found C 68.59, H 8.61;

(**4f**): Ethyl-2-((2-(4-acetylphenoxy)-2-methylpropanoyl)oxy)-2-methyl propanoate;. Melting point 199 °C. ^{1}H NMR (400 MHz, $CDCl_3$), §7.4-7.3(d, 9.5 Hz, 2H), 7.2-7.1(d, 10.1 Hz, 2H), 4.2(t, 2H), 2.7(s, 12H), 2.3(s, 3H), 1.8(t, 3H); m/z: 336, 293, 291, 256, 205, 201, 176, 160, 135, 131, 108, 74, 45, 43; Anal.Calcd for $C_{18}H_{24}O_6$:C 64.29, H 7.14; found C 64.30, H 7.17.

4. Conclusion

Synthesis and characterisation of α-phennoxy isobutyric acid and its derivative, esters and chloline salt were discussed. Biological activities and molecular docking studies of these compounds were found to be effective against various microbial and docking pathogens of human and plants.

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