Synthesis of amide conjugated oleic acid analogues

A Dissertation submitted
in partial fulfillment of the requirements for the award of degree of

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IN
CHEMISTRY

THAPAR INSTITUTE
OF ENGINEERING & TECHNOLOGY
(Deemed to be University)

Submitted By

Swati Garg
(301702037)

UNDER THE SUPERVISION OF

Dr. Ranjana Prakash
Professor

Dr. Amjad Ali
Professor and Head

SCHOOL OF CHEMISTRY & BIOCHEMISTRY
THAPAR INSTITUTE OF ENGINEERING AND TECHNOLOGY
PATIALA, 147004

JULY-2019

Dated: 15th July, 2019

TO WHOMSOEVER IT MAY CONCERN

It is certified that **Ms. Swati Garg** student of Master of Science in Chemistry of **"Thapar Institute of Engineering & Technology", Patiala**, has worked in the Bio-catalysis Lab., School of Chemistry and Biochemistry, T.I.E.T, Patiala from January 2019 to July 2019. During the period she has worked on **"Synthesis of amide conjugated oleic acid analogues"** and submitted her thesis in partial fulfillment of the requirements for the award of degree of **Master of Science** in **Chemistry**. The work was carried out by the candidate under my supervision and guidance.

Supervisor
Dr. Ranjana Prakash
Professor

Supervisor
Dr. Amjad Ali
Professor and Head

School of Chemistry and Biochemistry
Thapar Institute of Engineering and Technology
Patiala-147004

Candidate's Declaration

I hereby declare that the dissertation entitled **"Synthesis of amide conjugated oleic acid analogues"** is an authentic record of my work carried out as requirements for the award of degree of **Master of Science** in **Chemistry** at **Thapar Institute of Engineering and Technology, Patiala** under the supervision of **Dr. Ranjana Prakash (Professor) & Dr. Amjad Ali (Professor and Head)**, School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala from January, 2019 to July, 2019. No part of the matter embodied in this report has been submitted to any other university or institute for the award of any other degree.

Swati

Date: 15-07-2019 **Swati Garg**

It is certified that the above statement made by the student is correct and true to the best of my knowledge and belief.

Supervisor

Dr. Ranjana Prakash

Professor

Supervisor

Dr. Amjad Ali

Professor and Head

School of Chemistry and Biochemistry

Thapar Institute of Engineering and Technology

Patiala-147004

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Swati Garg

Abbreviations

OA	Oleic acid
FA	Fatty acid
MUFA	Monounsaturated Fatty Acid
PUFA	Polyunsaturated Fatty Acid
SCFA	Short chain Fatty Acid
LCFA	Long Chain Fatty Acid
BBB	Blood Brain Barrier
EPA	Eicosapentaenoic Acid
DHA	Docosahexaenoic Acid
CVD	Cardiovascular Disease
MCPBA	meta-Chloroperoxybenzoic acid
DCM (CH_2Cl_2)	Dichloromethane
$CDCl_3$	Deuterated chloroform
DMAP	Dimethylaminopyridine
EDCI.HCl	1-ethyl,3(3,3-dimethylaminopropyl)
TLC	Thin Layer Chromatography
^{1}H NMR	Proton Nuclear Magnetic Resonance
^{13}C NMR	Carbon Nuclear Magnetic Resonance
ω	Omega
α	Alpha
γ	Gamma
β	Beta
δ	Delta
ml	Milliliter
Hz	Hertz
h	Hour
TMS	Tetramethylsilane
s	Singlet
bs	Broad singlet

d	Doublet
dd	Double doublet
t	Triplet
m	Multiplet

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ABSTRACT

A series of amide derivatives of oleic acid epoxide were synthesized in this present work. An effective developed method was used for the conjugation of intermediate oleic acid epoxide **(2)** coupled with different aliphatic, heteroaromatic and heterocyclic amines in dichloromethane (DCM), dimethylaminopyridine (DMAP) and EDCI and synthesized compounds **3-8**. All the compounds were purified by column chromatography, identified by TLC and characterized by ^{1}H and ^{13}C NMR. As FAs can cross the blood brain barrier (BBB) and active against various neurological disorders, all synthesized amide conjugated oleic acid analogues will be evaluated for Alzheimer's/ Parkinson's diseases on the basis of preliminary screening results.

Chapter 1

INTRODUCTION

1.1 Fatty acids

Fatty acids (FAs) are aliphatic hydrocarbons with terminal carboxylic acid functionality obtained either in the free form or as a complex lipid part especially in fats or oils. They are either saturated (do not contain double bond) or unsaturated (contain one or more double bonds). Unsaturated FAs are generally denoted as omega (ω) FAs where ω indicates the position of unsaturation i.e. double bond position from the opposite end of the carboxyl group.[1] Odd chain FAs are having odd number of carbon atoms in the structure. Propionyl-CoA carboxylase, Methylmalonyl-CoA epimerase and Methylmalonyl-CoA mutase are three enzymes required for the oxidation of odd carbon FAs.[2, 3] However, most FAs contain even numbered carbon atoms, in which the chain length from 2-6 are called short chain, 8-10 as medium chain and 12-24 are considered as long chain FAs respectively. FAs such as palmitic, oleic, linoleic and stearic acids i.e. C16 and C18 species, are prevalent in higher plants and animals while, FAs having carbon atoms less than 14 and more than 20 are rare in nature. Because of free movement around C-C bond, molecules of saturated FAs show high flexibility and therefore assume to occupy a wide range of conformations.[4, 5] FAs having only one C-C double bond at various positions are termed as monounsaturated FAs (MUFAs). Most of them have cis-configuration which leads to bend structure having lower melting point and lesser thermodynamic stability than the trans configuration.[1] More than half of FAs residues of plants and animals, are found as unsaturated and often polyunsaturated. In polyunsaturated FAs (PUFAs), usually the double bonds tend to occur at every third carbon atom and so are not conjugated. PUFAs have many benefits in maintaining healthy life only if they are consumed in a balanced way.[3, 5, 6] ω-9 FAs are non-essential FAs as they can be produced by the body. They are considered to have more beneficial effects than PUFAs in regulating the metabolism of the body.[5] The unsaturated FAs such as oleic acid, linoleic acid, linolenic acid, arachidonic acids are usually available in nature.[3, 4]

1.2 Oleic acid

Oleic acid (18:1ω-9) is one of the most prevalent monoene acids widely distributed in nature and commonly used as human dietary FAs especially obtained from foods. Oleic acid is used as the prototype for all of the monoene acids and also for the ω-9 family of polyene acids.

Oleic acid can be found in olive oil and several nut oils such as almonds, filberts, cashews, pistachios, pecans and macadamia nuts.[3, 5] In olive oil, oleic acid contributes about 70% of the total FAs. Whereas, it contributes 60% in Low–erucic acid rapeseed oil (aka canola oil). In humans, oleic acid can be synthesized by desaturation of stearic acid.[3]

1.3 Natural and synthetic origin of FAs

There are two major sources of FAs, one is endogenous *de novo* lipogenesis (from carbohydrates) and the other is exogenous (from the diet). *De novo* lipogenesis involves the formation of diverse glycerides, which further leads to the generation of complex lipids in the process of FAs synthesis. The whole process of FA synthesis is quite similar as the utilisation of intermediates in glucose metabolism pathway.[7] Most of the cultured cells having distinct natural origin are found to be pretty active in the synthesis of FAs. Palmitate is one of the major fatty acid which is synthesized by *de novo* lipogenesis which helps in the formation of long chain saturated FAs by elongation and leads to the unsaturation at C4, C5, C6, or C9.[8, 9] Dairy and meat products, grains, fish and vegetable oils are the vital dietary sources of fatty acids and epitomize 30-35% of total energy intake.[8] Saturated FAs such as palmitic acid, stearic acid and myristic acid are mainly found in animals, plants, microorganisms and fungi. Saturated FAs with less number of carbon atoms mainly originate from milk and triacylglycerols. MUFAs like oleic acid (ω-9) and palmitoleic acid (ω-7) which are the key elements of many seed oils are most commonly found in plants and animals.[1, 10] PUFAs especially ω -3 and ω-6 are considered essential and must be obtained from the diet as they are not synthesised in the body. Both of them have many important structural and physiological functions.[11, 12] They are mainly present in the plant lipids and membrane phospholipids. α-Linolenic acid (ω-3) is basically obtained from higher plants and algae while eicosapentaenoic acid (EPA; ω-3) and docosahexaenoic acid (DHA; ω-3) are the major FAs mostly obtained from marine algae and other marine sources like fatty fish and fish oils.[1, 12]

1.4 Role of FAs in daily life

Various FAs are assumed to have diverse biological properties and hence show different pharmacological and metabolic effects such as neurological[13], cardiovascular[11, 14] etc. Different diets rich in different saturated FAs are assumed to have different health effects. It is suggested by various reports that high consumption of ω-3 and ω -9 FAs by humans and animals helps in reducing the risk of breast cancer in them.[15, 16]

ω-3 PUFAs are very beneficial in treating many cancers, inflammatory diseases such as eczema, psoriasis, inflammatory bowel disease and rheumatoid arthritis and also reducing cholesterol, diabetes as well as in cardiovascular diseases (CVDs).[12] They also prevent from colon cancer in rats and helps in decreasing the risk of colorectal cancer development.[17] Oleic acid which belongs to ω-9 family reduces the risk of Alzheimer's and Parkinson's disease, CVD and cancer.[10] It was observed that ω-9 FAs viz. oleic acid and ω-6 FAs viz. linoleic and linolenic acid indirectly aggravate the synthesis of a pro-inflammatory mediator, reactive oxygen species (ROS) superoxide. Oleic acid was identified in reducing the injury in adult male Sprague-Dawley rat cardiomyocytes and the effects of TNF-α-induced oxidative stress as well as decreases the inflammation in human endothelial cells induced by saturated FAs. Moreover, assimilation of milk high in oleic acid into the diet helps in decreasing the total cholesterol, triglyceride levels and LDL-cholesterol. Nervonic acid which is another ω-9 fatty acid has confirmed impact on CVD risk.[18]

Chapter 2

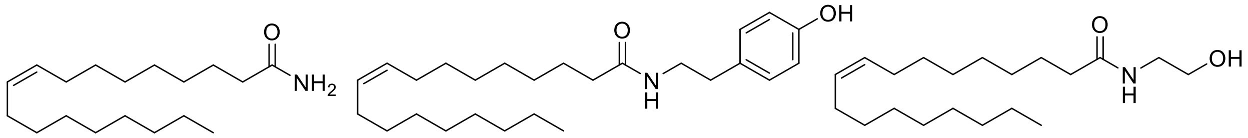
LITERATURE REVIEW

FAs, in any form, play an important role in metabolism[1] not only in humans but also in other animals.[19] They are very beneficial in curing many diseases like cancer[20], cardiovascular diseases[10], neurological disorders, inflammatory diseases, chronic diseases, Alzheimer's disease[9, 13] etc. Many evidences support the biological importance of short chain fatty acids (SCFAs)[21] as well as of long chain fatty acids (LCFAs).[19]

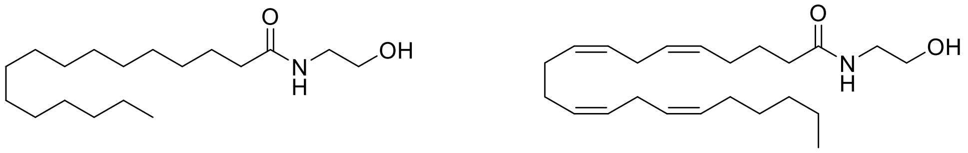
Puertollano et al. (2014) suggested the production of SCFAs from the metabolism of complex carbohydrates specially propionate and butyrate, which are found to be very beneficial in many therapies like cancer, diabetes, CVDs etc. They also play important role in maintaining intestinal epithelial cells and act as an energy source for it.[22] Various health benefits of LCFAs and their derivatives in cancer prevention as well as in treating the cancer were investigated. The report suggested that fatty acids and their derivatives help to inhibit the tumour cells as well as control the tumour cell response to anticancer agents. Recent studies have claimed that less consumption of omega-3 PUFAs in association with high consumption of omega-6 PUFAs increase the risk of breast cancer. In addition, various studies also support that PUFAs are very helpful in inhibiting the growth of tumour cells and its analogues have potential in the cancer treatment.[20] Kabara et al. (1972) studied the structural relationships of 30 unbranched FAs and their analogues for anti-biotic properties with 8 gram-negative and 12 gram-positive organisms by taking all the variables like functional group, unsaturation and chain length under consideration. They found that the activity of a straight chain fatty acid gradually increases on increasing the unsaturation and shows lesser activity when third double bond is added. Lauric acid was observed to show best inhibitory action amongst saturated fatty acids against gram-positive organisms. Esters of glycerol and alcohols were assumed to be active against gram-positive organisms only while amine derivatives tend to exhibit against both organisms.[23] Bruce et al. (1958) reported that alkanolamino-di-fatty acid amides and their salts either as free bases or as the acid addition salts show multiple pharmacological activities as well as it is very active in local anaesthesia in particular aliphatic or heterocyclic acid esters. They also tend to show antispasmodic activity.[24] In recent literature, some synthesized triazolyl fatty acid analogues were identified as anti-microbacterial activity, when tested against *Mycobacterium tuberculosis*.[25] Bezuglov et al.

(1998) investigated that amide derivatives of fatty acids of long chain saturated and unsaturated fatty acids are lipid bioregulators. The authors reported that ethanolamides of fatty acids including hydrolysis of N-acylated phosphatidylethanolamines by phospholipase D is an alternative way for their biosynthesis. They can act as endogenous ligands of the cannabinoid receptors after getting bind to them and show analogous pharmacological properties to that of cannabimimetics.[26] Serhan et al. (2002) reported bioactive 17R-hydroxy-containing di- and tri-hydroxy-docosanoids called 'resolvins' were formed in resolution phase when mice is treated with Aspirin (ASA) known for its uniqueness in recent remedies and docosahexaenoic acid (DHA) during lipidomic study of exudates. The family of protective compounds i.e. Resolvins are produced by the consumption of omega-3 DHA and EPA during multicellular events in resolution.[27] A previous study by Irby et al. (2017) suggested that the lipid conjugation with various small molecular or macromolecular drugs could increase the lipophilicity, varying the drug properties and hence generating higher stability and more drugs loading. These variations could increase tumour targeting and help in improving oral bioavailability. It was also observed that lipid-drug conjugates can overcome drug resistance in chemotherapy. Drugs conjugated to glycerides improved the absorption of drugs and increased the lymphatic targeting by taking the advantage of lymphatic transport pathway while phospholipid conjugated drugs increased the absorption of drugs into phospholipids. Lipid drug conjugation can also be done by amide bonding. The authors studied various delivery carriers such as micelles, liposomes, emulsions, lipid nanoparticles and polymer nanoparticles.[21]

Takao et al. (2015) synthesized a series of FA amides like Oleamide (a), N-oleoyltyramide (b), N-oleoylethanolamide (c), N-palmitoylethanolamide (d), N-arachidonoylethanolamide (e) (**Fig. 1**) by the condensation of oleoylchloride which is basically derived from oleic acid and oxalyl chloride with some biogenic amines. The authors evaluated the activation of peroxisome proliferator activated receptor α.[28] Baba et al. (2014) synthesized amide derivatives of long chain fatty acids by the condensation of palmitoyl chloride with three amino acids viz. glycine, β-alanine, γ-aminobutyric acid and evaluated the anti-inflammatory and anti-nociceptive effects.[29] Various authors reported the synthesis of FA amides catalysed by enzymes. Awasthi et al. (2007) reported the synthesis of FA amides by the ammonolysis of FAs and urea in organic solvent medium, catalysed by the enzyme Novozym 435.[30] Bachur et al. (1966) reported the synthesis of FA amides mainly ethanolamide catalysed by a microsomal system.[31]



Oleamide (a) N-oleoyltyramide (b) N-oleoylethanolamide (OEA) (c)



N-palmitoylethanolamide (PEA) (d) N-arachidonoylethanolamide (Anandamide) (e)

Figure 1: Examples of fatty acid amides

Various evidences about beneficial effects of fatty acids in human neurological health have been reported previously. Hamilton et al. (2007) discussed about the uptake and consumption of lipid and its contribution in neurological treatment and also highlighted the role of ω-3 and ω-6 FAs in the CNS, which is very important for understanding the physiological pathways of neurodegenerative diseases.[32] Kurotani et al. (2017) suggested that different saturated fatty acids (even- and odd-chain) have different association with adipokines. They concluded that adipokine profile of even-chain saturated fatty acids is favourable while that of odd-chain saturated fatty acids is unfavorable.[33] Farooqui et al. (2006) reported that lipid mediators can play important role in the initiation and modulation of oxidative stress and inflammation. Many neurological disorders like ischemia, Alzheimer's disease, spinal cord injury, epilepsy and prion diseases are responsible in changing metabolism of phospholipids, accumulation of lipid peroxides and increase in phospholipase A2 activity. They also investigated that phospholipase A2 inhibitors can act as anti-inflammatory agents as well as neuroprotectants in neurological disorders.[13] A previous report by Tabernero et al. (2001) suggested albumin could able to stimulate synthesis of oleic acid by astrocytes during the brain developmental stage and also plays an important role in the phospholipid synthesis which induces neuronal differentiation.[34]

From the available literature, it was observed that oleic acid and its mimics were found to be active against cancer, chronic diseases, neurological disorders like Alzheimer's disease and inflammatory diseases.[9, 13] As FAs can cross the blood brain barrier (BBB) and active against various neurological diseases, oleic acid was chosen to derivatize and all the synthesized derivatives will be evaluated for Alzheimer's/ Parkinson diseases on the basis of preliminary screening result.

Chapter 3

OBJECTIVES

1. Synthesis of Oleic acid epoxide from oleic acid.

2. Synthesis of amide derivatives of oleic acid epoxide.

Chapter 4

EXPERIMENTAL METHODS

4.1 Materials

All the chemical reagents required for the synthesis were purchased from Sigma Aldrich and the solvents (Rankem) used in the reactions were distilled and further dried before performing the present work. The coupling reactions were carried out by 1-ethyl,3(3,3-dimethyleaminopropyl)carbodiimide hydrochloride (EDCI.HCl).

4.2 Experimental Apparatus

The experimental set up for all reactions were carried out using round bottom flasks (RBF, 500 ml and 100 ml), a stand with clamps, magnetic beads and magnetic stirrer for stirring.

4.3 Analysis technique for synthesized compounds

All synthesized compounds were monitored by TLC on 0.25 mm silica gel 60 F254 plates (E. Merck) and EtOAc:Hexane as the mobile phase. These compounds were further visualized using UV illumination and the spots on the TLC plate were observed by using 2% ceric ammonium sulfate (spraying reagent). Purification of compounds was initially done by column chromatography using Silica gel 100-200 mesh (stationary phase) and EtOAc:Hexane (mobile phase). ^{1}H and ^{13}C NMR spectra were recorded on JEOL (400 MHz; JEOL JNM-ECS 400) instrument using $CDCl_3$ as the solvent with TMS as internal standard. The chemical shifts were represented in δ ppm and coupling constants in Hertz (Hz). The abbreviations used were as follows: s, singlet; bs, broad singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet. The spectral data were consistent with the assigned structures.

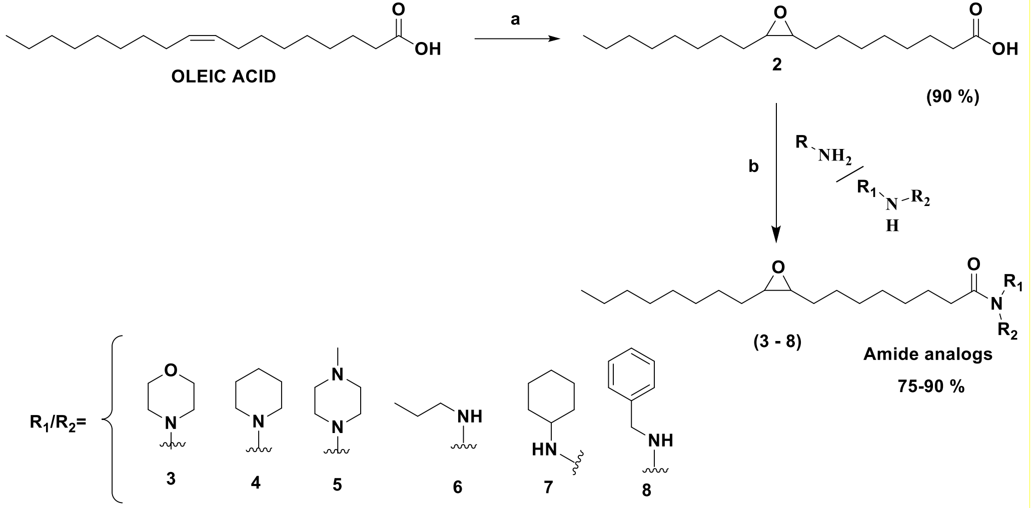
4.4 Methods

4.4.1 Synthetic Methodology

In the present study, the terminal acid functionality of oleic acid was targeted for synthetic modification. A series of amide derivatives were synthesized from the synthesized oleic acid epoxide intermediate as described in **Scheme I**.

The synthetic approach for the modification was initiated by converting OA **(1)** to oleic acid epoxide **(2)** using MCPBA in DCM. Condensation of intermediate oleic acid epoxide **(2)** in dichloromethane (DCM) in addition with dimethylaminopyridine (DMAP) and EDCI was coupled with different aliphatic, heteroaromatic and heterocyclic amines and synthesized compounds **3-8** in 75-90% yield (**Scheme-1).**

Scheme-I



Reagents and conditions: (a) m-CPBA, DCM, room temp.; (b) Substituted amines, dry CH_2Cl_2, EDCI, DMAP, room temp.

4.4.2 Preparation of Oleic acid epoxide (2)

To prepare oleic acid epoxide **(2),** the compound **1** (5 g, 17.7 mmol) was dissolved in DCM and MCPBA (3.054 g, 17.7 mmol) was portion wise added and stirred at room temperature for 2-3 h. After completion of the reaction, the reaction mixture was extracted in DCM and water for usual workup. The organic layer was dried over sodium sulphate and concentrated on rota evaporator. Purification was done through column chromatography with EtOAc:Hexane (2:8) as the eluent to afford product **2** white solid (90% yield).

4.4.3 General procedure for synthesis of amides of oleic acid epoxides (3-8)

To a mixture of oleic acid epoxide (**2**) (100 mg, 0.335 mmol) dissolved in dry dichloromethane (DCM) (2.0 ml), dimethylaminopyridine (DMAP) (8.19 mg,0.067 mmol) and EDCI.HCl (77.065 mg, 0.402 mmol) were added to the reaction mixture further to which different aliphatic, heteroaromatic and heterocyclic amines (0.503 mmol) were added. The reaction mixture was stirred for 6-10 h. The ongoing reaction was monitored using TLC at regular intervals. After completion, the reaction mixture was extracted with DCM and water. The collected organic layer was dried using anhydrous sodium sulfate and evaporated in rotary evaporator to yield amides of oleic acid epoxides (**3-8)**, which were further purified by column chromatography over silica gel (60-120 mesh) with yield of 75-90%

Chapter 5

RESULTS AND DISCUSSION

Chemistry

The synthesis of oleic acid epoxide **(2)** and its corresponding amide derivatives (**3-8**) were performed by the synthetic strategy as mentioned above in Scheme-1. Starting from OA in DCM as solvent and MCPBA was added portion wise to it and stirred at room temperature to obtain oleic acid epoxide **(2)** in good yield (90%). Conjugation of intermediate product **2** with diverse primary/secondary amines in addition with DMAP and EDCI in DCM as solvent used to obtain diverse amides of oleic acid epoxide (**3-8**). All the synthesized compounds (**3-8**) were initially identified as pure with the help of the TLC plate analysis. The formation of substituted groups of amide conjugated oleic acid epoxide were confirmed by ^{1}H NMR (**Fig. 2**) and results of ^{13}C NMR of all compounds are awaited. Here aliphatic, aromatic, heterocyclic amines were taken for amide conjugation of oleic acid epoxide synthesis in order to find out the structure activity relationship (SAR). The SAR study of all synthesized compounds will be generated after thorough bioevaluation study of all synthesized compounds which are now in process. Previous studies suggested that introduction of the nitrogen containing heterocyclic rings or amide linkage to fatty acids (N-palmitoylethanolamide[31], N-oleoylethanolamide[28], anandamide[35]) were identified for significant improvement in biological activities. N-palmitoylethanolamide (PEA) was reported for anti-inflammatory and analgesic activity.[31] Similarly, N-oleoylethanolamide (OEA) was reported as appetite-suppressant agent.[28] However, anandamide (arachidonoylethanolamide) is acting as a neurotransmitter in the brain signalling in the central nervous system (CNS) and considered as endocannabinoid as it binds to the cannabinoid receptors inside body.[35] Thereby, all these amide analogues of oleic acid epoxides will be evaluated against schizophrenia/ Alzheimer’s cell lines to obtain a preliminary biological profile of this series of newly synthesized compounds.

Synthesis of compound 3

The compound **3** was prepared by the reaction of oleic acid epoxide (**2**) (100 mg, 0.335 mmol) and morpholine (43.8113 µL, 0.503 mmol) in dry DCM as per the method described in Section 4.4.3 to furnish compound **3** as pale yellow liquid after purification by column chromatography (silica gel/EtOAc/Hexane 20/80) (80% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.65 (m, 4H), 3.60 (m, 2H), 3.45 (m, 2H), 2.88 (m, 2H), 2.29 (m, 2H), 1.63 (m, 4H), 1.47 (m, 6H), 1.33 (s, 6H), 1.26 (m, 10H), 0.87 (t, J = 6.9 Hz, 3H).

Synthesis of compound 4

The compound **4** was prepared by the reaction of oleic acid epoxide (**2**) (100 mg, 0.335 mmol) and piperidine (42.83 μL, 0.503 mmol) in dry DCM as per the method described in Section 4.4.3 to furnish compound **4** as light yellow liquid after purification by column chromatography (silica gel/EtOAc/Hexane 20/80) (80% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.52 (m, 2H), 3.37 (m, 2H), 2.88 (s, 2H), 2.29 (m, 2H), 1.60 (m, 4H), 1.52 (m, 10H), 1.32 (s, 8H), 1.25 (m, 10H), 0.86 (m, 3H).

Synthesis of compound 5

The compound **5** was prepared by the reaction of oleic acid epoxide (**2**) (100 mg, 0.335 mmol) and N-methylpiperazine (50.38 μL, 0.503 mmol) in dry DCM as per the method described in Section 4.4.3 to furnish compound **5** as brown liquid after purification by column chromatography (silica gel/EtOAc/Hexane 20/80) (80% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.63 (m, 2H), 3.47 (m, 2H), 2.88 (s, 2H), 2.40 (m, 4H), 2.31 (m, 6H), 1.60 (m, 2H), 1.47 (m, 6H), 1.32 (m, 6H), 1.26 (m, 6H), 1.23 (m, 4H), 0.86 (t, J = 6.6 Hz, 3H).

Synthesis of compound 6

The compound **6** was prepared by the reaction of oleic acid epoxide (**2**) (100 mg, 0.335 mmol) and N-propylamine (29.73 μL, 0.503 mmol) in dry DCM as per the method described in Section 4.4.3 to furnish compound **6** as white solid after purification by column chromatography (silica gel/EtOAc/Hexane 20/80) (80% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.44 (s, 1H), 3.18 (m, 2H), 2.88 (m, 2H), 2.13 (m, 2H), 1.61 (m, 2H), 1.50 (m, 6H), 1.28 (m, 20H), 0.88 (m, 6H).

Synthesis of compound 7

The compound **7** was prepared by the reaction of oleic acid epoxide (**2**) (100 mg, 0.335 mmol) and cyclohexylamine (68.22 μL, 0.503 mmol) in dry DCM as per the method described in Section 4.4.3 to furnish compound **7** as white solid after purification by column chromatography (silica gel/EtOAc/Hexane 20/80) (80% yield).

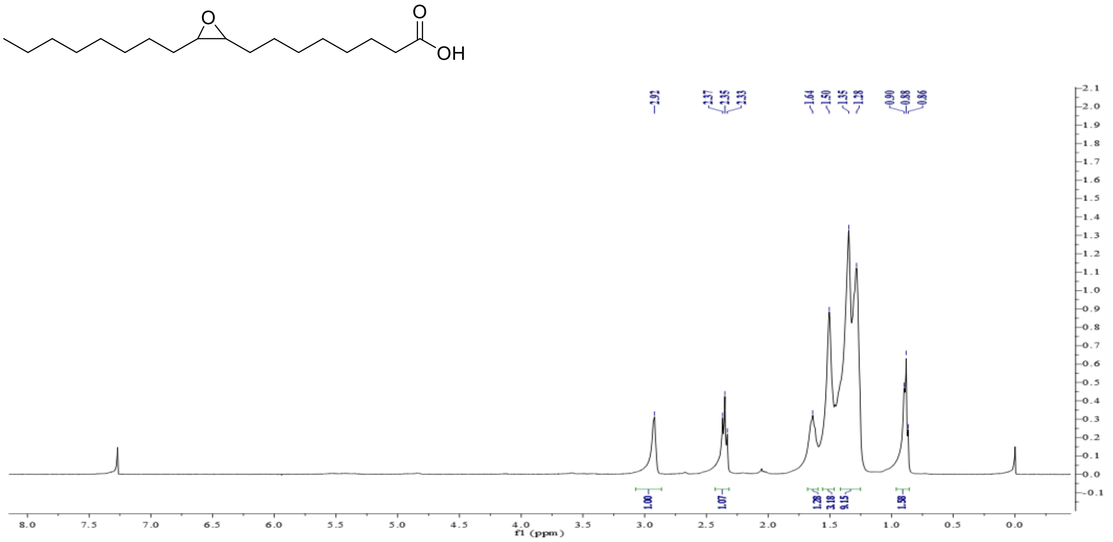
Synthesis of compound 8

The compound **8** was prepared by the reaction of oleic acid epoxide (**2**) (100 mg, 0.335 mmol) and benzylamine (53.89 μL, 0.503 mmol) in dry DCM as per the method described in Section 4.4.3 to furnish compound **8** as light yellow liquid after purification by column chromatography (silica gel/EtOAc/Hexane 20/80) (80% yield).

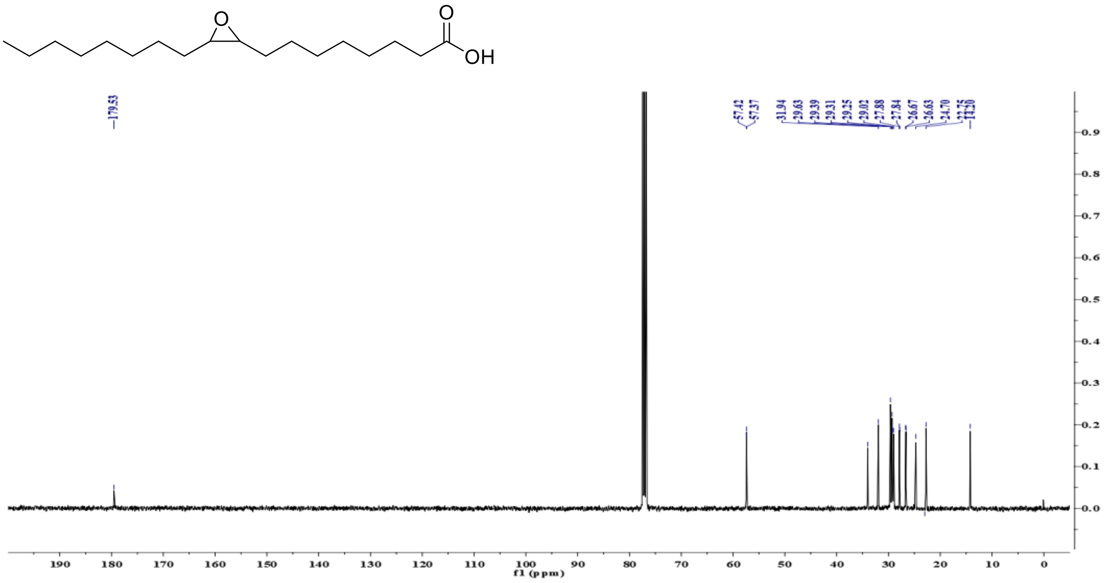
CONCLUSION

To conclude, a series of amide conjugated oleic acid analogues were synthesized and characterized by various spectroscopic techniques using ^{1}H NMR. However, ^{13}C NMR of all synthesized compounds are under process. All these derivatives were synthesized in order to explore these molecules for better biological importance comparing with OA. Thereby, these synthesized molecules will be evaluated for cytotoxic activity against schizophrenia/ Alzheimer's cell lines to obtain a preliminary biological profile of this series of compounds as our future research plan.

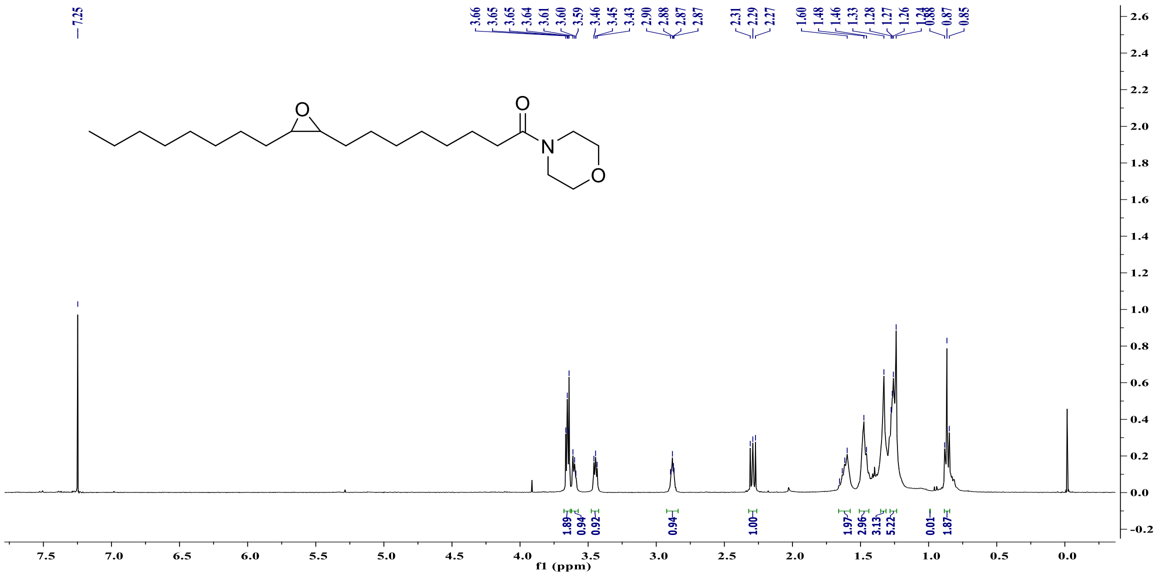
^{1}H NMR of compound 2



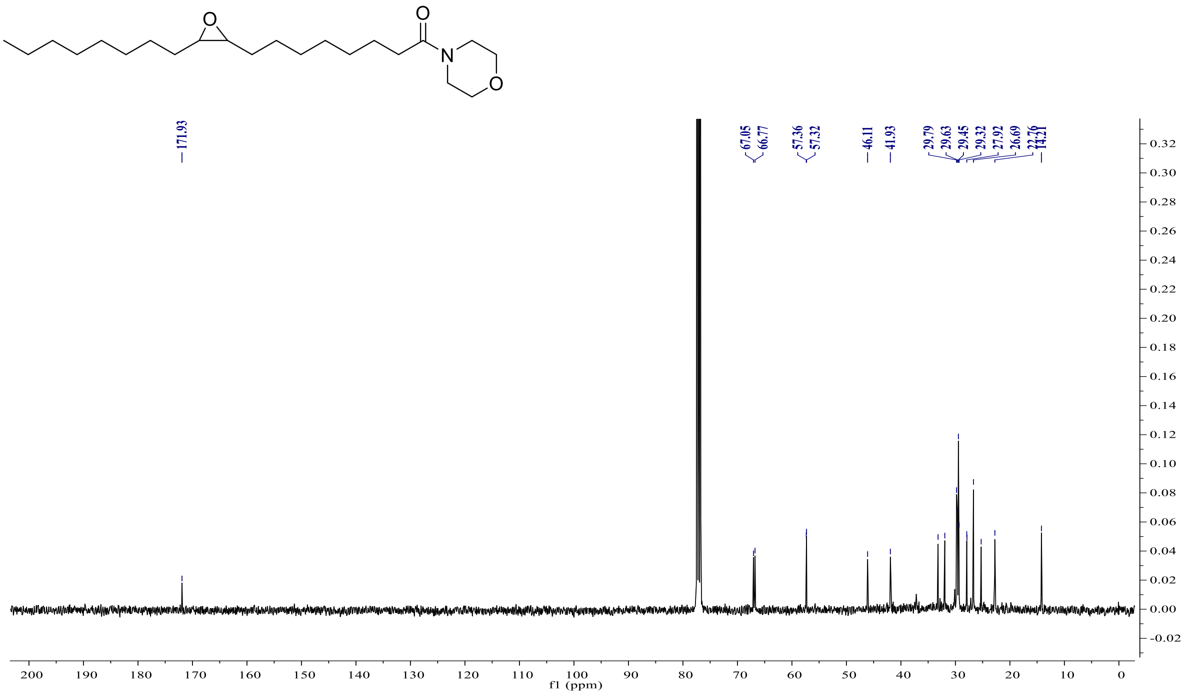
^{13}C NMR of compound 2



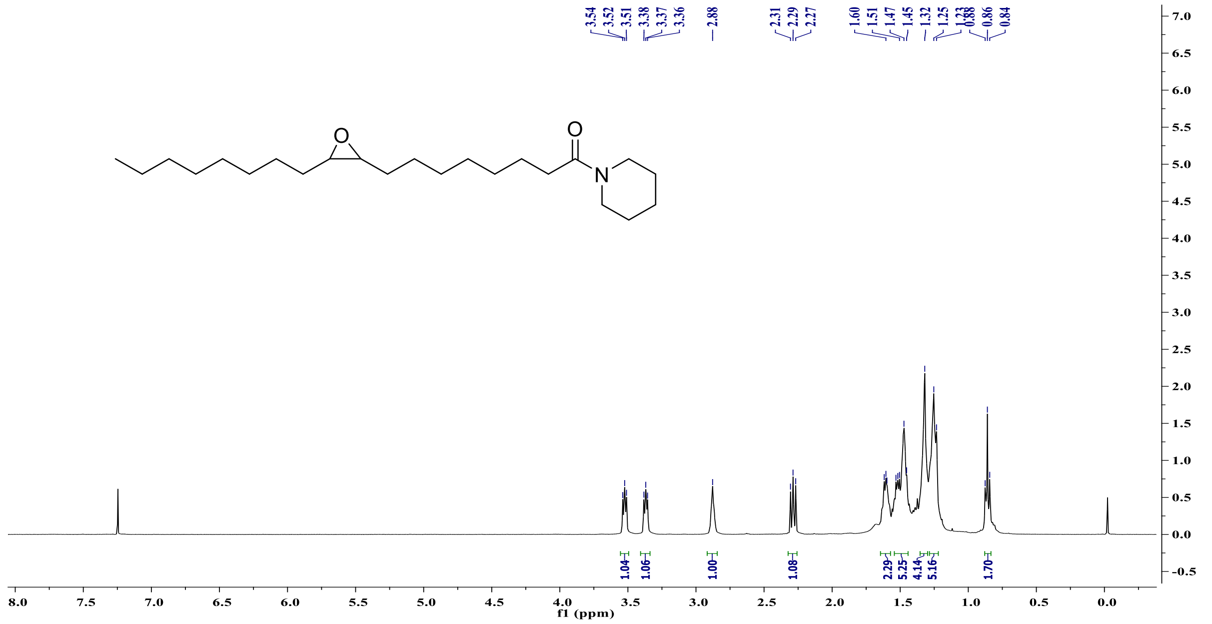
^{1}H NMR of compound 3



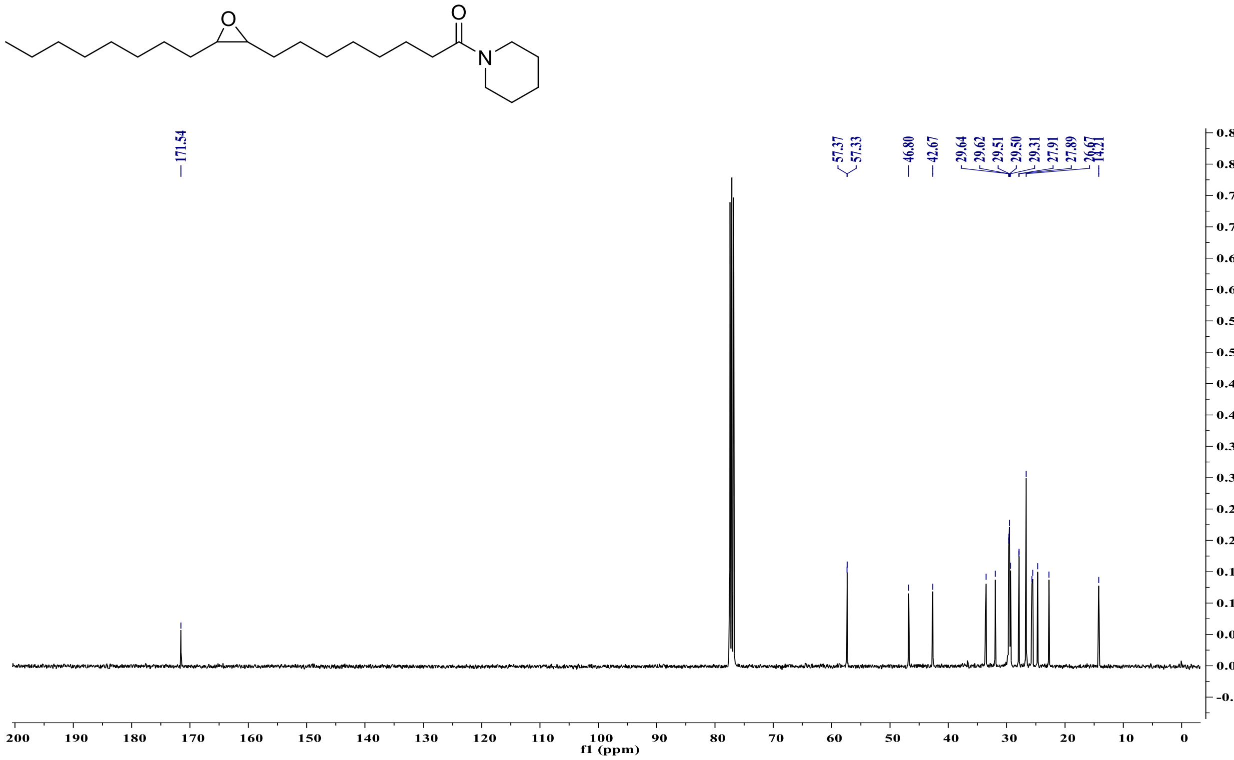
^{13}C NMR of compound 3



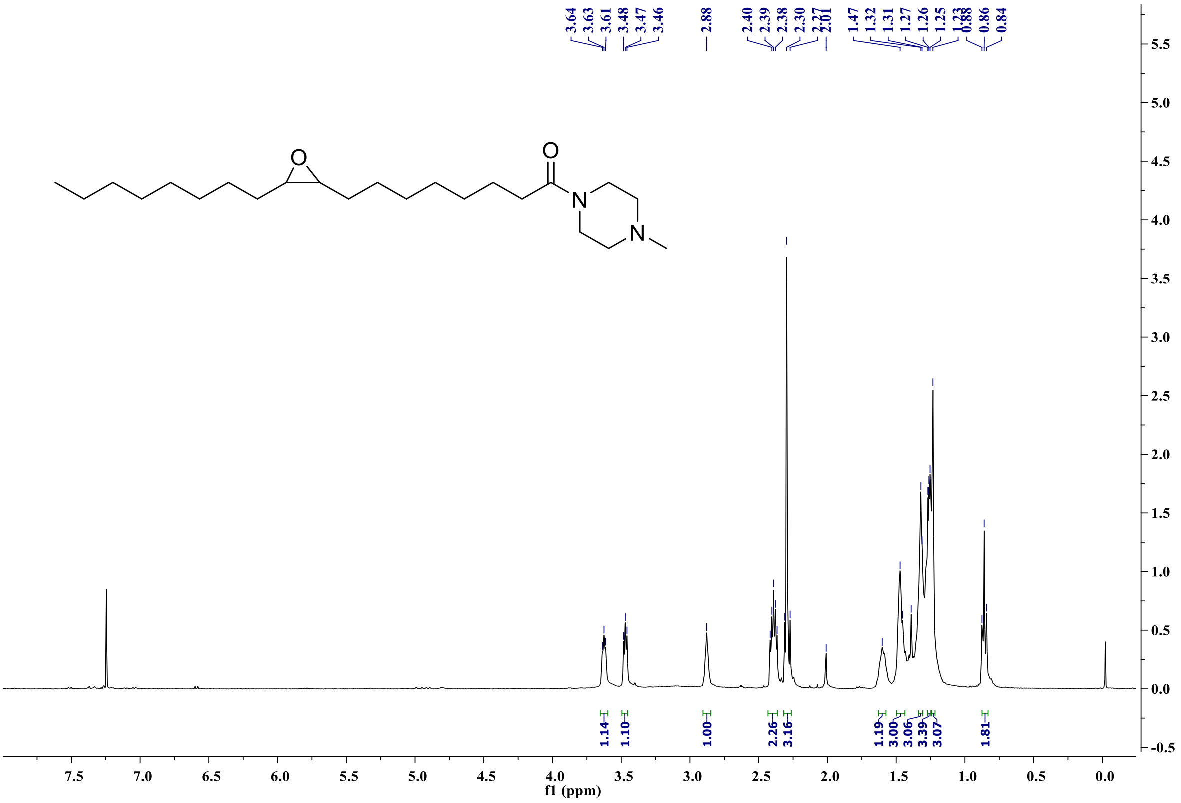
^{1}H NMR of compound 4



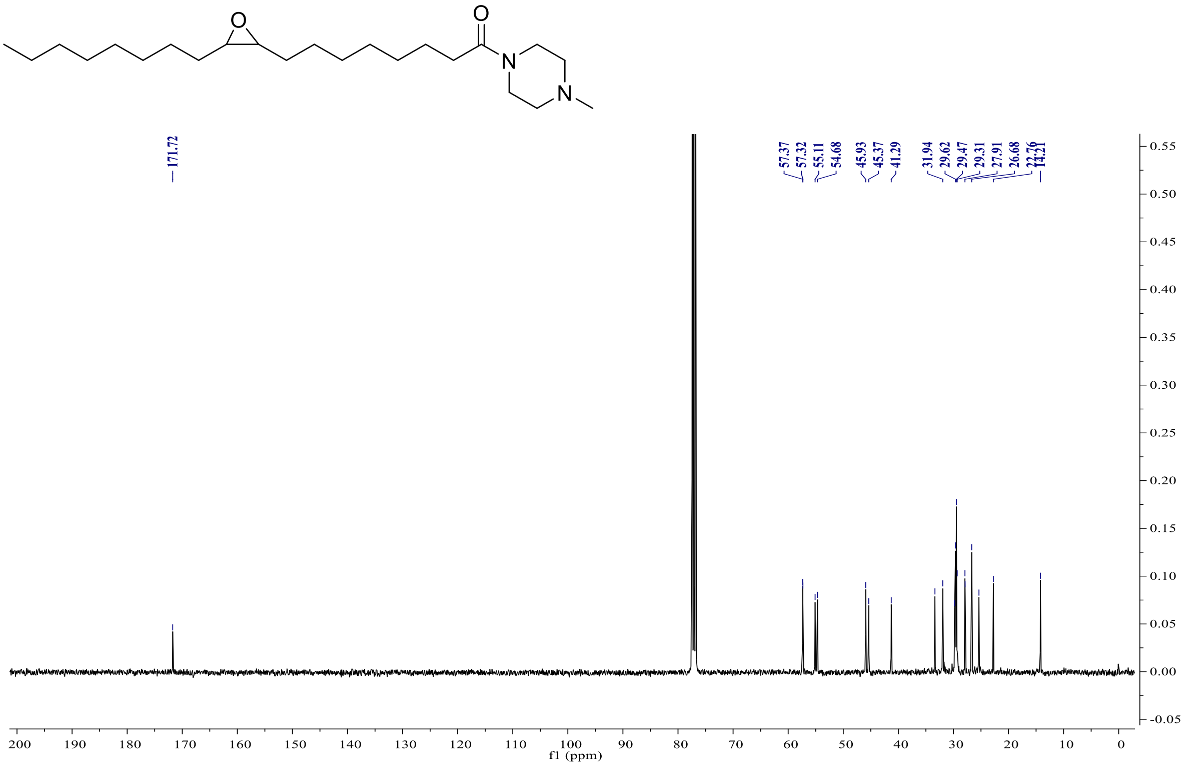
^{13}C NMR of compound 4



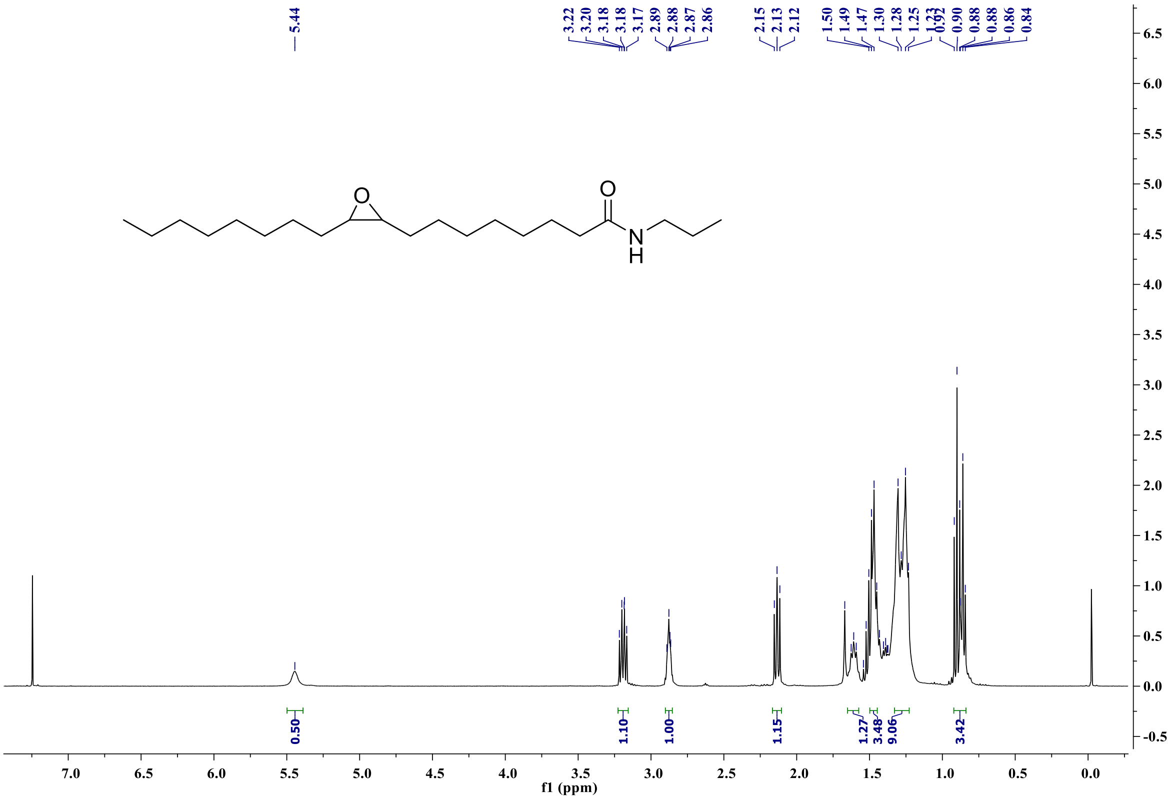
^{1}H NMR of compound 5



^{13}C NMR of compound 5



^{1}H NMR of compound 6



^{13}C NMR of compound 6

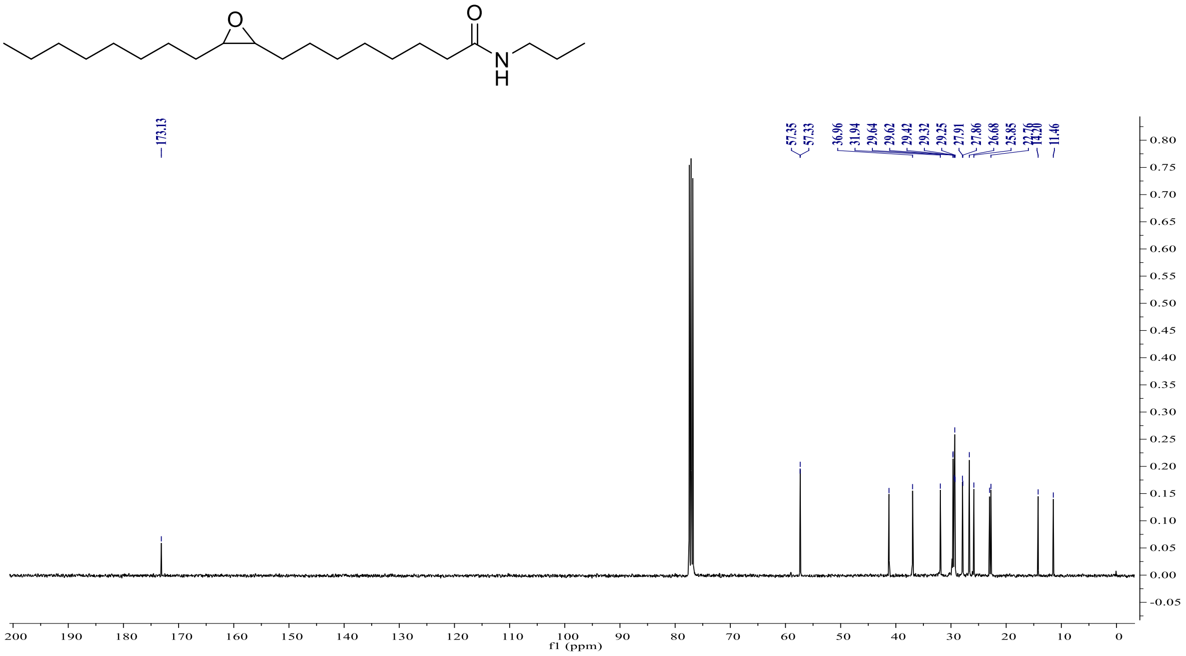


Fig. 2. ^{1}H NMR and ^{13}C NMR of compounds 2-6

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