LATE-STAGE TRANSFORMATION OF FIBRATES TOWARDS DEVELOPMENT OF NEW CHEMICAL ENTITIES.

A Thesis by

Greesha Majethia

Submitted in Partial Fulfillment of the Requirements

For the Degree of

Master of Science

in the

Chemistry

Program

YOUNGSTOWN STATE UNIVERSITY

May 2023

Late-Stage Transformation of Fibrates Towards Development of New Chemical Entities.

Greesha Nandlal Majethia.

I hereby release this thesis to the public. I understand that this thesis will be made available from the OhioLINK ETD Center and Maag Library Circulation Desk for public access. I also authorize the University or other individuals to make copies of this thesis as needed for scholarly research.

Signature:

Greesha Majethia Date

Approvals:

Dr. Ganesaratnam K Balendiran, Thesis Advisor Date

Dr. Josef Simeonsson, Committee Member Date

Dr. Sherri Lovelace-Cameron, Committee Member. Date

Dr. Salvatore A. Sanders, Dean of School of Graduate Studies Date

©

Greesha Nandlal Majethia
2023

Certificate of Registration

This Certificate issued under the seal of the Copyright Office in accordance with title 17, *United States Code*, attests that registration has been made for the work identified below. The information on this certificate has been made a part of the Copyright Office records.

United States Register of Copyrights and Director

Registration Number
TXu 2-363-424
Effective Date of Registration:
February 27, 2023
Registration Decision Date:
April 06, 2023

Copyright Registration for One Work by One Author
Registration issued pursuant to 37 CFR §202.3

Title

Title of Work: LATE-STAGE TRANSFORMATION OF FIBRATES TOWARDS DEVELOPMENT OF NEW CHEMICAL ENTITIES.

Completion/Publication

Year of Completion: 2023

Author

- **Author:** Greesha Nandlal Majethia
  Author Created: Dissertation
  Citizen of: India
  Domiciled in: India
  Year Born: 1999

Copyright Claimant

Copyright Claimant: Greesha Nandlal Majethia
830 Ohio Avenue, Apt 1, Younstown, OH 44504, United States

Rights and Permissions

Name: Greesha Nandlal Majethia
Email: majethiagreesha20@gmail.com
Telephone: (330)623-5659
Alt. Telephone: (330)623-5659
Address: 830 Ohio Avenue
Apt 1
Youngstown, OH 44504 United States

ABSTRACT

The research study included in the thesis aims to create novel Fenofibrate congeners that are simple to synthesize and are effective against hyperlipidemia. The research work carried out during the course of study is related to the development of synthetic methodology and characterization of new metabolites of fenofibrate. The synthesis of the new derivatives was achieved by late-stage derivatization strategy which yielded the desired novel derivatives by simple chemistry as well as shorter route of synthesis as compared to the traditional multi-step organic synthesis. New drug discovery is one of the major applications of the modern organic synthesis and with advancement new synthetic methods, the difficult task for the development of new chemical entities are progressing. Therefore, there is need to take actions for the evolution of new modes, catalysts, and strategies for the fast and economically viable routes for the new drugs and new drug molecules. The present work is related to the discovery of new chemical entities for the control and management of hyperlipidemia a major disorder in the human population. The present effort comprises late-stage modification of the fibrate class of drugs, viz fenofibrate with the objective to develop molecules with improved therapeutic and pharmacokinetic profiles. The work embodied in the thesis is divided into three chapters for the sake of clarity of understanding.

ACKNOWLEDGEMENTS

I would like to acknowledge my supervisor, Dr.Ganesaratnam K Balendiran, who made this work possible. His guidance, and advice improved me through all the stages of this project. I would also like to thank my committee members, Dr.Josef Simeonsson and Dr. Sherri-Lovelace-Cameron for their time, comments, and for pushing me intellectually. Special thanks to Dr. Wahajul Haq for his visit to Youngstown State University and helping me out throughout my entire research journey. I couldn't have made this far without his help. I thank Mr. Ray Hoff, for his assistance on various instruments in the chemistry department, especially NMR.

I am grateful to my closest friends Mitali, Vishva, Avinash, and Nikita for their support during my Master's program and constant encouragement to achieve better. Last but not least, I want to express my appreciation to my grandparents and my entire Indian family. My parents have always been supportive of my endeavors and have offered assistance when I most needed it since the moment I made the decision to attend graduate school. At my worst moments, my parents never stopped believing in me, listening to me, and offering me wise counsel. Without their help and blessing, I would not have been able to complete this degree. My appreciation to the instructors, staff, and students of the Youngstown State University Chemistry and Biochemistry Department for their assistance.

TABLE OF CONTENTS

Chapter **Page**

Table of Contents

CHAPTER 1: CURRENT STATUS OF LIPID-LOWERING DRUGS 6

1.1 Introduction 6

1.2 Fibrate as lipid controlling agent 8

1.3 Statins as a lipid-lowering agent 11

1.4 PCSK-9 inhibitors- a potential therapy for hyperlipidemia 19

1.4.1 Natural Products as PCSK9 inhibitors 25

1.4.2 Other small molecules as PCSK9 inhibitors 26

1.5 Recent Development in Anti-Hyperlipedmic therapy 30

1.6 Conclusions and future directions 31

CHAPTER 2: RECENT ADVANCES IN MEDICINAL CHEMISTRY OF FIBRATES.

....33

2.1 Introduction 33

2.2 Pharmacology and therapeutic applications of Fibrates and their derivatives 34

2.2.1 Hypolipidemic Activity of Fibrates 38

2.2.2 Antihyperglycemic activity of fibrate derivatives 40

2.2.3 Anti-Platelet Activity of Fibrate Derivatives 41

2.3 Fenofibrate as a potential anti-cancer agent 42

2.4 Conclusion and future directions 44

CHAPTER 3: LATE-STAGE DERIVATIZATION OF FENOFIBRATE 45

3.1 Introduction 45

3.2 Rationale of the proposed work: 46

3.3 Synthesis of Fenofibric Acid 50:... 49

3.3.1 Mechanism for the Hydrolysis of Fenofibrate 50

3.3.2 Experimental 51

3.3.3 Spectral Data 53

3.4 Synthesis of Reduced Fenofibrate (Fenfibritol) 55

3.4.1 Mechanism for the reduction of Fenofibratol 56

3.4.2 Spectral Data 59

3.5 Synthesis of Reduced Fenofibric Acid (52) 61

3.5.1 Sodium borohydride as a reducing agent 63

3.5.2 Synthesis of Reduced Fenofibric Acid: (Route-1: by the reduction of fenofibric acid)... 64

3.5.3 Experimental 65

3.5.4 Synthesis of Reduced Fenofibric Acid: (Route-2: by the hydrolysis of Fibritol) 66

3.5.5 Experimental 66

3.5.6 Spectral Data 68

Figure 3.9: ES-MS of Reduced Fenofibric Acid. 68

3.6 New derivatives of Reduced Fenofobirate (Fibritol) 71
3.6.1 The selection of Esterification Procedure 73
3.6.2 The Steglich Esterification 74
3.6.3 Synthesis of new Fenofibratol Esters 76
3.6.4 Experimental 77
4.0 References 89
5.0 Appendix 95

LIST OF FIGURES

Figure Page

Figure 1.1: Mechanism Action of Fibrates 9
Figure 1.2: Mechanism of action of statins 13
Figure 1.3: Mechanism of inhibition of PCSK9. 20
Figure 3.1: Possibilities of late stage functionalized Fenofibrate 47
Figure 3.2: Pharmacokinetics of Fenofibrate 49
Figure 3.3: ES-MS of Fenofibric Acid. 53
Figure 3.4: ^{1}H NMR of Fenofibric Acid. 54
Figure 3.5: ^{13}C NMR of Fenofibric Acid. 55
Figure 3.6: ES-MS of Fenofibritol 59
Figure 3.7: ^{1}H NMR of Fenofibratol 60
Figure 3.8: ^{13}C NMR of Fenofibratol 61

Figure 3.9: ES-MS of Reduced Fenofibric Acid. 68

Figure 3.10: ^{1}H NMR of Reduced Fenofibric Acid 69
Figure 3.11: ^{13}C of Reduced Fenofibric Acid. 70

Figure 3.12: Novel esters of Fenofibratol proposed 72

Figure 3.13: ES-MS Of (4-chlorophenyl)(4-((1-isopropoxy-2-methyl-1-oxopropan-2yl)oxy)phenyl)methyl cinnamate. (53) 80

Figure 3.14: ^{1}H NMR Of (4-chlorophenyl)(4-((1-isopropoxy-2-methyl-1-oxopropan-2-yl)oxy)phenyl)methyl cinnamate.(53) 81

Figure 3.15:: ^{13}C NMR Of (4-chlorophenyl)(4-((1-isopropoxy-2-methyl-1-oxopropan-2-yl)oxy)phenyl)methyl cinnamate.(53) 82

Figure 3.16: ES-MS Of (4-chlorophenyl)(4-((1-isopropoxy-2-methyl-1-oxopropan-2-yl)oxy)phenyl)methyl2-(4-(2-(4-chlorobenzamido)ethyl)phenoxy)-2methylpropanoate. 85

Figure 3.17:^{1}H NMR of (4-chlorophenyl)(4-((1-isopropoxy-2-methyl-1-oxopropan-2-yl)oxy)phenyl)methyl2-(4-(2-(4-chlorobenzamido)ethyl)phenoxy)-2methylpropanoate.(54) 86

Figure 3.18:^{13}C NMR Of (4-chlorophenyl)(4-((1-isopropoxy-2-methyl-1-oxopropan-2-yl)oxy)phenyl)methyl2-(4-(2-(4-chlorobenzamido)ethyl)phenoxy)-2methylpropanoate.(54) 87

LIST OF SCHEMES

Scheme 3.0.1:Synthesis of Fenofibric acid from Fenofibrate.. 50

Scheme 3.0.2:Mechanism for the hydrolysis of Fenofibric Acid. 51

Scheme 3.0.3:Synthesis of Fenofibritol 56

Scheme 3.0.4: Mechanism for the reduction of Fenofibratol 56

Scheme 3.0.5:Pharmacokinetics of Fenofibrate 62

Scheme 3.0.6:Synthesis of Reduced Fenofibric acid via Fenofibric acid. 64

Scheme 3.0.7: Synthesis of Reduced Fenofibric acid via Fibritol 66

Scheme 3.0.8: Synthesis of Fibritol esters 53 and 54 77

CHAPTER 1

CURRENT STATUS OF LIPID-LOWERING DRUGS.

1.1 Introduction:

Drug discovery is an integral part of the human civilization process to the human population in good health. The process of drug discovery research has witnessed several changes in view of the fast-growing science for the identification of new drug targets, molecular interactions, and signal transduction events as well as new insights into drug-target interactions. In addition to that the development of new molecules as candidate drugs are dropped during the clinical trials due to stringent safety norms. This scenario has warranted alternate strategies for the discovery of new druggable molecules that includes

the virtual screening of the molecules for their efficacy and toxic effects as well as target specificity, computer-assisted drug design approaches and QSAR. In addition to that, repurposing of the existing drugs has also been extensively under considerations. A more recent approach has been explored for the discovery of druggable molecules comprising late-stage derivatization of the bio-active molecules or existing drugs for further development of new therapeutic applications. Amongst several metabolic disorders responsible for health impairment lipid profile maintenance is very common and needs immediate attention. The present effort comprises late-stage modification of the fibrate class of drugs, viz fenofibrate with the objective to develop molecules with an improved pharmacokinetic profile.

Hyperlipidemia remains an important health problem for mankind. The disease is generally initiated with a higher level of lipids mainly the triglycerides which in turn results in hypercholesterolemia in advanced stages. Uncontrolled lipid profiles lead to the high cholesterol level particularly low-density lipoproteins resulted in cardiac disease and atherosclerosis. It is important to control the hyperlipidemic conditions by non- clinical management like monitoring the food intake constituents as well as physical exercise. Although the non-clinical modalities are often useful at the initial levels. However, clinical and therapeutic interventions become inevitable under chronic hyperlipidemic conditions. The control of hyperlipidemia is also critical because the elevated lipid profiles are responsible for stimulating other metabolic disorders like fatty liver, diabetics, and cardiovascular diseases.

The management and control of hyperlipidemia have been in practice for more than half of the century using small molecules prior to the discovery of small molecules as anti-

hyperlipidemic agents. Natural product-based therapies were being used and are still in use because of their beneficial effects as well as tolerance to the human body. However, under the clinical diagnosis of hyperlipidemia, there is a need for its clinical management by US FDA-approved therapies. The anti-hyperlipidemic drugs used for the management and control of hyperlipidemia are limited and generally comprise either the fibrate class of drugs or the statin group of medicine. In addition to that, there are some new therapies are being approved using unique mechanisms of action for specific and selective lipid control. A brief account of the lipid-lowering agent is summarized in the following text.

1.2 Fibrate as lipid controlling agent:

The major breakthrough in controlling blood triglycerides was witnessed in the year 1953 by the discovery of the imperial chemical company England that a compound accidentally observed to reduce blood lipid levels of farm workers.[**1**] The company further explored this discovery to indent for therapeutic useful anti- hyperlipidemic agents commonly referred to as fibrates. [**2**]. With the use of

Peroxisome proliferator-activated receptor (PPAR)- α, the fibrate class of drugs are capable to control hyperlipidemia. The family of nuclear receptors consists of a ligand-activated transcriptional factor known as the PPAR-α receptors [**3**] PPAR-α is a prime governor of energy homeostasis and it conducts protein expression which is involved in fatty acid beta-oxidation. The fibrate mostly acts as agonist to PPAR-α receptors as one of the most accepted modes of action as shown in Figure1.1:

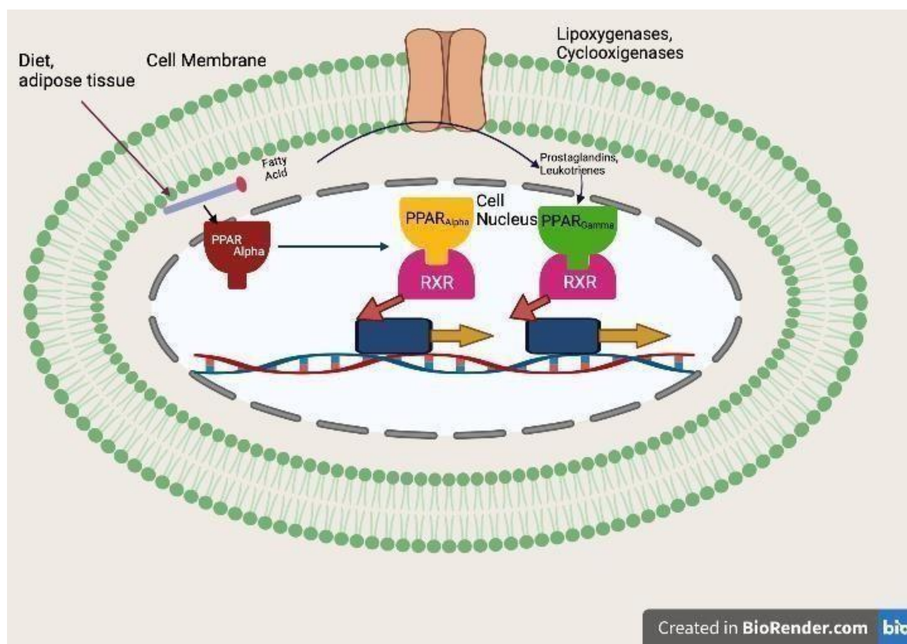
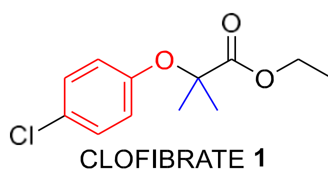
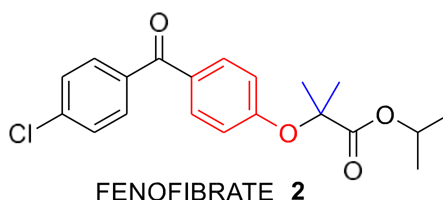


Figure 1.1: Mechanism Action of Fibrates.

The Clofibrate **1** was the first therapeutically useful fibrate approved for human applications and after several years of use, the clofibrate was discontinued in 2002 for human use in view of the toxic manifestations exhibited by the clofibrate.[4] In consideration of the promising and beneficial effects of the fibrate core structure, no efforts were made to improve the therapeutic profile and reduce the side effects as a result number of molecules were identified and developed for anti-hyperlipidemic drugs among the various molecules studied for clinical development. Three molecules viz. Fenofibrate **2**, Bezafibrate **3**, and Gemfibrozil **4** were approved for human applications [5]



Fenofibrate **2** is the first and the most extensively prescribed safe anti-hyperlipidemic agent discovered. It was approved for human applications in the year 1975 [6] The fenofibrate is used as a brand name- Tricor as oral tablets starting from 300mg to higher doses depending on the clinical conditions. It is considered the safest medicine in the fibrate class. The drug is still in the use as an oral anti-hyperlipidemic agent.



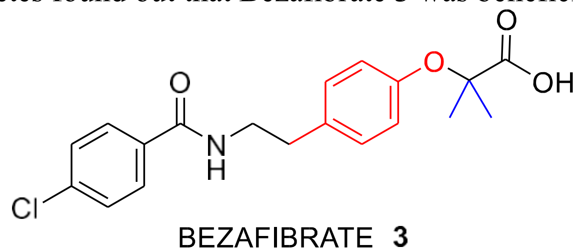
FENOFIBRATE **2**

Subsequently, another compound commonly known as Bezafibrate **3** has been granted permission for human use in 1958. [**4**] application as an oral anti-hyperlipidemic drug. It has several structural similarities to fenofibrate **2** by sharing the common area of 2- methyl-2 phenoxy propionic moiety. The bezafibrate **3** is marketed as bezalip and other brands in the dose of 200-400 mg as per the medical conditions. In addition to the significant reduction of triglyceride levels, it also reduces significantly LDL Levels and

Improves HDL Levels [7]

These improved properties of bezafibrate make the molecule the drug of choice for patients with the risk of cardiovascular disease. Patients with coronary artery disease and

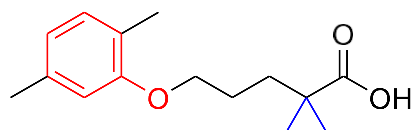
Type-II diabetes found out that Bezafibrate **3** was beneficial to them. [8]



BEZAFIBRATE **3**

In the year 1982, Gemfibrozil **4** was approved for clinical use as a brand name lipoid. This drug is approved in the form of an oral tablet of 600 mg dose for controlling abnormal blood lipid levels [9]. This drug belongs to the fibrate class with common 2- methyl-2 phenoxy propionic residue as a key structural feature. Currently, it is extensively used in the USA for controlling lipid levels and it is a highly prescribed drug in the recent past. So far, there are no noticeable side effects reported for gemfibrozil **4**.

This drug is not recommended in combination with statins. Gemfibrozil **4** helps to reduce blood cholesterol and triglycerides. Hence, effective in reducing cardiovascular indications. In some cases, gemfibrozil **4** is also recommended to reduce the risk of a heart attack.



GEMIFIBROZIL **4**

1.3 Statins as a lipid-lowering agent:

A group of compounds that can inhibit the function of hydroxyl methyl glutaryl Co-A reductase (HMG-CoA) are in use for several decades for the effective control and management of hyperlipidemia. There are several molecules with diversity in chemical structures that are approved by the FDA as anti-hyperlipidemic agents. This group of drugs is commonly referred to as statins. The statins comprise a huge market and are the most prescribed groups of drugs worldwide since they got marketing approval. Amongst various statins, some of the prominent marketed drugs are Lovastatin, Atorvastatin, Simvastatin, Fluvastatin, Rosuvastatin, and Pravastatin.

Cholesterol Biosynthesis consists of a rate-limiting step and also one of the key step where statins significantly inhibit HMG-CoA activity. The statin therapy resulted in the

decreasing of the low-density cholesterol LDL-C in the range of 20-40, as well as the triglyceride levels, which are reduced by up to 20%. Another important feature of statin therapy is the enhancement of high-density lipoprotein cholesterol HDL-C levels, good for cardiac health. The discovery of statins is an interesting scientific story and the discovery of the group of drugs called statins is related to the noble research of Akira Endo,[8, 10] a Japanese scientist who discovered a series of fundamental metabolites in the early seventies of the 19th century which made a basis for the novel pathways for controlling blood lipid levels. The isolation and characterization of the active metabolites from a blue-green mold, the fungus Penicillium citrinum Pen-51 and their further development led to the discovery of this breakthrough research [11-14]

The mechanism of Statins class of drugs is now well understood. This class of drugs functions by blocking the active site through competition of the first and key rate-limiting enzyme in the mevalonate pathway, HMG-CoA reductase. Conversion of HMG-CoA to mevalonic acid is blocked and substrate access is prevented by inhibition of this site. The percentage drop in LDL Cholesterol is fairly correlated with antiatherosclerosis effects of statins. In addition, there is no direct relation between antiatherosclerosis and hypolipidemic action. The typical mode of action the statin class of drugs is summarized in figure 1.2.

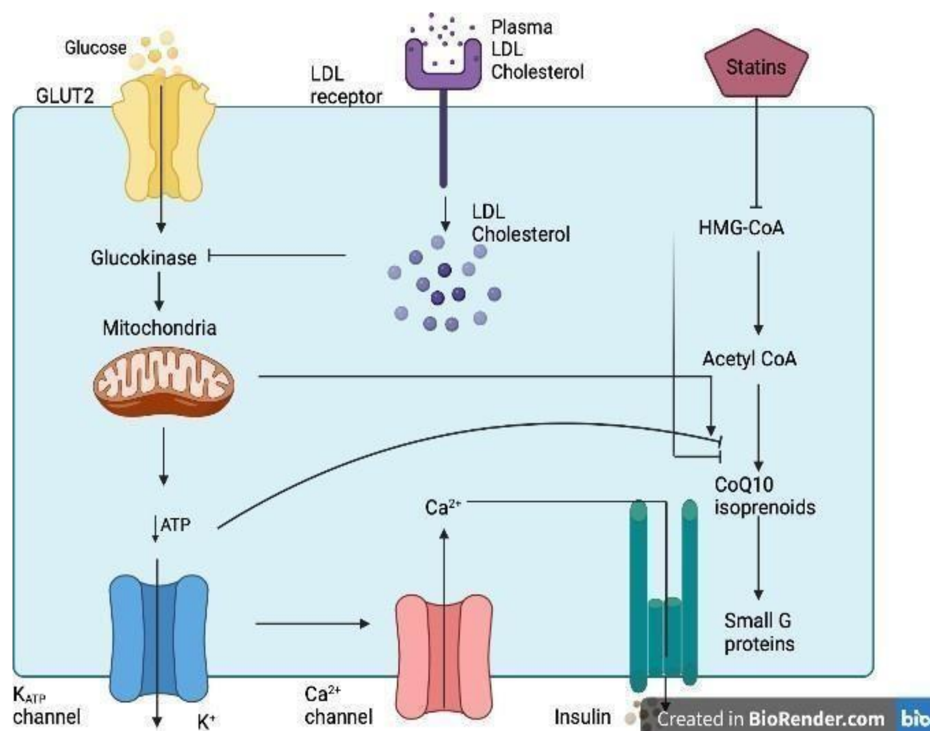


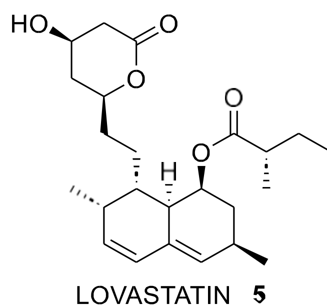
Figure 1.2: Mechanism of action of statins.

The statins are also known to exhibit pleiotropic effects that include improvement of endothelial dysfunction, increased nitric oxide bioavailability, antioxidant properties, inhibition of inflammatory responses, and stabilization of atherosclerotic plaques. In recent years the anticancer activity of statins is also reported [15] However, statins are also associated with certain side effects like a higher risk of diabetes and deleterious effects on skeletal muscles [16] and liver toxicity. [17] Nonetheless the statins group of medications used in ischemic heart disease, stroke, and peripheral vascular disease progression. A brief account of the discovery and chemical structure of prominent statins is given below [18]

1) Lovastatin:

Lovastatin was discovered as a naturally occurring active metabolite of a fungal origin. The development of lovastatin was completed in the year 1982 when lovastatin was

developed as a new class of antihyperlipidemic drugs with the potential for a dramatic reduction in LDL-C levels without any noticeable side effects. The drug was developed by the Merck group of companies which got FDA approval in the year 1987 [18-20] Lovastatin is the first statin that got FDA approval for clinical use as an oral medication for controlling the serum cholesterol levels. lovastatin is still one of the most commonly prescribed medications for millions of patients to date [21]



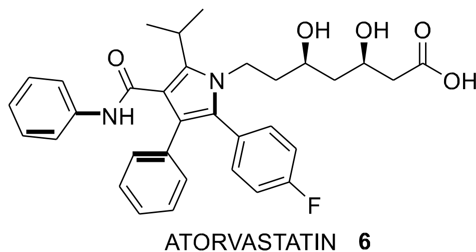
LOVASTATIN **5**

Since this is a fungal metabolite, several studies have been carried out on the understanding of biosynthetic pathways of the drug. However, the major supply of drugs comes from synthetic lovastatin. Lovastatin was first synthesized in the year 1980, before, its FDA approval by M. Hirama, the Japanese chemist. The work comprises the synthesis of Compactin, another metabolite, and the common intermediate was used for the synthesis of lovastatin. [22, 23]

2) Atorvastatin:

It is a purely synthetic compound designed and developed by Warner-Lambert in the year1982. The clinical studies demonstrated that Atorvastatin is highly potent with negligible side effects as a lipid-lowering agent particularly LDL-C which resulted in making Atorvastatin the bestselling drug under the brand name Lipitor [24] Atorvastatin

was approved by the FDA in the year 1996 for clinical use. Warner-Lambert joined hands with Pharma giant Pfizer for the marketing and the Pfizer earned record revenue from the Lipitor alone until the drug became generic in the fall of 2011 [25, 26]

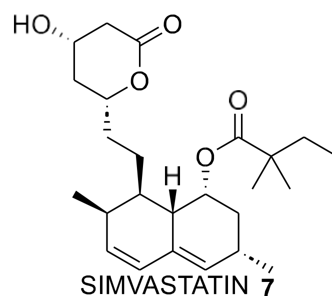


ATORVASTATIN **6**

The fundamental activity of Atorvastatin is attributed to the cure of Dyslipidaemia as well as prophylaxis for the cardiovascular indications. It is also used as the first-line therapy in cholanery heart diseases as well as co-therapy for myocardial infarction and Angina. The drug discovery of atorvastatin was an interesting discovery consisting of an enantiomers and required chiral chromatographic separation as it was racemic mixture and it took place at Parke-Davis. The stereochemistry of the first of the two alcohol functional group was rooted by a diastereoselective aldol reaction in a primary enantioselective root to atorvastatin [27, 28]

Simvastatin:

Simvastatin was developed around the same time when lovastatin was underdevelopment. The Merck group developed simvastatin from a metabolite of fermentation products Aspergillus terreus.[29] This drug has been very effective in reducing the mortality rates among patients with

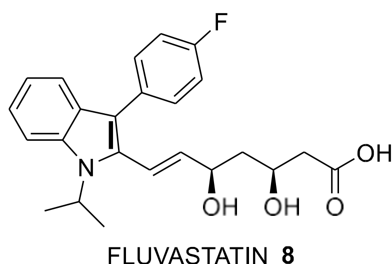


SIMVASTATIN **7**

coronary heart disease and also reduction of death incidence of revascularization procedures in the hospitalized patient. This drug was approved for medical use by FDA in the year 1992 and is currently marketed as a generic medication. Simvastatin is also amongst the highest prescription drugs. Before it became a generic drug, it was the largest-selling drug of the Merck group [30]

3) Fluvastatin:

Fluvastatin comes under the class of drug statins used as Anticholesteremic Agents and was developed in the year 1982 it become approved for medicinal use in the year 1994. It is commonly marketed under the brand name Lescol. Fluvastatin works on a very important step of Cholesterol synthesis by blocking the liver enzyme HMG-CoA reductase [32] where HMG-CoA reductase is used to increase the good cholesterol (HDLC) and lowering the level of bad cholesterol (LDL-C) in the body. HMG-CoA reductase works as the catalyst for the transformation of HMG-CoA to mevalonic acid.

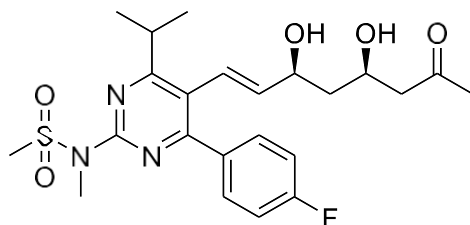


FLUVASTATIN **8**

It is a purely synthetic compound. Friedel- crafts acylation is a well- known technique for the synthesis of Fluvastatin in which there is an acylation of fluorobenzene with chloroacetyl chloride and further reaction with N-isopropyl aniline and $ZnCl_2$ catalyzed indole ring closure in ethanol.[33, 34]

4) Rosuvastatin:

Rosuvastatin is an oral antihyperlipidemic agent developed and marketed by the AstraZeneca group. The potential of rosuvastatin is not only limited to lipid- lowering but it is also prescribed to the patients without clinically evident coronary heart disease to reduce arterial revascularization, risk of stroke, and myocardial infarction. Since 2019, generic medicament of Rosuvastatin are available, while it was patented in 1991 and in 2003, it was approved in United States for medicinal use. Similar to other statins, rosuvastatin is also acted through HMG CoA inhibition pathways [35, 36] Low-density lipoprotein (LDL) cholesterol exhibited dose-related effects of rosuvastatin. Higher doses were more efficacious in improving the lipid profile of patients with hypercholesterolemia than milligram- equivalent doses of atorvastatin and milligram-equivalent or higher doses of simvastatin and pravastatin. The rosuvastatin is also recommended to be used together with dietary changes, exercise, and weight loss.

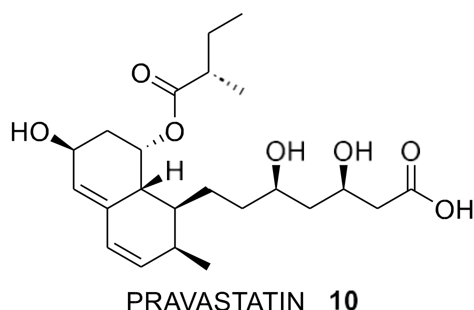


ROSUVASTATIN **9**

The rosuvastatin is a synthetic statin and the manufacturing process comprises the synthesis of key intermediates either by classical synthetic procedures or by bio- catalytical methods. The typical preparation of the intermediate ethyl 4-(4-fluorophenyl)- 6-isopropyl-2-(N-methyl-N-methylsulfonylamino) pyrimidine-5-carboxylate occurs according to standard chemical procedures. Reduction to the 5-hydroxymethyl derivative proceeds smoothly with diisobutylaluminum hydride (DIBALH) in toluene at -10 °C. The hydroxymethyl group is then converted to the Bromo derivative, which upon reaction with triphenylphosphine affords the Wittig reagent. The latter is treated with tert- butyl 2-[(4R,6S)-6-formyl-2,2-dimethyl-1,3-dioxan-4-yl] acetate and provides the protected rosuvastatin ester. Removal of the dioxane protecting group by HCl, ester hydrolysis with NaOH, and precipitation with CaCl2 give rosuvastatin. The 6-formyl side-chain intermediate is prepared by oxidation of the corresponding 6-hydroxymethyl compound, e.g., with DMSO-oxalyl chloride. [37, 38]

5) Pravastatin:

Pravastatin is used as an Anticholesteremic agent, and it is a bioactive metabolite of mevastatin which is isolated from Penicillium citrinum. It was developed by the research of Sankyo Pharma Inc [29] It is sold under the brand name Pravachol and is also used for the prevention of cardiovascular disease along with treating abnormal lipids. [39]Pravastatin was patented in 1980 and approved for medical use in 1989 [6]. FDA approved generic pravastatin for use in the United States on April 24, 2006. [40]



PRAVASTATIN **10**

The pravastatin exists in biological activity in following two pathways:

1) It works as a reversible competitive inhibitor, which inhibits the function of hydroxymethylglutryl-CoA (HMG-CoA) reductase.

2) Pravastatin inhibits the synthesis of very LDL which act as a precursor to low-density lipoprotein [6, 41]

1.4 PCSK-9 inhibitors- a potential therapy for hyperlipidemia:

In the previous sections, we have discussed fibrates and statins for the management and control of hyperlipidemia. However, the major course of concern is attributed to the development of atherosclerosis responsible for coronary heart disease and related cardiovascular diseases including hypertension. In the recent past, there are reports describing new approaches by targeting specific targets responsible for the biosynthesis of cholesterol more precisely for high density lipoprotein (HDL) and low-density lipoprotein (LDL), the latter being the causative for atherosclerosis. Among the various approaches under investigation, targeting PCSK9 function has been emerging as an attractive approach for the control of hyperlipidemia and cardiovascular diseases.

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a hydrolase/ serine protease produced by the gene of the same name. Its expression in the liver is initiated by sterol

regulatory aliment binding protein (SREBP-2) transcription factor and another activator, (HNFIA), which is a hepatic tissue-specific factor. Transcription followed by the splicing leads to the formation of PCSK9-mRNA. The translation of PCSK9-mRNA leads to the generation of the catalytic domain and other stages until the formation of active PCSK9 via pre-pro-PCSK9 and pro- PCSK9 intermediates [42-46] A diagrammatic representation of mechanism of action is presented in figure 3.1.

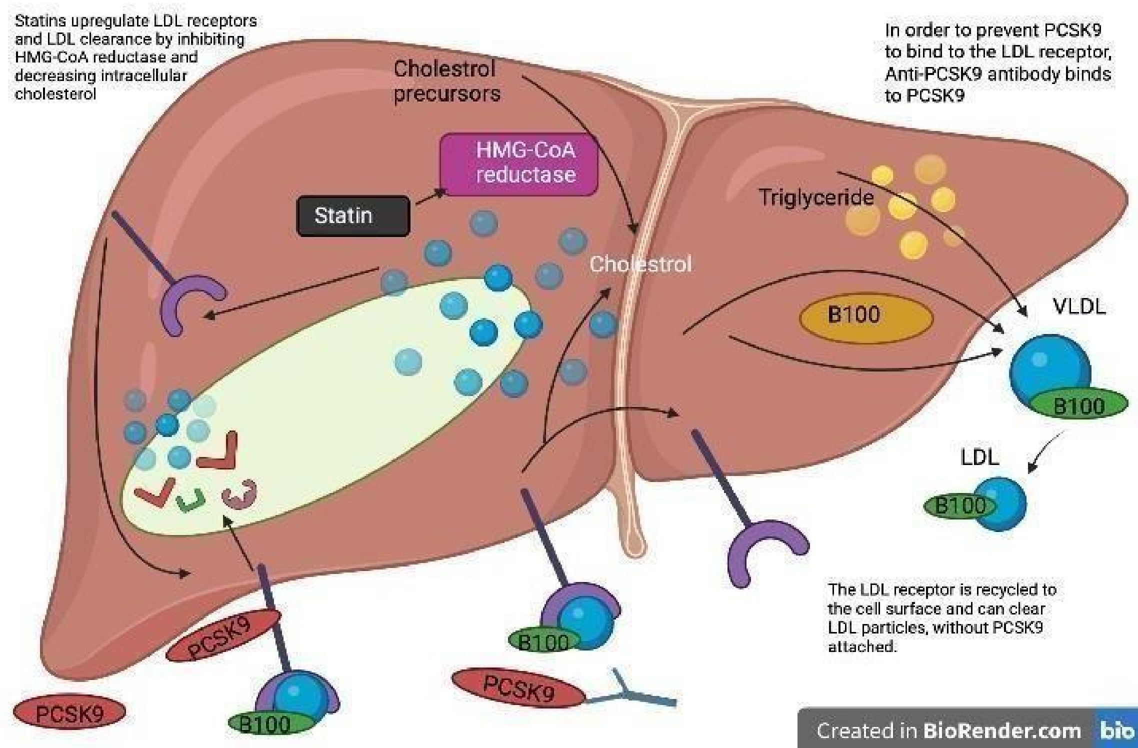


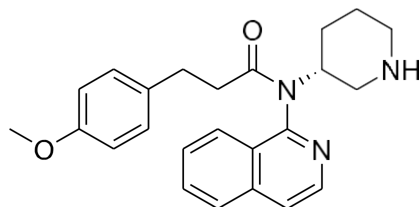
Figure 1.3: Mechanism of inhibition of PCSK9.

Inhibition of PCSK9 resulted in the function of LDL-R in the linear from its degradation. The inhibition of PCSK9 actively has been reported as a variety of agents comprising small molecules, peptides, and its conjugates and by monoclonal antibodies. The most important function of PCSK9 is considered the regulation of LDL-R expression. In other words, regulating the LDL levels in circulation.

The PCSK9 inhibitors also reduces LDL-R density on hepatocytes and the other hand, it captures the biosynthetically produced VLDL. It is also known, that the PCSK9 also interacts with other receptors. Thus, indicating their involvement in the regulation of other metabolic pathways. More clarity is expected from the research currently on this interesting target. A recent review summarizes the current status and future direction of the development in the area of specific inhibition of PCSK9 derived anti-hyperlipidemic agents [45, 47]

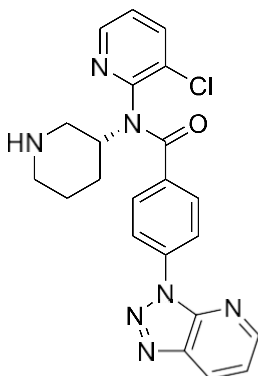
The literature available regarding the therapeutic advantage of PCSK9 inhibitors is a new class of lipid-lowering agents is becoming more acceptable. The PCSK9 antagonist may have a low molecular weight substance or macromolecular structures. However, the macromolecular structure cost remains a determining factor. Therefore, small molecules such as PCSK9 inhibitors are in high demand. There has been a rapid increase in the identification of a variety of small molecule, synthetic, and naturally occurring PCSK9 modulators.[46, 48] The beginning of discovery of small molecules as PCSK9 inhibitors started with the search for an inhibitor from the selection of [HTS] by the Pfizer group of researchers and the identification of PF-00932239 **11** from the phenotypic screening of approximately 2.5 million structures. [47, 49]

Another potent compound identified by the follow-up work improved the activity profile



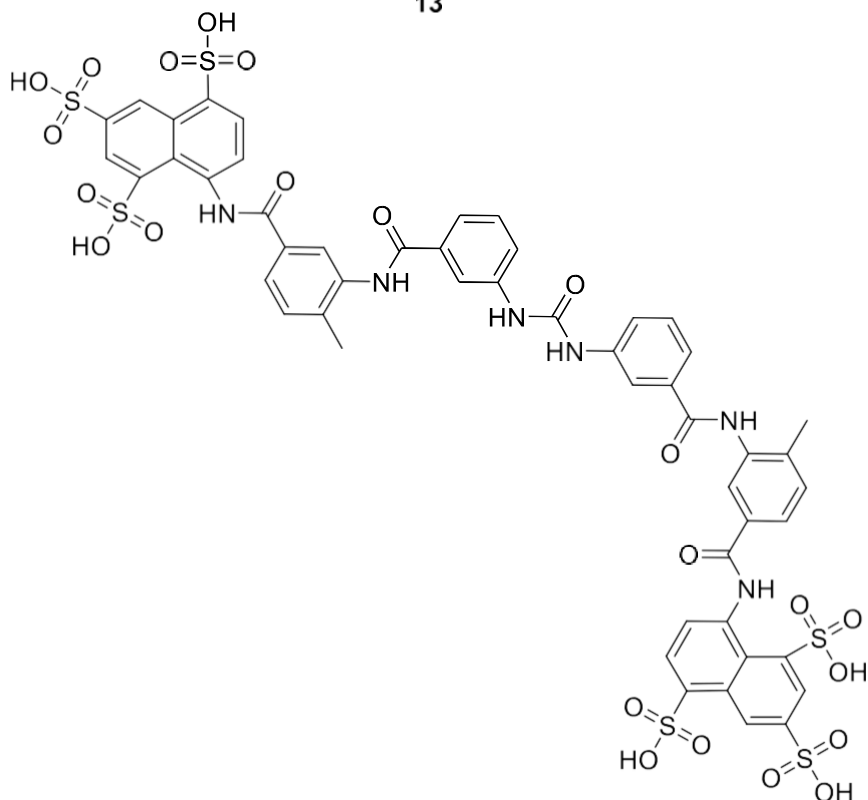
(*R*)-*N*-(isoquinolin-1-yl)-3-(4-methoxyphenyl)-*N*-(piperidin-3-yl)propanamide
11

named PF-06446846 **12** which has shown inhibition of sub-micromolecular compounds [50]



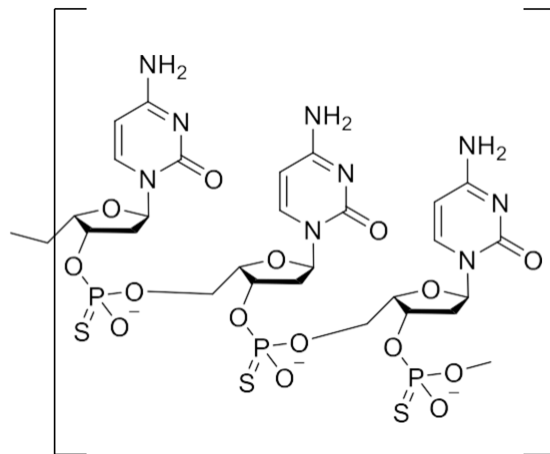
(*R*)-4-(3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-yl)-*N*-(3-chloropyridin-2-yl)-*N*-(piperidin-3-yl)benzamide
12

This is followed by optimization studies for the identification of a potent compound activity by blocking the ribosomal function. It is shown that PCSK-9 interacts with LDL- R and acts via hyperinsulfate proteoglycan receptor (SHPGS) inside the liver. Hence, hyper-in mimics were studied for the discovery of new PCSK-9 inhibitors. Some of the known hyperinmimics as PCSK9 inhibitors were identified viz **13, 14**[51-55]



13

8,8'-((3,3'-((3,3'-(carbonylbis(azanediyl))bis(benzoyl))bis(azanediyl))bis(4-methylbenzoyl))bis(azanediyl))bis(naphthalene-1,3,5-trisulfonic acid)



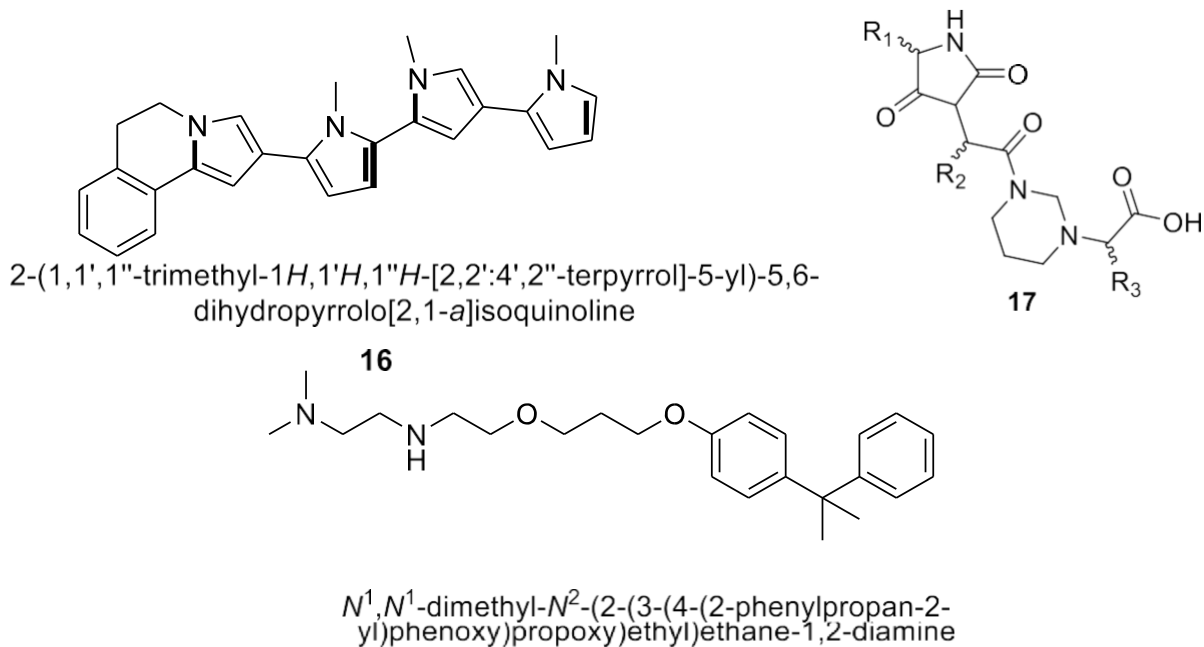
O-((2*S*,3*R*,5*R*)-5-(4-amino-2-oxopyrimidin-1(2*H*)-yl)-2-ethyltetrahydrofuran-3-yl) *O*-(((2*S*,3*R*,5*R*)-5-(4-amino-2-oxopyrimidin-1(2*H*)-yl)-3-(((((2*S*,3*R*,5*R*)-5-(4-amino-2-oxopyrimidin-1(2*H*)-yl)-3-((methoxyoxidophosphorothioyl)oxy)tetrahydrofuran-2-yl)methoxy)oxidophosphorothioyl)oxy)tetrahydrofuran-2-yl)methyl) phosphorothioate

14

The in-silico screening of the compounds for the target interaction was successfully explored for the discovery of a new small molecule PCSK9 inhibitor [56]. This is followed by the identification of a new compound showing potent inhibit reactivity named CB-35. Although these compounds exert the inhibitory effects mediated by direct binding to PCSK9. However, more work is required to establish the specificity of the compounds.

The peptidomimetics approach is now extensively explored for the identification of a small molecule peptidomimetic. It is worth mentioning here that several medium to small peptides has been identified as potent inhibitors of PCSK9. This prompted the research on

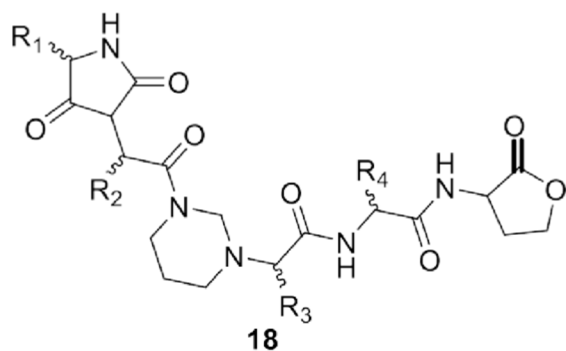
peptidomimetics considering the limitation of pharmacokinetic issues with the peptides.[57] Imidazole-based peptidomimetics were reported with significant activity as shown in the **15**.

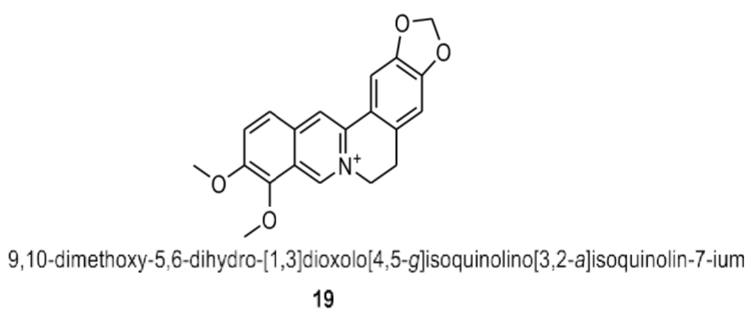


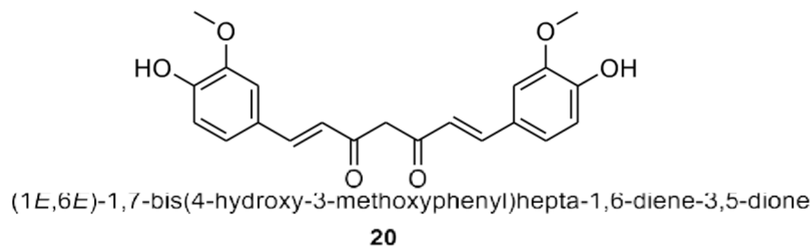
In another report, molecules having **16** and **17** were shown to be a potent inhibitor of PCSK9. These compounds were developed after several interactions of structural optimizations. [58, 59]

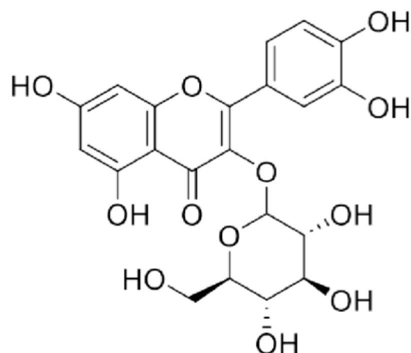
1.4.1 Natural Products as PCSK9 inhibitors:

Natural products remain an important class of compounds for providing leads and new directions for the discovery and development of new target-based therapies. As a result of extensive screening of natural products against PCSK9, a few natural products were identified to modulate the function of PCSK9. The mechanistic studies of these naturally occurring compounds are yet to be established. However, it is a consensus that most of the compounds are modulators of PCSK 9 secretion or expression. The structure of a few prominent PCSK9 modulators **18**-**22** is given below:



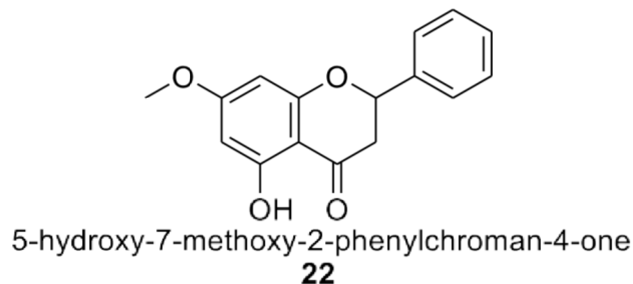






2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-(((3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)-4*H*-chromen-4-one

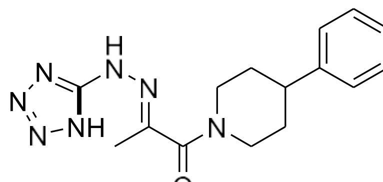
21



1.4.2 Other small molecules as PCSK9 inhibitors:

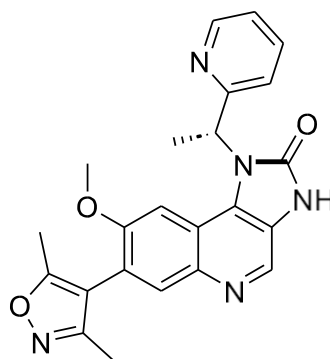
There are several molecules from various research groups from academia as well as industry working on the development of small molecule PCSK9 inhibitors. A set of synthetic peptides ranging from 3-8 amino acids binding to an allosteric site of PCSK9

and responsible for changing the conformation of PCSK9 protein resulting in the alteration of the kinetics of interaction between LDL-R and PCSK9. The most important peptide in this class was found to be SRX-55 **23** [60, 61]

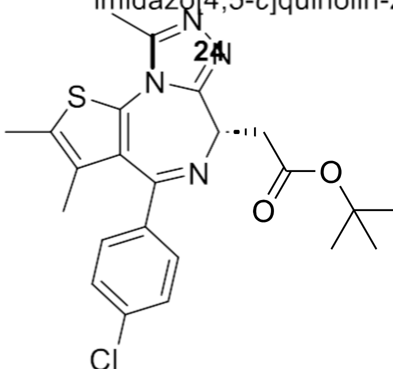


(*E*)-2-(2-(1*H*-tetrazol-5-yl)hydrazineylidene)-1-(4-phenylpiperidin-1-yl)propan-1-one
23

Another class of small molecule inhibitors is reported by a French group showing the mechanism of action by a novel pathway for inhibition of expression of PCSK9. Thus, reducing the circulating LDL levels. Several compounds of this series were reported with prominent activity. Whereas, the compounds IBET-151 **24** and JQ1 **25** were considered the most potent compounds of these series.[62]

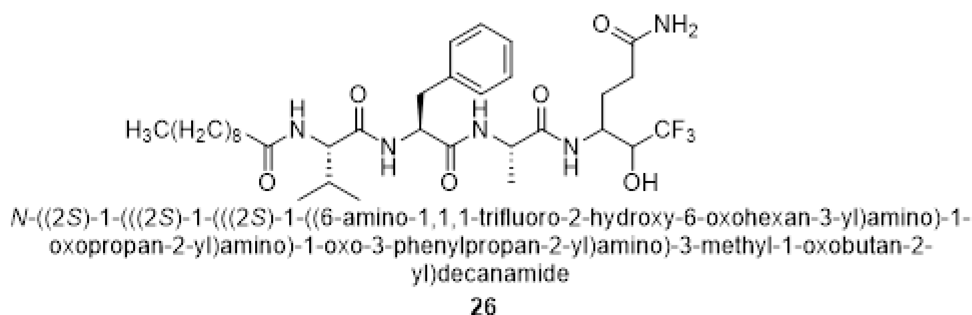


(*R*)-7-(3,5-dimethylisoxazol-4-yl)-8-methoxy-1-(1-(pyridin-2-yl)ethyl)-1,3-dihydro-2*H*-imidazo[4,5-*c*]quinolin-2-one
24



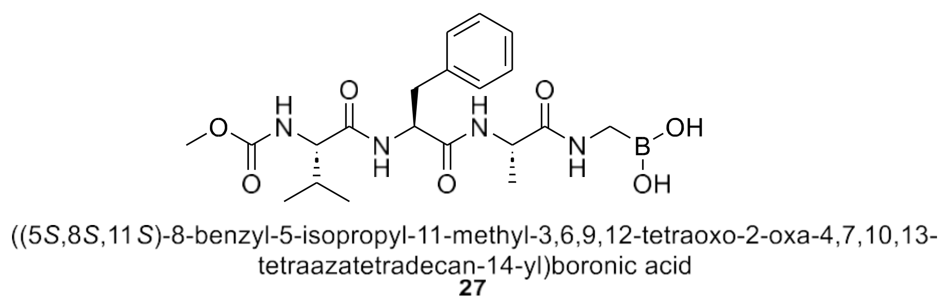
tert-butyl (*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetate
25

Several amino acid-based compounds are reported in the patent literature [63] demonstrating the compound belonging to peptide and peptidomimetics have shown highly potent activity for the inhibition of PCSK9 function. These compounds are showing activity in a dose-dependent manner and the structure of some of the prominent compounds **26, 27** are shown below:



N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide

26



((5*S*,8*S*,11*S*)-8-benzyl-5-isopropyl-11-methyl-3,6,9,12-tetraoxo-2-oxa-4,7,10,13-tetraazatetradecan-14-yl)boronic acid

27

1.4 Monoclonal Antibodies as a hyperlipidemic agent:

The clinical progression of monoclonal antibody-based medication for inhibiting PCSK9 and decreasing LDL levels renders it apparent that this approach is the most appropriate method. A variety of monoclonal antibodies, notably Alirocumab (formerly referred to as SAR236553/REGN727), Evolocumab (formerly referred to as AMG145), RG7 652, LGT209 (NCT01979601, NCT01859455), 1B20, and Bococizumab (in the past identified as RN316/PF-049 50615), have been granted approval for usage in hospitals and

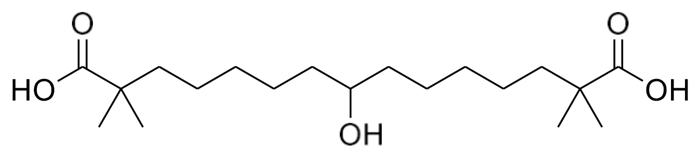
clinics. Evolocumab and Alirocumab have recently received approval by the FDA. The most current progress in this area of study is outlined in a new article. In order decrease the amount of LDL cholesterol and have a prolonged serum half-life and course of action, themAb bonocizumab uses pH-sensitive binding to PCSK9.[64-66] Similar to this, phase I clinical trials have been looking at adnectins (also known as monobody) and small peptide inhibitors for the reduction of LDL. The results indicated an ultimate dose-related decline in LDL-C by up to 48% across both healthy and hypercholesterolemic patients [64]. Adnectins have the added advantage of being significantly smaller than mAb, which renders them more affordable and easier to make. In preclinical creatures, their pharmacokinetics have been found to be favorable with a quick start in function; more studies will be conducted to assess how the medication matures. [65]

In the year 2015, the U.S. Food and Drug Administration (FDA) approved Sanofi and Regeneron Pharmaceuticals' Praluent (alirocumab) injection, the first lipid-lowering drug in a new class of drugs known as PCSK9 inhibitors for the treatment for patients with familial hypercholesterolemia (FH) or clinical atherosclerotic cardiovascular disease. The new class of drugs has the potential to offer millions of patients with high low-density lipoprotein cholesterol (LDL-C) an alternative treatment option to statins, which are associated with numerous side effects.

1.5 Recent Development in Anti-Hyperlipedmic therapy:

The research for the development of new therapeutic options for controlling the serum lipid profile is an ever-expanding area of drug research in consideration of the function of lipid metabolism in human health. The indiscriminate use of drugs like statins etc. is resulting in other complications. In the recent past few molecules are approved by FDA for clinical management of hyperlipidemia with specific and improved therapeutic applications.

Esperion otherwise known as, Nexletol, in 2002 was approved as non-statin pill with a unique formulation of both bempedoic acid and ezetimibe showing reduction in cholesterol by about 38 to 44%. It was anticipated by manufacturer, based on person consuming a statin and how much amount was consumed also mattered. But conclusion from FDA is still expected in coming time. Similarly, Inclisiran (Leqvio®; Novartis) approved in Europe in December 2020 for cholesterol-lowering small interfering RNA (siRNA) is first in class and it is associated with triantennary N-acetylgalactosamine carbohydrates (GalNAc). In addition to diet, it is used in combination with a statin or a stain with additional lipid-lowering therapies in patients with highly tolerated stain dose and which may not be able to reach low-density lipoprotein cholesterol goals. [64] Esperion Therapeutics came up with a non-statin antihyperlipidemic drug which they named it as Bempedoic acid **28**. Phase III CLEAR clinical trial program showed positive findings due to which, USA and EU approved Bempedoic acid fixed-dose combination with ezetimibe (NEXLIZET® in the USA, Nustendi® in the EU) and as monotherapy (NEXLETOL® in the USA, Nilemdo® in the EU). There is an invention of new class of drugs to cure low-density-lipoprotein which is also called as "bad" cholesterol and bempedoic acid is first in this class which helps in reducing oxygen flow and blood and it aims in an expansion of fatty deposits in the arteries.



Bempedoic Acid **28**

1.6 Conclusions and future directions:

Hyperlipidemia is one of the most common metabolic disorders prevalent amongst the human population across the world. Elevated lipid profiles is also a major cause of other

life-threatening disease like cardiovascular disease, the number 1 killer in the USA. The control of lipid profile has two major objectives, viz, the control of triglyceride, and second is the control of LDL-C. The main cause of arthocelurasis and cardiovascular disease. Amongst the various approaches for the control and management of hyperlipidemia discussed above, it can be seen that it started from the fibrate class of drugs, with the major objective to control Triglyceride levels but also provide the inhibition of LDL-C. The modern era is targeting the biosynthesis of LDL-C by targeting new pathways like PCSK9, etc. In addition to that, there are recent reports as well as approvals by FDA for monoclonal antibodies and other substances for a specific control. A meta-analysis of more than half a century of works reveals that the fibrate class of drugs is one of the safest and most efficacious drugs and therefore needs immediate attention for the discovery of improved anti-hyperlipidemic therapy.

CHAPTER 2

RECENT ADVANCES IN MEDICINAL CHEMISTRY OF FIBRATES.

2.1 Introduction:

Since the discovery of fibrate around the middle of 19th century, the fibrate class of molecules resulted in the identification of only three drugs that are in use for more than half a century without noticeable side effects. In spite of its safe and effective pharmacological profiles, much attention has not been paid towards the discovery of new chemical entities for drug development purposes. The fibrate class of molecules is continued to be a drug of choice for the management and control of hyperlipidemia, but on the other hand, the medicinal chemistry of fibrates was neglected to some extent and the major reason is attributed to the path-breaking discovery of statins. The indiscriminate

use of statins resulted in the identification of several side effects mainly muscle atrophy. In addition to that, with the advancement of molecular biology and systems biology research new target-based discoveries for the control of abnormal lipid profiles and related ailments at the molecular level. This includes monoclonal antibodies- based therapies, small interfering siRNA, and other biologics for the development of the new concept-based personalized medicine as well as gene therapy to some extent. The recent advances toward biologics are very specific and highly beneficial at some point, but the safety, delivery, and sustained production remain challenging. Further the cost of these medicines, making them unaffordable to the general patient population looking at this scenario the medicinal chemist turned their attention towards the fibrate class of molecules for the development of new chemical entities for the new chemical profiles as well as the discovery of safe therapeutic options for providing the unmet therapeutic need for various upcoming threats to human health. A brief account of the wide spectrum of pharmacological effects as well as the medicinal chemistry around the fibrate scaffold will be summarized in the following sections:

2.2 Pharmacology and therapeutic applications of Fibrates and their derivatives:

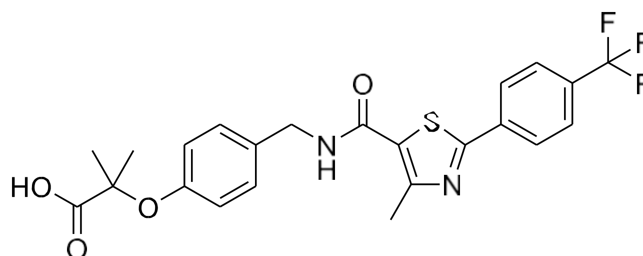
The fibrate class of molecules exerts lipid-lowering activity by agonistic binding to the PPAR receptors. The PPARs are the nuclear receptors and function as transcription factors for controlling the expression of vital cellular functions viz. glucose metabolism, and sugar metabolism. The receptors are also involved in insulin sensitivity and resistance and another important aspect for the function of PPAR is related to energy homeostasis. These nuclear receptors perform their functions by the activation of their subtypes by specific ligands. There are generally two major forms of PPARs: PPARα, and PPARγ. However, another group of receptors identified in the later stage is called PPARβ and PPARδ receptors. The PPARα class of receptors is generally involved in the lipid metabolism and expressed in organs liver, kidney, adipose tissues, etc.[65]

However, the PPARγ is generally attributed to the insulinotropic glucose homeostasis These sub-receptor types have different tissue distributions binding ligands and their activation and their suppression agents, The PPAR group of nuclear receptors has been an interesting target of research of metabolic disorders and the development of new therapies for the discovery of cardiovascular and metabolic syndrome [66, 67]

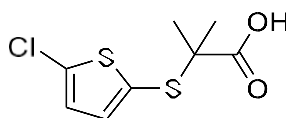
a) PPAR α agonist:

The PPARα is generally involved in the regulation of lipid metabolism in the event of weaker expression of PPARα the resulting in the manifestation of hyperlipidemia. Therefore, a specific activating ligand of PPARα will result in the reduction of blood lipid profiles. The fibrate class of molecules are specific ligands and activators of PPARα and therefore considered as PPARα agonists. The fibrates were developed as highly efficient

PPARα agonists. In the recent past, a number of new structures are discovered to have highly potent and selective PPARα activity **29-31**.[68] The classic fibrate head group commonly referred to as 2-methyl-2- phenoxy propanoic acid is considered to play an important role in the development of a highly potent and selective PPAR α agonist.

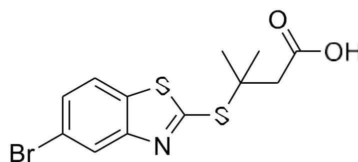


GW590735 **29**



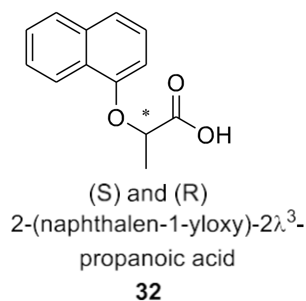
2-((5-chlorothiophen-2-yl)thio)-2-
methylpropanoic acid

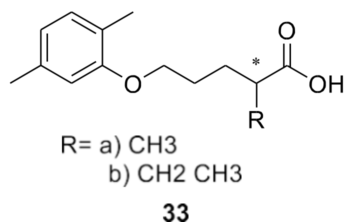
30



3-((5-bromobenzo[*d*]thiazol-2-yl)thio)-3-methylbutanoic acid
31

There are some chiral molecules reported in the literature as specific PPAR α agonists **32-33** with improved and specific agonistic activity.

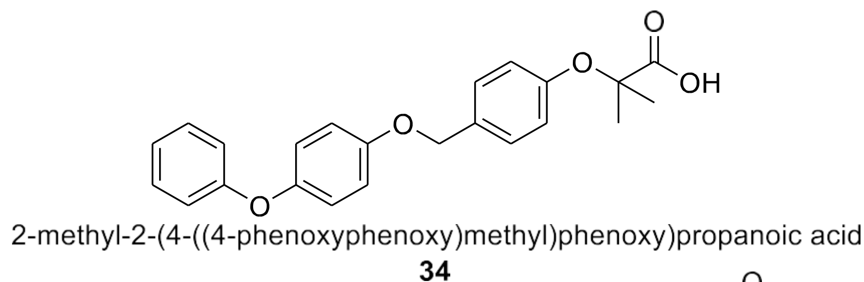


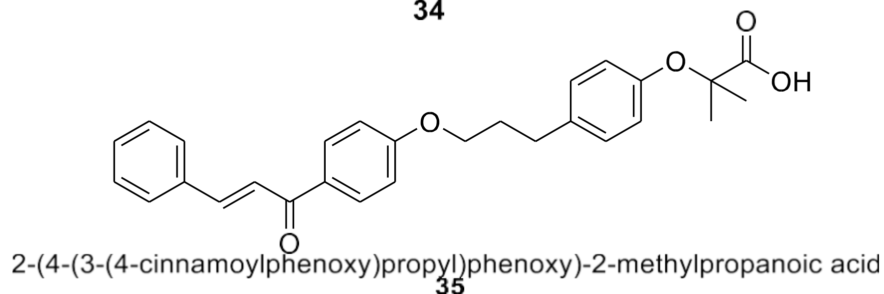


b) PPARγ agonists:

The PPARγ receptor is generally referred to as the regulation of blood glucose levels. However, recent studies revealed that the fibrate class of compounds has a partial affinity for the PPARγ receptors also. Further research in this direction resulted in the identification of a highly selective PPARγ agonist derived from fibrate core structure **34**,**35**.[69]

Since these molecules are derived from fibrate core structure and show strong PPARγ activity as well as antihyperglycemic activity. Comparable to the piogliatazone, classical PPARγ agonist anti-diabetic drug.[70]





c) PPAR β, δ agonist:

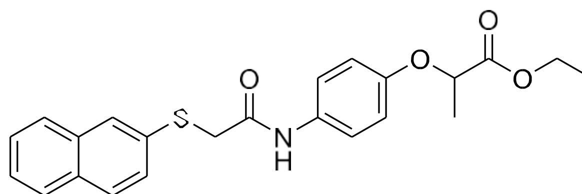
The fibrate class of molecules having PAN affinity to the group of nuclear receptor family PPAR. The PPAR β, δ agonists are widely distributed and control various functions like fatty acid oxidation, insulin sensitivity, muscle development, and related mitochondrial function. The β, and δ agonists have the potential for drug development and therefore a number of molecules are reported in the literature. Amongst various classes, a fibrate derivative **36** has been identified for drug development purposes.[71]

GW501516 **36**

In view of the broad spectrum of the agonistic activity by the fibrate class of molecules, the fibrates are interesting pharmacophores for the development of other therapeutic areas like diabetics and cardiovascular disorders in addition to their primary roles as anti-hyper lipidic drugs. The recent developments of the fibrates for alternative therapeutic targets will be briefly discussed in the following sections:

2.2.1 Hypolipidemic Activity of Fibrates:

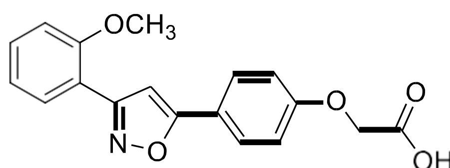
As discussed in the previous section, that clofibrate, Fenofibrate, Bezafibrate, and Gemfibrozil is the prominent drugs of the fibrate class used for controlling the blood triglyceride level also known for their influence on cardiovascular diseases. In the recent past, structure-activity relationship studies were carried out, and several new chemical structures were identified in addition to the existing drugs. There are two major structures reported viz hybrid molecules **37** [72] showed the improved hypolipidemic activity and also influence the cardiovascular functions.



ethyl 2-(4-(2-(naphthalen-2-ylthio)acetamido)phenoxy)propanoate

37

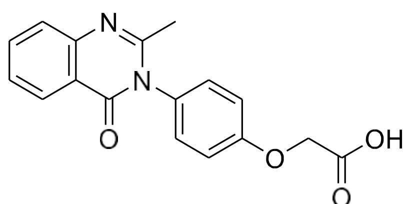
Another compound belongs to the oxazole spacer between the phenoxy and other aromatic groups **38**. [73]



2-(4-(3-(2-methoxyphenyl)isoxazol-5-yl)phenoxy)acetic acid

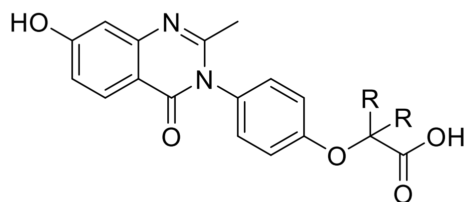
38

The compound has shown improved efficacy in controlling the total cholesterol Triglyceride and LDL- C [79] Similarly, other hybrid molecules are also reported with improved pharmacological activity **39, 40**, **41, 42.**[74]

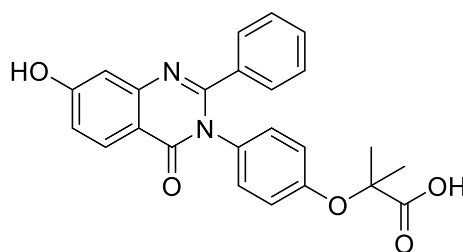


2-(4-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)phenoxy)acetic acid

39



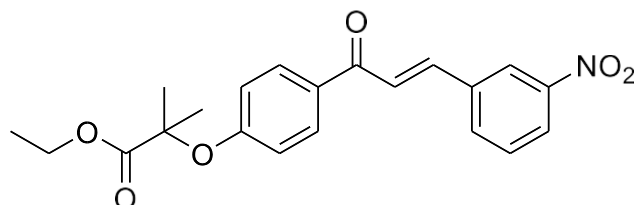
40 R=CH_3
41 R=H



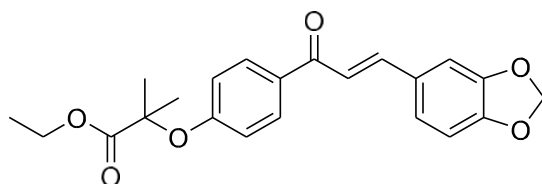
42

2-(4-(7-hydroxy-4-oxo-2-phenylquinazolin-3(4*H*)-yl)phenoxy)-2-methylpropanoic acid

A series of chalcones were reported with a broad spectrum of biological activity in addition to the lipid-lowering activity of the fibrate derivatives **43, 44**. [75, 76]



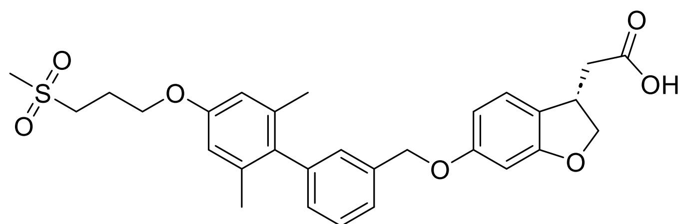
ethyl (*E*)-2-methyl-2-(4-(3-(3-nitrophenyl)acryloyl)phenoxy)propanoate
43



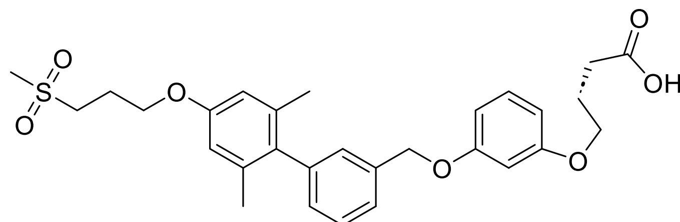
ethyl (*E*)-2-(4-(3-(benzo[*d*][1,3]dioxol-5-yl)acryloyl)phenoxy)-2-methylpropanoate
44

2.2.2 Antihyperglycemic activity of fibrate derivatives:

As mentioned in the previous section, the fibrate class of molecules, exert their



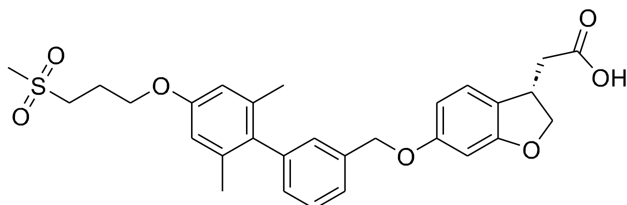
TAK-875 **45**



46

4-(3-((2',6'-dimethyl-4'-(3-(methylsulfonyl)propoxy)-[1,1'-biphenyl]-3-yl)methoxy)phenoxy)butanoic acid

activity via binding an agonist activity to PPARα receptors. The PPARs are the group of nuclear receptors responsible for controlling various metabolic and cellular activities. The PPARγ agonists are therefore classified as insulin sensitizers. A number of fibrates are now known with the agonistic activity to PPARγ also. These substances are classified as PPAR α/γ dual agonists. These molecules are very interesting in the sense that they can exert antihyperglycemic as well as antihyperlipidemic activity in view of their beneficial effects. Extensive research work is going on for the development of novel Type II diabetic therapy. Several molecules are reported in the literature TAK 875 **45**, **46**.[76, 77]

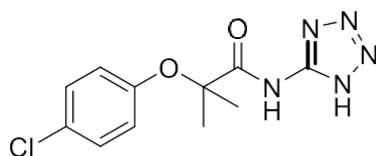


TAK-875

45

Unfortunately, these compounds have shown some undesired side effects during the developmental face hence were not continued. Towards these objectives, further structural optimization studies were carried out, and compounds analogous to (**46B)** were identified with high ligand efficacy and potent antidiabetic activity in an animal model.[78] The optimization work was continued, and other new structures were

identified with potent antidiabetic activities **47, 48**. [79, 80]



2-(4-chlorophenoxy)-2-methyl-*N*-(1*H*-tetrazol-5-yl)propanamide

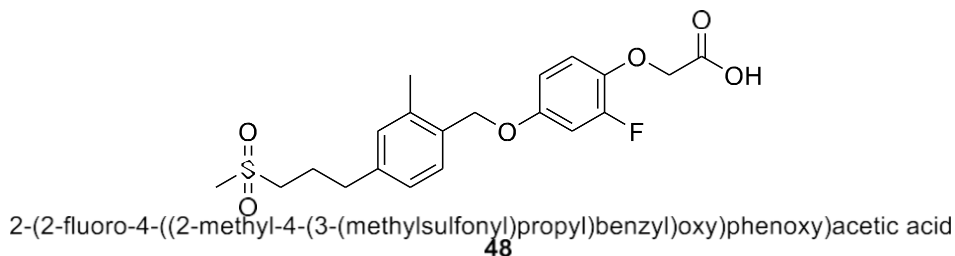
47

2.2.3 Anti-Platelet Activity of Fibrate Derivatives:

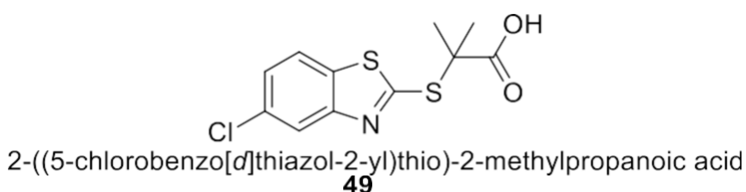
The fibrate class of molecules is known to control, hyperlipidemic situations generally led to cardiovascular diseases if remain uncontrolled. Since the basic scaffold of fibrate is well tolerant in human subjects the medicinal chemists explored other important targets by modulating fibrate core structures. Several new pharmacological activities are discovered around the fibrate core structures and the inhibition of platelet activation is an attractive area of research that is likely imputation to their influence on

PPAR activation.

A Clofibrate analog having heteroaromatic substitution, of a thio-isobutyrate side chain was identified as compound **49** a potent antiplatelet agent. This compound was able to influence, a significant effect on platelet aggregation using the platelet function as a benzothiazole derivative **49** expressing highly significant activity at millimole concentration amongst the series of the compound tested.[81]



2-(2-fluoro-4-((2-methyl-4-(3-(methylsulfonyl)propyl)benzyl)oxy)phenoxy)acetic acid
48



2-((5-chlorobenzo[*d*]thiazol-2-yl)thio)-2-methylpropanoic acid
49

2.3 Fenofibrate as a potential anti-cancer agent:

Since the clinical use of the fibrates, this class of molecules was in clinical use for controlling hyperlipidemia and hyper-cholesterolemia. However, in recent years, much attention has been paid for the wide spectrum of pharmacological activities of the available drugs; clofibrate, fenofibrate, bezafibrate, and gemfibrozil as well as their new synthetic congeners. It is interesting to mention that the fibrate class of drugs posses, significant pleiotropic activity.

The fibrates are showing a variety of pharmacological activities including the beneficial effect on Type II diabetics[82] and their associated ailments like Neuropathy,

Retinopathy, Cardiovascular, and Myocardial Protection.[83, 84]

In the recent past, the PPAR α specific agonists are reported for their ability to inhibit carcinoma growth in a number of human cancer types such as Acute Myeloid Leukemia[85,

86], Chronic lymphocytic leukemia [87], and in solid tumors including breast as well as ovarian cancers, etc [88, 89] Amongst the various substances studied, the fenofibrate and bezafibrate were identified as highly potent fibrates for anti-cancer activity[90]. Fenofibrate was studied against various cancer cell lines and found highly efficacious in reducing cell proliferation [91, 92]

Recently, a comprehensive review on the anti-cancer activity of fenofibrate has been published. The review has reported the in vitro and in vivo anticancer activity against various cancer types. In view of the anti-cancer potential of the fenofibrate, the authors recommend the use of fenofibrate as a repurposed drug to meet the available therapeutic gap for the treatment of human cancers. The recommendation was based on the clinical case reports of the investigational use of fenofibrate as an anti-cancer drug.[93]

The PPAR α agonists bezafibrate and fenofibrate have been evaluated for their anti- cancer potential in consideration to the ability of PPAR α agonists for their anti- angiogenic properties. This interesting activity was considered a rationale for the suppression of tumor growth of fibrates.[89] The bezafibrate has been reported as a potential for the treatment of lung adrino carcinoma[94] The Insilco docking event indicates that bezafibrate could target cyclin-dependent kinase (CDK2), which regulates the cell cycle. They have also demonstrated the in-vivo activity of bezafibrate lung adino carcinoma in mice xenograft Adenocarcinoma model [94]

The bezafibrate has also been evaluated for B-cell chronic lymphocytic leukemia (CLL) The bezafibrate alone or in combination with (MPA) is capable to induce Apoptosis and abrogate the proliferative activity of CD-40 without affecting the normal cells. The author emphasized the safety profile of bezafibrate for the development of novel therapy against CLL. [95]

It is worth mentioning that B-cells is one of the most common leukemia in human subjects lacking a proper therapy for the control and treatment of this fetal disease. The anti-leukemia activity of bezafibrate is highly encouraging for the development of fibratederived anti-cancer therapy.

2.4 Conclusion and future directions:

The fibrates are acting through activating the PPARα specifically for the control of hyperlipidemic situations. In the recent past, several groups have published structure-activity relationship (SAR) on fibrate core structure which resulted in the identification of novel PPAR α agonists on one hand and dual agonists like PPAR α,γ, and PPAR β,δ agonists. These dual agonists have been found to impact other pharmacological activities controlled by the group of PPAR nuclear receptors. The anti-diabetic, anti-platelet, and, anti-cancer activities are some of the important pharmacological activities identified as an outcome of SAR activity. The anti-cancer activities of fibrates are quite interesting in consideration of the safety of fibrates. The fibrates are also finding their utility in Cancer types like lung cancer and leukaemia where the therapies are thoroughly lacking. In view of the progress made in the area of fibrates. It is likely that new therapeutic options will be available as an outcome of the SAR or by the repurposing modes

CHAPTER 3

LATE-STAGE DERIVATIZATION OF FENOFIBRATE.

3.1 Introduction:

The Fibrate class of molecules are attracting the attention of medicinal chemist in view of the diversified pharmacological properties of fibrates reported in the recent past [96]. Another Interesting feature of the fibrates is the safety of the pharmacophore in human subjects. It is worth mentioning that the fibrates are in clinical use for more than five

decades without noticeable adverse effects. On the other hand, other therapeutic benefits of fibrates are reported in the past two decades viz. Antihyperglycemic activity, Antiplatelet activity, and more recently Anti-cancer Activity. All these ailments are suffering from inadequate therapy on the one hand and the toxicity of the available therapy on other hand. Therefore, the fibrates and related new chemical entities are under investigation as Investigational New Drugs (IND) or New Drug Applications (NDA) as a monotherapy as well as in combination with the available drugs.

Therefore, there is an urgent need for the development of new chemical entities derived from the fibrate core structure, which is a fruitful strategy for quick access to new drug like molecules. Amongst various approaches, we have opted to make the transformation of fenofibrate for the synthesis of new chemical entities which may have improved physicochemical properties as compared to the fenofibrate. The work carried out towards this objective is presented in the following sections:

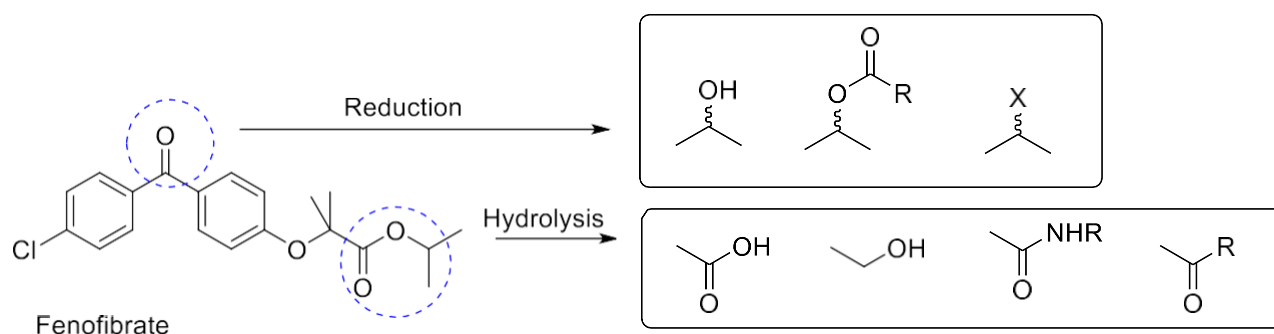
3.2 Rationale of the proposed work:

Late-stage diversification is an important approach for speedy discovery of new drugs from the biologically compatible chemical scaffolds. This approach comprises derivatization of the final compound (product) to obtain a variety of related sub-structures capable of modulation of physico-chemical and biological properties of the parent molecule which can also be approved drug molecules. The late-stage derivatization is evidenced in the literature as a fast and fruitful strategy for the discovery of new chemical entities. However, the late-stage diversification has been underexplored owing to the synthetic challenge of performing selective functionalization in the presence of diverse functional groups. The present work comprises the synthesis and characterization of new derivatives of Fenofibrate molecule using a Late-stage Functionalization approach. The aim of the present work for synthesis of new derivatives of Fenofibrate for obtaining quickly new drug

like molecules and possibly with improved therapeutically profiles. The late-stage functionalization has been beneficial, and it is obvious from the literature reports, that many university laboratories and pharmaceutical companies will have positive impact on drug development. [97] In the recent literature several success reports are published demonstrating the late-stage functionalization of natural products and synthetic drugs. The discovery of novel 3rd and 4th generations of Fluoroquinolone and macrolide antibiotics is a consequence of late-stage diversification. [98, 99] This example supports this approach for quickly discovery of new drugs with improved therapeutically profiles.

The late-stage functionalization is also challenging and offers limited options for chemists. However, this area of research is fast expanding. In view of the above discussion as well as the expertise available in our lab, the present work comprises of synthesis and characterization of new derivatives of Fenofibrate molecule using a Late- stage Functionalization approach.

The possibilities of late-stage functionalization of Fenofibrate according to the search of chemical literature are shown in figure-3.1. There are two sites for easy transformation are



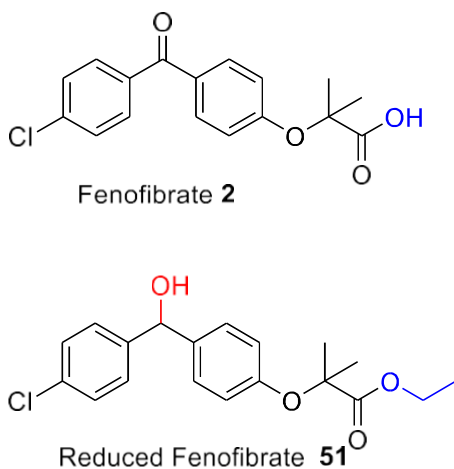
shown in the figure. Both the sites are already explored to some extent in our laboratories but there is a number of other possibilities still available for the chemists. The reduction of the ketonic carbonyl group resulted in secondary alcohol which can be converted to either, an ester or a halide, and the carboxylic group can be converted to free acid, reduced to

primary alcohols, substituted amides or ketones as per the reported methods. The proposed work for the present study will be summarized in the next

section.

Figure 3.1: Possibilities of late stage functionalized Fenofibrate.

The main objective of the present work comprises synthesis of new derivatives of fenofibrate with improved pharmacological activity. It has been demonstrated that fenofibrate regulates the lipid metabolism by augmenting the action of PPAR-α receptors and the drug is converted to its active metabolites for its mode of action.[100] These metabolites namely fenofibric acid, reduced fenofibrate and reduced fenofibric acid are reported in the literature.[Figure 3.2] The fenofibric acid and reduced fenofibrate are well studied and already prepared in our lab whereas the reduced fenofibric acid is not well explored for its activity because the synthesis of the reduced fenofibrtic acid is not yet reported. Therefore, the synthesis of the reduced fenofibric acid was envisaged for the present work. Since the new derivative is also reported as metabolite of fenofibrate, synthesis of reduced fenofibric acid may turn out to be more active as compared to the fenofbrate as well as its availability as synthetic compound will be of great help for understanding the role of reduced fenofibric acid in lipid metabolism.



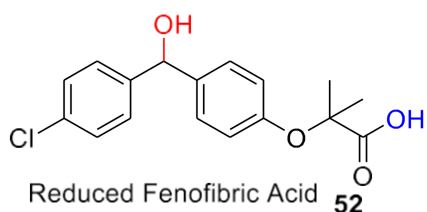


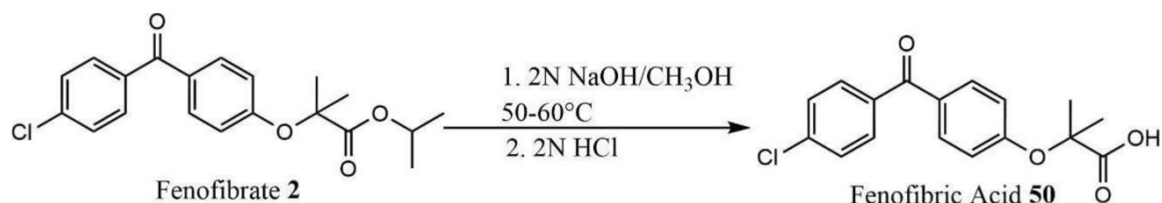
Figure 3.2: Pharmacokinetics of Fenofibrate.

The synthesis of reduced fenofibric acid **52**, the unexplored metabolite of fenofibrate, is challenging if the molecule is prepared by total synthesis approach. However, a facile expeditious synthesis of this molecule can be achieved by late-stage derivatization approach. The reduced fenofibric acid **52** can be prepared by either reduction of fenofibric acid **50** or by the hydrolysis of reduced fenofibrate **51**. It is important to note that starting material of the both the routes are not available commercially and therefore required to be prepared and fully characterized before these molecules are used as starting materials. Our lab has already standardized the synthesis of fenofibric acid **50** and reduced fenofibrate **51** Therefore, the synthesis of these molecules is developed and prepared in gram quantities in highly pure form. The following discussion comprising synthesis of fenofibric acid **50** and reduced fenofibrate **51** as starting material for the synthesis reduced fenofibrate **51**, a new synthetic metabolite of fenofibrate.

3.3 Synthesis of Fenofibric Acid 50:

Synthesis of Fenofibric acid **50** was carried out by the mild alkaline hydrolysis of fenofibrate according to the procedure reported in the literature Scheme 3.1. The fenofibrate was stirred in equimolar amount of aqueous 1N NaOH at 50-60 °C for 2-3 hrs. After completion of reaction as monitored by TLC the reaction mixture was evaporated under reduced pressure, acidified to pH-2 by aq. HCL and the precipitate is extracted in ethyl acetate. The organic layer is concentrated, and the desired compound is precipitated by

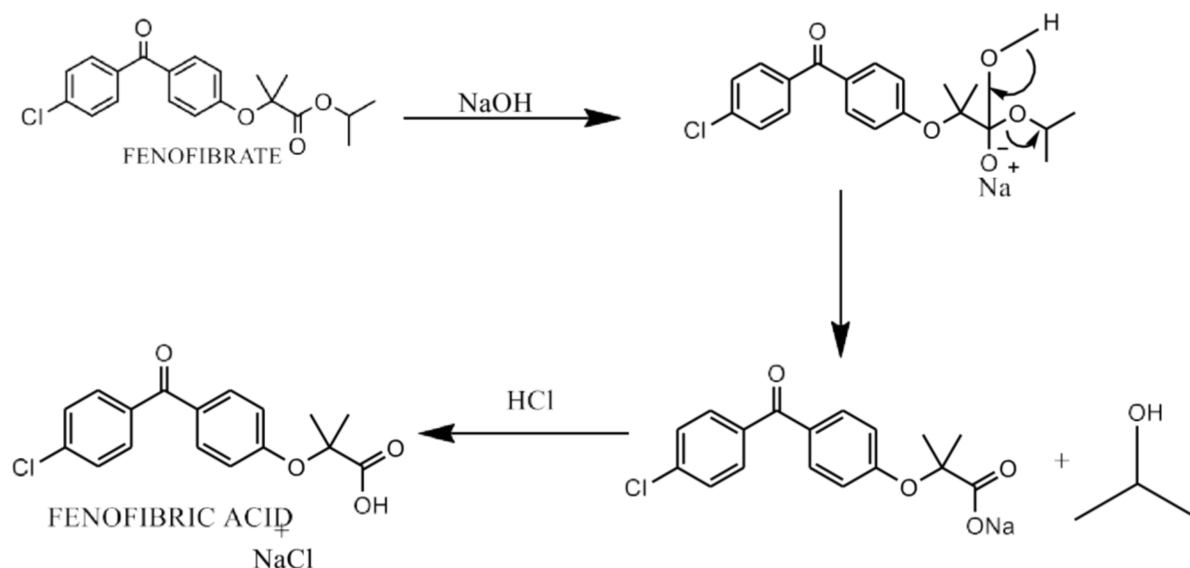
addition of hexane to get chromatographically homogeneous solid in excellent yield. The compound is characterized by spectroscopic methods.



Scheme 3.0.1: Synthesis of Fenofibric acid from Fenofibrate.

3.3.1 Mechanism for the Hydrolysis of Fenofibrate.

The hydrolysis of fenofibrate is carried out by the treatment of aqueous sodium hydroxide in molar stoichometry. The steps involved in the hydrolysis is shown in scheme 3.2



Scheme 3.0.2:Mechanism for the hydrolysis of Fenofibric Acid

3.3.2 Experimental:

The synthesis of **50** was carried out starting from Fenofibrate obtained from sigma USA. The Hydrolysis of Fenofibrate using NaOH in methanol was carried out according to the procedure established and published in our group An In-direct probe detection method

was used on a Bruker Advance II 400 MHz NMR to record the ^{1}H NMR and ^{13}C NMR. The method used to record the chemical shifts was using parts per million (ppm) from a standard of tetramethylsilane (TMS) in $CDCl_3$ and Hertz was used to measure coupling constants (J). Infrared spectrum was recorded on a Thermo Electron Corporation IR 200 spectrophotometer and analysis was done on EZ-OMNIC software. The mass spectrum was recorded on an Esquire-LC_00135 spectrometer. Stuart-SMP10 was the apparatus used to record melting point and it was reported uncorrected.

Yield 288 mg, (90%)
M.P. 192°C.
ES-MS: calculated for $C_{17}H_{15}ClO_4$ = 318; Observed [M+H] = 319 (Figure 3.3)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.71 (s, 6H), 6.95 (d, J=8.4 Hz,2H), 7.26 (s,4H), 7.45 (d, J=8.32,2H), 7.7 (m, J=8.5 Hz,2H). (Figure 3.4)

^{13}C NMR (100 MHz $CDCl_3$) δ: 21.54, 25.33, 68.98, 75.12, 76.69,77.01, 77.22,77.33,79.09,118.84,127.48, 127.79, 128.50, 133.12, 136.83, 142.27, 155.18, 173.64. (Figure 3.5)

The product was characterized by the absence of isopropyl protons in NMR spectroscopy. Similarly, the experiment was carried out for 2 mmol and 5 mmol scales to obtain a sufficient quantity of fenofibric acid for further use.

3.3.3 Spectral Data:

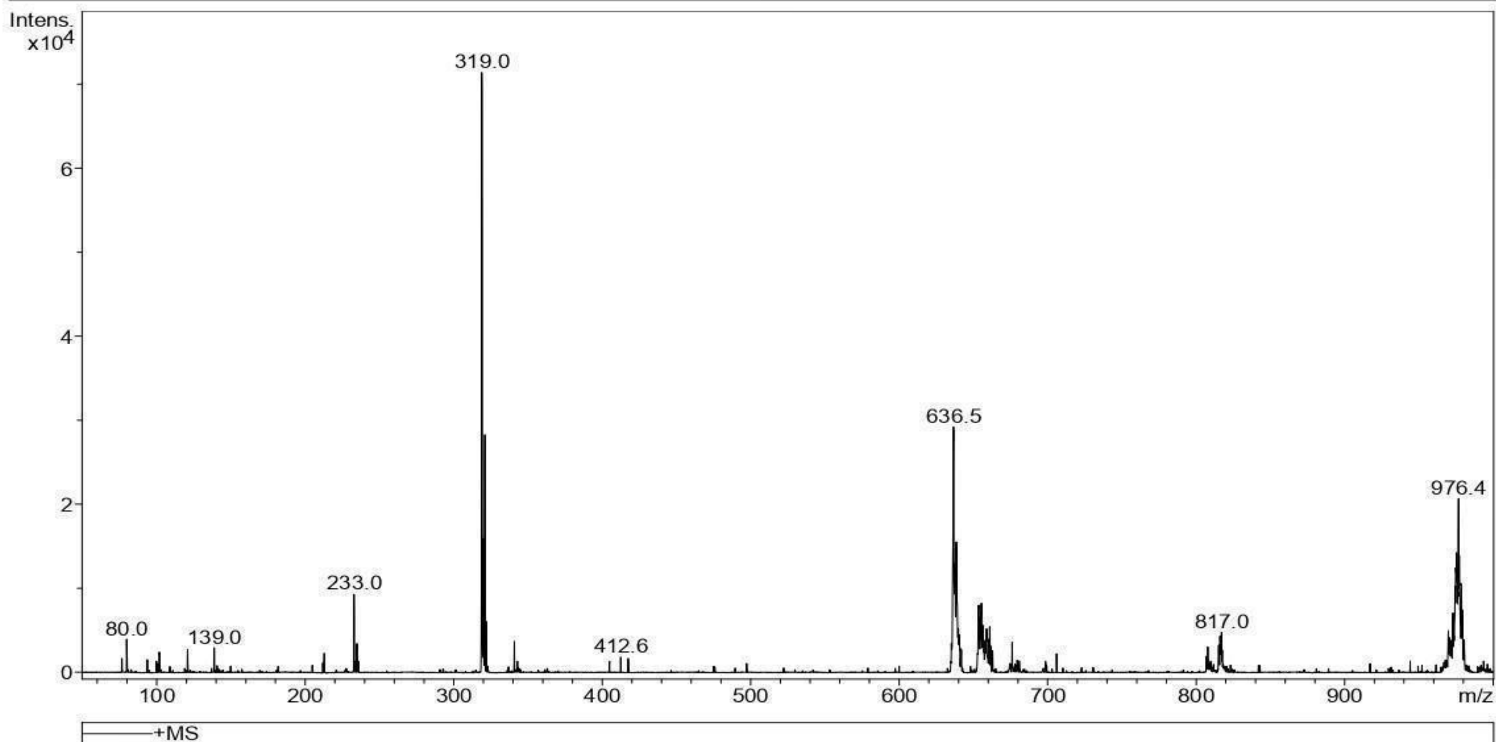


Figure 3.3: ES-MS of Fenofibric Acid.

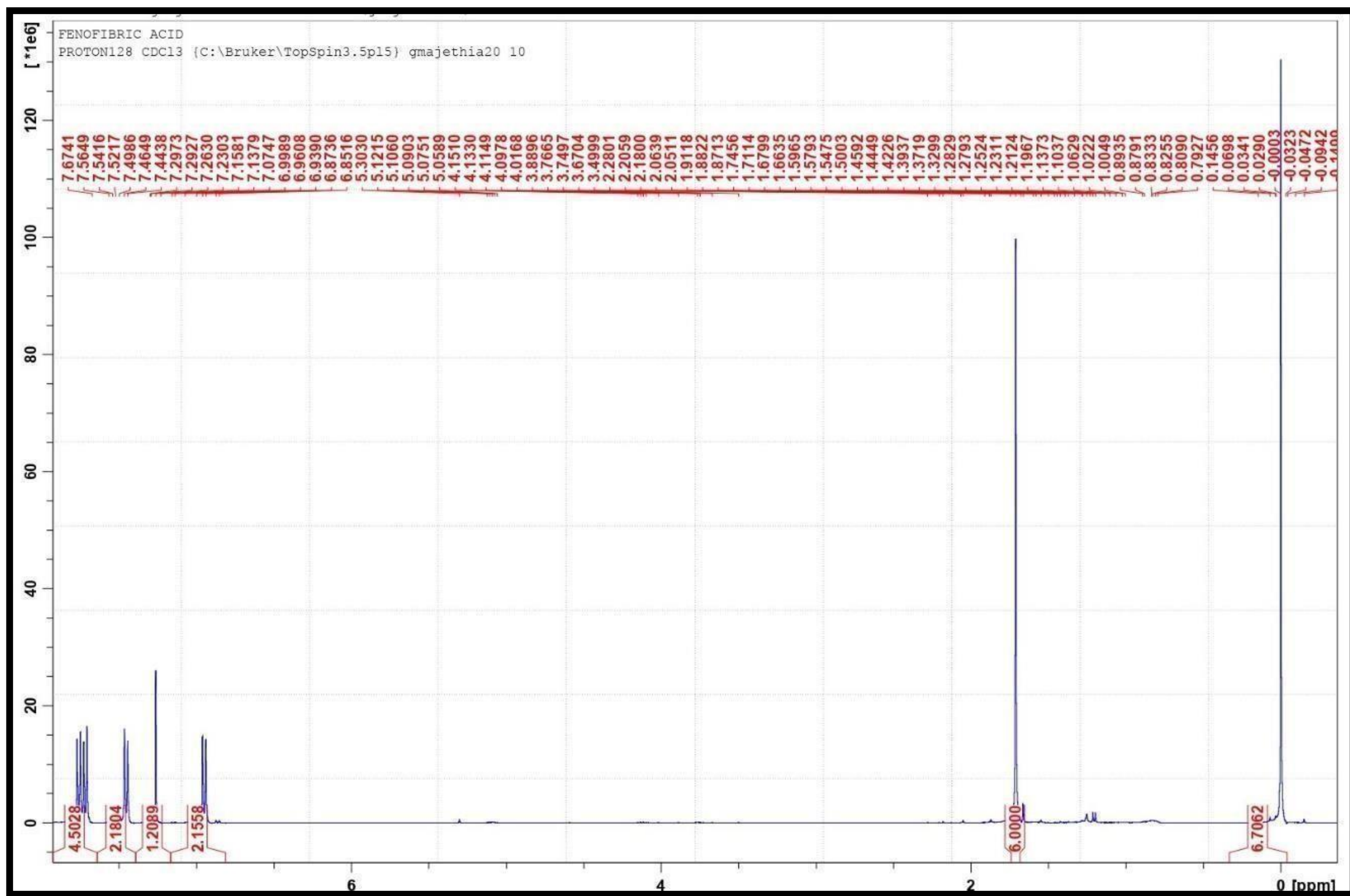


Figure 3.4: ^{1}H NMR of Fenofibric Acid.

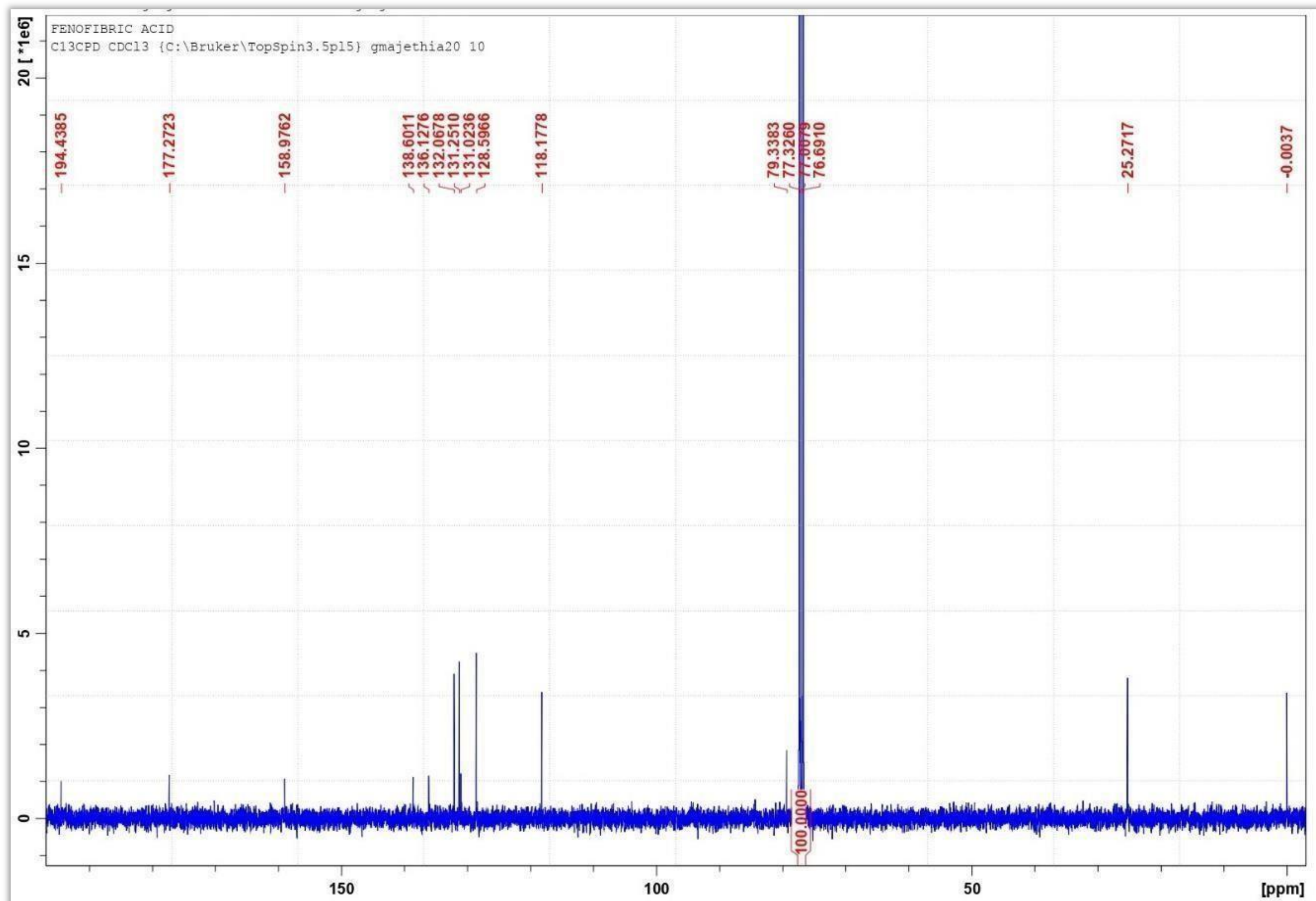
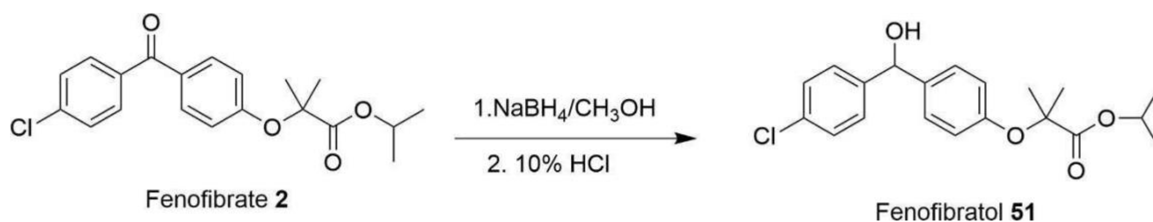


Figure 3.5: ^{13}C NMR of Fenofibric Acid.

3.4 Synthesis of Reduced Fenofibrate (Fenfibritol).

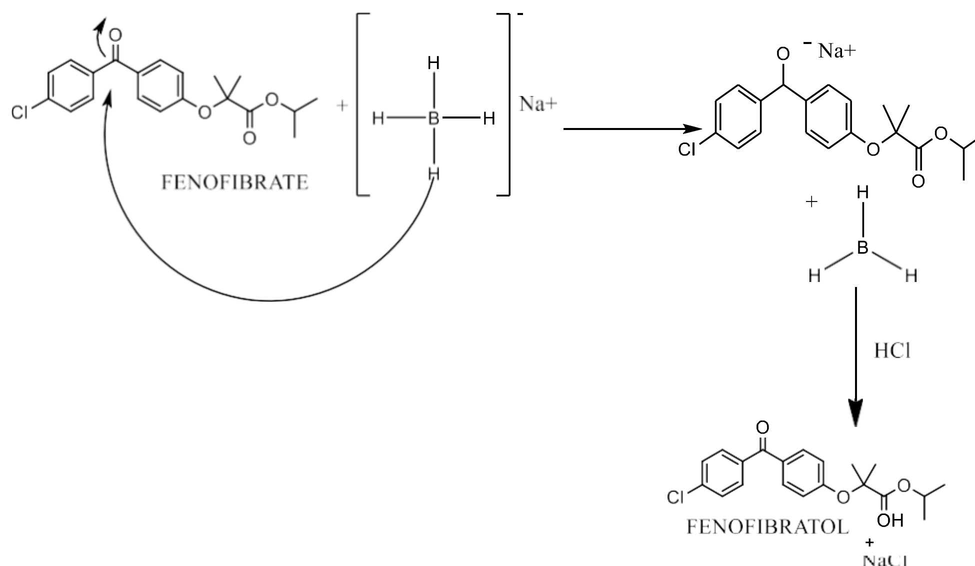
Synthesis of fenofibritol[101] was carried out by the reduction of fenofibrate with sodium borohydride in methanol at room temperature in line with procedure reported from our laboratory.(Scheme 3.3) The fenofibrate was stirred in methanol followed by the addition for 4-5 molar excess of sodium sodium borohydride at room temperature. After complete addition the reaction mixture was quenched by the addition of dilute hydrochloric acid and after usual work up the product is isolated as oily material in almost quantitative yield. The compound is characterized by spectroscopic methods.



Scheme 3.0.3:Synthesis of Fenofibritol.

3.4.1 Mechanism for the reduction of Fenofibratol:

The course of reaction for the reduction of ketonic group of fenofibrate is shown in scheme 3.4. The reduction was effected by the hydride transfer followed by the protonation of the intermediate. The reduction was very facile and completed in 4-5 mins.



Scheme 3.0.4: Mechanism for the reduction of Fenofibratol.

3.4.2 Experimental:

. In a typical experiment, a 100 mL round-bottomed flask with a magnetic stir bar was used, along with 360 mg (1.00 mmol) of fenofibrate that had been dissolved in 10 mL of methanol and 200 mg of NaBH4 that had been added to the same solution. After that, it was left on a stir plate for around 10 minutes. TLC (9:1 ethyl acetate/hexane) was used to monitor the reaction, which produced only one spot of the product. 15 mL of cold 10% HCl was added very slowly to the reaction mixture while it was submerged in water. The undiluted reaction mixture was poured onto 10 mL of water, allowed to separate, then the mixed organic layer was washed with brine after being extracted with 2 mL of ethyl acetate over MgSO4 until neutral and dry. The reaction mixture was filtered using a Whatman filter

pad, and the solvent was drained using a flask with a flat bottom. Utilizing TLC and NMR spectroscopy, it was identified. The item was distinguished by a singlet-shaped trademark peak. The chemical had a sticky character, and its purity was determined to be one spot-on TLC. **Yield 295 mg, (82%).**

ES-MS: calculated for $C_{20}H_{23}ClO_4$ = 362.85; Observed [M+Na] = 385 (Figure 3.6)

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.57 (s, 6H), 2.16 (s, 2H), 5.07 (m, 1H, J = 6.31 Hz), 5.76 (s, 2H), 6.79 (d J = 9.12 Hz, 2H), 7.19 (d, J = 8.84 Hz, 2H), 7.29 (d, J=8.8, 2H). (Figure 3.7)

^{13}C NMR (100 MHz, $CDCl_3$) δ: 1.54,25.33,68.98,75.12,76.69,77.01,77.22,77.33,79.09,118.84,127.48,127.79,128.50,133.12, 136.83, 142.278, 155.18,173.64. (Figure 3.8)

The experiments were carried out at 2 mmol and 5 mmol scales and a sufficient quantity of the product was obtained.

3.4.2 Spectral Data:

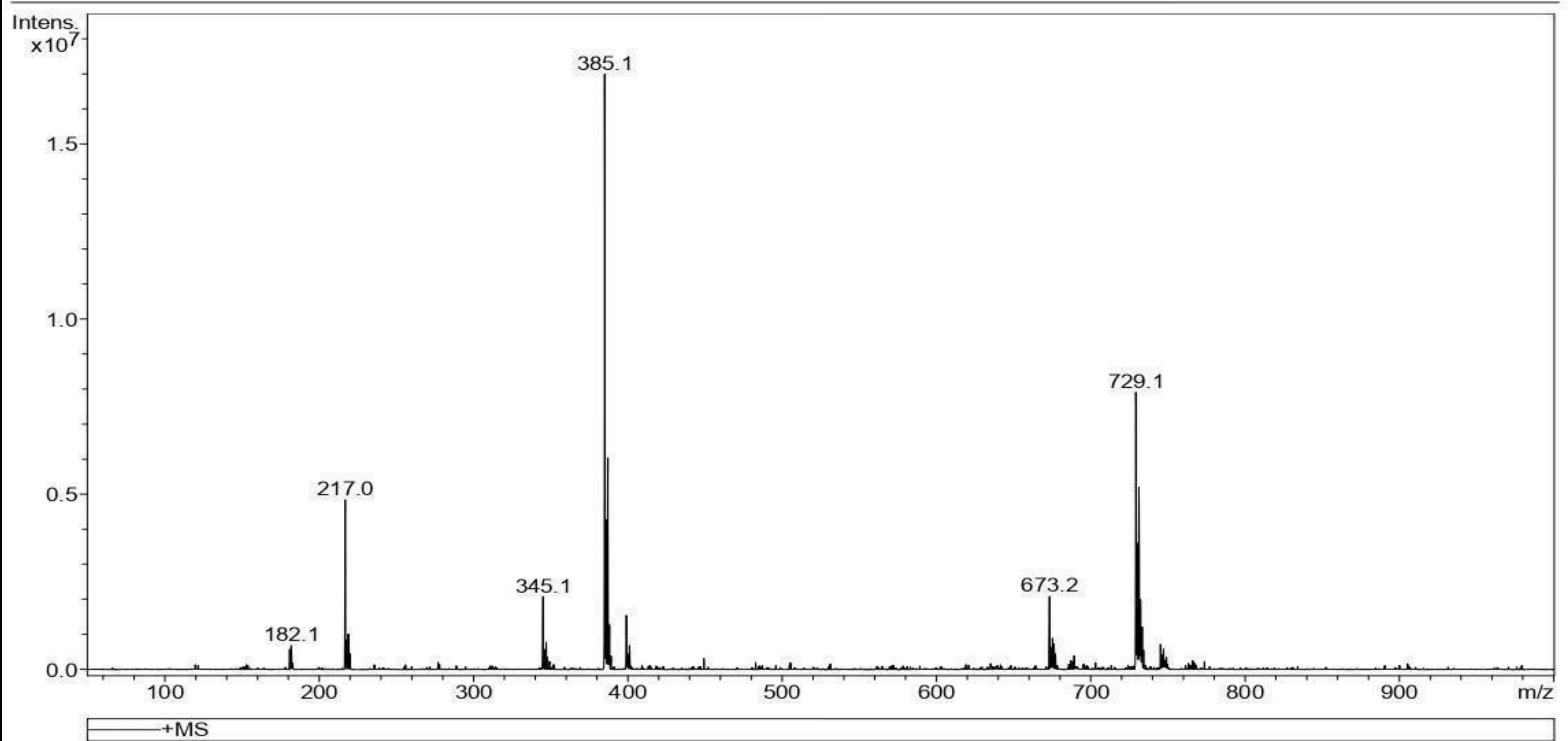


Figure 3.6: ES-MS of Fenofibritol.

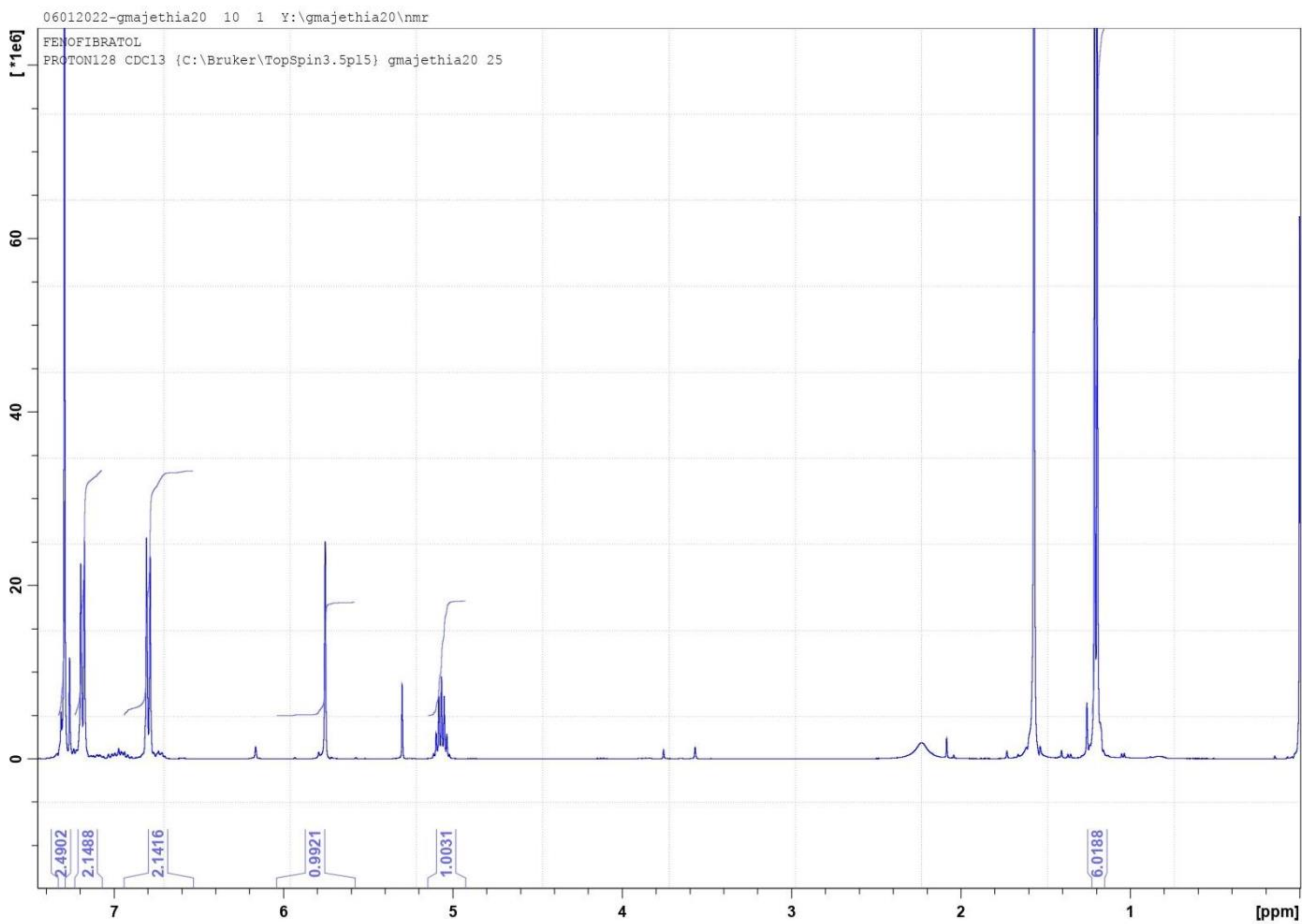


Figure 3.7: ^{1}H NMR of Fenofibratol.

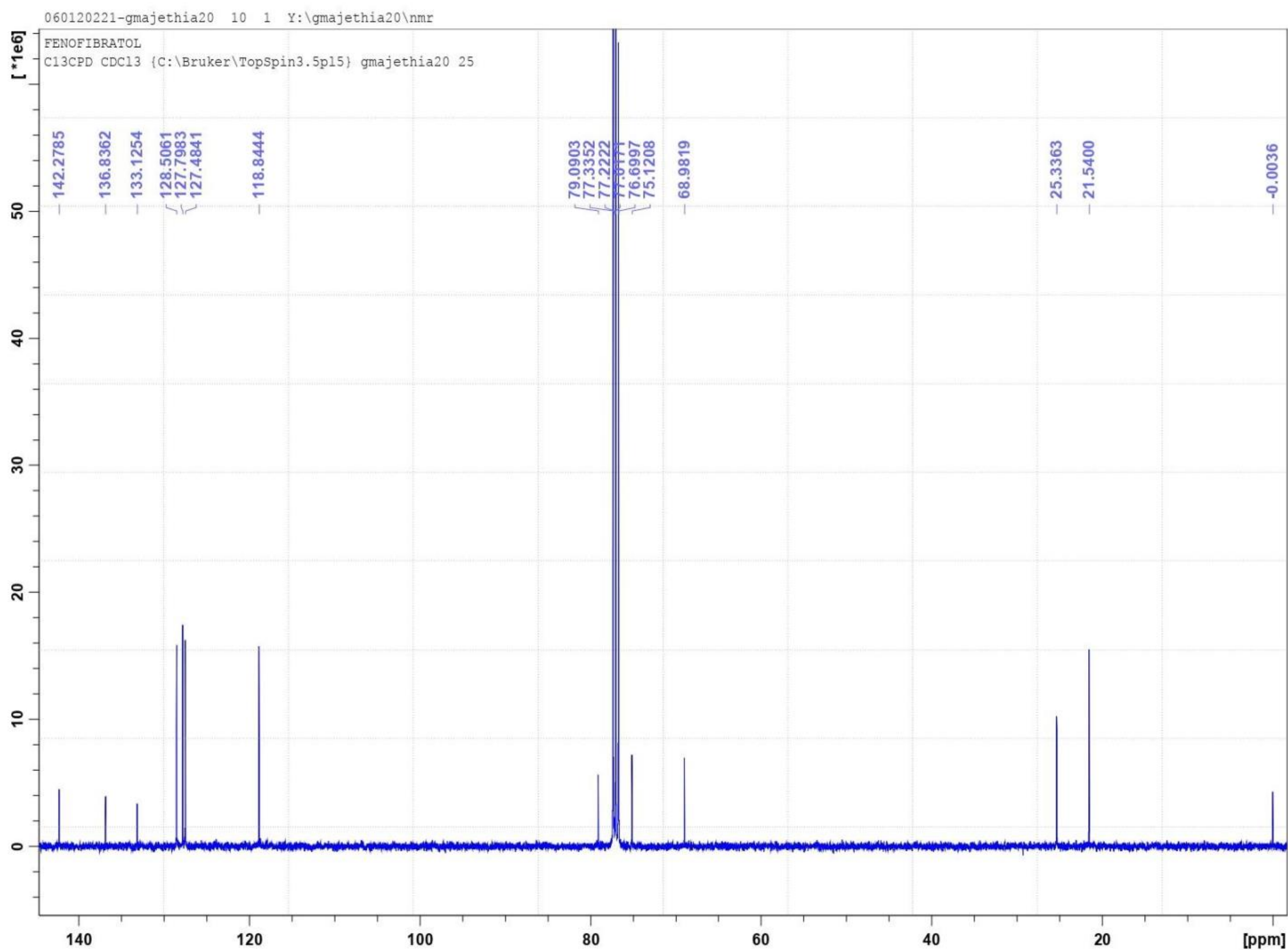
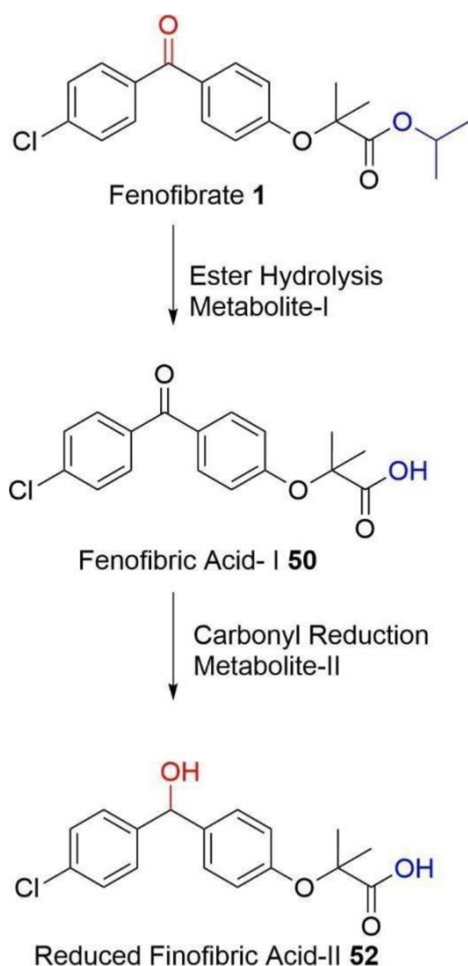


Figure 3.8: ^{13}C NMR of Fenofibratol

3.5 Synthesis of Reduced Fenofibric Acid (52).

It is now widely accepted that fenofibrate is predominantly eradicated in the urine as metabolites, mainly fenofibric acid and fenofibric acid glucuronide, after it has been absorbed from the gastrointestinal tract. [1]. The metabolization of fenofibrate occurs in stages. First, hydrolysis cleaves the carboxyl ester moiety, liberating fenofibric acid, which is the primary pharmacologically active component. The metabolite- reduced fenofibric acid is a pharmacologically active derivative of fenofibric acid once it undergoes carbonyl reduction. **Scheme 3.5** It is feasible to conjugate and excrete fenofibrate acid and lowered fenofibric acid as glucuronides.



Scheme 3.0.5: Pharmacokinetics of Fenofibrate.

As stated in previous section that the reduced fenofibric acid (is reported as a metabolite but the biological activity of this new metabolite was not studied as the synthetic compound was not available. For the present work we are reporting a facile synthesis of compound (**52**) in excellent yield and purity. The desired compound is prepared successfully by two routes of synthesis. The first method comprised the mild reduction of fenofibric acid by the use sodium borohydride and the other route comprises hydrolysis of fenofibritol. It is worth mentioning that the starting materials of both the routes are not commercially available and therefore prepared in lab and fully characterized before use as described in the preceding sections. The details of the synthesis and characterization of the desired compound is described in the following section.

3.5.1 Sodium borohydride as a reducing agent:

The reduction of carbonyl compound is an important synthetic transformation frequently carried out to achieve several synthetic transformations. The transformation of the carbonyl group to corresponding alcohol or alkene has been achieved by a variety of reducing agents that include catalytic hydrogenation or metal hydrides among various available methods.

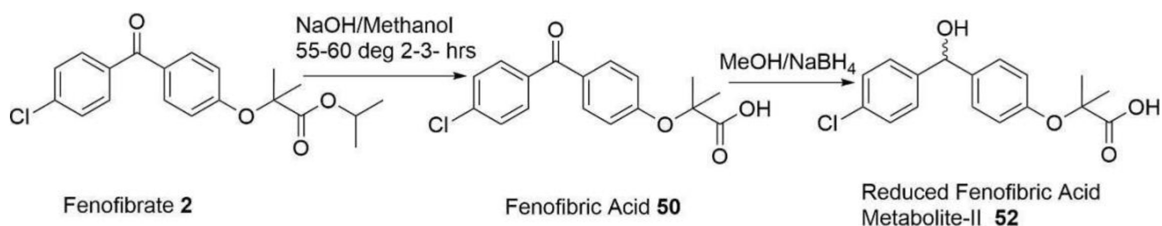
The metal hydrides are commonly used reducing agents for the carbonyl compounds. The metal hydrides depending upon the structure exhibit variety of reduction options depending on the reagent. $LiAlH_4$ are considered the most powerful hydrides where the reduction is uncontrolled and applicable to all kinds of carbonyl compounds. On the other hand, the borohydrides are mild as well as highly efficient reducing agents where the reduction can be controlled by manipulating the types of borohydrides viz $NaBH_3CN$ or more advanced soluble borohydride like vitroids and selectrides for stereoselective reduction. Another important feature of sodium borohydride is related to its stability at laboratory conditions and safer operations for reduction experiments. It is worth mentioning that $NaBH_4$ is a mild and efficient reducing agent where the carbonyl compounds like aldehydes and ketones can be reduced to the corresponding alcohols whereas carboxylic acids, esters, and, amides are unaffected. This feature of $NaBH_4$ makes it the reagent of choice for the reduction of the carbonyl group in highly functionalized organic compounds and also for the late-stage transformation reactions.

The present work comprises reduction of carbonyl group of benzophenone scaffold in presence of other functainility like acid group and halogen group in a molecule. Therefore, sodium borohydride in methanol was chosen as a reagent for reduction of carbonyl group of fibric acid. Since this fenofibric acid is not soluble in water the use of sodium borohydride in methanol at room temperature is considered as an ideal reagent for the

quantitative production of ketone to corresponding alcohol under mild conditions to obtain desired reduced compound.

3.5.2 Synthesis of Reduced Fenofibric Acid: (Route-1: by the reduction of fenofibric acid)

The Synthesis of Reduced Fenofibric acid was carried out from Fenofibrate as shown in scheme 3.5. The Fenofibrate was treated with aqueous sodium hydroxide in methanol at 55-60 °C as reported earlier in this laboratory. The resulting Fenofibric acid was subjected to sodium borohydride reduction in methanol to get the desired compound (Scheme 3.6).



Scheme 3.0.6: Synthesis of Reduced Fenofibric acid via Fenofibric acid.

3.5.3 Experimental:

The experiment was carried out at a 1 mmol scale using a 100 mL round-bottomed flask, fitted with a magnetic stir bar, Fenofibric Acid was added and then dissolved in 10 mL of methanol and 200 mg of $NaBH_4$ was added to the same solution. It was then placed on a stir plate for about 10 min. The reaction was monitored by TLC (9:1 ethyl acetate/hexane) and it resulted in one spot of the product. The reaction mixture was placed in a water bath and 15 mL of chilled 10% HCl was added very slowly. The crude reaction mixture was poured onto 10 mL water, separated, extracted with ethyl acetate (2 × 15 mL), and the combined organic layer was washed with brine until neutral and dried over $MgSO_4$. Using a Whatman filter pad, the reaction mixture was filtered, and the solvent was removed with a round bottom flask. It was characterized using TLC and NMR spectroscopy. Nature of compound is White solid. **Yield: 260 mg, (82%)**

Melting point: 133-135°C.
ES-MS: Calculated for C17H17ClO4 [M+H]+=320.77 Observed 320, [M+ Na]+=343.0.

(Figure 3.9)

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.58(S,6H), 5.76 (S,1H), 6.85-6.87 (m, J=8.6 Hz,4H),

7.21 (m, J= 8.16 Hz,2H), 7.30 (m, J= 7 Hz, 2H). (Figure 3.10)

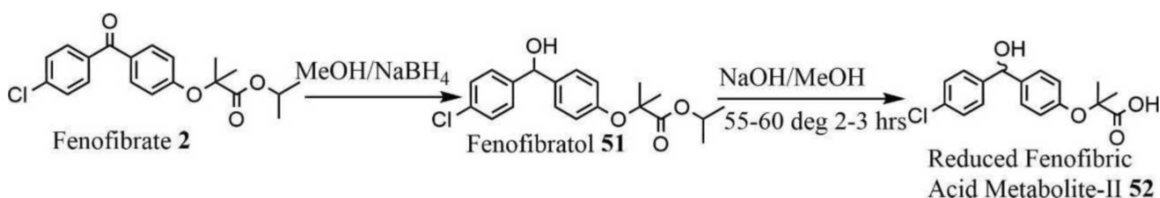
^{13}C NMR (100 MHz $CDCl_3$),: δ 23.03, 25.06, 25.08, 75.10, 76.70, 77.01, 77.33, 79.44,

120.10, 123.89, 127.44, 127.67, 127.83, 128.59, 133.29, 137.89, 142.03, 154.16, 177.96.

(Figure 3.11)

3.5.4 Synthesis of Reduced Fenofibric Acid: (Route-2: by the hydrolysis of Fibritol)

Synthesis of reduced fenofibric acid was carried out by the mild alkaline hydrolysis of fibritol in agreement with the procedure described in the literature. The fibritol (reduced fenofibrate) was treated with equimolar amount of aqueous 1N NaOH at 50-60 °C for 2-3 hrs. The confirmation of completion of reaction was given by performing TLC and the evaporated under reduced pressure, acidified to pH 2 by aq HCl and the precipitate is extracted in ethyl acetate. The organic layer is concentrated, and the desired compound is precipitated by addition of hexane to get chromatographically homogeneous oily material in almost quantitative yield.



Scheme 3.0.7: Synthesis of Reduced Fenofibric acid via Fibritol.

3.5.5 Experimental:

A suspension of fibritol (724 mg, 2 mmol) was added to 10 ml of methanol and agitated using a magnetic stirrer. This suspension was then combined with 2.5 ml of

1N NaOH, 2.5 mmol, and the reaction mixture was stirred at 50–60 degrees for two to three hours. Following the conclusion of the reaction, methanol was evaporated at reduced pressure using Rotavapor while the reaction mixture was being monitored on TLC. The residue was placed in water and brought to pH 2 by adding 2 N HCl. The reaction was extracted three times in 10 ml of ethyl acetate, and the combined organic layer was neutralized by neutralizing the brine before being dried over MgSO4 and filtered. Using Rotavapor, the ethyl acetate layer was condensed at low pressure to produce a solid that was chromatographically homogenous. The solid product was crystallized from ethyl acetate-hexane to get white powder.

Yield 580 mg (90%)

Melting point: 133-135°C.

The product was analyzed using spectroscopic data and found identical with the product obtained by the reduction fenofibric acid. Thus, it is evident that both Route1 and Route-2 gave similar product and new routes for the synthesis of reduced fenofibric acid, a metabolite of fenofibrate, are successfully developed.

3.5.6 Spectral Data:

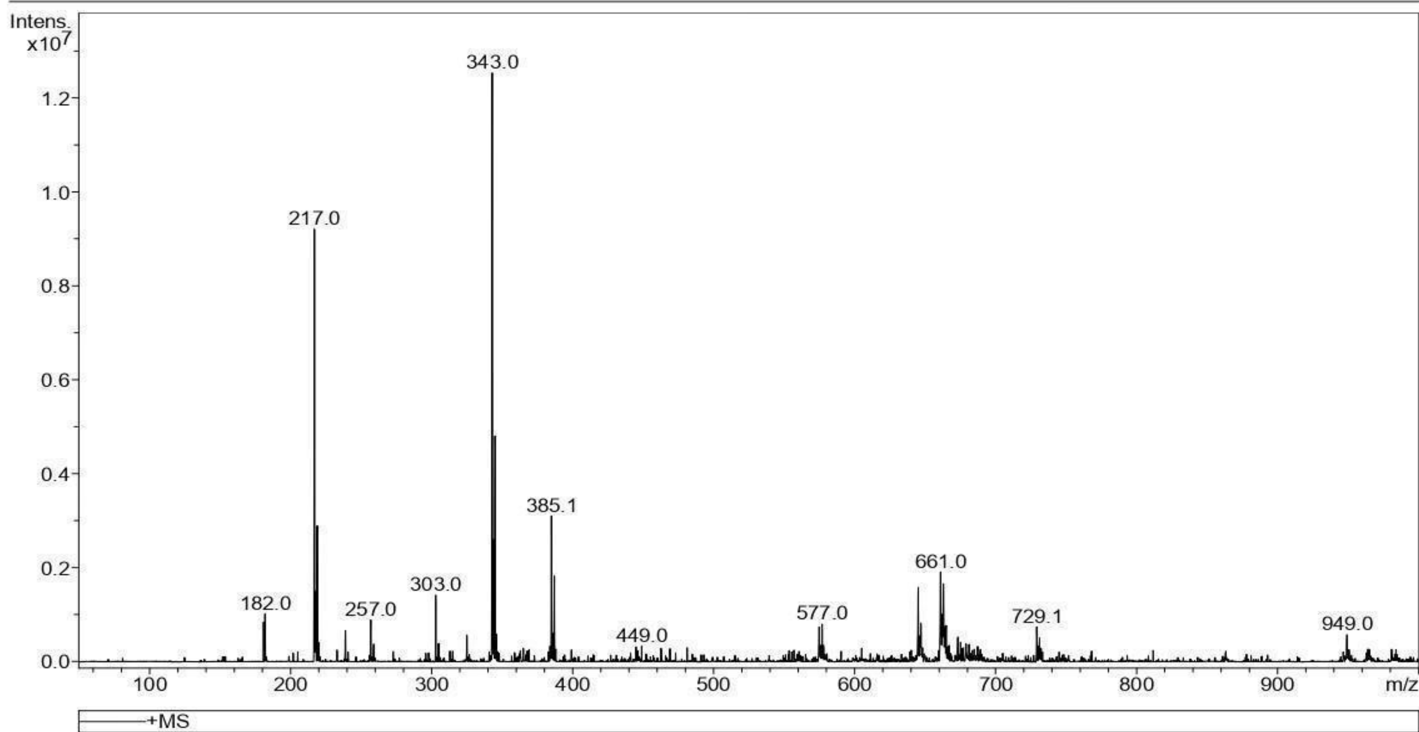


Figure 3.9: ES-MS of Reduced Fenofibric Acid.

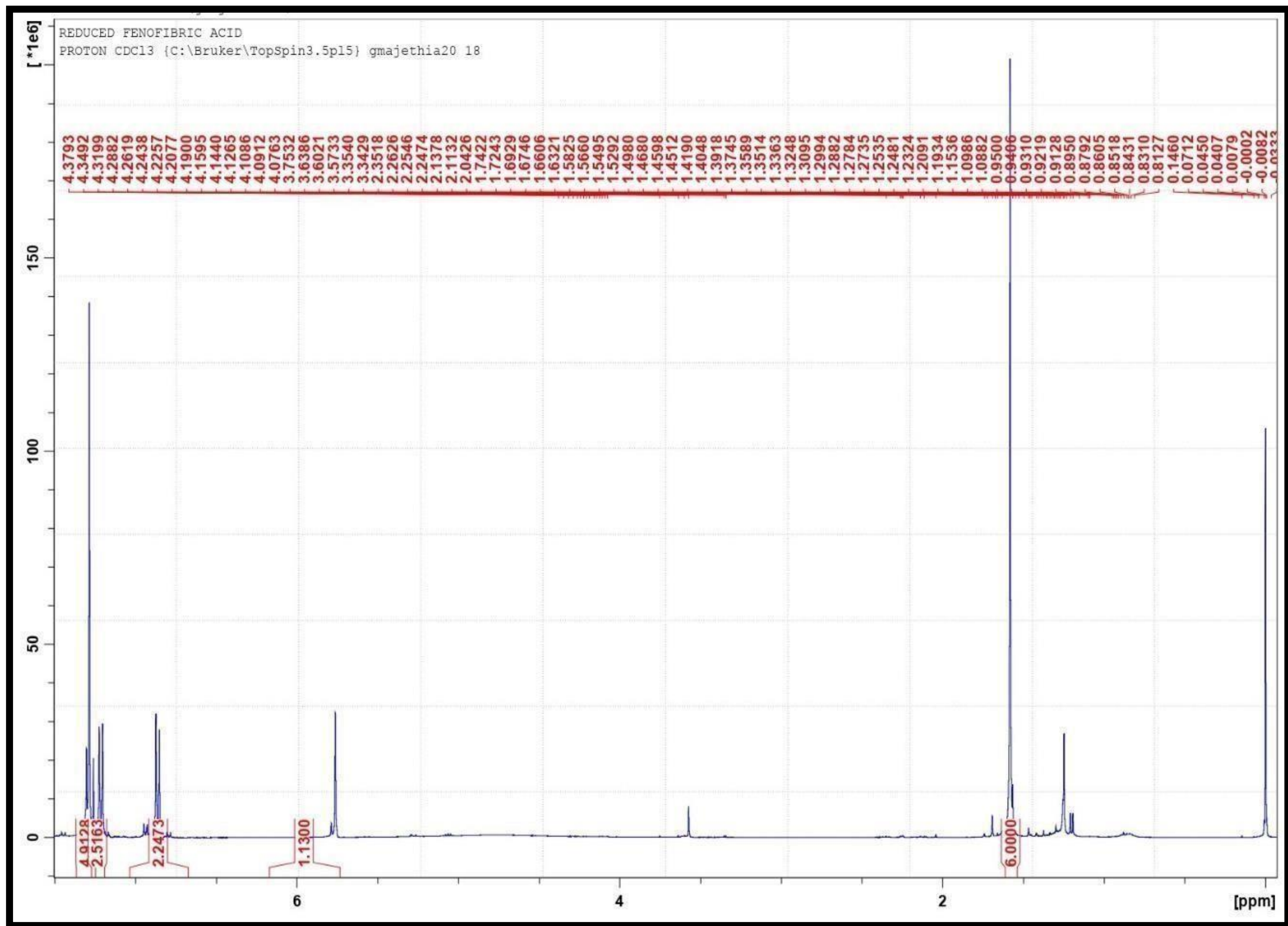


Figure 3.10: ^{1}H NMR of Reduced Fenofibric Acid.

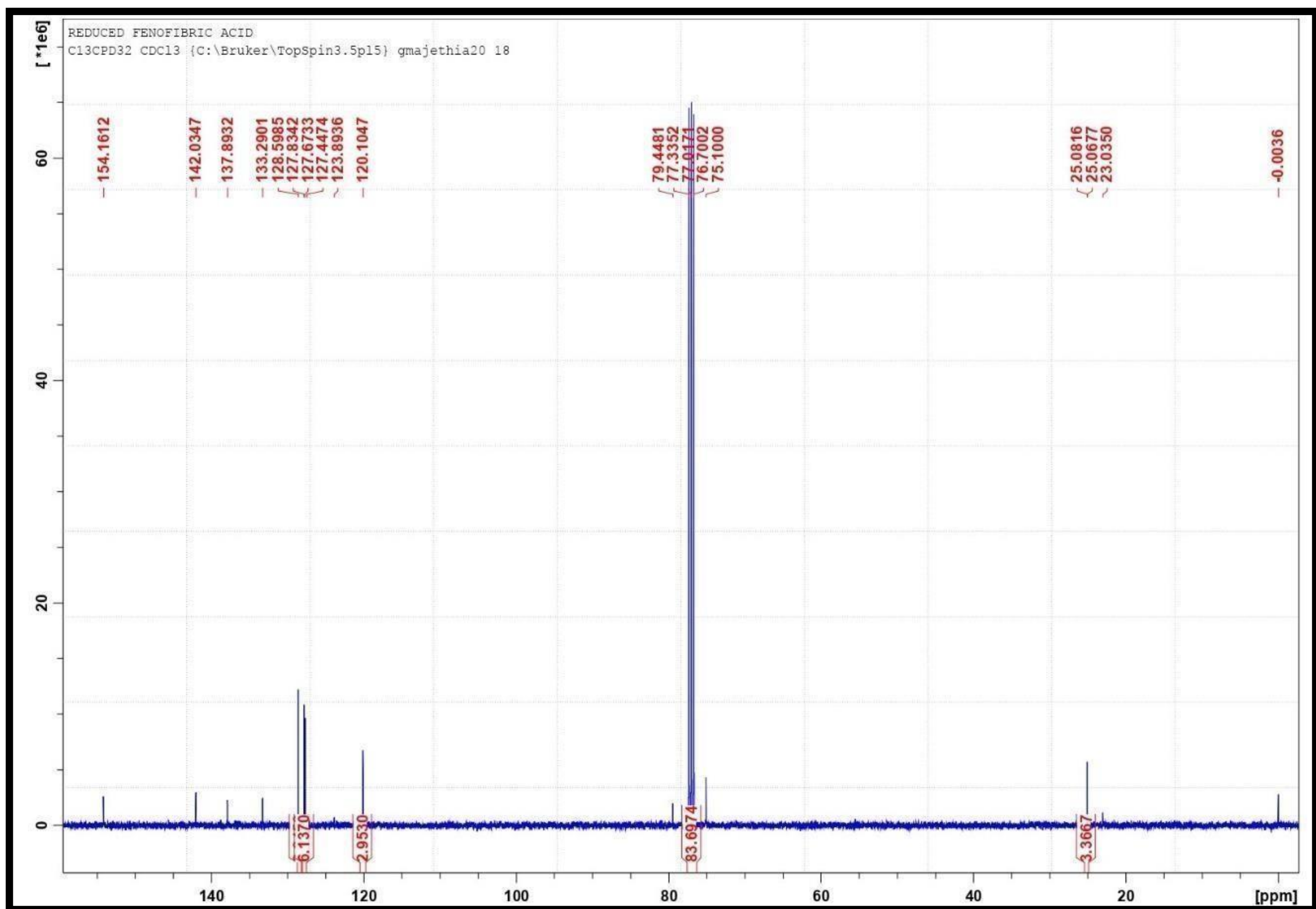


Figure 3.11: ^{13}C of Reduced Fenofibric Acid.

3.6 New derivatives of Reduced Fenofobirate (Fibritol):

In the previous section, synthesis of fenofibric acid, fibritol were reported as known compounds required for the synthesis of reduced fenofibric acid, a new metabolite of fenofibrate. The fenofibric acid and fibritol were the key intermediates for the preparation of reduced fenofibric acid. The fibritol prepared as starting material is in fact a unique compound and structurally similar to the reduced fenofibric acid but the isopropyl ester remains intact. The reduction of Fenofibrate with sodium borohydride in methanol at room temperature resulted in the isolation of corresponding reduced ketone as a racemic mixture. The product was analysed by HPLC using a chiral column. [102]

It is important to note that Fibritol is expected to exhibit improved biochemical and pharmacological activity because of the reduced hydrophobicity which remains a problem in Fenofibrate pharmaceutical preparation.

In line with the discussion in the previous section that there's a need for new structural variants for Fenofibrate in consideration of the identification of new pharmacological activities as well as the potential of Fibrates for repurposing for other indications. In a recent article, the authors have identified new drug candidates that have strong in vitro anti-glioblastoma activity based on Fibrate structure.[103]

In consideration of the advantage of late-stage derivatization of therapeutically tested molecules for the discovery of new chemical entities at a faster pace, it is proposed to undertake chemical diversification of Fibritol, the reduced Fenofibrate. The synthetic methods and analytical data are already standardized in our laboratory and the candidate has already repeated the synthesis on a gram scale and prepared a sufficient amount of reduced Fenofibrate for further diversification. The hydroxyl group of Fibritol is a reactive secondary hydroxyl group that can be transformed into ethers by the reaction of alkyl halides in presence of a strong base. There is the possibility that the hydroxyl group of the

Fibritol is esterified with carboxylic acids or acid chlorides to corresponding esters. Yet other feasible transformations are conversion of the hydroxyl group to halides by the reaction of appropriate reagent known in the literature or to the azide group, highly useful for click chemistry by Mitsunobu reaction.

Among these possible diversity options, the esterification is proposed for the present work consideration of wider scope, the possibility of new pro-drug development, and ease of operation in the laboratory facilities. It is worth mentioning that these ester derivatives of Fibritol are novel compounds and not yet reported. For the demonstration and standardization of synthetic procedures, it is proposed to prepare a couple of ester derivatives as shown in Figure-3.12.

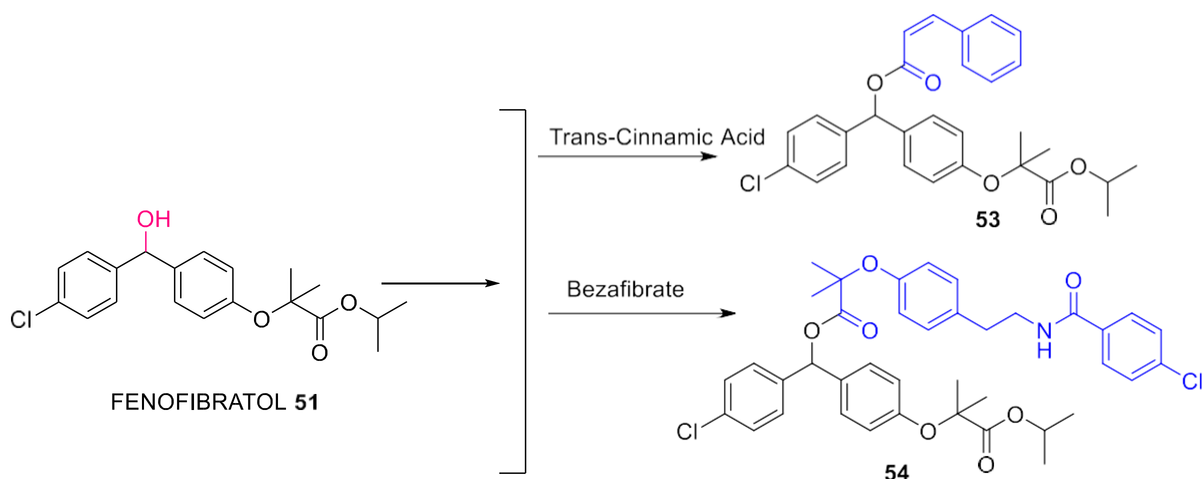


Figure 3.12: Novel esters of Fenofibratol proposed.

3.6.1 The selection of Esterification Procedure:

As the development of ester functional groups is vital for the synthesis of many economically available drugs and building blocks, esterification is often regarded as an essential switch within organic and medicinal chemistry. [1] Esters are also extensively employed in the industries of fragrances, flavor, and functional materials. They are also often found in natural goods, lipids, pheromones, and other physiologically active

substances. Consequently, synthetic techniques that enable for ester functional groups have gathered a lot of scientific attention. [102-104, 109-111]

The traditional acid-catalyzed Fischer-Speier approach, that utilizes a catalytic amount of mineral-based acids like HCl, H2SO4, etc., is usually employed in the processes for esterification. Employing multiple kinds of Lewis acid catalysts, such as scandium (III) triflate in acetic acid and ferric chloride in mesitylene, among others, various reagents and experimental circumstances for esterification are also reported in the literature.The major drawback in the procedure is related to experimental conditions nor suitable for complex and multifunctional molecules. The Fischer–Speier methodology require strong acidic conditions rendering acid sensitive moieties/molecules incompatible with this methodology. [104-106]

Therefore, there is need to select a robust and facile procedure for the esterification under mild conditions. Among several such procedures are reported in the literature Steglich esterification was selected for the present work in consideration to the experimental conditions and reagents used for the esterification reaction. The Steglich esterification is carried out using agent N,N′-dicyclohexylcarbodiimide as a major reagent in the presence of catalytic amount of 4-dimethylaminopyridine in aprotic organic solvents at ambient temperature. In view of the merits of the method we have chosen Steglich esterification for the preparation of compounds (Compound **53** and **54**) esters of fibritol.

3.6.2 The Steglich Esterification:

Steglich esterification can afford an extensive variety of esters consisting of laborious substituents such as sterically hindered tert-butyl group, acid liable group and it also proceeds under ambient temperatures, mild conditions, and often neutral pH whereas same esters under Fischer-Speier esterification would undergo elimination. An

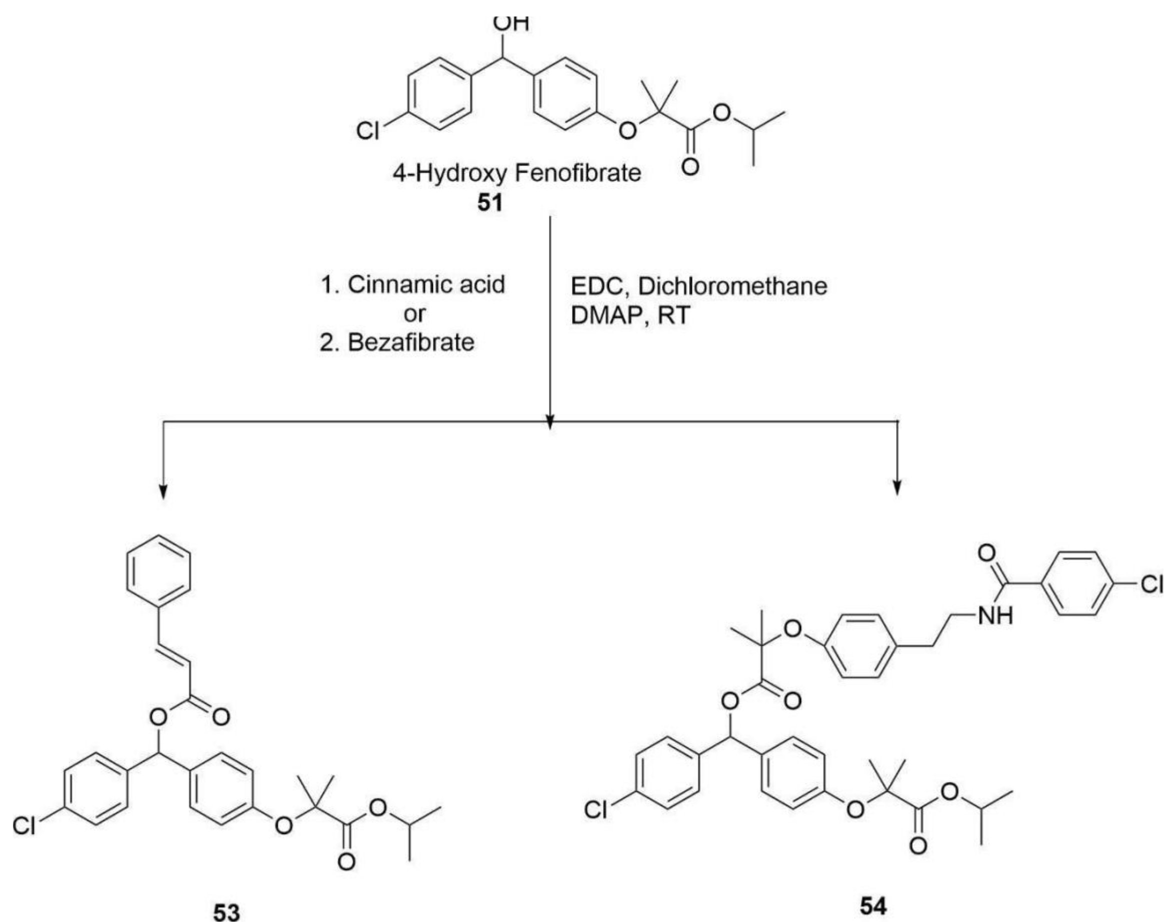
appropriate organocatalyst namely 4-dimethylaminopyridine (DMAP) and the amide coupling agent N,N'-dicyclohexylcarbodiimide (DCC) were reported in adapting appropriate esterification procedure of carboxylic acid and primary alcohols and it was first reported in 1978 by Wolfgang steglich and Bernhard Neises. This approach was emerged from amide coupling method which reported DCC as a fruitful coupling agent. DMAP has a greater nucleophilicity when compared to alcohol hence catalytic amount of DMAP is required for esterification. The DMAP accelerates the reaction rate by reacting with the O- acylisourea intermediate to form a highly activated electrophilic acylated pyridinium intermediate. By interacting with the O-acylisourea intermediate to generate a highly active electrophilic acylated pyridium intermediate, the DMAP speeds up the pace of the chemical reaction. Intramolecular 1,3-rerrangement of the O-acylisourea intermediate to an N-acylurea species is prevented as it is not able to react with alcohols. There are multiple challenges concerned with the application of DCC, as it is commonly employed as the coupling agent for the steglich

esterification given the fact it is so efficient at offering the active ester intermediate. Extensive precautionary measures must be observed when handling DCC given it is an allergy, an irritant, and it can damage internal organs. An additional drawback of using DCC is that one of its residue, N,N' dicyclohexylurea (DCU), is partially soluble in variety organic solvents and is insoluble in water. Despite the DCU by-products inadequate solubility enables it to be simple to filter it out of reaction mixtures, it can be troublesome to eliminate any residual traces, even using column chromatography, making purification cumbersome. Some of the issues mentioned previously can be overcome with the aid of using carbodiimide derivatives that are

organic solvents soluble, such as N,N'-diisopropylcarbodiimide (DIC). Despite the fact, there are multiple Steglich esterification variations (such as the Yamaguchi esterification that utilizes the coupling agent 2,4,6-ttrichlorobenzoyl chloride), the Steglich esterification continues to show one of the widely utilized synthetic processes to produce esters. [105, 107-111] Typical solvents employed in steglich esterification comprises of DCM, DMF,THF and CH3CN. Furthermore, due to EHS challenges, such as the fact that DIC is potentially dangerous when inhaled, coupling reagents like DCC and DIC are interpretated unfavorably by GlaxoSmithKline (GSK) reagent selection suggestions. [103] Considering the above situation, the steglich esterification tends to be challenging and intricate scenarios when safety and care are taken into thoughts.

3.6.3 Synthesis of new Fenofibratol Esters:

In view of the above discussion, we have prepared two new derivatives of Fibritol by esterification of the hydroxyl group at 4-position of fibritol. The esterification was carried out using Steglish esterification procedure which was considered most appropriate for the esterification fibritol. The fibritol (**51**) was treated with equimolar amount of either trans-cinnamic acid or fenofibric acid (**50**) in dichrorometane with diisopropylcarbodiimide (EDC) and catalytic anmount of 4-dimethyl amino pyridine (DMAP) at room temperature for a couple of hours. After complete reaction the new derivatives (**53**) and (**54**) were obtained in high yield and purity. The course of reaction and reagents used for the synthesis is shown in scheme 3.8.



Scheme 3.0.8: Synthesis of Fibritol esters 53 and 54 3.6.4

Experimental:

The synthesis of compound **53** and **54** was carried out using Cinnamic acid and Bezafibrate obtained from sigma USA and 4-Hydroxy Fenofibrate (fibritol) obtained by the synthesis from our lab and fully characterized before use as described in the previous section. An In-direct probe detection method was used on a Bruker Advance II 400 MHz NMR to record the ^{1}H NMR and ^{13} C NMR. The method used to record the chemical shifts was using parts per million (ppm) from a standard of tetramethylsilane (TMS) in $CDCl_3$ and Hertz was used to measure coupling constants (J). Infrared spectrum was recorded on a Thermo Electron Corporation IR 200 spectrophotometer and analysis was done on EZ-OMNIC software. The mass spectrum was recorded on a Esquire-LC_00135 spectrometer.

Stuart-SMP10 was the apparatus used to record melting point and it was reported uncorrected.

Synthesis of (4-chlorophenyl) (4-((1-isopropoxy-2-methyl-1-oxopropan-2- yl)oxy)phenyl)methyl cinnamate (53):

The starting material for this experiment was 4-Hydroxy fenofibrate 250 mg (0.7mM) and 148 mg (1mM) Trans Cinnamic Acid and it was dissolved in 4.5 ml of Dichloromethane in 50 mL oven-dried round- bottom flask fitted with a magnetic stir-bar and moisture guard tube. Slowly add 10mg of Dimethyl aminopyridine and then add 150 mg of EDC. Keep it on stirring for 2 hours and then monitor TLC. TLC was done in 10% ethyl acetate-hexane acetic acid and the results indicated that the reaction was completed. The product was extracted in Ethyl Acetate. In the product add 25ml of ethyl acetate and 10ml 2N HCl and transfer it to a separatory funnel. Washing was done with Brine and the second washing was done in sodium bicarbonate and again with brine. Anhydrous magnesium sulfate is used to dry organic layer followed by filtration. Residue crude product was obtained by concentrating product under the reduced pressure. TLC was monitored again in 10% ethyl acetate-hexane acetic acid and the results indicated that the reaction is complete. The product obtained was gummy in nature.

Yield: 330 mg, (90%).

ES-MS: Calculated for C29H29ClO5 [M+H] = 493 Observed [M+ Na]+= 515 (Figure-3.13)

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.19 (d, J=6.3 Hz,6H), 1.57 (s,6H), 5.06 (m, J=6.12 Hz,1H), 6.53(d,J=16 Hz,1H),6.81(d,J=9.2 Hz,2H), 7.21-7.74 (m, 9H) (Figure-3.14)

^{13}C NMR (100 MHz CDCl3): δ 21.56,25.37,25.41,69.01,75.87,76.73,77.05,77.36,79.14,117.79,118.70,128.19,128.26,128.48,128.67,128.94,130.51,132.98,133.68,134.24,138.95,145.63,155. 48,165.94,173.58 (Figure-3.15)

3.6.4.1 Spectral Data:

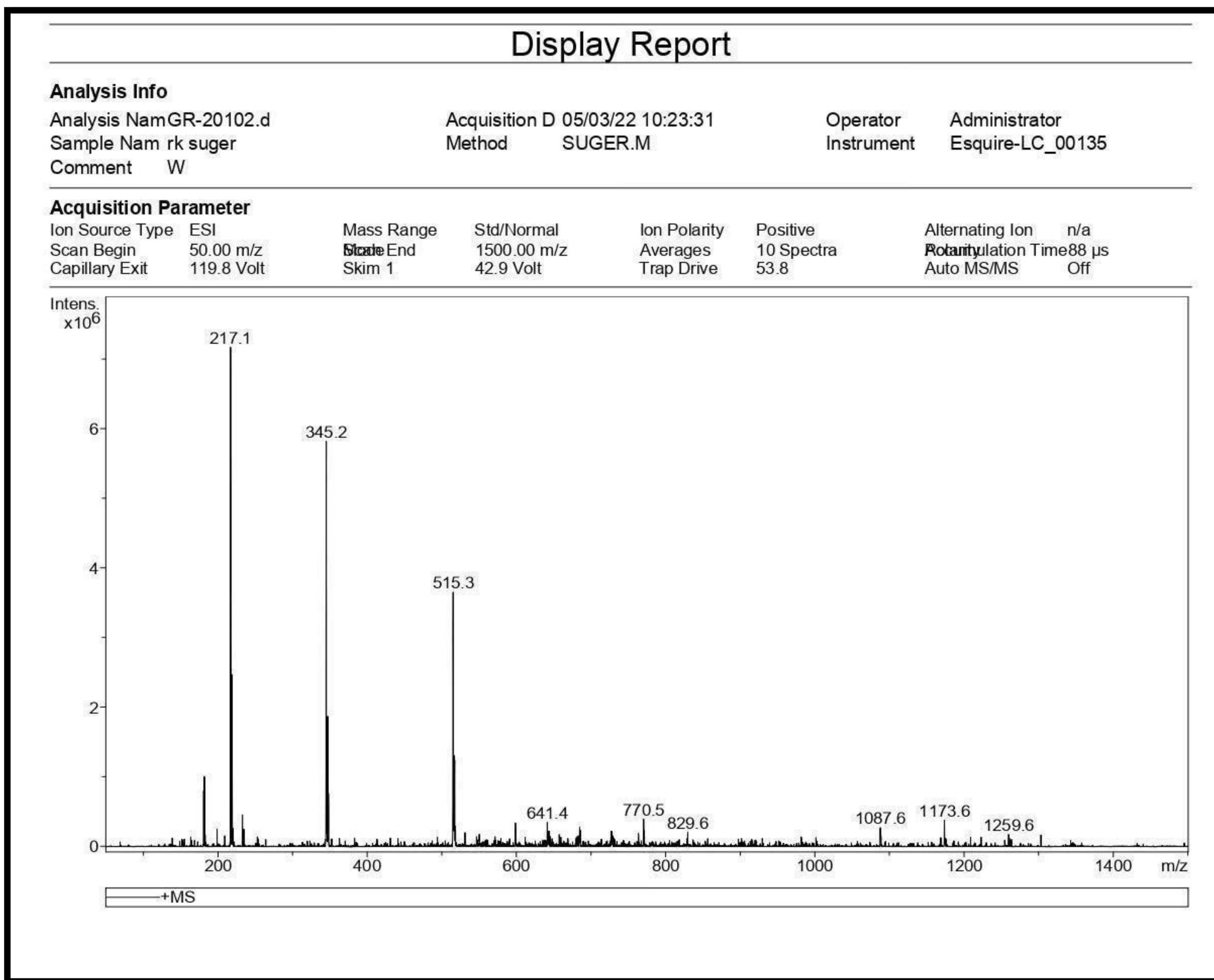


Figure 3.13: ES-MS Of (4-chlorophenyl)(4-((1-isopropoxy-2-methyl-1-oxopropan-2yl)oxy)phenyl)methyl cinnamate. (53)

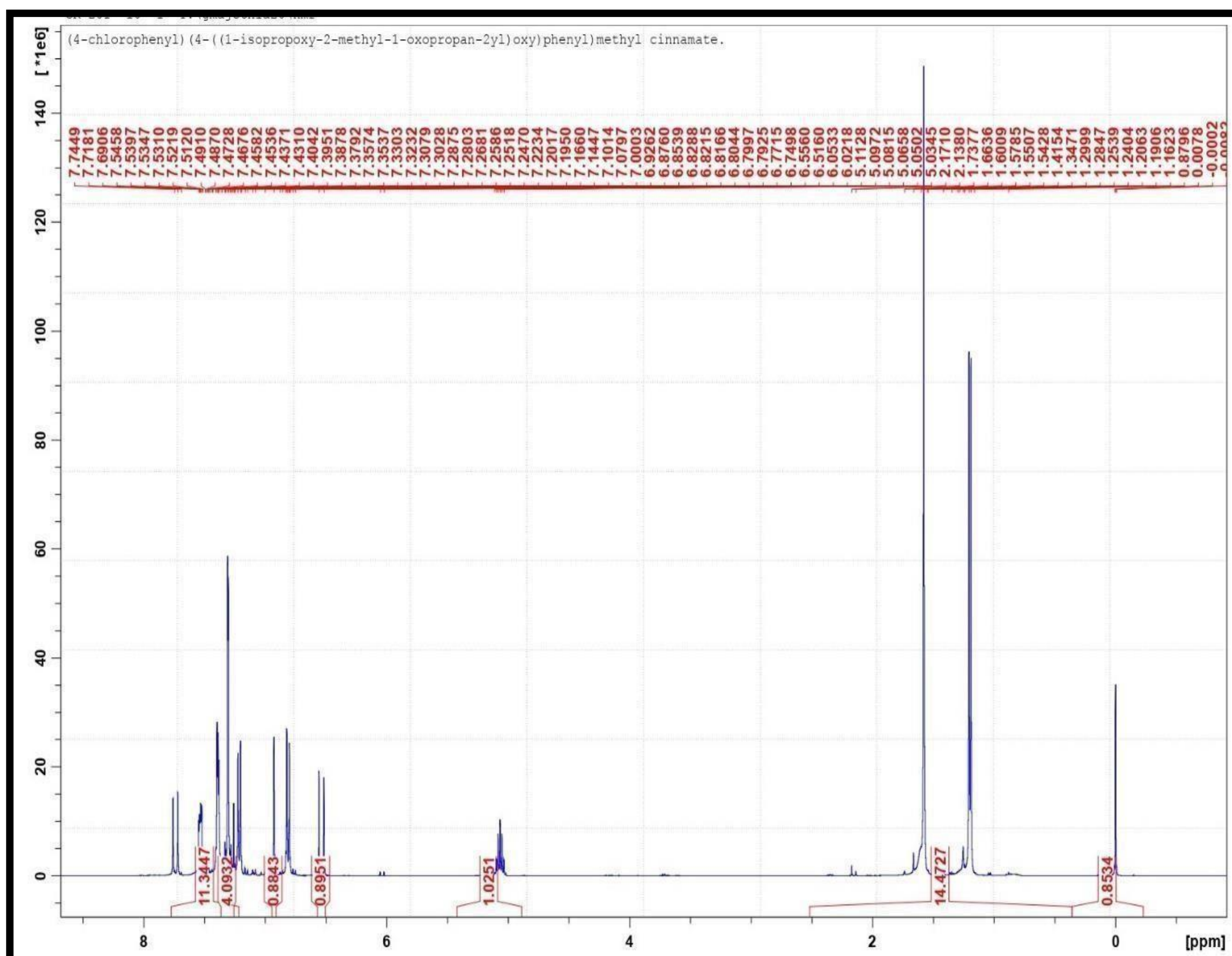


Figure 3.14: ^{1}H NMR Of (4-chlorophenyl)(4-((1-isopropoxy-2-methyl-1-oxopropan-2-yl)oxy)phenyl)methyl cinnamate.(53)

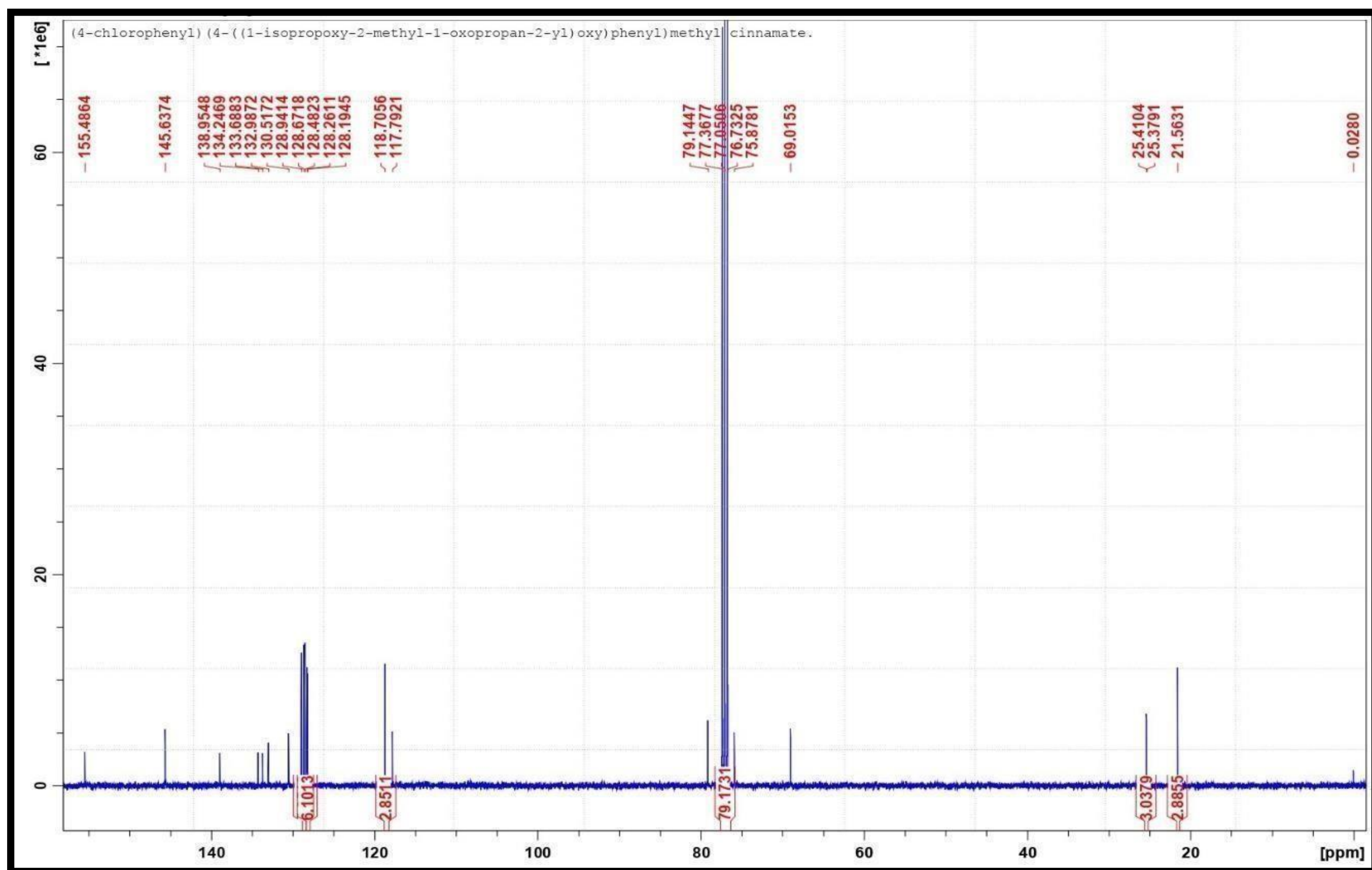


Figure 3.15:: ^{13}C NMR Of (4-chlorophenyl)(4-((1-isopropoxy-2-methyl-1-oxopropan-2-yl)oxy)phenyl)methyl cinnamate.(53)

3.6.4.2 Synthesis of (4-chlorophenyl)(4-((1-isopropoxy-2-methyl-1-oxopropan-2-yl)oxy)phenyl)methyl 2-(4-(2-(4-chlorobenzamido)ethyl)phenoxy)-2-methylpropanoate (54):

Typically, the experiment was carried out with 4-Hydroxy fenofibrate 250 mg (0.7 mM) and 362 mg(1 mM) Bezafibrate dissolved in 4.5 ml of Dichloromethane in 50mL round bottom flask fitted with magnetic stir-bar which is oven dried and consisted of moisture guard tube. Slowly add 10mg of Dimethyl aminopyridine and then add 150 mg EDC. Keep it on stirring for 2 hours and then monitor TLC. TLC was done in 10% ethyl acetate-hexane acetic acid and the results indicated that reaction is done. The product was extracted in Ethyl Acetate. In the resulting product, add 25ml of ethyl acetate and 2N HCl, 10 ml and transfer it to a separatory funnel. Washing was done with Brine and the second washing was done in sodium bicarbonate and again with brine. Anhydrous magnesium sulfate was used in order to dry the organic layer followed by filtration. In order to get the residue crude product, it was concentrated under reduced pressure. TLC was monitored again in 10% ethyl acetate-hexane acetic acid and the results indicated that reaction is done. The product obtained was gummy in nature.

Yield: 400 mg, 86%

ES-MS: Calculated for $C_{39}H_{41}Cl_2NO_7$ [M+H]= 706 Observed $[M+Na]^+$= 728 (Figure- 3.16)

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.56-1.59 (d, J=3.8 Hz,18H), 1.56 (s), 2.83(t,J=6.7 Hz,2H), 3.64 (t,J=6.6 Hz,2H), 5.04 (sept,J=6.28 Hz,1H), 6.09 (s,1H), 6.6-7.0 (m,8H), 7.1-7.6 (m, 9H) (Figure-3.17)

^{13}C NMR :δ 21.54,25.26, 25.43, 34.65, 41.22, 69.05, 76.71, 77.03, 77.35, 79.10, 79.13, 118.57, 119.05, 128.26, 128.50, 128.58, 128.81, 129.43, 132.28, 133.00, 133.79, 137.6,1154.04, 155.51, 166.35, 173.53. (Figure-3.18)

3.6.4.4 Spectral Data:

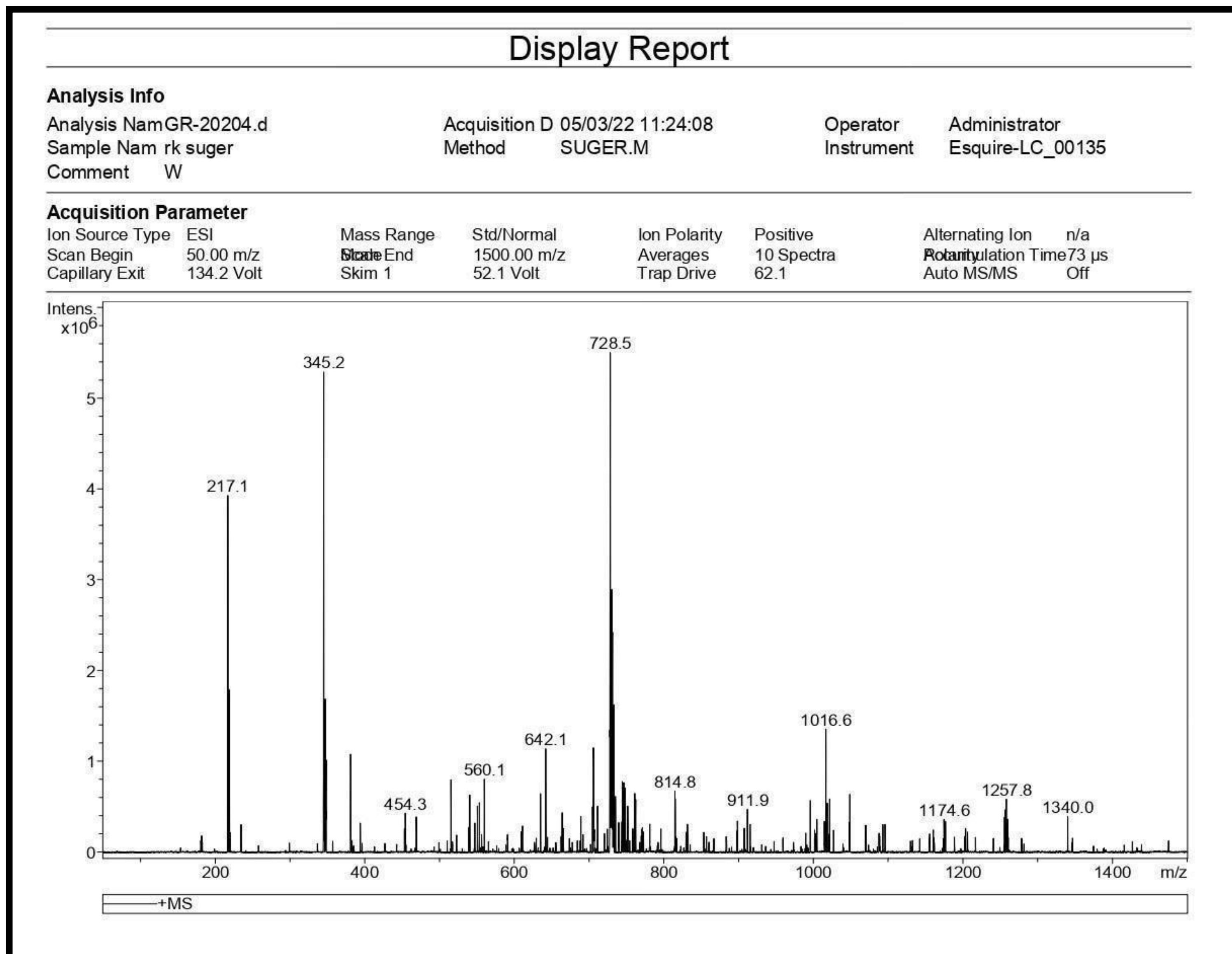


Figure 3.16: ES-MS Of (4-chlorophenyl)(4-((1-isopropoxy-2-methyl-1-oxopropan-2-yl)oxy)phenyl)methyl2-(4-(2-(4-chlorobenzamido)ethyl)phenoxy)-2methylpropanoate.

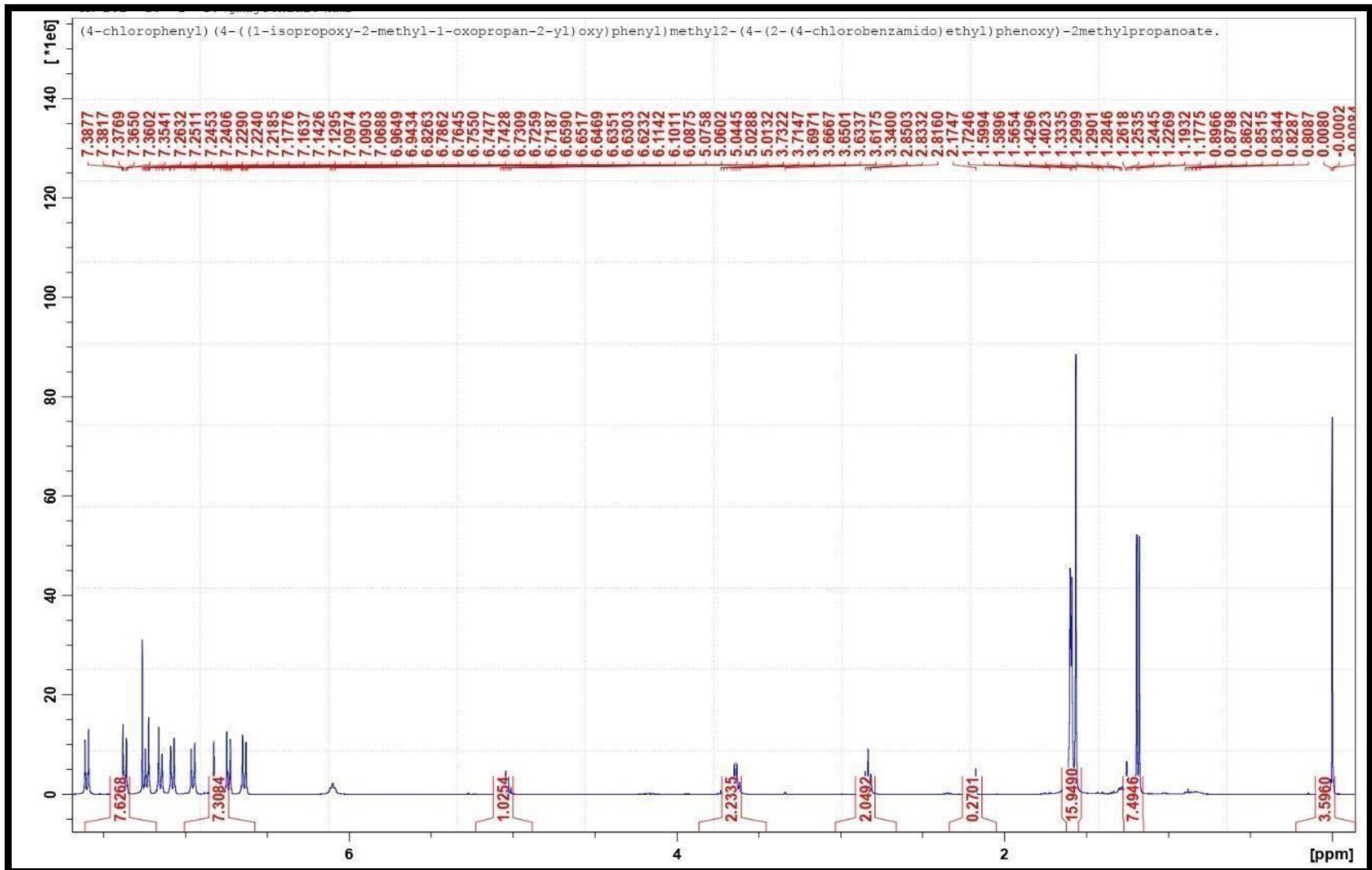


Figure 3.17: ^{1}H NMR of (4-chlorophenyl)(4-((1-isopropoxy-2-methyl-1-oxopropan-2-yl)oxy)phenyl)methyl2-(4-(2-(4-chlorobenzamido)ethyl)phenoxy)-2methylpropanoate.(54)

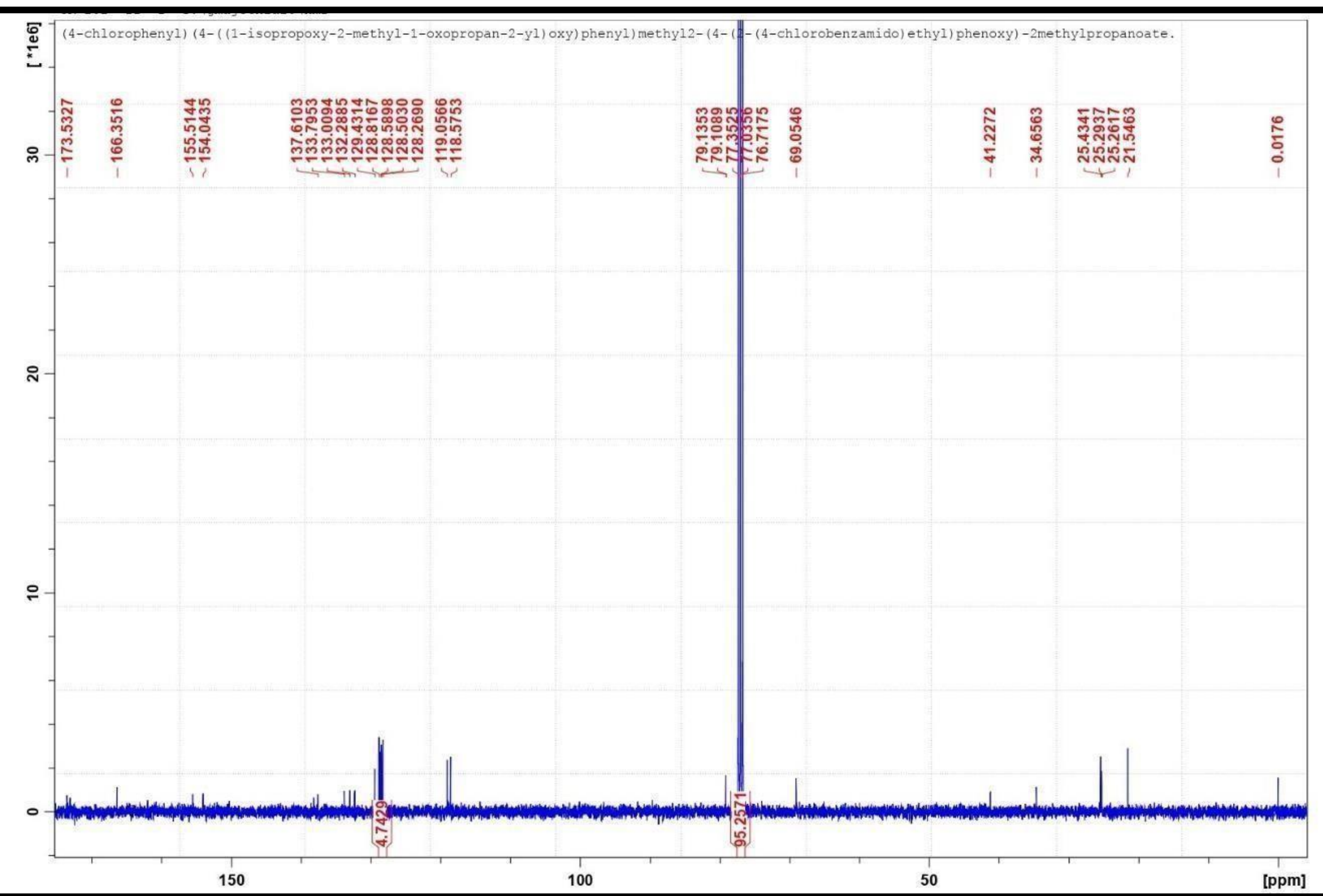


Figure 3.18: ^{13}C NMR Of (4-chlorophenyl)(4-((1-isopropoxy-2-methyl-1-oxopropan-2-yl)oxy)phenyl)methyl2-(4-(2-(4-chlorobenzamido)ethyl)phenoxy)- 2methylpropanoate.(54)

4.0 References:

1. Mathivat, A. and J. Cottet, *[Clinical trials on the hypocholesteremia-producing effect of 2-phenylbutyric acid].* Bull Mem Soc Med Hop Paris, 1953. **69**(30-31): p. 1030-48.
2. Thorp, J.M. and W.S. Waring, *Modification of metabolism and distribution of lipids by ethyl chlorophenoxyisobutyrate.* Nature, 1962. **194**: p. 948-9.
3. Balendiran, G.K. and B. Rajkumar, *Fibrates inhibit aldose reductase activity in the forward and reverse reactions.* Biochem Pharmacol, 2005. **70**(11): p. 1653- 63.
4. Oliver, M., *The clofibrate saga: a retrospective commentary.* Br J Clin Pharmacol, 2012. **74**(6): p. 907-10.
5. Balendiran, G.K., M. Verma, and E. Perry, *Chemistory of Fibrates.* Curr Chem Biol, 2007. **1**(3): p. 311-316.
6. Fischer, J.G., C.Robin, *Analogue based Drug Discovery*, J.W. Sons, Editor. 2006.
7. Bezafibrate Infarction Prevention, s., *Secondary prevention by raising HDL cholesterol and reducing triglycerides in patients with coronary artery disease.* Circulation, 2000. **102**(1): p. 21-7.
8. Tenenbaum, A., et al., *Peroxisome proliferator-activated receptor ligand bezafibrate for prevention of type 2 diabetes mellitus in patients with coronary artery disease.* Circulation, 2004. **109**(18): p. 2197-202.
9. Formulary, B.N. 2018, Pharmaceutical Press.
10. Endo, A., *A historical perspective on the discovery of statins.* Proc Jpn Acad Ser B Phys Biol Sci, 2010. **86**(5): p. 484-93.
11. Endo, A., M. Kuroda, and Y. Tsujita, *ML-236A, ML-236B, and ML-236C, new inhibitors of cholesterogenesis produced by Penicillium citrinium.* J Antibiot (Tokyo), 1976. **29**(12): p. 1346-8.
12. Endo, A., *The discovery and development of HMG-CoA reductase inhibitors.* J Lipid Res, 1992. **33**(11): p. 1569-82.
13. Brown, M.S., S.E. Dana, and J.L. Goldstein, *Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in human fibroblasts by lipoproteins.* Proc Natl Acad Sci U S A, 1973. **70**(7): p. 2162-6.
14. Kaneko, I., Y. Hazama-Shimada, and A. Endo, *Inhibitory effects on lipid metabolism in cultured cells of ML-236B, a potent inhibitor of 3-hydroxy-3- methylglutaryl-coenzyme-A reductase.* Eur J Biochem, 1978. **87**(2): p. 313-21.
15. Jiang, W., et al., *Statins: a repurposed drug to fight cancer.* J Exp Clin Cancer Res, 2021. **40**(1): p. 241.
16. Yandrapalli, S., et al., *Statins and the potential for higher diabetes mellitus risk.* Expert Rev Clin Pharmacol, 2019. **12**(9): p. 825-830.
17. Rzouq, F.S., et al., *Hepatotoxicity fears contribute to underutilization of statin medications by primary care physicians.* Am J Med Sci, 2010. **340**(2): p. 89-93.
18. Shrestha, A., A. Mulmi, and R. Munankarmi, *Statins and Abnormal Liver Enzymes.* S D Med, 2019. **72**(1): p. 12-14.
19. Vagelos, P.R., *Are prescription drug prices high?* Science, 1991. **252**(5009): p. 1080-4.
20. Vagelos, P.R.a.G., L, *Medicine Science and Merk*. 2004, Cambridge University Press: United Kingdom.

21. SP., K., *Lovastatin, ClinCalc DrugStats Database*, in http://clincalc.com/DrugStats/Drugs/Lovastatin. 2022.
22. Hirama M, V.M., *" A chiral total synthesis of compactin".* J.Am. Chem Soc., 1982. **104 (15)**: p. 4251-4253.
23. Hirama M, I.I., Mitsuko, *"Synthesis of (+)- Mevinolin starting from Naturally occuring building blocks and using an asymmetry inducing reaction.".* Tetrahedron Lett. 24 (17), 1983: p. 1811-1812.
24. J, A., *"The fall of the world's best-selling drug."*. 28 November 2009, Financial Times.
25. *"Lipitor becomes world's top-selling drug"*. 28 December 2011, Crain's New York Bussiness via Associated Press.
26. *" Lipitor loses patent, goes generic."*. 30 November 2011, CNN.
27. Roth, B.D., *The discovery and development of atorvastatin, a potent novel hypolipidemic agent.* Prog Med Chem, 2002. **40**: p. 1-22.
28. Roth, B.D., et al., *Inhibitors of cholesterol biosynthesis. 3. Tetrahydro-4-hydroxy- 6-[2-(1H-pyrrol-1-yl)ethyl]-2H-pyran-2-one inhibitors of HMG-CoA reductase. 2. Effects of introducing substituents at positions three and four of the pyrrole nucleus.* J Med Chem, 1991. **34**(1): p. 357-66.
29. Tobert, J.A., *Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors.* Nat Rev Drug Discov, 2003. **2**(7): p. 517-26.
30. A, B., *"Merk Loses Protection for Patent on Zocor"*. 23 June 2006, The New York Times.
31. J. Pharm. Appl. Chem, C.B.a.J.P., *Synthetic Methods for Simvastatin- an Overview.* 2019. **1**: p. 23-43.
32. Apothekerverlag, V.Ö., *Haberfeld H. Austria-Codex (in German).* . 2015.
33. Sikorski, J.A., *Comprehensive Medicinal Chemistry II.* 2007.
34. Zacharia, J.T., T. Tanaka, and M. Hayashi, *Facile and highly enantioselective synthesis of (+)- and (-)-fluvastatin and their analogues.* J Org Chem, 2010. **75**(22): p. 7514-8.
35. *"Rosuvastatin Calcium Monograph for Professionals"*, Drugs.com, Editor. 24 December 2018, American Society of Health-System Pharmacists (AHFS).
36. Wolfe, S., *Rosuvastatin: winner in the statin wars, patients' health notwithstanding.* BMJ, 2015. **350**: p. h1388.
37. Metzner, R., et al., *Integrated Biocatalysis in Multistep Drug Synthesis without Intermediate Isolation: A de Novo Approach toward a Rosuvastatin Key Building Block.* Organic Process Research & Development, 2015. **19**(6): p. 635-638.
38. METZNER, B.K.W.G.,*Process for enantioselective synthesis of 3-hydroxy-glutaric acid monoesters and usethereof.*, in *WO2014/140006*. 2014.
39. *"Pravastatin Sodium Monograph for Professionals".* AHFS, Editor. 23 December Drugs.com.
40. *"FDA Approves First Generic Pravastatin".* 6 March 2010, Food and Drug Administration (FDA).
41. Vaughan, C.J. and A.M. Gotto, Jr., *Update on statins: 2003.* Circulation, 2004. **110**(7): p. 886-92.
42. Denis, M., et al., *Gene inactivation of proprotein convertase subtilisin/kexin type 9 reduces atherosclerosis in mice.* Circulation, 2012. **125**(7): p. 894-901.
43. Nishikido, T. and K.K. Ray, *Non-antibody Approaches to Proprotein Convertase*

Subtilisin Kexin 9 Inhibition: siRNA, Antisense Oligonucleotides, Adnectins, Vaccination, and New Attempts at Small-Molecule Inhibitors Based on New Discoveries. Front Cardiovasc Med, 2018. **5**: p. 199.

44. Strom, T.B., K. Tveten, and T.P. Leren, *PCSK9 acts as a chaperone for the LDL receptor in the endoplasmic reticulum.* Biochem J, 2014. **457**(1): p. 99-105.
45. Benjannet, S., et al., *The proprotein convertase (PC) PCSK9 is inactivated by furin and/or PC5/6A: functional consequences of natural mutations and post-translational modifications.* J Biol Chem, 2006. **281**(41): p. 30561-72.
46. Cameron, J., et al., *Mutation S462P in the PCSK9 gene reduces secretion of mutant PCSK9 without affecting the autocatalytic cleavage.* Atherosclerosis, 2009. **203**(1): p. 161-5.
47. Kuzmich, N., et al., *PCSK9 as a Target for Development of a New Generation of Hypolipidemic Drugs.* Molecules, 2022. **27**(2).
48. Pettersen, D. and O. Fjellstrom, *Small molecule modulators of PCSK9 - A literature and patent overview.* Bioorg Med Chem Lett, 2018. **28**(7): p. 1155- 1160.
49. Disney, M.D., *Inhibiting Translation One Protein at a Time.* Trends Biochem Sci, 2017. **42**(6): p. 412-413.
50. E. Darout, R.D., L. Hawkins, A.T. Londregan, P.M. Loria, B. Maguire, and D.N.P. K.F. Mcclure, D.W. Piotrowski, *N-piperidin-3-ylbenzamide Derivatives for Treating Cardiovascular Diseases.* 23 October, 2014.

.

51. Shulman, A.G., *Heparin for prevention of atherosclerosis.* N Engl J Med, 1988. **319**(17): p. 1154-5.
52. Neri Serneri, G.G., et al., *Effectiveness of low-dose heparin in prevention of myocardial reinfarction.* Lancet, 1987. **1**(8539): p. 937-42.
53. Engelberg, H., R. Kuhn, and M. Steinman, *A controlled study of the effect of intermittent heparin therapy on the course of human coronary atherosclerosis.* Circulation, 1956. **13**(4): p. 489-98.
54. Bottiger, L.E., et al., *Long-term heparin treatment in ischaemic heart disease. Effects on clinical condition and plasma lipids.* Acta Med Scand, 1967. **182**(2): p. 245-57.
55. Sayen, J.J., et al., *Unstable angina, myocardial infarction, heparin and death: medium dose heparin (not exceeding 20,000 units/day) in the treatment of patients with acute coronary event--first year and long-term comparative mortality.* Trans Am Clin Climatol Assoc, 1983. **94**: p. 141-53.
56. Min, D.K., et al., *In silico Screening of Chemical Libraries to Develop Inhibitors That Hamper the Interaction of PCSK9 with the LDL Receptor.* Yonsei Med J, 2015. **56**(5): p. 1251-7.
57. Alghamdi, R.H., et al., *LDL-R promoting activity of peptides derived from human PCSK9 catalytic domain (153-421): design, synthesis and biochemical evaluation.* Eur J Med Chem, 2015. **92**: p. 890-907.
58. Taechalertpaisarn, J., et al., *Small Molecule Inhibitors of the PCSK9.LDLR Interaction.* J Am Chem Soc, 2018. **140**(9): p. 3242-3249.
59. Ko, E., et al., *Exploring key orientations at protein-protein interfaces with small molecule probes.* J Am Chem Soc, 2013. **135**(1): p. 167-73.
60. M.M. Muehlemann, T.E.B., K.D. Monroe, J.W. Bourne, M.T. Reece, and V.

Nevalainen., *Proprotein Convertase Subtilisin/kexin Type 9 (PCSK9) Allo- steric Binding Ligands to Modulate Serum Low Density Lipoprotein (LDL) Levels.* 21 August, 2014.

.

61. M.R. Arkin, Y.T., J.A. Wells, *Small-molecule inhibitors of protein-protein interactions: progressing toward the reality.* Chem. Biol. , (2014)
62. R. Scharfmann, L.H., *Methods and Compositions for Reducing PCSK9 Expression.* 08 February, 2018. .
63. D. Guay, S.C., N. Lachance, J. Chiasson, V.L. Truong, P. Lacombe, K. Skorey,N.G. Seidah, *Small Molecule Modulators of PCSK9 and Methods of Use Thereof.* 18 September, 2014. .
64. *Drugs.* . 2021 Jun;81(9):1129.
65. Tyagi, S., et al., *The peroxisome proliferator-activated receptor: A family of nuclear receptors role in various diseases.* J Adv Pharm Technol Res, 2011. **2**(4): p. 236-40.
66. Berger, J.P., T.E. Akiyama, and P.T. Meinke, *PPARs: therapeutic targets for metabolic disease.* Trends Pharmacol Sci, 2005. **26**(5): p. 244-51.
67. Giampietro, L., et al., *Novel Phenyldiazenyl Fibrate Analogues as PPAR alpha/gamma/delta Pan-Agonists for the Amelioration of Metabolic Syndrome.* ACS Med Chem Lett, 2019. **10**(4): p. 545-551.
68. Yamazaki, Y., et al., *Design and synthesis of highly potent and selective human peroxisome proliferator-activated receptor alpha agonists.* Bioorg Med Chem Lett, 2007. **17**(16): p. 4689-93.
69. Giampietro, L., et al., *Synthesis and structure-activity relationships of fibrate-based analogues inside PPARs.* Bioorg Med Chem Lett, 2012. **22**(24): p. 7662-6.
70. Cox, R.L., *Rationally designed PPARdelta-specific agonists and their therapeutic potential for metabolic syndrome.* Proc Natl Acad Sci U S A, 2017. **114**(13): p. 3284-3285.
71. Sashidhara, K.V., et al., *Discovery of amide based fibrates as possible antidyslipidemic and antioxidant agents.* Eur J Med Chem, 2012. **57**: p. 302-10.
72. Wahle, K.W., *Atherosclerosis: cell biology and lipoproteins.* Curr Opin Lipidol, 2002. **13**(3): p. 347-9.
73. Mokale, S.N., et al., *Synthesis and in-vivo hypolipidemic activity of some novel substituted phenyl isoxazol phenoxy acetic acid derivatives.* Bioorg Med Chem Lett, 2014. **24**(9): p. 2155-8.
74. R. Amoroso, L.L., I. Cacciatore, L. Marinelli, A. Ammazzalorso, I. Bruno, B. De Filippis, M. Fantacuzzi, C. Maccallini, L. Menghini, A. Di Ste- fano, L. Giampietro, , 2018: p. 1230 –1238.
75. H. Wei, J.R., X. Zhang, , 2016. **6**: p. 10846– 10860.
76. Sahu, N.K., et al., *Exploring pharmacological significance of chalcone scaffold: a review.* Curr Med Chem, 2012. **19**(2): p. 209-25.
77. Defossa, E. and M. Wagner, *Recent developments in the discovery of FFA1 receptor agonists as novel oral treatment for type 2 diabetes mellitus.* Bioorg Med Chem Lett, 2014. **24**(14): p. 2991-3000.
78. Li, Z., et al., *Design, synthesis and structure-activity relationship studies of novel phenoxyacetamide-based free fatty acid receptor 1 agonists for the treatment of type 2 diabetes.* Bioorg Med Chem, 2015. **23**(20): p. 6666-72.

79. Webster, S.P., et al., *Discovery and biological evaluation of adamantyl amide 11beta-HSD1 inhibitors.* Bioorg Med Chem Lett, 2007. **17**(10): p. 2838-43.
80. Navarrete-Vazquez, G., et al., *Discovery, synthesis and in combo studies of a tetrazole analogue of clofibric acid as a potent hypoglycemic agent.* Bioorg Med Chem Lett, 2013. **23**(11): p. 3244-7.
81. Ammazzalorso, A., et al., *Synthesis and antiplatelet activity of thioaryloxyacids analogues of clofibric acid.* Eur J Med Chem, 2005. **40**(9): p. 918-21.
82. Noonan, J.E., et al., *An update on the molecular actions of fenofibrate and its clinical effects on diabetic retinopathy and other microvascular end points in patients with diabetes.* Diabetes, 2013. **62**(12): p. 3968-75.
83. Zhang, J., et al., *Fenofibrate increases cardiac autophagy via FGF21/SIRT1 and prevents fibrosis and inflammation in the hearts of Type 1 diabetic mice.* Clin Sci (Lond), 2016. **130**(8): p. 625-41.
84. Balakumar, P., A. Rohilla, and N. Mahadevan, *Pleiotropic actions of fenofibrate on the heart.* Pharmacol Res, 2011. **63**(1): p. 8-12.
85. Laurora, S., et al., *Peroxisome proliferator-activated receptor ligands affect growth-related gene expression in human leukemic cells.* J Pharmacol Exp Ther, 2003. **305**(3): p. 932-42.
86. Scatena, R., et al., *Bezafibrate as differentiating factor of human myeloid leukemia cells.* Cell Death Differ, 1999. **6**(8): p. 781-7.
87. Liu, H., et al., *Growth inhibition and apoptosis in human Philadelphia chromosome-positive lymphoblastic leukemia cell lines by treatment with the dual PPARalpha/gamma ligand TZD18.* Blood, 2006. **107**(9): p. 3683-92.
88. Shigeto, T., et al., *Peroxisome proliferator-activated receptor alpha and gamma ligands inhibit the growth of human ovarian cancer.* Oncol Rep, 2007. **18**(4): p. 833-40.
89. Panigrahy, D., et al., *PPARalpha agonist fenofibrate suppresses tumor growth through direct and indirect angiogenesis inhibition.* Proc Natl Acad Sci U S A, 2008. **105**(3): p. 985-90.
90. Balendiran, G.K., et al., *Cancer biomarker AKR1B10 and carbonyl metabolism.* Chem Biol Interact, 2009. **178**(1-3): p. 134-7.
91. Li, T., et al., *Fenofibrate induces apoptosis of triple-negative breast cancer cells via activation of NF-kappaB pathway.* BMC Cancer, 2014. **14**: p. 96.
92. Chang, N.W., et al., *Fenofibrate exhibits a high potential to suppress the formation of squamous cell carcinoma in an oral-specific 4-nitroquinoline 1- oxide/arecoline mouse model.* Biochim Biophys Acta, 2011. **1812**(4): p. 558-64.
93. Poupon, R., D. Cazals-Hatem, and L. Arrive, *Fenofibrate-induced massive regression of mutiple inflammatory hepatocellular adenoma.* Clin Res Hepatol Gastroenterol, 2016. **40**(1): p. e1-3.
94. Liu, X., et al., *Expression Profiling Identifies Bezafibrate as Potential Therapeutic Drug for Lung Adenocarcinoma.* J Cancer, 2015. **6**(12): p. 1214-21.
95. Hayden, R.E., et al., *Treatment of primary CLL cells with bezafibrate and medroxyprogesterone acetate induces apoptosis and represses the pro-proliferative signal of CD40-ligand, in part through increased 15dDelta12,14,PGJ2.* Leukemia, 2009. **23**(2): p. 292-304.
96. Ertl, P., E. Altmann, and J.M. McKenna, *The Most Common Functional Groups in Bioactive Molecules and How Their Popularity Has Evolved over Time.* J Med Chem, 2020. **63**(15): p. 8408-8418.

97. R. J. Ouellette and J. D. Rawn, e.R.J.O.a.J.D.R., *Organic Chemistry,*. 2014, Elsevier,: Boston, . p. 699-745.
98. A. D. Godwin, e.C.D.C.a.C.E.C., *Applied Polymer Science*. 2000, Oxford: Pergamon. p. pp. 157–175.
99. M. Tsakos, E.S.S., L. L. Clement, N. L. Villadsen and T. B. Poulsen, Nat. Prod. Rep., , *RSC.* 2015. **32**: p. 605-632.
100. Liu, A., et al., *Fenofibrate metabolism in the cynomolgus monkey using ultraperformance liquid chromatography-quadrupole time-of-flight mass spectrometry-based metabolomics.* Drug Metab Dispos, 2009. **37**(6): p. 1157-63.
101. Kotheimer, A.E., W. Haq, and G.K. Balendiran, *Synthesis and Chiral Separation of Fibratol, Isopropyl 2-(4-((4-chlorophenyl)(hydroxyl) methyl)-phenoxy)-2-methylpropanoate.* Int J Org Chem (Irvine), 2018. **8**(2): p. 201-206.
102. Majethia, G.N., W. Haq, and G.K. Balendiran, *Chemoselective Reduction of Fenofibric Acid to Alcohol in the Presence of Ketone by Mixed Anhydride and Sodium Borohydride.* Int J Org Chem (Irvine), 2022. **12**(2): p. 116-125.
103. Jordan, A. and H.F. Sneddon, *Development of a solvent-reagent selection guide for the formation of thioesters.* Green Chemistry, 2019. **21**(8): p. 1900-1906.
104. Höfle, G., W. Steglich, and H. Vorbrüggen, *4-Dialkylaminopyridines as Highly Active Acylation Catalysts. [New synthetic method (25)].* Angewandte Chemie International Edition in English, 1978. **17**(8): p. 569-583.
105. Neises, B. and W. Steglich, *Simple Method for the Esterification of Carboxylic Acids.* Angewandte Chemie International Edition in English, 1978. **17**(7): p. 522-524.
106. Jordan, A., et al., *A solvent-reagent selection guide for Steglich-type esterification of carboxylic acids.* Green Chemistry, 2021. **23**(17): p. 6405-6413.
107. El-Faham, A. and F. Albericio, *Peptide coupling reagents, more than a letter soup.* Chem Rev, 2011. **111**(11): p. 6557-602.
108. Gibson, F.S., M.S. Park, and H. Rapoport, *Bis[[4-(2,2-dimethyl-1,3-dioxolyl)]methyl]- carbodiimide (BDDC) and Its Application to Residue-Free Esterifications, Peptide Couplings, and Dehydrations.* The Journal of Organic Chemistry, 1994. **59**(24): p. 7503-7507.
109. J. Inanaga, K.H., H. Saeki, T. Katsuki and M. Yamaguchi, , Bull. Chem. Soc. Jpn., 1979,. **52**.
110. Dhimitruka, I. and J. Santalucia, Jr., *Investigation of the Yamaguchi esterification mechanism. Synthesis of a lux-s enzyme inhibitor using an improved esterification method.* Org Lett, 2006. **8**(1): p. 47-50.
111. Jordan, A., P. Stoy, and H.F. Sneddon, *Chlorinated Solvents: Their Advantages, Disadvantages, and Alternatives in Organic and Medicinal Chemistry.* Chem Rev, 2021. **121**(3): p. 1582-1622.

5.0 Appendix:

International Journal of Organic Chemistry, 2022, 12, 116-125 https://www.scirp.org/journal/ijoc
ISSN Online: 2161-4695 ISSN Print: 2161-4687

Chemoselective Reduction of Fenofibric Acid to Alcohol in the Presence of Ketone by Mixed Anhydride and Sodium Borohydride

Greesha N. Majethia, Wahajul Haq, Ganesaratnam K. Balendiran

Department of Chemistry, Youngstown State University, Youngstown, OH, USA
Email: gkbalendiran@ysu.edu

How to cite this paper: Haq, W. and Balendiran, G.K. (2022) Chemoselective Reduction of Fenofibric Acid to Alcohol in the Presence of Ketone by Mixed Anhydride and Sodium Borohydride. *International Journal of Organic Chemistry*, **12**, 116-125.
https://doi.org/10.4236/ijoc.2022.122010

Received: March 22, 2022
Accepted: June 27, 2022
Published: June 30, 2022

Copyright © 2022 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative
Majethia, G.N.,

Abstract

A highly efficient and facile protocol for the selective reduction of carboxylic acid of Fenofibric acid to corresponding alcohol was developed. The selective reduction was carried out by activation of carboxylic acid by mixed anhy- dride followed by the reaction of sodium borohydride in presence of metha- nol. This is the first example of chemoselective reduction of carboxylic acid to alcohol in presence of a ketone without any external catalyst or ligand in a single step. The reaction offers wide applicability for the selective carboxy- lic group reduction methodology. The chemoselective reduction was demon- strated by the reduction of Fenofibric acid, an active metabolite of the drug Fenofibrate, to corresponding alcohol in excellent selectivity, yield, and puri- ty.

Commons Attribution International

License (CC BY 4.0). http://creativecommons.org/licenses/by/4.0/

Keywords

Open Access Chemoselective, Reduction of Carboxylic Acid, Sodium Borohydride, Mixed Anhydride

1. Introduction

The chemistry of the carbonyl group has been extensively used in various syn- thetic transformations. The carbonyl chemistry comprises a repertoire of several name reactions and reagents used for a specific purpose. The reduction of car- bonyl compounds to other functional groups offers new synthetic applications and the identification of novel chemical molecules. The conversion of a car- bonyl group to corresponding alcohol is one of the important steps studied by various researchers. Several reagents have been developed for this purpose, such

Jun. 30, 2022

as LiAlH4, NaBH4, and DIBAL-H, Red-Al, and other combinations [1]-[7]. There are reports of selective reduction of aldehydes in presence of other carbonyl compounds including ketones leading to atom economy and overall yield im- provement [8] [9] [10] [11].

The reduction of carboxylic group to alcohols is tricky and requires strong con- ditions for the reduction as compared to aldehydes, ketones and ester groups. The functional group tolerance is also compromised and therefore there is a need for highly efficient reducing agent under mild reaction conditions. This has been achieved by the transformation of carboxylic group to a highly reactive interme- diate which in turn is reduced by a mild reducing agent like sodium borohydride. It has been reported that the activated carboxylic acid can be successfully re- duced to corresponding alcohols by the reaction of sodium borohydride at room temperature in excellent yield. The reduction of an activated carboxylic acid is re- ported using sodium borohydride by carbonates or mixed anhydrides, O-acylurea generated by *in situ* action of carbodiimides, and activated esters and boronic esters [12]-[22].

Selective and orthogonal reduction of carbonyl compounds remains an im- portant area of active research. Fairly good chemoselectivity has been achieved with the advancement of methodology however, there is still a need for robust methodology to achieve the selectivity for many combinations for industrial ap- plication. Among various combinations, the orthogonal reduction of carboxylic acids is reported in the presence of derivatives like esters and amides, to mention a few. Catalytic hydrosilylation has been shown to be a very useful approach for the reduction of carboxylic acid to alcohol. The available methodology and fu- ture prospects were reviewed in consideration of the significance of orthogonal selectivity and

chemoselectivity by catalytic hydrosilylation of carboxylic acid derivatives under mild conditions [23].

Recently, a chemoselective reduction was reported using hydrosilylation and combination with [Ni-OH] complex as a catalyst for the reduction of amide to amines under base-free conditions with key functional group tolerance [24]. The hydroboration reduction of carboxylic acids using sodium amino diborane as a catalyst has been reported in excellent selectivity of functional group tolerance [25]. Therefore, a facile reduction of carboxylic acid with functional group to- lerance is highly desirable.

Herein we report a facile and chemoselective reduction of a carboxylic group, more challenging carbonyl group, to corresponding alcohol in presence of a ke- tone under mild condition without any external ligand or catalyst in excellent yield and selectivity. In our ongoing research project on Fibrates we envisaged the synthesis of alcohol derivatives(4-chlorophenyl)(4-((1-hydroxy-2-methylpropan- 2yl) oxy)phenyl)methanone (**3**) [26] [27] [28]. The synthesis of the title com- pound can be achieved by the two step reaction as reported recently that in - volves double hydride reduction of the Fenofibrate, an agent against diabetes and that lowers lipid levels [29] [30] [31] [32] [33]. The isopropyl group of the

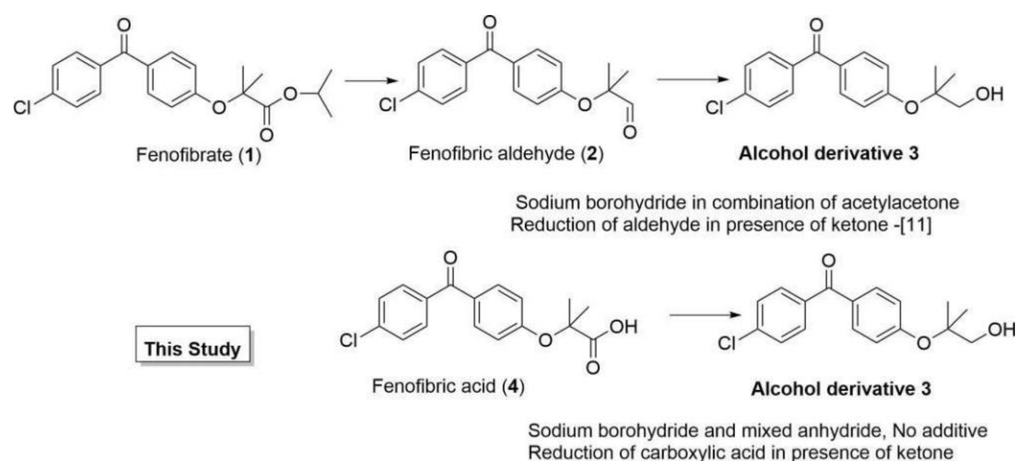


Figure 1. Direct reduction of carboxylic acid in presence of ketone.

Fenofibrate was reduced to corresponding aldehyde **2** by the reduction with DIBAL-H and subsequently the chemoselective reduction of the aldehyde group was achieved to obtain **3** using sodium borohydride in presence of ketone group using acetylacetone as ligand masking the ketone group of benzophenone moie- ty [11] **Figure 1**.

In order to develop a facile and direct synthesis of **3** under mild conditions, we surmised that reduction of an activated carboxylic group by commonly ac- cessible and bench stable sodium borohydride may be useful for a chemoselec- tive reduction in the presence of another carbonyl group, a ketone, in the cur- rent study. The

chemoselectivity may be attributed to the relative reactivity of an activated carboxylic acid and other co-existing carbonyl groups. For the present study, we have selected mixed anhydride using isobutyl chloroformate in pres- ence of a tertiary amine at optimum temperature for mixed anhydride reaction ranging from −15˚C to −10˚C. The selective reactivity of sodium borohydride on the activated carboxylic group and other co-existing carbonyl groups may be more evident at the low-temperature range used for mixed anhydride reactions. The mixed anhydride is generated by the reaction of Fenofibric acid (**4**) with iso- butyl chloroformate and N-methyl morpholine in dry Tetrahydrofuran at −15˚C to −10˚C. The mixed anhydride thus obtained was treated with 1.5 to 2 molar equitant of sodium borohydride at −15˚C followed by the addition of methanol. The reaction was quenched after 5 min by the addition of 1 N hydrochloric acid below −10˚C and the product was isolated by a usual workup.

The reaction product came out as per the expectation as evident from the thin layer of chromatography on the silica gel plate. The reaction product showed a chromatographically homogeneous single spot and the starting material was completely consumed. The reaction product was characterized by the distinctive peak corresponding to alcoholic methylene singlet in NMR as desired for com- pound **3** and the absence of carboxylic carbonyl in the ^{13}C NMR (see experimental).

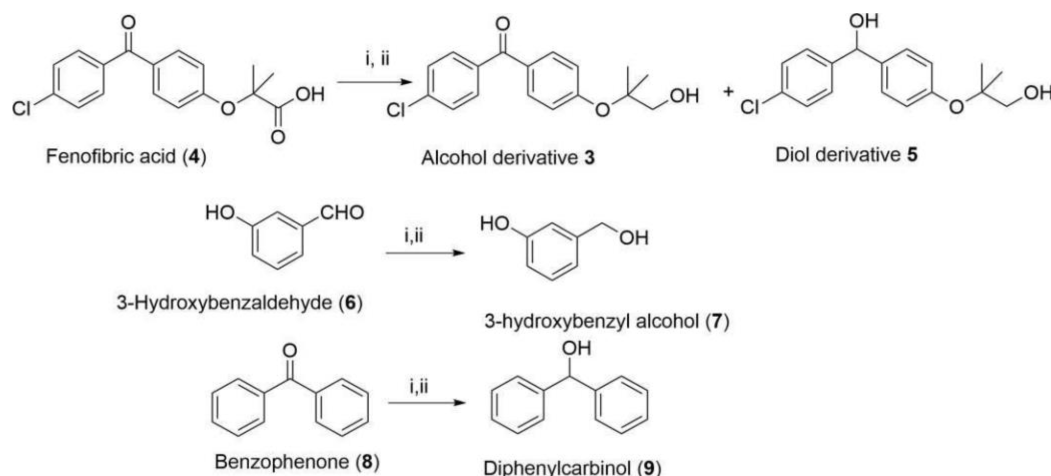


Figure 2. Reactions for optimization studies.

Reagents and Conditions

i) N-methyl morpholine and isobutyl chloroformate −15˚C, 5 min.

ii) Stoichiometric $NaBH_4$ in methanol −15˚C, 5 min.

The details of experimental conditions of the reaction optimization studies are summarized in **Figure 2** and **Table 1**. It is apparent from the results that activa- tion of the carboxylic group by the mixed anhydride followed by the reaction of sodium borohydride resulted in the formation of the desired alcohol 3 as the ex- clusive product. The stoichiometric excess of sodium borohydride has little im- pact as the

use of excess equivalents has an insignificant effect on the selectivity of the reaction and the best combination was the use of a 2 molar excess of so- dium borohydride (Entry-2).

The reaction time did not impact on selectivity of the reaction as no change was observed when the reaction was allowed to continue extra time with main- taining the temperature below −10˚C as shown in entries 5 and 6 in the optimiza- tion table. The reduced product corresponding to diol was not visible on the TLC plate but can be seen in NMR as one additional singlet at 5.89 ppm correspond- ing to reduced ketone. The quantities given in table for the % yield are based on the proton NMR of the crude products without purification and the values are drawn by the signal intensity of the signature peak of hydroxyl methylene pro- tons as singlet. It is important to note that the reaction temperature seems to play a significant role in maintaining selectivity. The formation of diol was only observed when the reaction was quenched at room temperature. It is clear from the data that the use of a 2 molar excess of sodium borohydride at −15˚C for 5 min is the most appropriate experimental condition for the selective reduction of carboxylic acid to the alcohol. Subsequently, we performed the sodium boro- hydride reduction of aldehyde and ketone containing compounds in order to validate the role of temperature on the reducing ability of sodium borohydride as shown in **Figure 2**. The reaction was performed at the optimized reaction condition viz. use of 2 molar excess of sodium borohydride at −15˚C for 5 mins.

Table 1. Optimization of reaction conditions.

Entry	Substrate 1 mM	Mole of $NaBH_4$ (mM)	Time (min)	Temperature (˚C)	(Derivative) % yield	Diol* % yield
1	**1**	1.1	5	−15 to −10	**(3)** 60	**(5)** 0
2	**1**	2.0	5	−15 to −10	**(3)** 100	**(5)** 0
3	**1**	4.0	5	−15 to −10	**(3)** 98	**(5)** Trace*
4	**1**	5.0	5	−15 to −10	**(3)** 98	**(5)** Trace*
5	**1**	2.0	10	−15 to −10	**(3)** 100	**(5)** 0
6	**1**	2.0	15	−15 to −10	**(3)** 100	**(5)** 0
7	**1**	2.0	15	−15 to −0	**(3)** 100	**(5)** 0
8	**1**	2.0	15	−15 to −0	**(3)** 95	**(5)** ~5*
9	**6**	2.0	5	−15 to −10	**(6)** 0	**(7)** 100
10	**8**	2.0	5	−15 to −10	**(8)** 60	**(9)** 40

*by NMR.

During these reactions, we have observed mixed results. The aldehyde **6** was completely reduced to corresponding alcohol **7** whereas the reduction of ketone **8** was incomplete and the conversion was around 50% only (Entries 9 & 10). The data of **Table 1** that temperature plays a significant role in selectivity but the reactivity of the mixed carbonic anhydride seems to play a decisive role in che- moselectivity.

Synthesis of (4-chlorophenyl)(4-((1-hydroxy-2-methylpropan-2-yl)oxy) phenyl) methanone (**3**) has recently been reported in a recent communication by chemoselective reduction of aldehyde in presence of a ketone [11]. Chemoselective reduction of aldehydes was reported with a combination of acetylacetone and $NaBH_4$ under mild conditions by a double selective reduction procedure. How- ever, in the present study, we have developed a highly efficient and direct route to alcohol **3** by the chemoselective reduction of carboxylic acid, more difficult to reduce, under mild conditions. The Chemoselective reduction of carboxylic acid in presence of a ketone has potential of wide application in the area of medicinal chemistry for the synthesis of complex molecules.

2. Experimental

The synthesis of **3** was carried out starting from the Fenofibrate obtained from Sigma USA. The Fenofibrate was converted the Fenofibric acid (**4**) by alkaline hydrolysis according to the method reported in the literature. Fenofibric acid (4) 318 mg (1 mmole) was taken in dry THF (8 ml) in an oven-dried, 50 mL round- bottomed flask with a magnetic stir-bar and moisture guard tube. The solution was kept under stirring at −15˚C in acetone: dry ice bath. To this chilled and stirred solution N-methyl morpholine, 0.11 ml (1.1 mmole) was added followed by the addition of isobutyl chloroformate 0.14 ml (1.1 mmole). The stirring was continued for 4 - 5 min maintaining the reaction temperature at −15˚C. After 5 minutes sodium borohydride 80 mg (2 mmole) was added to the reaction mix- ture followed by the dropwise addition of methanol (1 ml). The reaction was stirred maintaining the reaction temperature strictly at −15˚C for 5 minutes fol- lowed by the quenching of the reaction by the addition of 2 ml of 1N hydroch- loric acid to the reaction mixture. The reaction mixture was allowed to reach room temperature and the solvent was removed under reduced pressure. The re- sidue was taken in ethyl acetate 20 ml and washed with brine until neutral to pH. The organic layer was dried over anhydrous magnesium sulfate and filtered us- ing a Whatman #1 filter paper. The solvent was removed under reduced pressure to obtain the chromatographically homogeneous product as white solid and the spectral data was in agreement with the literature [11].

3. Synthesis of (4-Chlorophenyl)(4-((1-Hydroxy-2-Methylpropan-2-Yl) Oxy)Phenyl)Methanone (3)

The synthesis of **3** was carried out starting from the Fenofibrate obtained from Sigma USA. The Fenofibrate was converted to the Fenofibric acid by alkaline hydrolysis according to the method reported in the literature [34]. Fenofibric acid (**4**) 318 mg (1 mmole) was taken in dry THF (8 ml) in an oven-dried, 50 mL round-bottomed flask with a magnetic stir-bar and moisture guard tube. The solution was kept under stirring at −15˚C in acetone: dry ice bath. To this chilled and stirred solution N-methyl morpholine, 0.11 ml (1.1 mmole) was added fol- lowed by the addition of isobutyl chloroformate 0.14 ml (1.1 mmole). The stir- ring was continued for 4 - 5 min maintaining the reaction temperature at −15˚C. After 5 minutes Sodium Borohydride 80 mg (2 mmole) was added to the reac- tion mixture followed by the dropwise addition of methanol (1 ml). The reaction was stirred maintaining the reaction temperature strictly at −15˚C for 5 minutes followed by the quenching of the reaction by the addition of 2 ml of 1N hy- drochloric acid to the reaction mixture. The reaction mixture was allowed to reach room temperature and the solvent was removed under reduced pressure. The residue was taken in ethyl acetate 20 ml and washed with brine until neutral to pH. The organic layer was dried over anhydrous magnesium sulfate and fil- tered using a Whatman #1 filter paper. The solvent was removed under reduced pressure to obtain the chromatographically homogeneous product as white sol- id. Yield 285 mg, 94%. M.P. 137˚C - 138˚C, ^{1}H NMR (400 MHz, $CDCl_3$): δ =
7.75 - 7.71 (m, 4H), 7.45 (d, J = 8.6 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 3.63 (s, 2H), 1.37 (s, 6H). ^{13}C NMR: (100 MHz, $CDCl_3$) δ = 23.0, 59.20, 62.06, 70.58, 77.02, 77.33, 81.88, 122.32, 122.65, 128.59, 131.60, 131.25, 131.72, 131.99, 136.15, 138.57, 159.31, 194.48. IR spectrum (cm^{-1}): 2976, 1757, 1245, 1146, 901. Mass Calculated for $C_{17}H_{17}ClO_3$ $[M + H]^+$ = 305.7 Observed 305, $[M + Na]^+$ = 327.

For reaction optimization studies the reactions were carried out in the same manner as described above but the molar excess of sodium borohydride was taken and reaction time and reaction temperature was adjusted according to **Ta- ble 1**. The crude products were analyzed by TLC and NMR for the quantitation of signature peaks for the formation of compound **3** and compound **5** respec- tively.

4. Synthesis of 3-Hydroxy Benzyl Alcohol (7)

3-hydroxy benzaldehyde (**6**) 112 mg (1 mmole) was taken in dry THF (5 ml) in an oven-dried, 50 mL round-bottomed flask with magnetic stir-bar and moisture guard tube. The solution was kept under stirring at −15˚C in acetone: dry ice bath. To this chilled and stirred solution Sodium Borohydride 80 mg (2 mmole) was added to the reaction mixture followed by the dropwise addition of metha- nol (1 ml). The reaction was stirred maintaining the reaction temperature strict- ly at −15˚C for 5 minutes followed by the quenching of the reaction by the addi- tion of 2 ml of 1N

hydrochloric acid to the reaction mixture. The reaction mix- ture was allowed to reach room temperature and the solvent was removed under reduced pressure. The residue was taken in ethyl acetate 20 ml and washed with brine until neutral to pH. The organic layer dried over anhydrous magnesium sulfate and filtered using a Whatman #1 filter paper. The solvent was removed under reduced pressure to obtain the chromatographically homogeneous prod- uct gummy solid. The TLC showed complete conversion of the compound **6** to the reduce product **7** which was obtained as exclusive product. 120 mg, 97%; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.25 (t, 2H, J = 7.7 Hz), 6.90 (t, 1H J = 7.7 Hz), 6.76
(m, 1H), 4.65 (s, 2H). ^{13}C NMR: (100 MHz, $CDCl_3$) δ = 155.83, 142.68, 129.87, 119.16, 114.59, 113.74, 77.32, 77.01, 76.69, 65.07. Mass Calculated for $C_7H_8O_2$ 124.14, Observed $[M + H]^+$ = 125.2, 107.

5. Synthesis of Diphenyl Carbinol (9)

Benzophenone (**8**) 182 mg (1 mmole) was taken in dry THF (5 ml) in an oven- dried, 50 mL round bottomed flask with magnetic stir-bar and moisture guard tube. The solution was kept under stirring at −15˚C in acetone: dry ice bath. To this chilled and stirred solution Sodium Borohydride 80 mg (2 mmole) was added to the reaction mixture followed by the dropwise addition of methanol (1 ml).
The reaction was stirred maintaining the reaction temperature strictly at −15˚C for 5 minutes followed by the quenching of the reaction by the addition of 2 ml of 1N hydrochloric acid to the reaction mixture. The reaction mixture was al- lowed to reach room temperature and the solvent was removed under reduced pressure. The residue was taken in ethyl acetate 20 ml and washed with brine until neutral to pH. The organic layer dried over anhydrous magnesium sulfate and filtered using a Whatman #1 filter paper. The solvent was removed under reduced pressure to obtain the chromatographically homogeneous product gum- my matter solid. The TLC showed partial conversion of compound **8** to the re- duced product **9** as evident from the thin layer chromatography and the signa- ture peaks in the proton NMR. 73 mg, 40%. ^{1}H NMR (400 MHz, $CDCl_3$): δ =

7.55 - 7.20 (m, 10H Aromatic protons), 5.84 (s, 2H, hydroxyl methylene). ^{13}C NMR: (100 MHz, $CDCl_3$) δ = 143.75, 128.50, 122.57, 126.51, 77.33, 77.01, 76.69. 76.25. Mass Calculated for $C_{13}H_{12}O$ 184.09, Observed $[M + H]^+$ = 185.

The synthesis of the similar compound exists, however, the compound is pre- pared from Fenofibrate by the hydrolysis of the isopropyl group followed by the reduction of both ketonic group as well as carboxylic group by BH_3 in THF fol- lowed by the oxidation by Dess-Martin but no selectivity was reported.

In summary, we have developed a facile and highly chemoselective direct re- duction of carboxylic acid to alcohol in presence of ketone. The reaction is car- ried out using easily accessible and cost effective reagents under extremely mild

conditions. The reduction is complete within five minutes in excellent yield and purity. The facile and chemoselective reduction is attributed to the high reactiv- ity of the carbonic anhydride at a low temperature. This study is the first report for the direct reduction of a carboxylic acid to corresponding alcohol in presence of a ketone group. The study offers a new chemoselective protocol for the or- thogonal and chemoselective reduction useful for the development of new syn- thetic methodologies.

Acknowledgements

This work is supported by NIDDK of National Institute of Health Grant. The authors are thankful to Ray Hoff for technical assistance.

Conflicts of Interest

The authors declare no competing financial interest.

References

[1] Brown, H.C. and Krishnamurthy, S. (1979) Forty Years of Hydride Reductions. *Tetrahedron*, **35**, 567-607. https://doi.org/10.1016/0040-4020(79)87003-9

[2] Sasano, Y., Kogure, N., Agasawa, S., Nagasawa, S., Kasabata, K. and Iwabuchi, Y. (2018) 2-Azaadamantane N-oxyl (AZADO)/Cu Catalysis Enables Chemoselective Aerobic Oxidation of Alcohols Containing Electron-Rich Divalent Sulfur Functionalities.
Organic Letters, **20**, 6104-6107. https://doi.org/10.1021/acs.orglett.8b02528

[3] Dyer, R.M.B., Hahn, P.L. and Hilinski, M.K. (2018) Selective Heteroaryl N-Oxidation of Amine-Containing Molecules. *Organic Letters*, **20**, 2011-2014. https://doi.org/10.1021/acs.orglett.8b00558

[4] Shenvi, R.A., O'Malley, D.P. and Baran, P.S. (2009) Chemoselectivity: The Mother of Invention in Total Synthesis. *Accounts of Chemical Research*, **42**, 530-541. https://doi.org/10.1021/ar800182r

[5] Trost, B.M. and Salzmann, T.N. (1973) New Synthetic Reactions. Sulfenylation-Dehydrosulfenylation as a Method for Introduction of Unsaturation. *Journal of the American Chemical Society*, **95**, 6840-6842. https://doi.org/10.1021/ja00801a058

[6] Trost, B.M. (1983) Selectivity: A Key to Synthetic Efficiency. *Science*, **219**, 245-250. https://doi.org/10.1126/science.219.4582.245

[7] Yahata, K., Minami, M., Watanabe, K. and Fujioka, H. (2014) Selective Transformations of Carbonyl Functions in the Presence of α,β-Unsaturated Ketones: Concise Asymmetric Total Synthesis of Decytospolides A and B. *Organic Letters*, **16**, 3680-3683. https://doi.org/10.1021/ol501463p

[8] Kim, J.Y., Shin, W.K., Jaladi, A.K. and An, D.K. (2018) Chemoselective Reduction of Aldehydes and Ketones by Potassium Diisobutyl-*t*-Butoxy Aluminum Hydride (PDBBA). *Tetrahedron*, **74**, 4236-4241. https://doi.org/10.1016/j.tet.2018.06.048

[9] Osako, T., Torii, K., Hirata, S. and Uozumi, Y. (2017) Chemoselective Continuous-Flow Hydrogenation of Aldehydes Catalyzed by Platinum Nanoparticles Dispersed in

an Amphiphilic Resin. *ACS Catalysis*, **7**, 7371-7377. https://doi.org/10.1021/acscatal.7b02604

[10] Gribble, G.W. (1998) Sodium Borohydride in Carboxylic Acid Media: A Phenomenal Reduction System. *Chemical Society Reviews*, **27**, 395-404. https://doi.org/10.1039/a827395z

[11] Sui, G., Lv, Q., Song, X., Guo, H., Dai, J., Ren, L., Lee, C.-S., Zhou, W. and Hao, H.-D. (2019) Chemoselective Reduction of Aldehydes via a Combination of $NaBH_4$ and Acetylacetone. *New Journal of Chemistry*, **43**, 15793-15796. https://doi.org/10.1039/C9NJ03210J

[12] Kokotos, G. (1990) A Convenient One-Pot Conversion of N-Protected Amino Ac- ids and Peptides into Alcohols. *Synthesis*, **299**, 299-301. https://doi.org/10.1055/s-1990-26857

[13] Rodriguez, M., Llinaero, M., Doulet, S., Heitz, A. and Martinez, J. (1991) A Facile Synthesis of Chiral N-Protected β-Amino Alcohols. *Tetrahedron Letters*, **32**, 923-926. https://doi.org/10.1016/S0040-4039(00)92121-X

[14] Bandgar, B.P., Modhave, R.K., Wadgaonkar, P.P. and Sande, A.R. (1996) Selective Reduction of Mixed Anhydrides of Carboxylic Acids to Alcohols Using Borohydride Exchange Resin (BER)-Nickel Acetate. *Journal of the Chemical Society*, *Perkin Transac- tions* 1, **1**, 1993-1994. https://doi.org/10.1039/P19960001993

[15] Soai, K., Yokoyama, S. and Mochida, K. (1987) Reduction of Symmetric and Mixed Anhydrides of Carboxylic Acids by Sodium Borohydride with Dropwise Addition of Methanol. *Synthesis*, **1987**, 647-648. https://doi.org/10.1055/s-1987-28035

[16] Naqvi, T., Bhattacharya, M. and Haq, W. (1999) An Efficient Method for the Reduction of *N*-Protected Amino Acids and Peptides to the Corresponding Alcohols. *Journal of Chemical Research*, *Synopese*, **7**, 424-425. https://doi.org/10.1039/a902069a

[17] Papavassilopoulou, E., Christofis, P., Terzoglou, D. and Moutevelis-Minakakis, P. (2007) Reduction of Pentafluorophenyl Esters to the Corresponding Primary Alco- hols Using Sodium Borohydride. *Tetrahedron Letters*, **48**, 8323-8325. https://doi.org/10.1016/j.tetlet.2007.09.124

[18] Tale, R.H., Patil, K.M. and Dapurkar, S.E. (2003) An Extremely Simple, Convenient and Mild One-Pot Reduction of Carboxylic Acids to Alcohols Using 3,4,5-Trifluorophenylboronic Acid and Sodium Borohydride. *Tetrahedron Letters*, **44**, 3427-3428. https://doi.org/10.1016/S0040-4039(03)00035-2

[19] Srivastava, T., Srivastava, T.K., Haq, W. and Katti, S.B.J. (2003) A Facile and Simple Method for the Reduction of N-Protected Amino Acids and Peptides to the Corresponding Alcohols. *Journal of Chemical Research*, **8**, 516-517. https://doi.org/10.3184/030823403103174777

[20] Falorni, M., Giacomelli, G., Porcheddu, A. and Taddei, M.A. (1999) A Simple Me- thod for the Reduction of Carboxylic Acids to Aldehydes or Alcohols Using H_2 and Pd/C. *The Journal of Organic Chemistry*, **64**, 8962-89642. https://doi.org/10.1021/jo991112f

[21] Bhaskar Kanth, J.V. and Periasamy, M. (1991) Selective Reduction of Carboxylic Acids into Alcohols Using Sodium Borohydride and Iodine. *The Journal of Organic Chemistry*, **56**, 5964-5965. https://doi.org/10.1021/jo00020a052

[22] Morales-Serna, J.A., García-Ríos, E., Bernal, J., Paleo, E., Gaviño, R. and Cárdenas, J. (2011) Reduction of Carboxylic Acids Using Esters of Benzotriazole as High-Reactivity Intermediates. *Synthesis*, **9**, 1375-1382. https://doi.org/10.1055/s-0030-1259988

[23] Addis, D., Das, S., Junge, K. and Beller, M. (2011) Selective Reduction of Carboxylic Acid Derivatives by Catalytic Hydrosilylation. *Angewandte Chemie International Edition*, **50**, 6004-6011. https://doi.org/10.1002/anie.201100145

[24] Pandey, P. and Bera, J.K. (2021) Hydrosilylative Reduction of Primary Amides to Primary Amines Catalyzed by a Terminal [Ni-OH] Complex. *Chemical Communications*, **57**, 9204-9207. https://doi.org/10.1039/D1CC03537A

[25] Wang, J., Ju, M.-J., Wang, X., Ma, Y.-N., Wei, D. and Chen, X. (2021) Hydrobora- tion Reaction and Mechanism of Carboxylic Acids using $NaNH_2(BH_3)_2$, a Hydro- boration Reagent with Reducing Capability between $NaBH_4$ and $LiAlH_4$. *The Jour- nal of Organic Chemistry*, **86**, 5305-5316. https://doi.org/10.1021/acs.joc.1c00302

[26] Kotheimer, A. (2010) Design, Synthesis and Characterization of Novel Chiral Inhibitors for Aldose Reductase. MSc, Youngstown State University, Youngstown.

[27] Kotheimer, A.E., Haq, W. and Balendiran, G.K. (2018) Synthesis and Chiral Separation of Fibratol, Isopropyl 2-(4-((4-chlorophenyl)(hydroxyl)methyl)-phenoxy)-2-methylpropanoate. *International Journal of Organic Chemistry*, **8**, 201-206. https://doi.org/10.4236/ijoc.2018.82015

[28] Kotheimer, A.E., Haq, W. and Balendiran, G.K. (2019) Fibrane the Reduced Derivative of Fenofibrate. *Results in Chemistry*, **2**, Article ID: 100018. https://doi.org/10.1016/j.rechem.2019.100018

[29] Balendiran, G.K. and Balakrishnan, R. (2004) In Search of Aldose Reductase Inhibitors. Purdue University Press, West Lafayette, Vol. 12, 276-283.

[30] Balendiran, G.K. and Rajkumar, B. (2005) Fibrates Inhibit Aldose Reductase Activi- ty in the Forward and Reverse Reactions. *Biochemical Pharmacology*, **70**, 1653-1663. https://doi.org/10.1016/j.bcp.2005.06.029

[31] Klemin, S., Calvo, R.Y., Bond, S., Dingess, H., Rajkumar, B., Perez, R., Chow, L. and Balendiran, G.K. (2006) WY 14,643 Inhibits Human Aldose Reductase Activity. *Journal of Enzyme Inhibition and Medicinal Chemistry*, **21**, 569-753. https://doi.org/10.1080/14756360600720887

[32] Balendiran, G.K., Verma, M. and Perry, E. (2007) Chemistory of Fibrates. *Current Chemical Biology*, **1**, 311-316. https://doi.org/10.2174/2212796810701030311

[33] Sawaya, M.R., Verma, M., Balendiran, V., Rath, N.P., Cascio, D. and Balendiran, G.K. (2016) Characterization of WY 14,643 and Its Complex with Aldose Reductase. *Scientific Reports*, **6**, Article No. 34394. https://doi.org/10.1038/srep34394

[34] Rath, N.P., Haq, W. and Balendiran, G.K. (2005) Fenofibric Acid. *Acta Crystallographica Section C*, **61**, o81-o84. https://doi.org/10.1107/S0108270104032573

Results in Chemistry 4 (2022) 100417

Contents lists available at ScienceDirect

Results in Chemistry

journal homepage: www.sciencedirect.com/journal/results-in-chemistry

A facile and chemoselectivity in synthesis of 4-chloro-*N*-(4-((1-hydroxy-2-methylpropan-2-yl)oxy)phenethyl) benzamide, the alcohol derivative of Bezafibrate

Greesha N. Majethia , Wahajul Haq , Ganesaratnam K. Balendiran *

Department of Chemistry, Youngstown State University, One University Plaza, Youngstown, OH, USA

ARTICLEINFO

Keywords:
Bezafibrate
Chemoselectivity
Reduction of carboxylic acid
Sodium borohydride
Mixed anhydride

ABSTRACT

A facile method for the reduction of carboxylic acid group of Bezafibrate, an approved drug, is described. The selective reduction of carboxylic acid group to corresponding alcohol was carried out by activation of the car boxylic acid moiety via mixed anhydride followed by the addition of stoichiometric amount of $NaBH_4$ and

methanol to obtain the first alcohol variant of Bezafibrate. The reaction was completed in 5 −10 min in excellen yield and purity. The new alcohol derivative was characterized by spectroscopic methods. This is the first repor on this new molecule.

-

t
t

Introduction

Bezafibrate, a member of fibrate class of compounds, is in clinical use as an anti-diabetes agent and for lowering lipid levels. It is believed that fibrates are agonist of peroxisome proliferator-activated receptors (PPARs) [1,2]. In addition, molecular action of fibrates includes, aldo–keto reductase family of proteins, aldose reductase and AKR1B10 targets [3–6]. Though members of fibrate class share noticeable physi- ological and clinical outcomes their chemical scaffold contains signifi- cantly divergent fragment(s). Though most of the fibrates contain several fragments that are same, amide group is present only in Beza- fibrate. Moreover, Bezafibrate is clinically administrated as carboxylic acid derivative rather than an ester. Therefore, Bezafibrate may occur in different ionic states in the initial stage soon after its administration because the pH is 4–7 in small intestine, and it is 7.3–7.45 in blood. This causes Bezafibrate to have a shorter half-life in biophase and excreted by kidney rapidly. Here we present the synthesis of Bezol, a new structural variant of Bezafibrate, 4-chloro-*N*-(4-((1-hydroxy-2-methylpropan-2-yl) oxy)phenethyl)benzamide (**I**) Fig. 1.

Materials and method

The required reagents and solvents were procured from Sigma-Aldrich and used without further purification. The reaction of the carboxylic group reduction was carried out at —15 °C in acetone dry ice bath. The ^{1}H NMR and ^{13}C NMR were recorded on a Bruker Advance II 400 MHz NMR spectrometer with an indirect detection probe. Chemical shifts were reported in parts per million (ppm) from a standard of tetramethylsilane (TMS) in $CDCl_3$ (0.1% w/v TMS) and coupling constants (J) are reported in Hertz. The mass spectrum was recorded on Esquire-LC_00135 spectrometer and Infrared spectrum was taken on a Thermo Electron Corporation IR 200 spectrophotometer and analyzed using EZ-OMNIC software. Melting point is recorded on Stuart-SMP10 melting point apparatus and reported uncorrected.

The synthesis of compound **I** was carried out starting from the Bezafibrate obtained from Sigma-Aldrich chemical company, USA. A solution of Bezafibrate 1.5 gm (4.3 mmol) in dry THF (15 ml) was placed in a flame-dried, round-bottomed flask fitted with magnetic stirring bar and moisture guard tube. The reaction mixture was kept under stirring at — 15 ◦C in acetone: dry ice bath. To this chilled and stirred solution N-methyl morpholine (0.5 ml, 5 mmol) was added followed by the addition of isobutyl chloroformate (0.7 ml, 5 mmol). The stirring was continued for 4–5 min and the reaction temperature was maintained at —15 ◦C. After 5 min, solid sodium borohydride (0.35 g, 9 mmole) was added to the reaction mixture followed by the dropwise addition of methanol (2 ml). The reaction was stirred maintaining the reaction temperature strictly at — 15 ◦C for additional 5 min followed by the quenching of the reaction by the addition of 2 ml of 1 M hydrochloric acid to the reaction mixture. The reaction mixture was allowed to reach room temperature and the solvent was removed under reduced pressure. The residue was

* Corresponding author at: Department of Chemistry, Youngstown State University, One University Plaza, Youngstown, OH, USA. *E-mail addresses:* pl_note@yahoo.com, gkbalendiran@ysu.edu (G.K. Balendiran).

https://doi.org/10.1016/j.rechem.2022.100417
Received 25 April 2022; Accepted 26 June 2022 Available online 29 June 2022
2211-7156/© 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

taken in ethyl acetate (35 ml) and washed with brine until neutral to pH. The organic layer was dried over anhydrous magnesium sulfate and filtered using a filter paper. The filtrate was concentrated under reduced pressure to obtain chromatographically homogeneous white solid product. Yield 1.37 gm, 91 %. M.P. 120–121 ◦C, ^{1}H NMR: δ = ^{1}H NMR (400 MHz $CDCl_3$): δ 1.49 (s, 6H, isopropyl), 2.88 (t, 2H J = 7.0 Hz, methylene-tyramine), 3.75 (s, 2H, Hydroxy methylene), 3.47 (q, 2H, J = 6.7 Hz, NH-methylene), 6.12 (bs, 1H, NH proton), 6.7 (d, 2H, Ar J = 8.10 Hz, Ar-Tyramine), 6.92 (d, 2H Ar, J = 8.00 Hz Ar-Tyramine), 7.5 (d, 2H Ar J = 8.10 Hz), 7.82 (d, 2H Ar J = 8.10 Hz). ^{13}C NMR: (100 MHz, $CDCl_3$) δ = 34.88, 41.02, 41.29, 50.88, 81.54, 121.55, 128.25, 128.84, 129.54, 132.95, 133.57, 137.70, 152.99, 166.42, 174.71. ESI Mass Calculated for $C_{19}H_{22}ClNO_3$ = 347.13, $[M + Na]^+$ = 370.1 observed.

Results and discussions

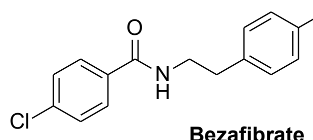
The reduction of carboxylic group of Bezafibrate is not straightforward due to the presence of amide carbonyl moiety in the molecule. However, new methodology is being developed for high chemo-selectivity reduction of amides [7]. In addition the direct reduction of the carboxylic acid can be done by the use of strong and sophisticated reagents, to name a few, reagents with appropriate catalyst [8], by using Vitride reagent [9], by catalytic hydrogenation [10]. Another successful approach comprises the reduction after the activation of carboxylic acid to its corresponding alcohol using easily accessible and bench stable catalyst like sodium borohydride under mild experimental conditions. The activation of the carboxylic group can be achieved prior to reduction or reduction is performed by *in situ* activation of carboxylic acid [11–14]. Recent reports indicate that the catalytic hydrogenation require specific catalyst otherwise uncontrolled side reactions will be generated [15,16].

Synthesis of 4-chloro-N-(4-((1-hydroxy-2-methylpropan-2-yl)oxy) phenethyl)benzamide (I)

In the present study we have developed a facile method for the reduction of Bezafibrate via activating the carboxylic group by mixed anhydride followed by the reductions of the activated mixed anhydride using sodium borohydride and methanol as a reducing agent under mild condition. The choice on the use of the mixed anhydride is based on the consideration of the selective —15 ◦C reduction of carbonyl in the Bezafibrate molecule. The mixed anhydride/borohydride/methanol

system is greener approach as it does not create any solid byproducts and does not require tedious purification procedures. The reduction is very facile and completed in few minutes in almost quantitative yield as summarized in Scheme 1.

The procedure shown in the Scheme 1 utilizes very commonly available reagents for the development of an exceptionally facile, expeditious and cost effective method for the preparation of compound **I** in high yield and purity. The mixed anhydride is generated *in situ* by reaction of Bezafibrate with isobutyl chloroformate and N-methyl morpholine at —15 ◦C. After five minutes 2 M equivalent of sodium borohydride was added maintaining the temperature around—15 ◦C followed by the addition of methanol to the reaction mixture. After the addition of methanol, the cooling bath is removed and allowed to come



to the room temperature. The reaction mixture was quenched by the addition of 1 M hydrochloric acid and followed by usual work up resulted in the formation compound-**I** as chromatographically homo- geneous glassy solid. The reduced product was characterized by spec- troscopic methods as 4-chloro-*N*-(4-((1-hydroxy-2-methylpropan-2-yl) oxy)phenethyl)benzamide in excellent yield and purity without chromatographic purification. Additional singlet presence at 3.58 ppm in ^{1}H NMR corresponding to hydroxy methylene protons and absence of carboxylic acid carbonyl region between 180 and 185 ppm in ^{13}C NMR confirms the formation of compound (I).

Strategy implemented in this study emerges from "Late-Stage Diversification" concept that is known for repurposing molecules and natural products efficiently for drug development [17,18]. This notion has also been successful in the discovery of many drugs which include: 1) New antibiotics that are needed to meet the challenges of acquired bacterial resistance [19,20]. 2) Advanced macrolide derivatives of a highly potent Mechanistic target of rapamycin (mTOR) inhibitor and immunosuppressive drug, Rapamycin, produced and have received clinical approval due to improved therapeutic potential [21,22]. Such functionalization has resulted in the identification of novel rapalogs, Temsirolimus and Everolimus as new drugs for treatment [21,22]. The potency of Rapamycin has significantly improved by attaching additional hydroxyl groups which enhance the hydrophilicity of the highly hydrophobic Rapamycin. 3) Discovery of octreotide, a highly potent derivative of Sandostatin obtained by the conversion of C-terminal carboxylic group of threonine to corresponding alcohol [23] was developed by similar approach. 4) An alpha-glucosidase inhibitor, Voglibose is used clinically to lower post-prandial blood glucose levels in patients with Diabetes Mellitus. 5) Luseogliflozin, Sodium-glucose Cotransporter-2 (SGLT2) inhibitor has been used in clinical trials for the treatment of Diabetes Melltius, Type 2.

As reflected by growing number of drugs that contain hydroxyl fragment and are in clinical use or trials, combining hydroxyl group has been beneficial for the improvement of biological activity of hydrophobic molecules. In view of the above prospective compound **I** is designed as a new Bezafibrate derivative with reduced lipophilicity as well eliminated the acidic character of the molecule. Synthesis of newly designed compound takes advantage of late-stage derivatization strategy to obtain drug like molecule with minimal structural alteration from the parent molecule. The alcohol derivative of the Bezafibrate may have interesting biochemical properties because of its neutral character.

Conclusion

In summary, a facile method for the selective reduction of carboxylic acid group of Bezafibrate was developed. The reduction of carboxylic acid to corresponding alcohol was selectively carried out by activation of carboxylic acid via mixed anhydride followed by the addition of stoichiometric amount of sodium borohydride and methanol to obtain the new alcohol variant of Bezafibrate without any changes to the amide carbonyl group. The reaction was completed in 5–10 min in excellent yield and purity as evident from the spectroscopic data of the compound. The new alcohol derivative is an example of late-stage derivatization for obtaining novel therapeutically useful molecules. The new alcohol variant is expected to exert interesting chemical and biological

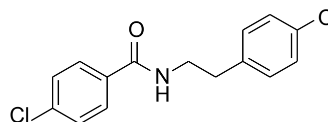
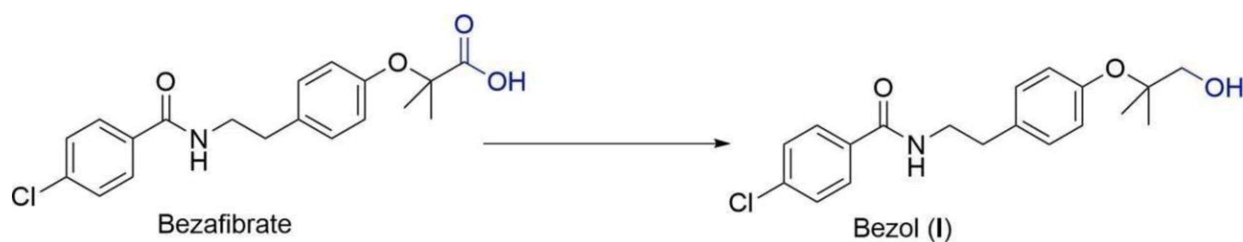


Fig. 1. Chemical structure of Bezafibrate ($C_{19}H_{20}ClNO_4$) and its reduced derivative, 4-chloro-*N*-(4-((1-hydroxy-2-methylpropan-2-yl)oxy)phenethyl)benzamide (**I**) ($C_{19}H_{22}ClNO_3$).



Scheme 1. Synthesis of 4-chloro-*N*-(4-((1-hydroxy-2-methylpropan-2-yl)oxy)phenethyl) benzamide (**I**).

properties that are different from parent compound.

CRediT authorship contribution statement

Greesha N. Majethia: Conceptualization, Methodology, Investigation, Resources, Data curation. **Wahajul Haq:** Conceptualization, Methodology, Investigation, Resources, Data curation. **Ganesaratnam K. Balendiran:** Conceptualization, Methodology, Investigation, Resources, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors gratefully acknowledge the financial support from NIDDK of National Institute of Health for the research grant.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rechem.2022.100417.

References

[1] I. Issemann, S. Green, Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators, Nature 347 (1990) 645–650, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db =PubMed&dopt =Citation&list_uids =2129546.

[2] G.K. Balendiran, M. Verma, E. Perry, Chemistory of fibrates, Curr. Chem. Biol. 1 (2007) 311–316, https://doi.org/10.2174/2212796810701030311.

[3] G.K. Balendiran, R. Balakrishnan, In search of aldose reductase inhibitors, Vol. 12, Purdue University Press, West Lafayette, IN, 2004/06.. ISBN 9781557533845.

[4] G.K. Balendiran, B. Rajkumar, Fibrates inhibit aldose reductase activity in the forward and reverse reactions, Biochem. Pharmacol. 70 (2005) 1653–1663, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db =PubMed&dopt =Citation&list_uids =16226225.

[5] M. Verma, H.-J. Martin, W. Haq, T.R. O'Connor, E. Maser, G.K. Balendiran, Inhibiting wild-type and C299S mutant AKR1B10; a homologue of aldose reductase upregulated in cancers, Eur. J. Pharmacol. 584 (2008) 213–221, https://doi.org/10.1016/j.ejphar.2008.01.036.

[6] G.K. Balendiran, H.J. Martin, Y. El-Hawari, E. Maser, Cancer biomarker AKR1B10 and carbonyl metabolism. Chem. Biol. Interact., 2009; 178: 134 137. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=19028477.

[7] M. Szostak, M. Spain, A.J. Eberhart, D.J. Procter, Highly chemoselective reduction of amides (primary, secondary, tertiary) to alcohols using SmI2/amine/H2O under mild conditions, JACS 136 (2014) 2268–2271, https://doi.org/10.1021/ ja412578t.

[8] A. Harinath, J. Bhattacharjee, T.K. Panda, Facile reduction of carboxylic acids to primary alcohols under catalyst-free and solvent-free conditions, Chem. Commun. 55 (2019) 1386–1389, https://doi.org/10.1039/c8cc08841a.

[9] P. Juszczyk, R. Kasprzykowska, A.S. Kolodziejczyk, Simple and efficient synthesis of chiral amino alcohols with an amino acid-based skeleton, Lett. Pept. Sci. 10 (2004) 79–82, https://doi.org/10.1023/b:lips.0000032366.28662.1d.

[10] J. Ullrich, B. Breit, Selective hydrogenation of carboxylic acids to alcohols or alkanes employing a heterogeneous catalyst, ACS Catalysis 8 (2018) 785–789, https://doi.org/10.1021/acscatal.7b03484.

[11] K. Soai, S. Yokoyama, K. Mochida, Reduction of symmetric and mixed anhydrides of carboxylic acids by sodium borohydride with dropwise addition of methanol, Synthesis 1987 (07) (1987) 647–648.

[12] J.A. Morales-Serna, E. García-Ríos, J. Bernal, E. Paleo, R. Gavi˜no, J. C´ardenas, Reduction of carboxylic acids using esters of benzotriazole as high-reactivity intermediates, Synthesis 9 (2011) 1375–1382, https://doi.org/10.1055/s-0030- 1259988.

[13] T. Naqvi, M. Bhattacharya, W. Haq, Efficient method for the reduction of N- protected amino acids and peptides to the corresponding alcohols. J. Chem. Res. (S) 1999: 424–425. https://doi.org/10.1039/a902069a.

[14] R.H. Tale, K.M. Patil, S.E. Dapurkar, An extremely simple, convenient and mild one-pot reduction of carboxylic acids to alcohols using 3,4,5-trifluorophenylboronic acid and sodium borohydride, Tetrahedron Lett. 44 (2003) 3427–3428, https://doi.org/10.1016/S0040-4039(03)00035-2.

[15] E. Antico, P. Schlichter, C. Werle, W. Leitner, Reduction of carboxylic acids to alcohols via manganese(I) catalyzed hydrosilylation, JACS Au 1 (2021) 742–749, https://doi.org/10.1021/jacsau.1c00140.

[16] B. Groemer, S. Yoshioka, S. Saito, Selective reduction of carboxylic acids to alcohols in the presence of alcohols by a dual bulky transition-metal complex/Lewis acid catalyst, ACS Catal. 12 (2022) 1957–1964, https://doi.org/10.1021/acscatal.1c04392.

[17] S. G¨or¨og, The changing face of chemical derivatization in pharmaceutical and biomedical analysis, Fresenius' J. Anal. Chem. 362 (1) (1998) 4–8.

[18] B.L. Tekwani, L.A. Walker, 8-Aminoquinolines: future role as antiprotozoal drugs, Curr. Opin. Inf. Dis. 19 (2006) 623–631, https://doi.org/10.1097/ qco.0b013e328010b848.

[19] B. Hong, T. Luo, X. Lei, Late-stage diversification of natural products, ACS Central Sci. 6 (2020) 622–635, https://doi.org/10.1021/acscentsci.9b00916.

[20] V.M. D'Costa, K.M. McGrann, D.W. Hughes, G.D. Wright, Sampling the antibiotic resistome, Science 311 (2006) 374–377, https://doi.org/10.1126/ science.1120800.

[21] Martens UM, Small Molecules. Third ed.; Heilbronn, Baden-Württemberg, Germany; Vol. 211, 2018.

[22] L.-H. Meng, X.F.S. Zheng, Toward rapamycin analog (rapalog)-based precision cancer therapy, Acta Pharmacol. Sin. 36 (2015) 1163–1169, https://doi.org/10.1038/aps.2015.68.

[23] X.X. Wang, T. Ye, M. Li, X. Li, O. Qiang, C.W. Tang, R. Liu, Effects of octreotide on hepatic glycogenesis in rats with high fat diet-induced obesity, Mol. Med. Rep. 16 (2017) 109–118, https://doi.org/10.3892/mmr.2017.6586.

–