Synthesis, Antibacterial, Antioxidant, and Molecular Docking of Tetrahydro-β-Carboline Derivatives

Nigatu Bejigo Aboye

A Thesis Submitted to

Department of Applied Chemistry

School of Applied Natural Sciences

Presented in Partial Fulfillment of the Requirement for the Degree of Master's in Chemistry (Synthetic Organic Chemistry)

Office of Graduate Studies

Adama Science and Technology University

May, 2021

Adama, Ethiopia

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Co-Advisor: Dr. Milkyas Endale

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Approval of Board of Examiners

We, the advisors of the thesis entitled "**Synthesis, Antibacterial, Antioxidant, and Molecular Docking of Tetrahydro-*β*-Carboline Derivatives**" and developed by Nigatu Bejigo Abuye, hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

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We, the undersigned, members of the Board of Examiners of the thesis by Nigatu Bejigo Abuye have read and evaluated the thesis entitled "**Synthesis, Antibacterial, Antioxidant, and Molecular Docking of Tetrahydro-*β*-Carboline Derivatives**" and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Synthetic Organic Chemistry.

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DECLARATION

I hereby declare that this thesis entitles "**Synthesis, Antibacterial, Antioxidant, and Molecular Docking of Tetrahydro-*β*-Carboline Derivatives**" submitted to Adama Science and Technology University for the award of degree of Masters of Science in Synthetic Organic Chemistry is the result of work carried out under the guidance of Dr. Yadessa Melaku and Dr. Milkyas Endale. This thesis is my original work and has not been presented for a degree or diploma in any other University. All sources or materials used for this thesis have been duly acknowledged.

Nigatu Bejigo

Signature: _____

APPROVAL SHEET

This is to certify that the thesis entitled "**Synthesis, Antibacterial, Antioxidant, and Molecular Docking of Tetrahydro-*β*-Carboline Derivatives**" submitted in partial fulfillment of the requirements for the degree of Masters of Science in Applied Chemistry with specialization in Synthetic Organic Chemistry School of Applied Natural Science MSc program of Department of Applied Chemistry, Adama Science and Technology University and is a record of original research carried out by Nigatu Bejigo (ID No. PGR/18249/11) under our supervision, and no part of the thesis has been submitted for any other degree or diploma in any other University.

Name of major Advisor	Signature	Date
Name of Co-advisor	Signature	Date

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LIST OF ACRONYMS AND ABBERVIATIONS

AAPH	2,2'-Azobis(2-amidinopropane) dihydrochloride
d	doublet
dd	doublet of doublet
DEPT	Distortionless Enhancement by Polarization Transfer
DH*β*C	Dihydro *beta* carboline
DMSO	Dimethyl Sulfoxide
DPPH	1,1-diphenyl-2-picryl-hydrazyl
FC	Friedel-Crafts
FIS	Fischer indole synthesis
Hz	Hertz
m	Multiplate
MHA	Muller Hinton agar
MW	Micro-wave
NCS	Nocroclaurine synthase
NMR	Nuclear magnetic resonance
ppm	Parts per million
PSR	Pictet-Spengler reaction
R_f	Retention factor
ROS	Reactive Oxygen Species
s	Singlet
SAR	Structure activity relationship
STRs	Strictosidine synthases
t	Triplet
TFA	Trifluoracetic acid
TFA	Trifluoroacetic acid
THBC	Tetrahydro-*beta*-carboline
TH*β*C	Tetrahydro-*beta*-carboline
TLC	Thin layer chromatography
UV-vis	Ultraviolet-visible
*β*c	*Beta* carboline

ABSTRACT

1,2,3,4-Tetrahydro-β-carbolines (THβCs) are a large group of natural and synthetic indole alkaloids that possess a common tricyclic pyrido[3,4-b] indole scaffold with wide spectrum of biological activities including antibacterial, antimalarial, antifungal, antioxidant, anticancer, anti-inflammatory and anti-leishmanial activities. In this study, four tetrahydro-β-carboline derivatives were prepared from tryptophan and aromatic or aliphatic aldehydes using Pictet-Spengler reaction catalyzed by AcOH and TFA. Structure of these compounds were determined using spectroscopic methods UV-Vis and NMR (^{1}H-NMR, ^{13}C-NMR, and DEPT-135-NMR). The antibacterial activity of the synthesized compounds was screened against two Gram-positive bacterias (Staphylococcus pyogenes and Staphylococcus aureus) and two Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa) using Agar disc diffusion. Results showed that all the studied compounds displayed good antibacterial activity compared to ciprofloxacin which was used as a positive control. The best activity was displayed by compound ***142c*** *against P. aeruginosa, E. coli, and S. aureus with mean inhibition zone of 15.0 ± 1mm, 14.67 ± 1.5 mm, and 13.3 ± 1.5mm mm at 1.0 mg/mL, respectively whereas compound* ***142b*** *showed higher activity against E. coli (14.3±2.1mm), P. aeruginosa (14.3±1.53mm), S. aurous (13±2.0mm), and S. pyogenes (14.3±1.53mm). DPPH radical scavenging activity displayed 90%, 92%, 91%, and 95% inhibition for compounds* ***140***, ***142a***, ***142b***, ***142c***, *respectively, at 100 μg/mL. In silico molecular docking analysis revealed that compound* ***142c*** *exhibited better docking efficiency with DNA gyrase displaying binding affinity of -6.9kcal/mol close to ciprofloxacin (−7.2 kcal/mol). Molecular docking study was also examined using human peroxiredoxin 5 enzymes for compound* ***142c*** *scoring binding affinity of -5.4 kcal/mol compared with standard antioxidant ascorbic acid (-4.*9 kcal/mol). *The results of in silico molecular docking study of the compounds against E. coli DNA gyrase B and human peroxiredoxin 5 enzymes were also in good agreement with in vitro antibacterial and antioxidant analysis. The in vitro antibacterial activity of compound* ***142b*** *and antioxidant activity of compound* ***142c*** *suggest the potential use of these compounds as potential drug lead candidates.*

Keywords*: antibacterial activity, antioxidant, Pictet-Spengler reaction, tetrahydro-β-carboline, molecular docking.*

CHAPTER 1

INTRODUCTION

1.1. Background

The *β*-carboline alkaloids are a large group of natural and synthetic indole alkaloids that possess a common tricyclic pyrido[3,4-*b*] indole ring structure [1]. They can be categorized according to the saturation of their nitrogen containing, six-membered ring. Unsaturated members are named as fully aromatic *β*-carbolines **1** (*β*Cs), whereas the partially or completely saturated ones are known as dihydro-*β*-carbolines **2** (DH*β*Cs) and tetrahydro-*β*-carbolines **3** (TH*β*Cs), respectively [2, 3]. The three rings in TH*β*C are referred to as A, B (pyrrole ring), and C-ring (piperidine moiety) (Fig. 1). Since the first time in 1841, *β*-carboline alkaloid harmaline **4** was recognized, originally isolated from *Peganum harmala*, extensive investigations have focused on the detection and identification of *β*-carboline alkaloids [4].

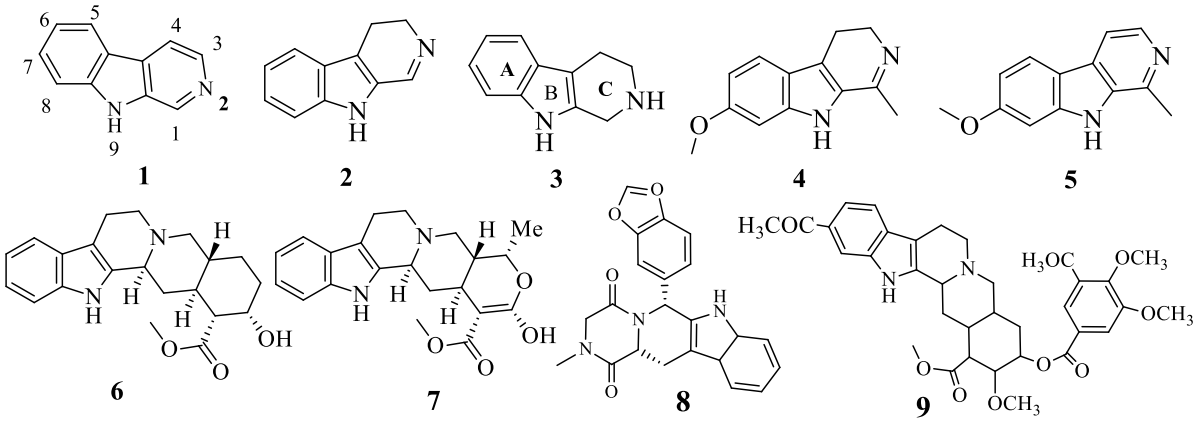


Figure 1: Chemical structure of *β*-carboline.

Next to the most representative *β*-carboline such as harmaline **4,** and harmine **5,** the tricyclic 1,2,3,4-tetrahydro-*β*-carboline (TH*β*C) ring system is a key structural element in a range of biologically and pharmacologically important alkaloids isolated from a variety of natural sources [5, 6] such as in biological tissues and fluids [7], plants [8, 9], red algae, in fruit [10]

and meat-derived products [11, 12] such as juices, jams and sausages [13]. They are traditionally used as emmenagogues, narcotics, abortifacients and in treatment of fever, rheumatism and asthma as well for recreation and as a stimulant of the central nervous system [14, 15]. These sources were reported to have antimicrobial [16, 17], antifungal [1], anticancer [18], and antioxidant [11] properties.

Recently, the TH*β*Cs alkaloids continue to be promising lead compounds for discovering and developing novel clinical drugs. TH*β*Cs, tadalalif **6**, yohimbine **7**, ajmalicine **8**, and reserpine **9** (Fig. 1) are the representative analogs of the carboline alkaloid isolated from a variety of natural sources with a range of biological and pharmacological activities [5, 19].The SAR studies have demonstrated that the introduction of appropriate substituents into the positions -1, -2, -3 and -9 of the *β*-carboline nucleus play a crucial role in determining their multiple pharmacological function [20].

1.2. Statement of the Problem

In recent years, the continuous emergence of resistant or multi-resistant strains of microorganisms towards the drugs used clinically raises the need for the search of new antimicrobial substances that could be used for the treatment of the infections produced by those strains. Several researches have been carried out on synthesis and biological studies of *β*-carboline derivatives and results suggest that substituents in positions-1, -3, and -9 of the *β*-carboline skeleton alter the biological activity. The development of modified antibacterial agents with different and efficient mechanisms of action is definitely an urgent medical need.

β-carboline alkaloid and its saturated analogue, TH*β*Cs, are common structural motifs in natural products and pharmaceuticals originally isolated from *P. harmala* L. and found to exhibit various biological activities [21, 22, 23]. Many synthetic methods have been developed for the preparation of the scaffold and derivatives of tetrahydro-*β*-carboline with alkyl C-1 constituents, and improvements in the synthesis methodology are still an active research issue [16]. Therefore, there is a need to evaluate the antibacterial and anti-oxidant activities of tetrahydro-*β*-carboline using different C-1 substituted derivatives.

The current research project is therefore designed to synthesize some derivatives of tetrahydro-*β*-carboline with alkyl functionalization at C-1 from substituted aryl. Computational *de novo* design approach was used to confirm mode of binding for antibacterial activity and the drug-likeness of synthesized compounds. Docking studies was performed with DNA-Gyrase (6F86) and LasR binding domain (2UVO) employing flexible ligand docking approach by using AutoDock Vina software.

1.3. Significance of the Study

Currently, tetrahydro-*β*-carboline and their derivatives have attained much attention because of their extensive biological activities including antimicrobial, antifungal, antioxidant, anti-cancer, and anti-inflammatory. It is expected that this work will contribute to a better understanding of the effects of the modification of the tetrahydro-*β*-carboline at C-1 from substituted aryl in the antimicrobial and antioxidant activities of the compounds. The research can also provide an information on molecular docking analysis so as to have better understanding of the molecular mechanisms of interaction between the compounds with promising antibacterial activity and selected protein domains. Furthermore, report obtained here will provide documentary information that can be referred by other researchers interested to work on the identification of lead compound and synthesis of related compounds.

1.4. Objectives of the Study

1.4.1. General Objective

- To synthesize and evaluate the antibacterial, antioxidant and molecular docking analysis of TH*β*-carboline derivatives.

1.4.2. Specific Objectives

- To synthesize 1,2,3,4-Tetrahydro *β*-carboline derivatives using Pictet-Spengler reaction (PSR) bearing different substituted group at C-1 and C-3 positions.
- To characterize the 1,2,3,4-Tetrahydro *β*-carboline derivatives using meting point, UV, and NMR (^{1}H-NMR and ^{13}C-NMR).

- To evaluate the antibacterial activities of 1,2,3,4-Tetrahydro-*β*-carboline derivatives using disc diffusion method against *Staphylococcus aureus*, *Staphylococcus pyogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa.*
- To evaluate the radical scavenging activities of 1,2,3,4-Tetrahydro-*β*-carboline derivatives using DPPH assay.
- To examine molecular docking study of synthesized derivatives using Autodoc version 4.2 with DNA-Gyrase (6F86), LasR binding (2UVO) and against human peroxidoxin 5 (PDB ID: 1HD2) protein domains.

CHAPTER 2

LITERATURE REVIEW

2.1. Synthetic Approaches Towards TH*β*Cs Derivatives

There are numerous methods that have been developed for the synthesis of TH*β*C and their derivatives. The most well-known and straightforward route for the construction of TH*β*C moiety is Pictet-Spengler reaction (PSR) discovered in 1911 [24]. Another classical and established protocol is Bischler-Napieralski cyclization (BN) where the product is a DH*β*C, which can then be further reduced to form the corresponding TH*β*C [25].

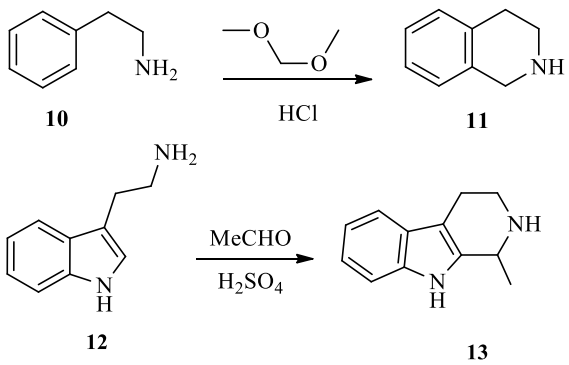
Although the classical version of these reactions is well established as a method of choice for construction of TH*β*C frameworks, original strategy has been modified over the past decades associated with the requirement of stereochemistry, regiochemical selectivity and efficiency [26] including conventional method [27, 28], biocatalytic methods [15], transition metal catalyzed [5], ionic-liquid methods [16], microwave assisted methods [17] have been employed. In addition to these two classical cyclization methods, several other methods such as Fischer-indole reaction [29], Friedl-Craft indole alkylation reaction [30], and Simultaneous ring B and ring C closing methods [31] were reported.

2.1.1. Pictet-Spengler reaction (PSR)

The Pictet-Spengler reaction was first discovered by Pictet and Spengler in 1911 where they synthesized 1,2,3,4-tetrahydroisoquinoline (THIQ) **11** from *β*-phenylethylamine **10** and formaldehyde dimethylacetal under heating in the presence of concentrated hydrochloric acid. After the discovery of the PSR it took nearly 20 years before Tatsui used tryptamine **12** as the amine component, which paved the way for the first synthetic creation of the 1-Methyl-1,2,3,4-tetrahydro-*β*-carboline **13** skeleton in the year 1928 [32] (Scheme 2).

A typical PSR reaction is a two-step process that involves the condensation of an aliphatic amine (electron-rich aryl or heteroaryl groups: *β*-arylethylamine or tryptamine/tryptophan) with aldehyde or ketone to form an imine or iminium ion, which is most commonly activated by

Bronsted acids. Final intramolecular cyclization between a sufficiently reactive, electron-rich aromatic ring and the activated iminium ion results in a N-heterocyclic ring via a new C-C bond [33, 9]. From the mechanistic view, it is well recognized that an acidic catalyst usually an excess of a Brønsted acid/Lewis acids ranging from catalytic to stoichiometric amounts [16], in the presence of non-aqueous protic or aprotic solvent activates the imine intermediate before cyclization into the tetrahydro-*β*-carboline [34, 35].



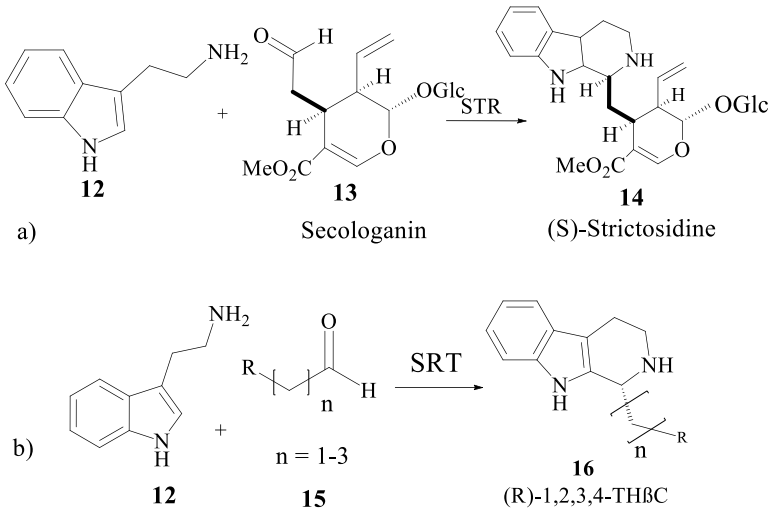
Scheme 1: The first PSR; the synthesis of THIQ **11** and THβC **13**.

A variety of efficient catalytic systems were used for the synthesis of TH*β*Cs in PSR. Some examples of conventional catalysts are TFA [36], conc. H_2SO_4 [37, 38], AcOH [9], p-TsOH [32] and Lewis acids such as TMSCl [27] have been reported as catalysts for the PSR. Moreover, biocatalytic, ionic-liquid solvent promoted PSR, and micro-wave irradiated PSR have been used to modify the original route with the requirement of efficient catalysis and higher yields which is discussed in detail under the next sections.

2.1.2. Biocatalytic PSR

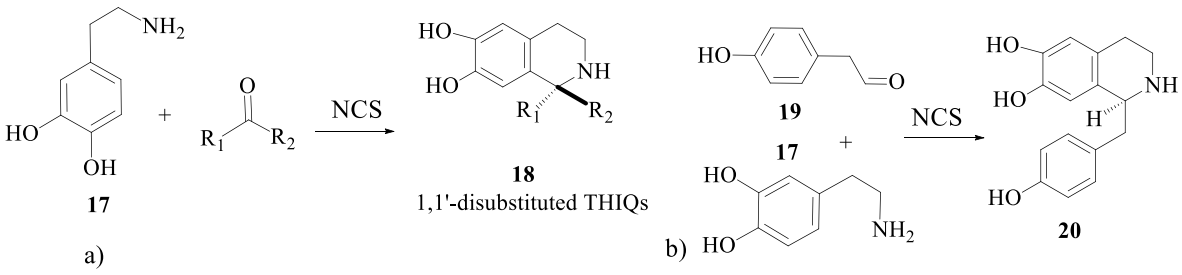
In the use of enzyme-mediated synthesis of TH*β*Cs, in the indole alkaloid field, the most prominent and best characterized members of the highly substrate-specific Pictet-Spenglerases, the norcoclaurine synthases (NCS) [39] and the strictosidine synthases (STRs) [40] have been applied effectively in *vivo* and in *vitro* to catalyze Pictet-Spengler reaction (PSR) [41]. In this natural reaction, the STR condenses tryptamine (**12**) and secologanin **13** to generate an intermediate Schiff base that cyclizates to give the (S)-configured 1,2,3,4-tetrahydro-*ß*-carboline (S)-strictosidine **14** which is the key intermediate of indole alkaloid biosynthesis in

plants (Scheme 1) [42, 28]. In this regard, various biomimetic approach has been developed on the basis of the first known enzyme-catalyzed pathway of PSR leading to TH*β*Cs [43].



Scheme 2: a). STR condenses tryptamine **12** and secologanin **13** to give the (*S*)-configured TH*ß*C (S)-strictosidine **14**. b). Strictosidine synthase catalyzed PSR between tryptamine **12** and aliphatic aldehydes.

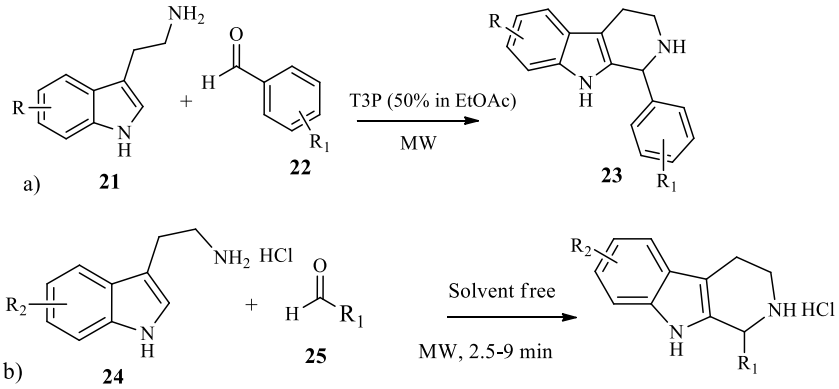
Benjamin group have described that not only STR but also norcoclaurine synthase (NCS) from *Thalictrum flavum* (TfNCS) can catalyze the PSR between dopamine **17** and unactivated ketones for the first-time, thus facilitating the facile biocatalytic generation of 1,1'-disubstituted THIQs **18** (Scheme 3a) [39]. The mechanistic studies on Norcoclaurine Synthase are discussed in detail by Louis and his co-worker where NCS catalyzes an asymmetric PS condensation of dopamine **17** and 4-hydroxyphenylacetaldehyde **19** to give (S)-norcoclaurine **20** (Scheme 3b) [44].



Scheme 3: a) Biocatalytic route to **18**, from dopamine and ketones, via a PSR, catalysed by NCS. b) The PSR catalyzed by norcoclaurine synthase to give **20**.

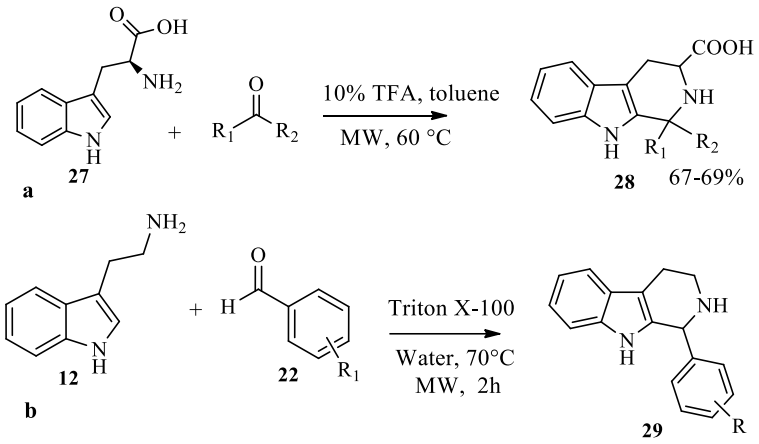
2.1.3. Micro-wave/Ultrasound assisted PSR

In recent years, an efficient and environmentally friendly synthesis of tetrahydro-*β*-carboline via PSR employing micro-wave (MW)/ultrasonic irradiation has tremendously increased due to its simplicity, short reaction time, high yield and green nature of the reactions [45], and several studies had been reported [46, 47, 48, 49]. In 2013, Matthieu *et al*., synthesized and reported various tetrahydro-*β*-carbolines **23** from a mixture of substituted tryptamine **21** and a variety of substituted aldehydes **22** in the presence of Propane phosphonic acid anhydride (T3P) using the optimized conditions under microwave irradiation [T3P (2.5 equiv.), MW, 10 min., 110 °C] (Scheme 4a). It was also confirmed that T3P was required for this cyclization and ketones are often problematic in the Pictet-Spengler reaction and sometimes fail to react even [34]. Under neat reaction system, without additional catalyst Fei and Qi-Dong developed the synthesis of 1,6,7-substitiuted-1,2,3,4-Tetrahydro-*β*-carbolines **26** from tryptamine hydrochlorides **24** and different **25** aldehydes (Scheme 4b). The study used to compare the reaction result between conventional heating (90 min, 100°C, AcOH, and 80% max) and microwave irradiation (2-3 min, < 100°C, neat, and 95%) where dramatic reduction in the reaction time and higher product yield was achieved [50].



Scheme 4: a) T3P catalyzed microwave-assisted PSR; b) Synthesis of tetrahydro-*β*-carboline hydrochlorides under catalyst-free and neat conditions with the aid of microwave irradiation.

In 2004, Fu-Ming *et al*., demonstrated that though intrinsically slow in reaction rates (which had taken 13h to 15.5 days *via* conventional method at rt; 74-84% yield), ketone reactions (specifically cyclic ketones such as cyclohexanone and cyclopentanone) with tryptophan **27** can be accelerated (hours to minutes) using microwaves (60 °C and 150 W) in an open vessels with high isolated yields of 1,3-disubstituted-1,2,3,4-tetrahydro-*β*-carboline **28** (67–99%) (Scheme 5a) [36]. By using non-ionic surfactant catalyst Triton X-100 (10mol%) in aqueous media under ultrasound irradiation, Venkata group reported a highly efficient procedure for the preparation of tetrahydro-*β*-carbolines in good yields (89-94%; 2-4 hrs.) compared to conventional heating methods (65-75%; 9-12 hrs.) by the condensation of tryptamine **12** and aryl/ heteroaryl aldehydes **22** having both electron-donating and electron-withdrawing substituents to furnish tetrahydro-*β*-carbolines **29** via PSR (Scheme 5b) [45].

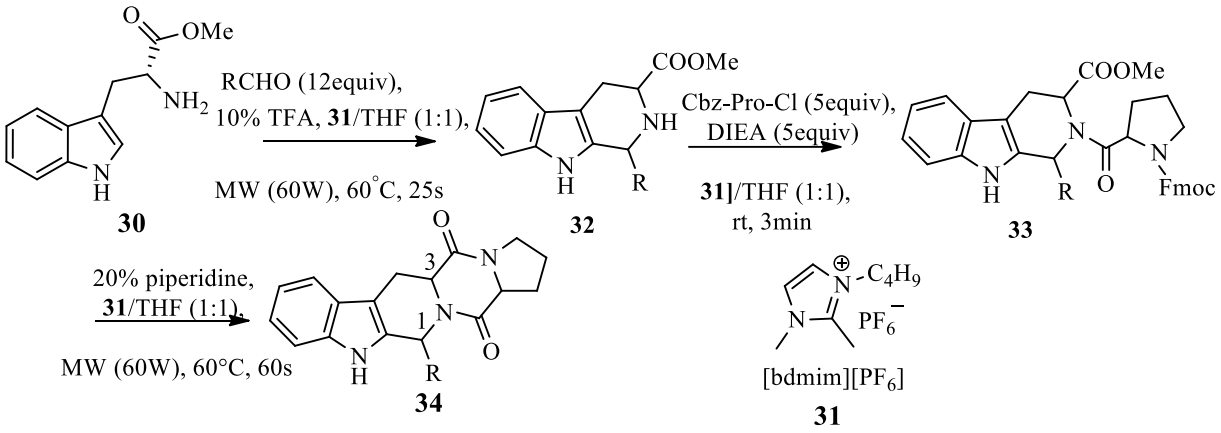


Scheme 5: a) MW assisted PSR to afford 1,3-disubstituted-1,2,3,4-tetrahydro-*β*-carboline and compound **29**.

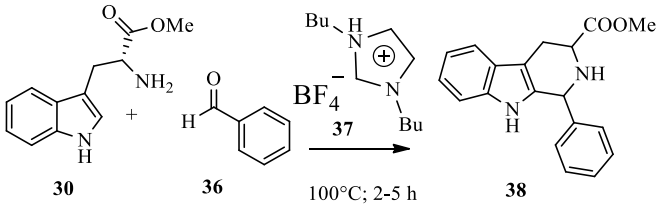
2.1.4. Ionic-liquid catalyzed PSR

Nowadays, ionic liquids are used as catalysts as well as ecofriendly solvents in organic synthesis due to unique physical and chemical properties (non-volatile, recyclable, non-explosive) which can also be applied in the synthesis of TH*β*Cs via PSR [51]. In 2004, Ya-Hew and Yen-Ho synthesized tetrahydro-*β*-carbolinediketopiperazines **34** from tryptophan methyl ester **30** and various aldehyde all with higher total isolated yields under microwaves (49–69%; 60 min) than at rt (20–41%; 2h) in the 1-Butyl-3-methylimidazolium hexafluorophosphate **31** ([bdmim][PF_6]) [bdmim][PF_6]/THF solvent system with temperature controlled at 60°C (Scheme 6a) [52]. In

2006, Muthukrishnan *et al*. also reported an ionic liquid promoted Pictet-spengler reaction of D-tryptophan ester **30** with different aldehydes in different imidazolium based ionic liquids like [bbim]BF_4, [bbim]PF_6, and [bbim]Br in the presence of trifluoroacetic acid (TFA) as an acid catalyst. Here, [bbim]BF_4 **37** was found to be superior in terms of yield, reaction time and easy isolation of products as compared with other ionic liquids at 100 °C for 2 h to afford the corresponding 1,3-disubstituted 1,2,3,4-tetrahydro-*β*-carboline **38** between 70-90% yield (Scheme 7) [16].



Scheme 6: [bdmim][PF4] promoted synthesis of tetrahydro-*β*-carbolinediketopiperazines **34**.

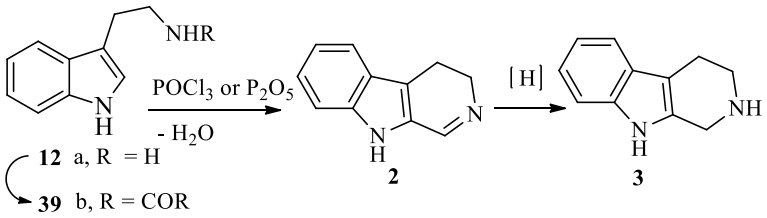


Scheme 7: [bbim] BF_4 promoted synthesis of tetrahydro-*β*-carboline **38**.

2.2. Bischler-Napieralski Cyclization Reaction

Bischler-Napieralski reaction/cyclization (BNR) is another plausible and classical reaction for TH*β*Cs formation from *β*-indolylamides **39** after the acylation of tryptamine **12** where the cyclization starts from tryptamides **39** (Scheme 8) and usually requires reagents that are harsh, dangerous and difficult to handle, for example $POCl_3$ [53] and/or P_2O_5 [54] in benzene or

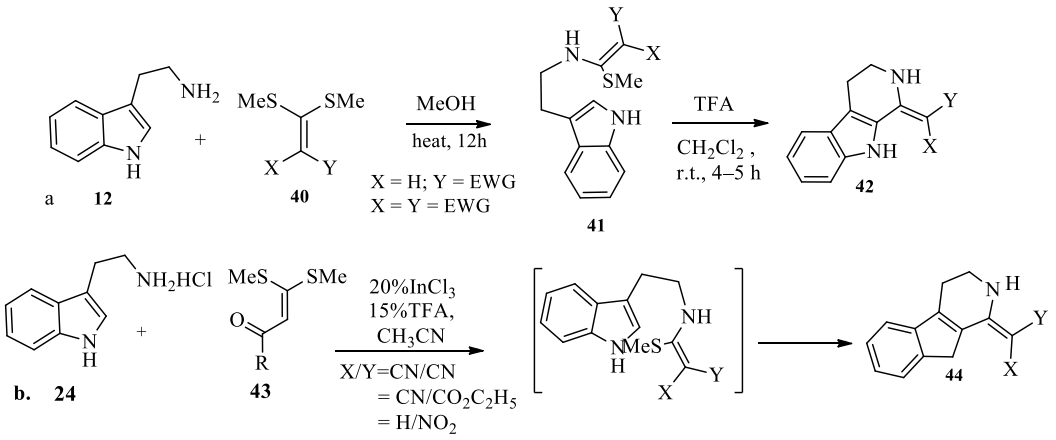
toluene at high temperature. The first product of the BNR is a DHβC **2**, followed by reduction to form the corresponding THβC **3** [25].



Scheme 8: The general reaction scheme for Bischler-Napieralski reaction/cyclization.

However, it is an established classical protocol, the synthetic methods of THβCs using BNR have been reported very rarely since the reaction involves multi steps, results in poor yields [55], often-long reaction times, harmful and toxic catalysts, tedious workup, and production of great amount of wastewater [56]. In order to overcome these problems, researches have been conducting using a modified reaction conditions either by using different dehydrating reagents, microwave irradiation [57, 49], and suitable catalysts such as T3P® reagent [58], zeolites [56].

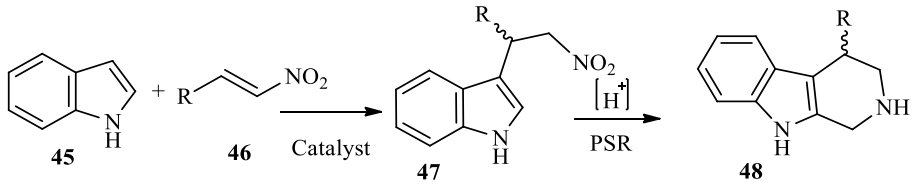
In (2005), Sriparna *et al*., have developed a synthesis of novel functionalized enamines **42**. The desired N,S-acetals **41** were easily accessible in high yields via direct displacement on the appropriate polarized ketene dithioacetals **40** with tryptamine **12** in refluxing ethanol (Scheme 9a). However, attempted Bischler-Napieralski type intramolecular cyclization of the N, S-acetals **41** in the presence of various Lewis/protic acids ($SnCl_4$, H_3PO_4 or PTSA) under varying conditions furnished only complex mixtures of products. Here, the TFA/CH_2Cl_2 combination was found to be the best in terms of yields, cleaner work-up and isolation procedure (Scheme 9a) [59]. Similarly, in recent year, Thokchom and Okram reported a novel 1-substituted tetrahydro-β-carbolines by cyclocondensation of ketene S, S-acetals **43** with tryptamine **12** in presence of $InCl_3$ and TFA as co-catalysts by Bischler-Napieralski cyclization. Based on the established classical mechanism of Bischler-Napieralaski, the electron rich nitrogen atom of tryptamine attacks the electrophilic carbon of ketene S, S-acetal, thereby forming a new C-N bond initially. Finally, the elimination of one molecule of methanethiol may generate an iminium intermediate and a subsequent intramolecular electrophilic elimination of one more molecule of methanethiol gives the final desired product **44** (Scheme 9b) [55].



Scheme 9: a) TFA/CH_2Cl_2 catalyzed Bischler-Napieralski cyclization to give functionalized enamines **41**. b) InCl3-TFA catalyzed synthesis of functionalized THβCs derived enamines **44**.

2.3. Micheal addition: Friedele-Crafts allylation of indoles with nitroalkene

The Friedel-Crafts (FC) reaction of aromatic compounds with electron-deficient alkenes are used widely in synthetic organic chemistry [60, 61]. Particularly, FC reaction of indoles **75** with various electrophiles is one of the most straightforward methods to afford indole derivatives and much effort has been expended. In this regard, the FC alkylation between indoles (nucleophiles) and β-nitroalkenes **46** (electrophile) being promoted by metal complex ligands [62], Lewis acids [63] and/or organocatalysts [30] is significant as it gives access to indole-based alkaloids such as THβCs **48** ([64]. In this reaction, FC adduct **47** is the first intermediate (found to be valuable synthetic precursor) which is further reduced to amine that undergo a stereo-controlled Pictet-Spengler cyclization to give a cyclized product **48** (Scheme 10).

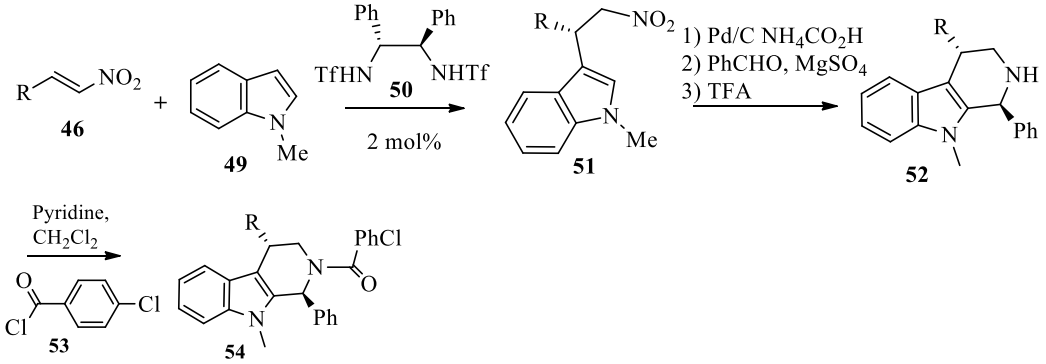


Scheme 10: Friedele-Crafts alkylation of indoles with nitroalkene to give THBC **48**.

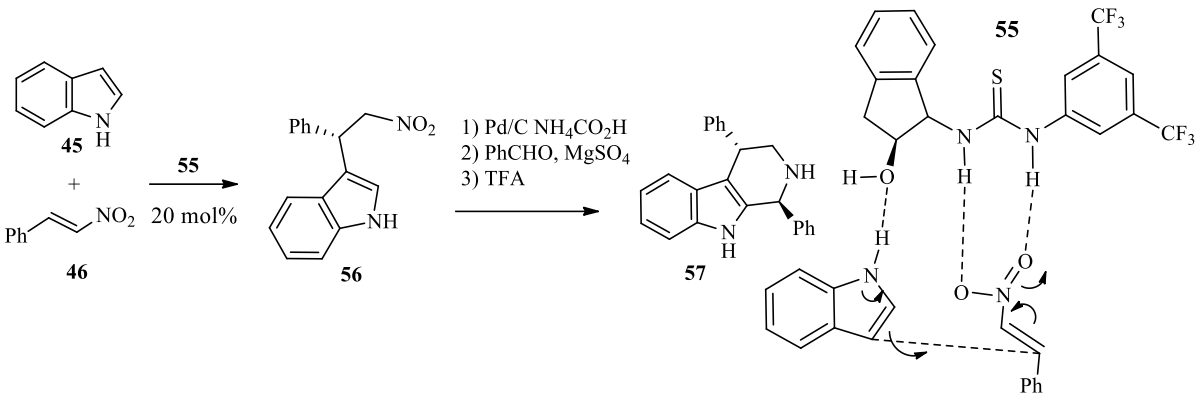
However, the construction of THβCs with substitution at positions 1 and 3 can be conveniently obtained by using the PS cyclization from tryptamine **12** and tryptophan **27** respectively,

obtaining 4-functionalized THβCs remains more challenging. One merit of Friedel-Crafts alkylation of indoles with nitroalkene is to give 4-substitiuted THβCs. In 2005, the enantioselective Friedel-Crafts addition of indoles to nitro-olefins using chiral hydrogen-bonding bis-sulfonamides as the catalysts has been developed by Wei and his co-workers [65]. It was showed that without catalyst no reaction was observed between β-nitrostyrene **46** and N-methyl indole **49** and proceeded with good yield and moderate enantioselectivity for nitrostyrene having electron-withdrawing groups (R= *p*-Br-Ph, *o*-NO_2-Ph). However, introduction of electron-rich substituents on the phenyl group in the nitrostyrene resulted in lower enantioselectivity (R= *o*-OMe-Ph) (Scheme 11).

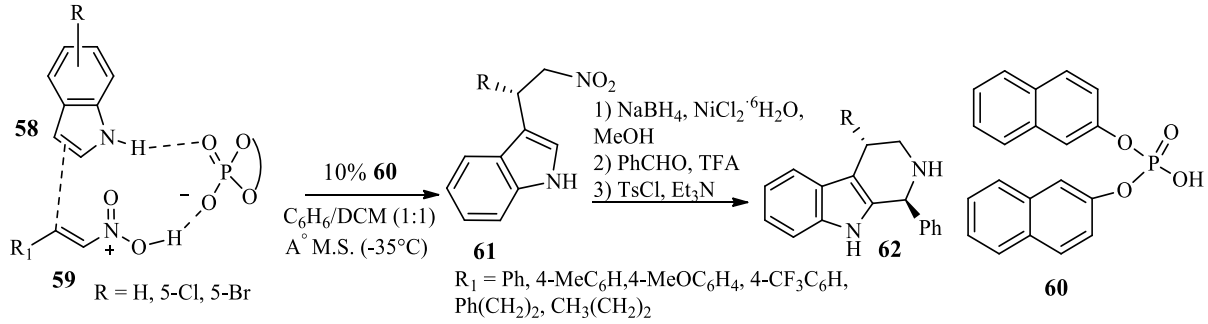
In the same year, Raquel *et al*., synthesized previously unreported 1,4-diphenyl-substituted THβC using thiourea **55** catalyzed FC alkylation [30]. The study revealed that thiourea promote the FC additions of indoles **45** to nitroalkenes **46** by forming a reversible complex involving a double hydrogen bond between the thiourea hydrogen atoms and the two oxygen atoms of the nitroalkene (Scheme 12). Recently, the detailed reaction mechanism of aminoindanol based thiourea derivative containing bifunctional organocatalyst **55** was reviewed by Isaac group in 2016 to develop organocatalytic enantioselective FC alkylation of indoles, employing nitroalkenes as versatile electrophile [66].



Scheme 11: 1,2-diphenyltrifluoromethanesulfonamide **50** catalyzed FC reaction to afford **54**.



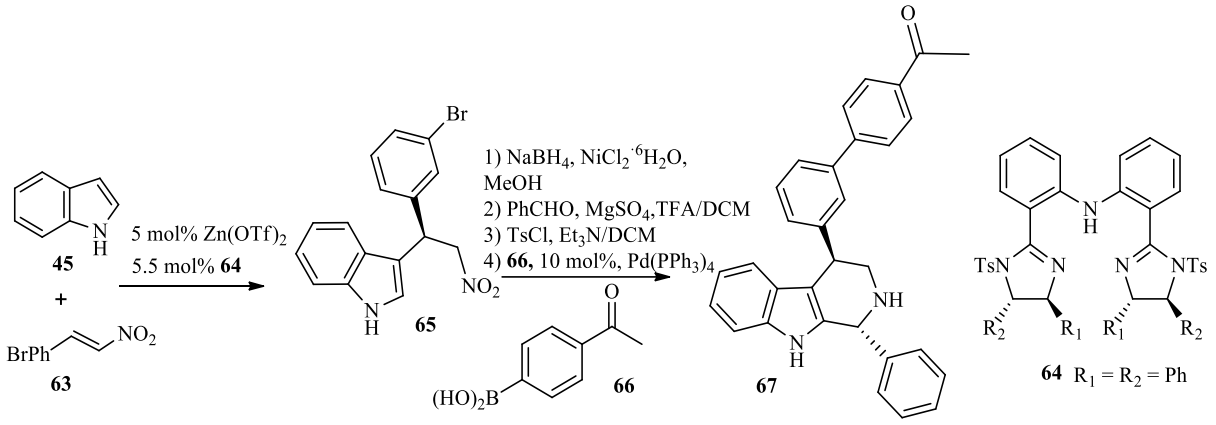
Scheme 12: Friedel-Crafts alkylation of indoles **45** with nitroalkenes **46** catalyzed by thiourea **55** to provide TH*β*C **57**.



Scheme 13: Chiral Brønsted acid catalyzed FC alkylation of indoles with nitroalkenes.

The first Chiral phosphoric acid (R)-3 reported by Junji *et al*., to provide the best enantioselectivity in the Friedel-Crafts alkylation of indole **58** with nitroalkene **59** (2equiv) bearing electron-donating, electron-withdrawing, and hetero-aromatic groups underwent the Friedel-Crafts alkylation reaction to afford Friedel-Crafts adducts **61** with excellent enantioselectivitie in benzene/DCM (1:1) at -35°C transforming into amine and 1,2,3,4-tetrahydro-*β*-carboline derivative **62** [67]. In this catalysis, the phosphoric acid activates the nitro moiety and at the same time the phosphoryl oxygen atom forms a hydrogen bond with the hydrogen atom of the indole N-H moiety wherein the phosphoric acid worked as a bifunctional catalyst (Scheme 13). The detailed reaction mechanism of chiral Bronsted acid catalyzed FC type reactions of indole and its derivatives with various carbon-centered electrophiles (electron deficient olefins, carbonyls, and imine) was briefly reviewed [68].

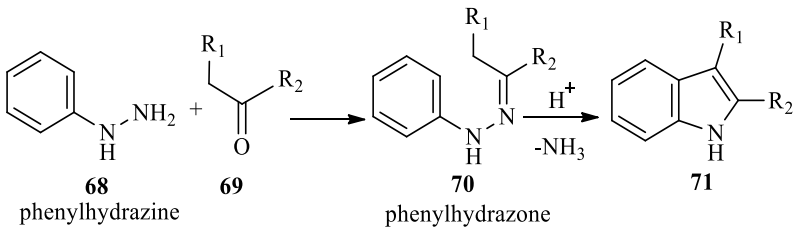
In additions, novel ligands were used as a promoter in the asymmetric Friedel-Crafts alkylation of indole derivatives with nitroalkenes to afford TH*β*Cs. For example, in 2010, Han and Da-Ming designed and tested for the asymmetric Friedel-Crafts method thus provides a way for the construction of a chiral 1,2,3,4-tetrahydro-*β*-carboline **67** library [69]. In the asymmetric Friedel–Crafts alkylation of indole **45** with nitroalkenes **63**, the complex of ligand **64** with Zn $(OTf)_2$ gave good reactivity and excellent enantioselectivity. The chiral adduct **65** derived from 3-Br-substituted nitrostyrene **63** was transformed to chiral TH*β*C (Scheme 14). The tosylated Pictet-Spengler product further undergo Suzuki-Miyaura coupling with 4-acetylphenylboronic acid **66** gave the desired product **67** in 70% yield (97% ee).



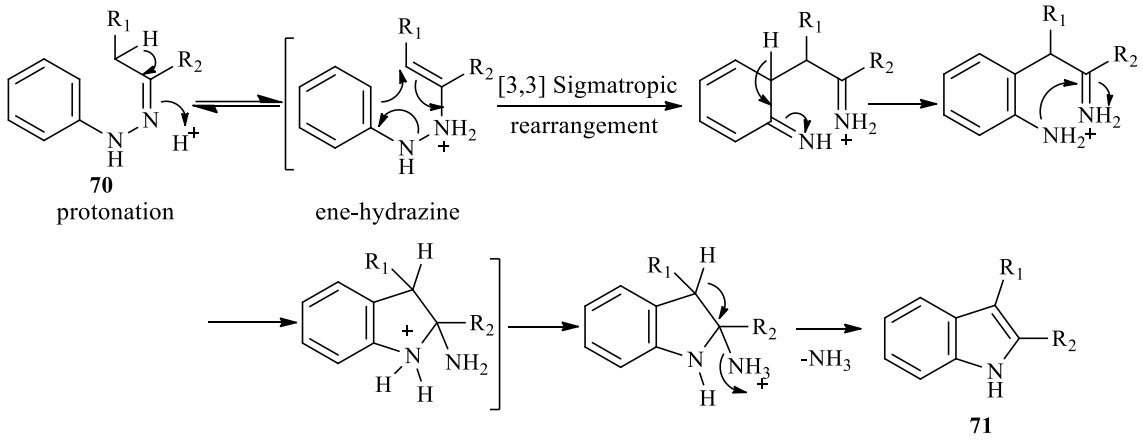
Scheme 14: Asymmetric FC alkylation of indole **45** with nitroalkenes **63** to give TH*β*C product **67**.

2.4. Fischer-indole Reaction

The Fischer indole synthesis (FIS) is a classical and the most widely used method for indole scaffolds based natural and synthetic products. David, and Majid *et al*., extensively reviewed the reaction protocol and the total synthetic products using FIS [70, 71]. From the various indole-based products, TH*β*Cs (via the formation of fused pyrrole moiety, ring B, Fig. 1) has been reported well [72, 73]. Previously discussed approaches involve cyclization of tryptamine, tryptophan, or allylic indole (fused ring A and ring B together) whereas FIS involves late-stage indole introduction. The FIS converts ayrlhydrazones **70** of aldehydes or ketones **69** into indoles **71** in the presence of an acid catalyst (Scheme 15) [74, 75, 76].

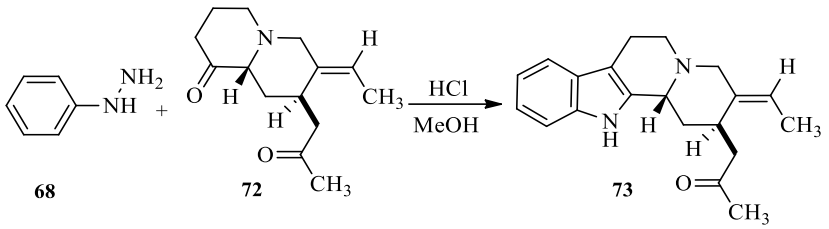


Scheme 15: General reaction for Fischer indole synthesis.

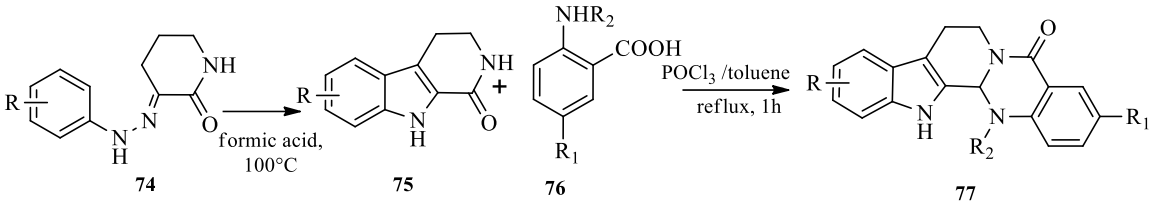


Scheme 16: The reaction mechanism for Fischer indole synthesis.

In a very early report by Richard *et al*., Fischer indole cyclization was employed for the synthesis of tetracyclic TH*β*C **73**. It was accomplished based on HCl-catalyzed Fischer indole reaction with phenyl hydrazine **68** which cleanly afforded deformyl-isogeissoschizine **73** in 64% isolated yield from ketone **72** (Scheme 17) [77]. Two years later, Bipul *and* his co-workers described the synthesis of a novel fused TH*β*Cs, quinazolino-*β*-carboline-5-one derivatives **77** using FIS. The main intermediate, the formation of substituted TH*β*C **75** was prepared from substituted hydrazone **74** by using formic acid as acidic catalyst [78]. The TH*β*C was then treated with substituted anthranilic acid derivatives **76** in the presence of $POCl_3$ in toluene under reflux to provide the products **77** in almost 80-90% yield (Scheme 18). Another report on the synthesis of a series of tetrahydro-*β*-carboline-1-one **81** was accomplished based on Fischer indole reaction starting from substituted aniline **139** by Jiang-Ping group. Here, compound **79** was reacted with a diazonium intermediate derived from substituted aniline **78** to generate hydrazone **80**, which was refluxed in formic acid to provide a *β*-carboline analog **81** (Scheme 19) [79].

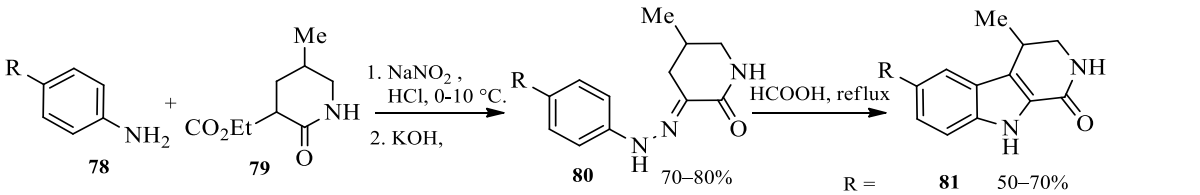


Scheme17: HCl-catalyzed Fischer indole synthesis to afford tetracyclic THβC **73**.



a R = Br, , R_1 , R_2 = H;
b. R = Cl, , R_1 , R_2 = H
c. R = Cl, , R_1 = H, R_2 = CH_3 ;
d. R = Br, , R_1 = H, R_2 = CH_3
e. R = H, , R_1 = H, R_2 = CH_3 ;
f. R = F, , R_1 = H, R_2 = CH_3
g. R = OCF_3 , R 1 , R_2 = H;
h. R = CF_3 , R_1 , R = H

Scheme18: Synthesis of novel quinazolino-β-carboline-5-one derivatives **77** using FIS.

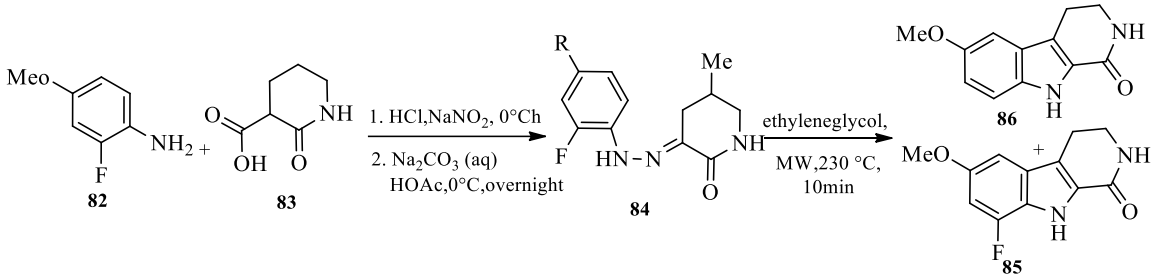


a. H , b. Me, c. Ph, d. Br, e. HO, f. MeO
g. BnO, h. AcNH, i. $H_2N(CO)$

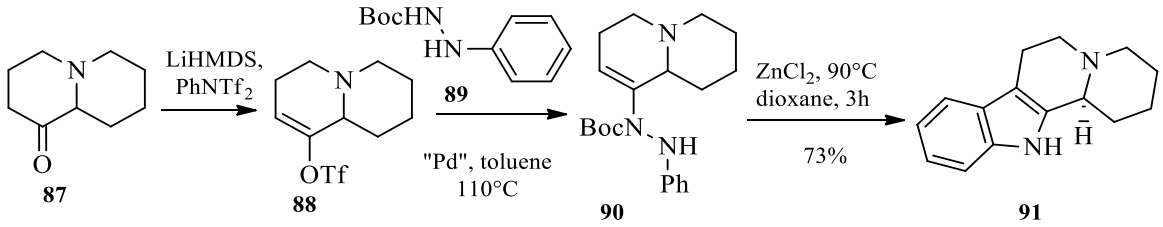
Scheme19: 6-substitiuted-4-methyl-1-oxo-1,2,3,4-terahydro-β-carboline.

In 2006, Jorge *et al*. reported two possible sites for new carbon-carbon bond formation by FIS during cyclization of fluorinated hydrazone. Here, under MW conditions, Fischer cyclization of hydrazone **84** from 2-fluoro-4-methoxy aniline **78** via diazonium intermediate produces 8-fluoro-6-methoxy-1-oxo-1,2,3,4-terahydro-β-carboline **85** (yield, 34%). But, unexpected and comparable amount of non-fluorinated carboline 6-methoxy-1-oxo-1-4-terahydro-β-carboline **86** (yield, 32%) was accompanied. Thus, the cyclization also occurred on the fluorine-substituted position, with loss of fluorine (Scheme 20) [80]. In another work, Byeong-Yun *et al*. have demonstrated that enol triflate **88** prepared from the corresponding bicyclic ketone was

readily coupled with aryl hydrazide **89** to give ene-hydrazide **90** in good yield. Here, the resulting ene-hydrazide undergoes the Fischer indolization reaction, affording the corresponding natural product desbromoarborescidine A **91** in 73%. According to the study, among the Lewis acids used, $ZnCl_2$ provided the best results when heated in 1,4-dioxane under reflux (Scheme 21) [29].



Scheme 20: MW-assisted synthesis of fluorinated and non-fluorinated TH*β*Cs using FIS.



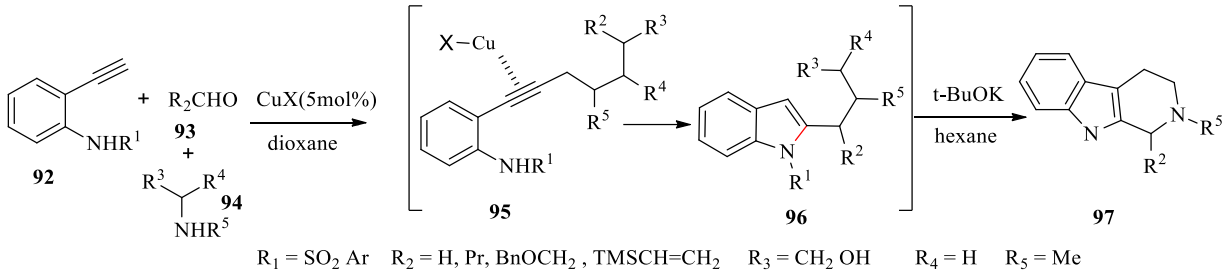
Scheme 21: $ZnCl_2$-catalyzed Fischer indolization reaction to Desbromoarborescidine **91** from enol triflate **88**.

2.5. Simultaneous pyrido[2,3-b]indole ring formation

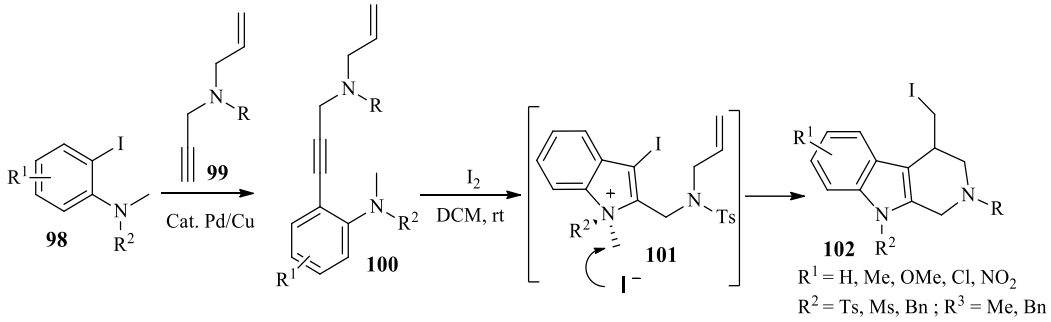
The simultaneous construction of the three rings of the TH*β*C core is difficult to achieve efficiently from a naked scaffold. This reaction follows *via* catalytic cascade reactions to form concurrently fused indole-pyridines rings, pyrido[2,3-b]indole [81, 82, 83, 84].

In 2011, Ohta *et al*., generated by copper-catalyzed indole formation TH*β*Cs **168** *via* a Cu-catalyzed domino three-component coupling-cyclization reaction using ethynylanilines **92**, aldehydes **93** and secondary amines **94**. This was achieved by a second cyclization at the C-3 position followed by t-BuOK/hexane mediated cyclization of intermediate 2 (aminomethyl)indole **96** (Scheme 22) [85]. Hongjian *et al.,* described an iodine-mediated domino electrophilic cyclization reaction of substituted 2-(3-(Allylamino)prop-1-ynyl)anilines

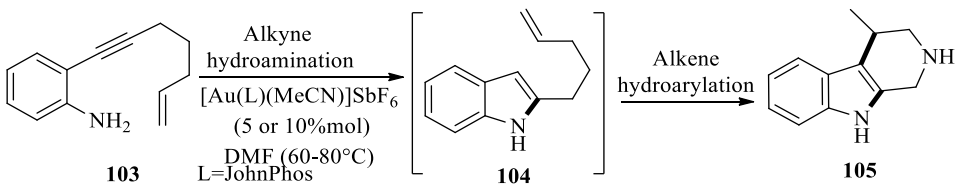
100 for the preparation of 4-iodomethyl substituted tetrahydro-*β*-carbolines **102** (yield, 90-95%). First, compound **100** was readily prepared by Sonogashira coupling of the **98** and corresponding alkyne **99.** Here, the iodine served as a Lewis acid, coordinates to the triple bond to promote cyclization which produces the intermediate **101** followed by the removal of the alkyl group by iodide via an S_N2 reaction and final cyclization (Scheme 23) [38]. Recently, in 2015, Ana and his co-workers explored the gold-catalysed hydroaminative/arylative cascade cyclization (Scheme 39) of 2-aminoaryl-1,7-enyne **103** as an expeditious route to 2,3-fused indole rings **105** via unactivated alkene and 1,3-unsubstituted indole intermediates **104** (Scheme 24) [31].



Scheme 22: One-pot three component synthesis of tetrahydro-*β*-carbolines using t-BuOK.



Scheme 23: Iodine-mediated domino electrophilic cyclization to give 4-iodomethyl substituted tetrahydro-*β*-carbolines **102**.



Scheme 24: Gold(I)-catalysed synthesis of 2,3-fused indole derivatives **105**.

2.6. Biological Activities of TH*β*Cs

TH*β*Cs derivatives have attracted attention because of their biological and pharmaceutical properties such as antimicrobial [86, 21], antioxidant [87], anticancer [18], antimalarial [88], anti-inflammatory [89], and anti-leishmanial activities [90, 90]. Therefore, in view of growing importance of various TH*β*C derivatives, a brief account of recently reported TH*β*C alkaloids and their bioactivities is reported in the next sections.

2.6.1. Antibacterial Activity

In 2014, Hong-jian group reported the activity of a series of tetrahydro-*β*-carboline-3-carboxylic acid derivatives and the compound **106** exhibited more than 70% fungicidal activities against 14 kinds of phytopathogens at 50 mg/kg. The study showed that, the compound containing butyl ester **163** on 3-position was much higher than that of compound containing N-butylamide **107** on 3-position. In the same year, this group reported the fungicidal activities of tetrahydro-*β*-carboline derivatives containing acylhydrazone moiety (−CONHN=CH−) by adopting the tactics of active fragment stitching and using compound **106** as the lead compound. The result revealed that the derivatives showed good fungicidal activities against 14 kinds of phytopathogens; especially compounds **108**, **109**, and **110** exhibited desirable fungicidal activities against each of the phytopathogens [25]. Additionally, tetrahydro-*β*-carboline derivatives exhibited higher activities than analogues *β*-carboline derivatives [91]. In another work, recently reported series of 1-aryl-2,3,4,9-tetrahydro-1H-*β*-carbolines compared the substitution effect on microbial effect on a simple tryptoline **2**. Hence, compounds **111a**, **111f**, **111g**, **111i**, **111j** and **111k** are found to effectively inhibit the growth of microbial cultures of *E. coli, S. aureus, A. niger, and H. oryzae* in good comparison to the standard drug Penicillin and they have successfully improved the activity of basic 2,3,4,9-tetrahydro-1H-*β*-carboline scaffold (Fig. 2) [22].

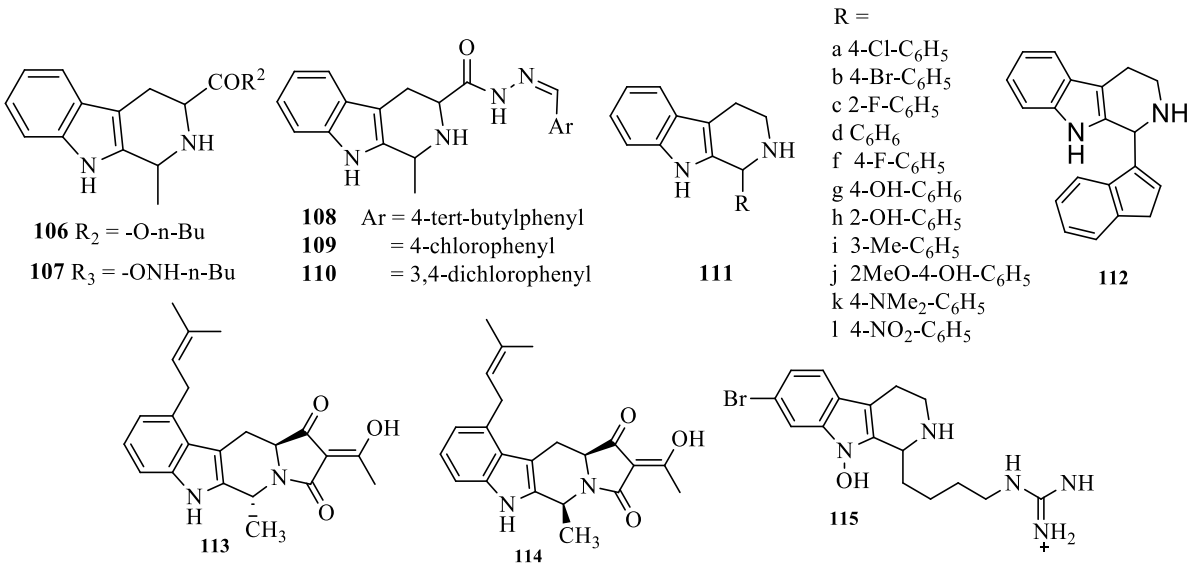


Figure 2: Chemical structure of active antimicrobial THBC derivatives.

In 2018, Alexandra and Olga, described a more detailed biological profile of the eudistomin U **112** (indole scaffold linked to *β*-carboline; orginally isolated from *Caribbean Lissoclinum* fragile). Hence, it was shown that the Gram-positive bacteria (*S. pyogenes, S. aureus, and M. smegmatis*) were most susceptible to the treatment with the compound **112**. Accordingly, the corresponding IC_{50} values (3.4-6.4 μg/mL) were nearly two-fold more potent than Gram-negative bacteria (*E. coli and P. aeruginosa*; 12.3-27.7 μg/mL) [92]. Recently, Xuan and Zhanzhu disclosed antibacterial activities of naturally occurring Griseofamine A **113** and its diastereomer 16-epi-griseofamine A **114** for the first time. Griseofamine A **113** exhibited in vitro activities against a panel of drug-resistant Gram-positive bacteria (*S. aureus, S. epidermidis, E. faecalis, and E. faecium*) with MIC values of 8-16 μg/mL. while 16-epi-griseofamine A **114** was 2-3 times more potent than griseofamine A with MIC values of 2-8 μg/mL. The result suggests the crucial role of the stereochemistry in the antibacterial activity [93]. Furthermore, Jiayi *et al*. reported the methanol extract of N-hydroxylated 1,2,3,4-tetrahydro-*β*-carboline **115** constituents of the New Zealand ascidian *Pseudodistoma opacum* and tested against a chloroquine-resistant strain (FcB1-Colombia) of Plasmodium falciparum and found to exhibit an IC_{50} value of 3.8 μM (±0.2, n = 3) (Fig. 2) [94]. Currently, however,

only a few studies have been published on the antimicrobial activities of *β*-carboline alkaloids in general.

2.6.2. Anticancer Activity

Since a few years, *β*-carboline alkaloid (*β*C and TH*β*C) ring system has attracted significant attention due to their effective anticancer activities [95, 96, 97, 98, 99]. In 2014, Ying group in, tetrahydro-*β*-carboline/hydroxylcinnamic acid hybrids linked with different substituted nitrogen-containing heterocycles at the positions-N9 **116** were synthesized and screened for their antitumor activities against six human cancer cells including hepatoma cells (30.08-5.93μM), gastric cancer cells (32.09-6.63μM), colon carcinoma cells (28.36-9.32μM), breast adenocarcinoma cells (24.37-7.76μM), ovarian cancer cells (35.91-8.21μM), and SMMC-7721(31.69-6.45μM). Here, the analysis of SAR revealed that the antiproliferative activities suggested the electron-donating substituent (OCH_3) on the ferulic acid derivatives were able to confer antitumor activities to these molecules [100]. In the same year, Cong *et al*. reported various 2-benzoyl-1,3,4,9-tetrahydro-*β*-carboline **117a-e** through substitution at different positions to define the SAR resulted in the discovery of potent inhibitors of the transforming growth factor-β (TGFβ) signaling pathway (pivotal oncogenic pathways in most advanced cancers). Among them, compound **117d** (n=1, Ar= phenyl), one of the tested compounds, not only showed potent inhibition of lung cancer cell proliferation in vitro but also strongly suppressed growth of lung cancer and breast cancer in vivo [96].

Furthermore, novel N-substituted tetrahydro-*β*-carboline-imidazolium salt derivatives **118** were evaluated for their *in vitro* antitumor activity against a panel of human tumor cells lines (HL-60, SMMC-7721, A-549, MCF-7, SW480) and proved to be potent antitumor agents. The imidazolium salt derivatives bearing a 2-ethyl-imidazole (12.81-2.77μM), benzimidazole (15.03-3.24μM) or 5,6-dimethyl-benzimidazole ring and a 3-naphthylmethyl or 1-(naphthalen-2-yl)ethan-1-one at position-3 (17-13-2.61μM) of the imidazole ring, were found to be the most potent compounds [101].

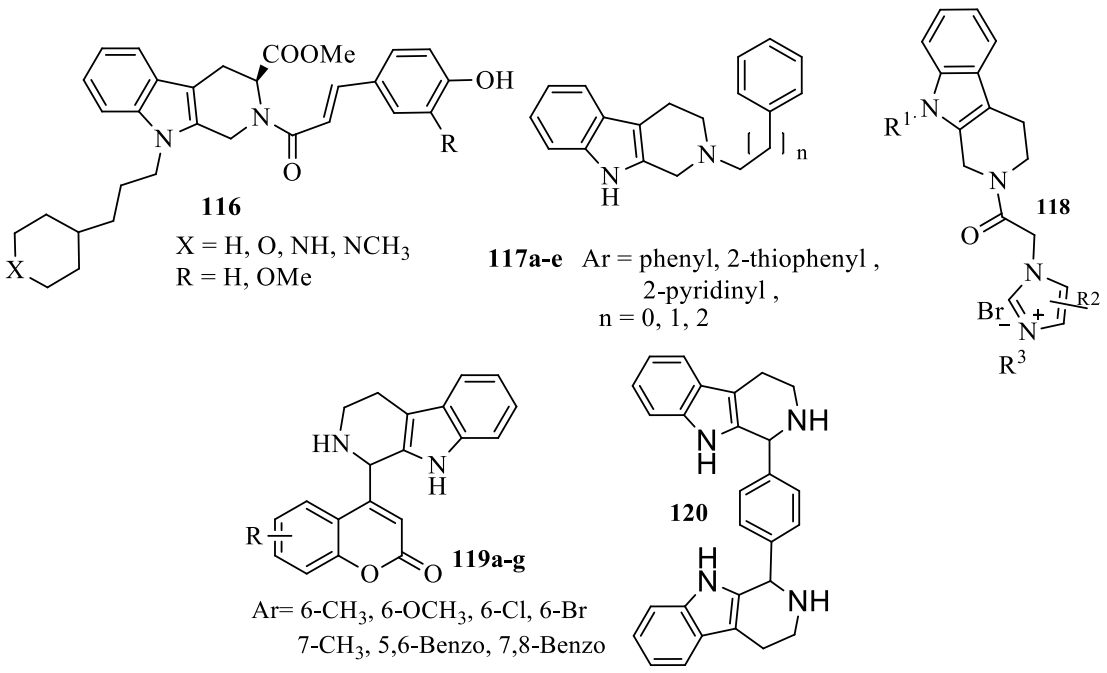


Figure 3: The chemical structure of active anticancer compounds.

Additionally, in 2016, Samundeeswari group reported C_6- and C_7-substitiuted coumarin THβCs 190a-g on coumarin moiety and only **119e** (C_7-CH_3**)** and **119f** showed appreciable activity screened for their growth inhibitory activity against 60 human cancer cell lines. Here, C_7-CH_3 substituted coumarin **119e** showed moderate activity with < 50% Growth inhibition (GI) for all human cell lines, whereas, compound **119f** (R= 5,6-Benzo) exhibited better activity with > 50% GI for nearly 15 cell lines which included renal cancer cell lines. The study concluded that substituent at C_6 and C_7 positions on coumarin enhances the anticancer activity [102]. Recently, this Samundeeswari group again come up with a promising anticancer THβC-hybrid due to their inhibition of DNA topoisomerase or CDK. Among these phenyl-1,4-bis-THβCs the racemic mixture **120** which shows a broad spectrum of growth inhibition with GI_{50} values ranges from 1.0 μM to 4.5 μM against most of the cancer cell lines (45 cell lines out of 60) (Fig. 3) [18].

2.6.3. Antioxidant Activity

Oxidative stress (which cause the generation of Reactive Oxygen Species, ROS), β-amyloid (Aβ) deposits, mitochondrial dysfunction, and low levels of acetylcholine has been implicated as a core contributor to the initiation and progression of multiple neurodegenerative diseases including Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Parkinson disease

(PD), multiple sclerosis (MS) and stroke [103, 104]. Here, the pathogenesis of several neurodegenerative diseases, including PD, AD, and MS involving the generation of reactive oxygen species and the properties of antioxidants are extensively reviewed [105, 106]. Studies have also shown that natural and synthetic TH*β*Cs possess a wide range of antioxidant activity [107, 108, 109, 110].

In 2016, Nicole *et al*., evaluated TH*β*Cs for their radical scavenging activity by monitoring their interaction with 2,2-diphenyl-1-picrylhydrazyl (DPPH). Here, compounds **121a-e** (EC_{50} = 9.17-3.17), **f** (EC_{50} = 1.83) and **g** (EC_{50} = 1.82) revealed radical scavenging activity, ranging from 50% to 74% compared to that of *α*-tocopherol [111]. Based on the topology of the active site of cholinesterases and other target proteins involved in the pathogenesis of AD, Gerard *et al*., have synthesized tacrine-trolox and tacrine-tryptoline hybrids with various linker chain lengths. The result discovered that free radical scavenging activities (studied using 1,1-diphenyl-2-picrylhydrazyl, DPPH) were not significantly affected by varying linker chain lengths and the hybrid compound containing the tryptoline moiety linked with a 7 carbons spacer to tacrine **122** displayed the best AChE and BuChE inhibitory activity (IC_{50} = 17.37 and 3.16nM) [87]. With same concept, Yifan *et al*., synthesized bivalent *β*-carboline derivatives modified by several series of hydrophobic moieties as potential neuroprotective agents for AD. The result showed **123** (R_1=CH_3, n=2) and (R_1=CH_3, n=3) exhibited the good selectivity potency on butyrylcholinesterase (BuChE) inhibition (IC_{50} =1.7 and 2.7 μM, respectively) and resulted in a marked decrease in cell viability (57.2%) due to the neuroprotective potential of the compounds on H_2O_2-induced oxidative stress on neuronal cell line SH-SY5Y [112].

Recently, Yihang and his co-workers reported the first *in vivo* (into the striatum of Wistar rats) evaluation of the neurotoxic effects of TaClo **124** causing aggressive PD from the perspective of mitochondrial dysfunction. When the changes in the mitochondrial membrane potential were measured by incubating the tissues with 5,5′6,6′-tetrachloro-1,1′3,3′-tetraethylbenzimidazole-carbocyanine iodide (JC-1 stain), TaClo impairs the function of mitochondrial complex by causing oxidative stress which is known to occur at the early stage of cell apoptosis (Fig. 4) [113]. Very recently, Ahmad *et al*., described novel TH*β*C, 2-benzoyl-6-methoxy-9-methyl-1-phenyl-1,2,3,4-tetrahydro-*β*-carboline **125**, and evaluated for *in vitro* acetylcholinesterase (AChE) inhibitory activity which showed potential AChE inhibitor with an IC_{50} value of 26.52

± 0.79 mM with relatively low cytotoxicity profile (80% of the cells were viable at all concentrations) at cellular level which can be potential candidate for AD therapeutic agents (Fig. 4) [114].

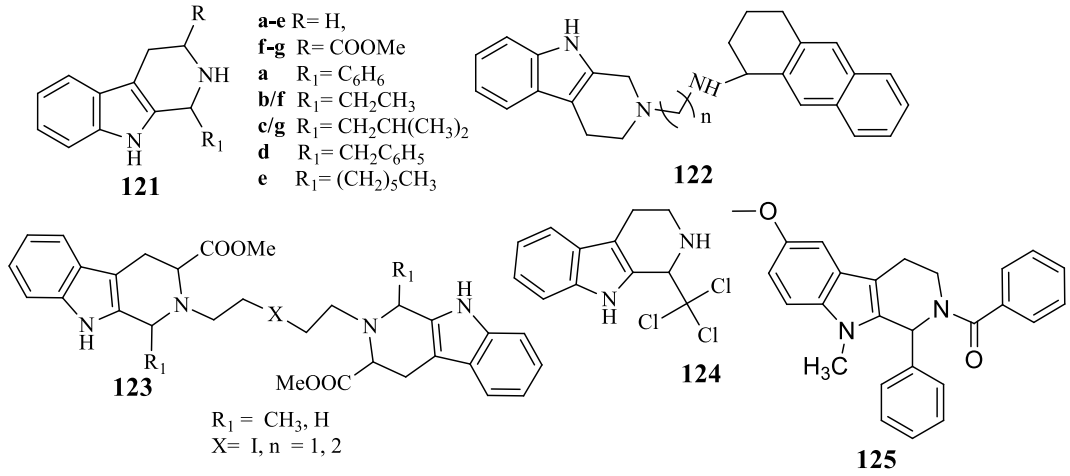


Figure 4: The chemical structure of active antioxidant compounds.

2.6.4. Anti-leishmanial Activity

Leishmaniasis is caused by intracellular protozoan *Leishmania* spp parasites and is considered as one of the most neglected tropical diseases. Due to no effective vaccines, the treatment of leishmaniasis relies on the chemotherapy approach [115, 116, 117]. From the literature quest, it was revealed that several natural and synthetic product scaffolds, including the class of *β*-carbolines have shown potential anti-leishmanial agents [118, 119].

In 2014, Sudhakar *et al*., identified tetrahydro-*β*-carboline analogs **126a-f** possessing significant antileishmanial activity against *Leishmania donovani* promastigotes. The thiophen-2-yl linked analog **126e** and naphthyl linked analog **126f** were most promising antileishmanial agents, exhibiting IC_{50} values of 9.1 and 22.1 μM, respectively [90]. Similarly, Penta *et al*. screened for 1-phenyl-N2-substitiuted tetrahydro-*β*-carboline derivatives **1271a-p** against both promastigote and amastigote forms of *L. infantum.* Here, the two analogues (R = H, $R_1 = CH_3$) and (R = *m*-NO_2, R_1 = H) exhibited selective and potent inhibition of amastigotes with IC_{50} values 0.67 and 0.87 mM respectively and potency was comparable with amphotericin B. Here, the SAR study

suggested that, substitution on *meta, ortho* positions showed favorable effect, while replacement with bulkier group had minimal effect on activity and *para* substitution was not desirable [120].

In another study, N2-substitiuted tetrazole hybrids 1,2,3,4,9-tetrahydro-*β*-carboline **128a-u** identified as potential antileishmanial chemotypes. From the analogues, compound with (R = 3,4,5 tri-OMe, R_1 = Cyclohexyl) was found to be the most active in the series having IC_{50} = 1.57 ± 0.12 μM. However, in this study, no obvious trend of activity with respect to the substituent was observed [24]. Surprisingly, results obtained from examination of anti-leishmanial potential of tetrahydro-*β*-carbolines-peptide hybrid **129** showed represent a new structural lead for anti-leishmanial chemotherapy. Most of the screened derivatives exhibited significant *in vitro* anti-leishmanial activity against promastigote and intracellular amastigotes (IC_{50} ranging from 2.43 to 7.61 μM) than the control, miltefosine (IC_{50} = 8.2 μM), with less cytotoxicity [121] (Fig. 5).

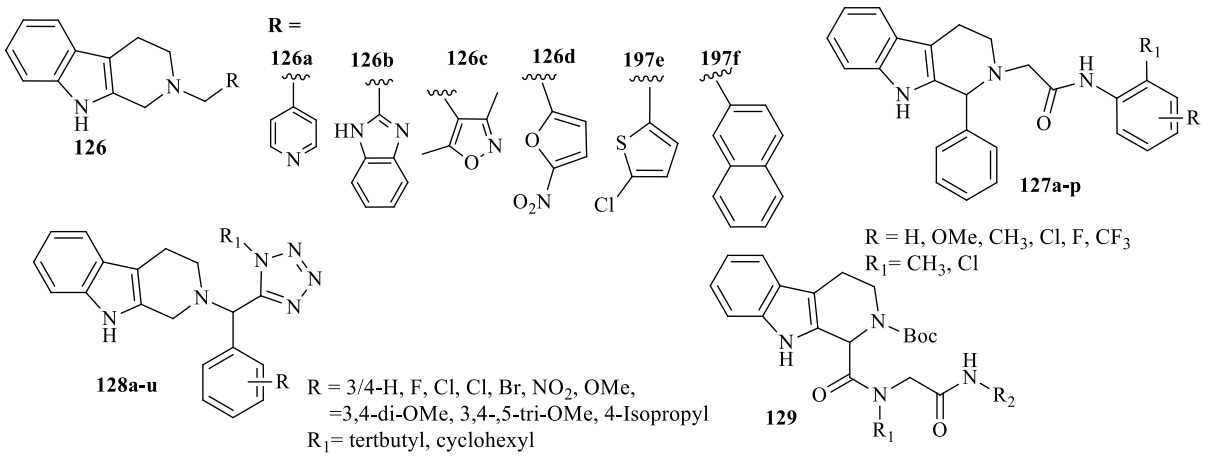


Figure 5: The structure of active antileishmanial compounds.

2.6.5. Anti-malarial Activity

The *β*-carbolines and TH*β*Cs are also reported to have a very significant anti-malarial activity [122, 123, 124, 125]. In 2014, Lydia *et al*., evaluated for antimalarial activity of 1,2,3,4-tetrahydro-*β*-carboline analogues against a chloroquine-resistant strain of *P. falciparum*. Amongst the analogues **130a** (9.6 μM) and **130b** (17.2 μM) exhibited either comparable or enhanced antimalarial activity versus the corresponding fully aromatic *β*-carboline structure [126].

Varun and his co-workers reported *in vivo* antimalarial potency of two novel TH*β*Cs **131 a** and **131b** (5 μg/mL) against *Plasmodium berghei*. Based on the results, the compounds were categorized as highly active against the chloroquine (CQ) sensitive (NK-65) strain of rodent malaria parasite *P. berghei* with IC_{50} = 5 μg/mL, a comparable inhibitory activity with the standard drug CQ (10 μM) and leucovorin (5μg/mL) exhibited [127]. Recently, in 2020, Scott *et al*. evaluated a library of tetrahydro-*β*-carboline derivatives **132** by appending various aromatic substitutions in order to make additional SAR study against *P. falciparum*. Among the series, the lead compound, a 5-chloro-TH*β*C derivative (R = Me), displayed modest activity and low toxicity against human cells. According to the SAR study, replacing the methyl group of the lead compound with a phenyl ring (R = C_1-Ph) allows for additional hydrophobic interactions, giving most phenyl-substituted derivatives improved activity [128]. In additions, Bharvi *et al.* investigated anti-malarial activity of TH*β*C-Quinoline conjugates linked via either 1H-1,2,3-triazole **133a-b** (which has a favorable influence on the anti-plasmodial activity) or a substituted acyl hydrazide-core **133a-b** for their *in vitro* anti-plasmodial evaluation on CQ resistant W2 strain of *P. falciparum*. However, the introduction of hydrazine core not only diminished the activities but also resulted in increased cytotoxicity against mammalian Vero cells [129](Fig. 6).

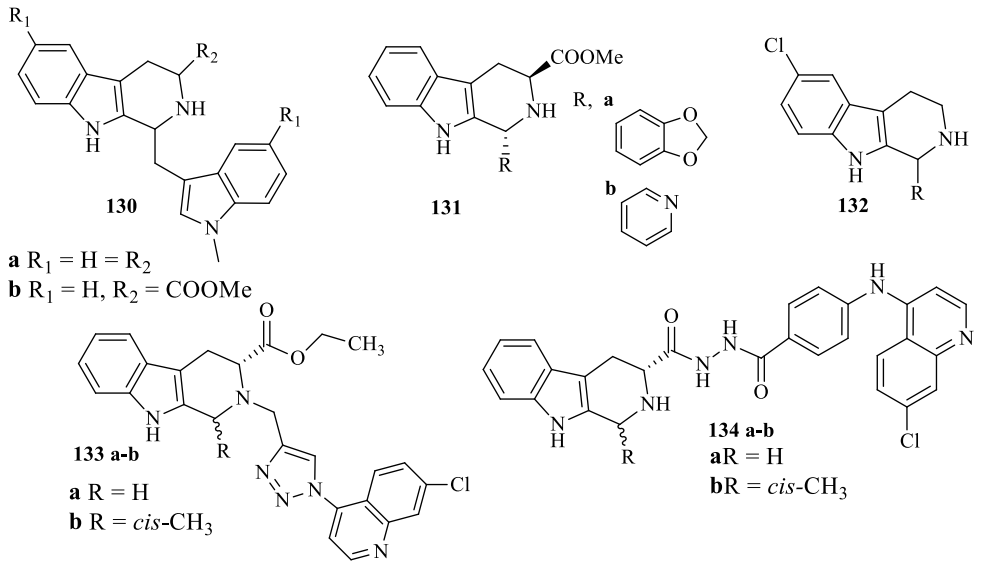


Figure 6: The structure of some anti-malarial compounds.

2.6.6. Anti-inflammatory Activity

However poorly characterized, naturally occurring and synthetic *β*-carboline alkaloids are reported to have a significant inhibitory activity [130, 131, 132]. In 2014, Samir and his co-workers reported anti-inflammatory activity of **135** and found to exhibit potent histone deacetylase (HDAC) inhibition (IC_{50} = 43.9nM) due to their effects on cell death acting through acetylation of non-histone proteins [133]. *In vivo* anti-inflammatory activity of (2′S,5′S)-tetrahydropyrazino[1',2':1,6]-di{2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole}-1',4'-dione **136** was assayed on xylene induced ear edema mouse model. The minimal effective dose of THPDTPI inhibiting the inflammation was 0.001 μmol/kg [134].

Recently, Michel group described the tetracyclic TH*β*C derivatives as highly potent histone deacetylase 6 (HDAC6) inhibitors for its anti-inflammatory activity. Inhibitor potency was determined in an invitro assay on purified human HDAC6 protein. Here, compounds **137a-d** exhibited low nanomolar IC_{50} -values while **138** displayed an IC_{50} value lower than 1 nM. These outcomes indicated that the 6S,12aR **138** configuration is preferential and lead to greater inhibitory potency [135]. Le-Ling and his co-workers identified the pentacyclic TH*β*C from *Nauclea officinalis* plant and evaluated its *in vitro* anti-inflammatory activity on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW264.7 cell. compounds showed significant anti-inflammatory activity. According to the result, compounds **139** showed a significant anti-inflammatory activity with an IC_{50} value of 2.85±0.67μM [136](Fig. 7).

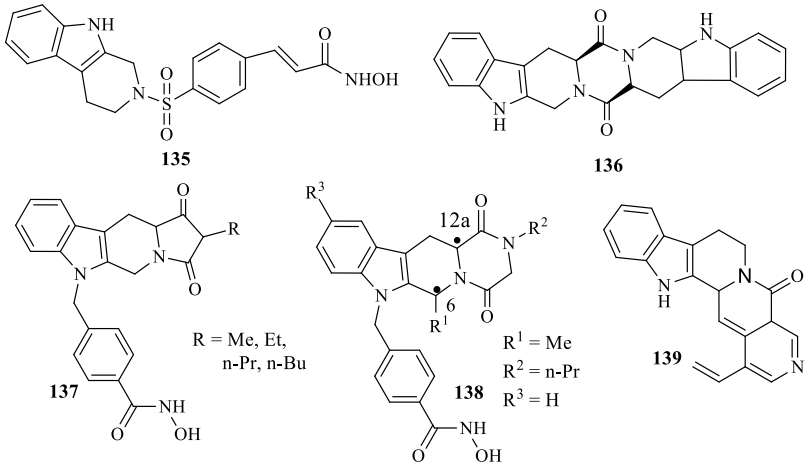


Figure 7: The structure of some active anti-inflammatory compounds.

CHAPTER 3

3. MATERIALS AND METHOD

3.1. Reagents and Chemicals Used

Solvents and chemicals were purchased from commercially available suppliers (Fine chemical and Firmament chemical supplier, Addis Abeba) and were used as received without further purification. Distilled water, ethyl acetate (98.0%), sodium bicarbonate, methanol (99.8%), petroleum ether (>99.9%), dichloromethane (99.5%), silica gel (60-120 mesh), L-tryptophan (98.0%), formaldehyde (37.0%), benzaldehyde(99.0%), *p*-hydroxybenzaldehyde(99.0%), 4-hydroxy-3 methoxybenzaldehyde, acetic acid, trifluoro acetic acid(99.5%), sulfuric acid, ammonium hydroxide, 2, 2-dipphenyl-1 picrylhydrazyl (DPPH), and anhydrous sodium sulfate were chemicals used in this research work.

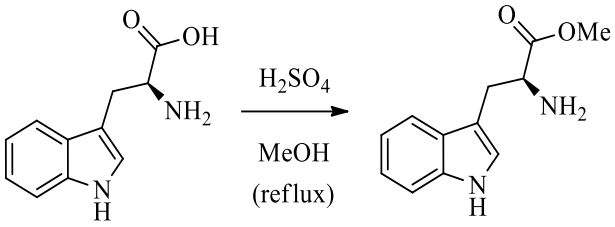
3.2. Apparatus and Instruments

Melting points were determined in an open capillary using digital melting point apparatus, expressed in $^{\circ}$C. Reaction progress was checked on pre-coated TLC plates and spots were visualized using UV lamp at 254 and 365 nm. Silica gel (60-120 mesh, Merck grade) has been used for silica column chromatography. The column was subjected to gradient elution by increasing gradient of ethyl acetate in petroleum ether and dichloromethane and spots were visualized under UV lamp (254 nm). The synthesized compounds were characterized on the basis of physical and spectral analysis. The UV-Vis spectrum of synthesized compound **30** was recorded on Double-beam UV-Vis spectrophotometer using DCM and MeOH as blank solvents and λ_{max} values were expressed by nm. The ^{1}H and ^{13}C NMR spectra of the compounds were recorded on Bruker avance 400 MHz NMR spectrophotometer using Chloroform-*d* or Methanol-d_4 as the solvent and the values are expressed in δ ppm.

3.3. Experimental Procedures

3.3.1. Synthesis of L-tryptophan methyl ester

In a three-neck round bottom flask, to a suspension of commercial DL-tryptophan **27 (**2.00 g, 24.5 mmol) in methanol (70 mL, 98%) dropwise concentrated sulfuric acid was added until complete solubilization. The reaction mixture was kept under reflux (60-65 ºC) for 24h and its progress monitored by TLC (EtOAc: MeOH: NH_3/ 5: 2: 1). The solution was allowed to cool to room temperature and neutralized with a solution of 5% sodium bicarbonate ($NaHCO_3$), and extracted with ethyl acetate (2 x 30 mL). The organic phase was dried over anhydrous sodium sulfate (Na_2SO_4) and after filtration the solvent was removed in a rotary evaporator to afford **30** [23].



27 **30**

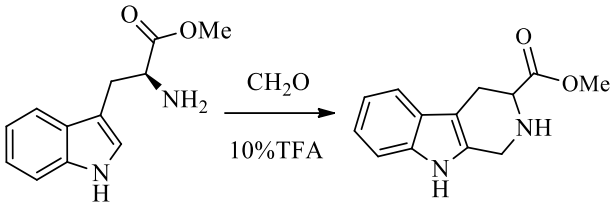
Scheme 25: Synthesis of L-tryptophan methyl ester **30**.

3.3.2. General procedure for the synthesis of 1,2,3,4-tetrahydro-*β*-carboline-3-carboxylic acid methyl esters

L-tryptophan methyl ester (240 mg, 1.1 mmol), and formaldehyde was dissolved in CH_2Cl_2 (70 mL) and cooled to 0 °C in an ice bath. To this solution, 5% triflouroacetic acid (TFA, 0.5mL) was added dropwise and the mixture was stirred at room temperature for about 24h and completion of the reaction was monitored by TLC. The reaction mixture was then basified with dilute NH_4OH solution and extracted with CH_2Cl_2 (3×50 mL). The organic layer was washed with water, brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure to afford compound **140** [137].

Spectral data of compound **140**:

[1]H NMR (400 MHz, Chloroform-*d*) spectrum δ_H 3.94–3.86 (m, 2H), 2.10 (brs, NH), 3.68 (dd, J = 5.7, 3.6 Hz, 1H), 3.15-3.18 (dd, J = 7.9, 3.0 Hz, 2H), 7.53 (d, J = 7.6 Hz, 1H), 7.78 (d, J = 8.1 Hz, 1H), 7.18 (t, J = 9.1 Hz, 1H), 7.46–7.38 (dd, 1H), 3.62 (s, 3H) and **[13]**C-NMR (100 MHz, $CDCl_3$) δ_H 43.6, 59.1, 24.7, 107.2, 127.2, 117.9, 119.6, 121.9, 109.3, 137.1, 131.6, 55.7, 173.5.



30 **140**

Scheme 26: Synthesis of 1,2,3,4-tetrahydro-*β*-carboline-3-carboxylic acid methyl ester.

3.3.3. General procedure for the preparation of 1-substitiuted 1,2,3,4-tetrahydro-*β*-carboline-3-carboxylic acids (142a-c).

L-Tryptophan (2.0 g, 10 mmol) was added to a 250 mL round-bottom flask containing 40 mL acetic acid. This was followed by the addition of aromatic aldehydes (10 mmol, benzaldehyde for **142a**, *p*-hydroxybenzaldehyde for **142b,** and vanillin for **142c**). The mixture was heated to reflux for 2-3 hrs under until all of the tryptophan was consumed as indicated by TLC. The reaction mixture was then cooled to rt, and adjusted pH to 5 with concentrated ammonium hydroxide, the precipitated product was collected by filtration and washed well with water and then dried [138]. The residue was chromatographed on silica gel to furnish compound **142a**, **142b**, and **142c**.

Spectral data of this compound **142a**:

[1]H NMR (400 MHz, Methanol-d_4) δ_H 8.43 (s, COOH), 8.23 (d, *J* = 8.0 Hz, 1H, H-5), 8.12 – 8.03 (m, 1H, H-8), 7.63 (dd, *J* = 7.0, 10.7 Hz, 1H, H-7), 7.62 – 7.59 (m, 1H, H-6), 7.13 (d, *J* = 8.0 Hz, 2H, H-2'6'), 6.93 (dd, *J* = 11.1, 7.5 Hz, 1H, H-3'5'), 6.81 – 6.74 (m, 1H, H-4'), 5.21 (s, 1H, H-1), 4.20 – 4.05 (m, 1H, H-3), 3.46 – 3.45 (m, 2H, H-4), 2.12 (s, 1H, NH, piperidine) and

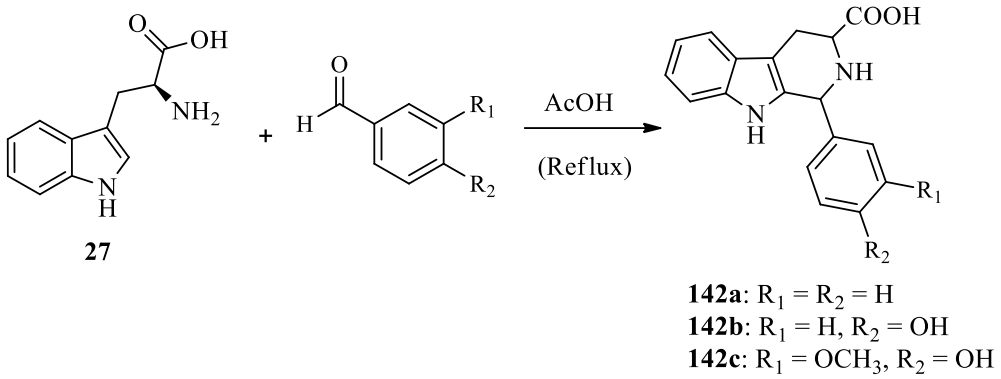
^{13}C NMR (100 MHz, Methanol-d_4) δ_c 58.5, 55.5, 22.3, 108.3, 127.5, 118.1, 119.7, 122.9, 111.7, 136.9, 137.5, 132.9, 130.0, 130.3, 129.2, 173.9

Spectral data of this compound **142b**:

^{1}H NMR (400 MHz, Methanol-d_4) $\delta_{\mathbf{H}}$ 10.74 (s, 1H), 10.27 (s, 1H), 7.85 (d, J = 7.9 Hz, 1H), 7.65 (d, J = 8.1 Hz, 2H), 7.46 (t, J = 6.6 Hz, 1H), 7.41 – 7.37 (m, 1H), 7.24 (d, J = 8.2 Hz, 1H), 6.27 (d, J = 7.0 Hz, 2H), 4.91 (s, 1H), 3.91 – 3.81 (m, 1H), 3.61 (d, J = 5.7 Hz, 2H), 1.60 (s, 1H, NH, piperidine) and **^{13}**C-NMR (101 MHz, Methanol-d_4) $\delta_{\mathbf{c}}$ 56.7, 54.4, 20.6, 105.3, 124.1, 116.1, 117.7, 120.7, 109.6, 138.76, 129.7, 135.7, 126.5, 114.0, 157.7, 167.9.

Spectral data of this compound **142c**:

^{1}H NMR (400 MHz, Methanol-d_4) δ_H 9.84 (s, 1H), 8.30 (s, 1H), 8.10 (d, J = 8.0 Hz, 1H), 7.96 (d, J = 12.8 Hz, 1H), 7.53 (dd, J = 7.8, 1.9 Hz, 1H), 7.29 (d, J = 6.5 Hz, 1H), 7.21 – 7.05 (m, 1H), 7.01 (d, J = 6.9 Hz, 1H), 6.81 (dd, J = 11.1, 7.5 Hz, 1H), 5.09 (s, 1H), 4.08 (dd, J = 7.1 Hz, 1H), 3.78 (s, 3H), 3.36 – 3.29 (m, 2H), 2.00 (s, 1H, NH, piperidine) and **^{13}**C-NMR (101 MHz, Methanol-d_4) $\delta_{\mathbf{c}}$ 56.7, 56.2, 29.6, 108.0, 127.8, 118.0, 119.3, 121.6, 112.2, 134.6, 137.0, 131.0, 113.9, 149.7, 147.2, 111.4, 120.5, 171.8, 54.2



Scheme 27: Synthesis of substituted THβ-carboline derivatives via conventional method.

3.4. Spectroscopic Techniques

Physical and spectroscopic techniques were used to characterize the synthesized compounds. NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer operating at 400 MHz

using Methanol-d_4 and Chloroform-*d*. A region from 0 to 12 ppm for ^{1}H and 0 to 190 ppm for ^{13}C was employed for scanning. Chemical shifts are reported in δ units and coupling constants (*J*) in hertz (Hz). Multiplicities of ^{1}H NMR signals are indicated as *s*, *d*, *dd*, *t* and *m*. UV-Vis spectrum were recorded in range of 200-700 nm.

3.5. Determination of Antibacterial Activity

In vitro antibacterial susceptibility test was determined by disc diffusion method [139] against the bacterial species representing Gram negatives (*E. coil, and P. aeruginosa*) and Gram positives (*S. aureus* and *S. pyogenes*). The bacterial strains were obtained from Adama regional microbiology laboratory. The medium was prepared by dissolving 38 g of Mueller Hinton agar medium in 1000 mL of distilled water and autoclaved at 121 °C for 15 min. The autoclaved medium was poured into sterile plates (20-25 mL/plate) and the plates were allowed to solidify under sterile condition at room temperature. After solidification, the plates were seeded with overnight grown culture approximately 1.5 X 10^8 CFU/mL by swabbing evenly on to the surface of the medium with a sterile cotton swab.

The synthesized compounds at the concentrations of 0.5 mg/mL and 1.0 mg/mL per disc was prepared by dissolving in DMSO. Whatman filter paper no. 1 was used to prepare discs approximately 6 mm in diameter. The sterile discs were infused with the synthesized compounds and position on the surface of the medium with sterile forceps and gently pressed down to ensure contact with the MHA. Control experiments were carried out under similar conditions using ciprofloxacin for antibacterial activity as a standard drug. The plates were inverted and then incubated at 37 °C for 24 hrs. After incubation the inhibition zone produced by the synthesized compounds were evaluated by measuring the diameter (mm) of the clear zone around the disc against the test organisms using a ruler. The results were expressed as mean value ± standard deviation. The antibacterial activities of synthesized compounds were done in collaboration with the Department of Applied Biology, Adama Science and Technology University.

3.6. Antioxidant activity

DPPH radical-scavenging assay (RSA): The DPPH assay was used to assess the free radical scavenging activity of the synthesized compounds. The free radical scavenging activity of the

synthesized compounds were measured by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) method [140]. With this method, it is possible to determine the radical scavenging power of an antioxidant by measuring the decrease in the absorbance of DPPH at 517 nm.

The synthesized compounds were dissolved in four vials containing methanol to give 100, 50, 25 and 12.5µg/mL. To 1 mL of each concentration, 4 mL DPPH (0.004% DPPH in MeOH) was added to give 20, 10, 5 and 2.52 µg/mL. The resulting solution was placed in an oven at 37^{o}C for 30 minutes and subjected to UV-Vis spectrophotometer to record absorbance at 517 nm. Ascorbic acid was used as a reference standard and dissolved in methanol to make the same concentration. For the control experiment, methanol was added with DPPH. Finally, the absorbance values were measured and the antioxidant activities were calculated using the following equation [141].

$$\% \text{ of Radical scavenging activity} = \frac{\text{Abcontrol} - \text{Abanalyte}}{\text{Abcontrol}} x\ 100$$

; where $Ab_{control}$ is the absorbance of the control, and $Ab_{analyte}$ is the absorbance of samples.

Results obtained were expressed as percentage decrease with respect to DPPH control results.

3.7. Molecular docking study of synthesized compounds

To have a better understanding about the inhibitory mechanism as well as the mode of interactions of the synthesized compounds, *in silico* antibacterial and antioxidant molecular docking analysis will be accomplished using the ADT version 1.5.2 and AutoDock version 4.2 docking program. The crystal structure of the enzyme (PDB code 1KZN) with resolution 2.3 Å will be chosen as the protein model. The structures of ligands will be optimized using the HyperChem 7.0 software. Auto Dock version 4.2 will be used to prepare the molecules and parameters before submitting it for docking analysis with Auto Dock. Polar hydrogen atoms will be added while non-polar hydrogen atoms will be merged and then, Gasteiger partial atomic charges will be assigned to the ligands. All rotatable bonds of ligands, defined by default of the program, will be allowed to rotate during the automated docking process and then prepared protein and ligand structures will be saved in the PDBQT format suitable for calculating energy grid maps. A grid box size of 46×46×46 Å points with a grid spacing of 0.375 Å will be considered. Lamarckian genetic algorithm (LGA) program with an adaptive whole method

search in the Auto Dock will be chosen to calculate the different ligand conformers. After 200 independent docking runs for each ligand, a cluster analysis will be done. In according to the root mean square deviation (RMSD) tolerance of 2.0 Å conformations will be clustered and will be ranked by energy of which the conformation with the best scored pose with the lowest binding energy will be selected for these ligands [142, 143]. The molecular docking study was done in Adama Science and Technology University by Rajalakshaanan Eswaramoorthy (PhD).

CHAPTER 4

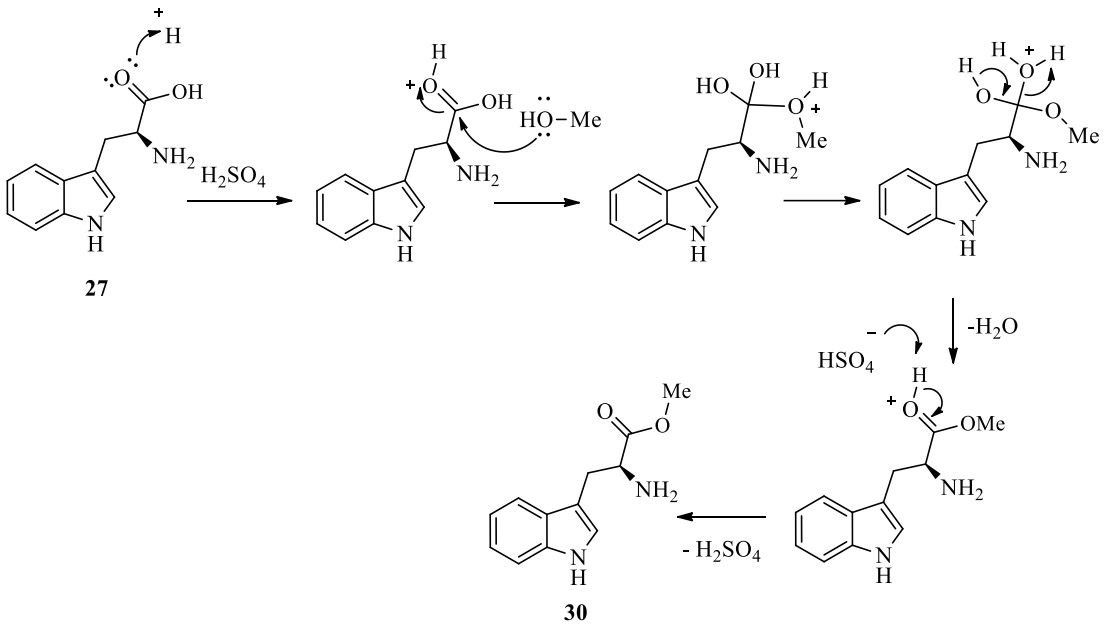
4. RESULTS AND DISCUSSION

4.1. Synthesis of the target compounds

The target compounds were synthesized according to schemes 28-30. L-tryptophan methyl ester **30**, the starting material for 1,2,3,4-tetrahydro-*β*-carboline-3-carboxylic acid methyl ester **140** was prepared by methylating L-tryptophan using methanol activated by acid under reflux for 24 hrs.

To drive the equilibrium to L-tryptophan methyl ester, excess alcohol is added following Le-Chatelier's Principle. In addition, the reaction was catalyzed by an acid which enhances the electrophilic nature of the carbonyl carbon of the carboxylic acid moiety. The tetrahedral intermediate formed by the attack of the alcohol can then isomerize by means of proton migration, to allow water to behave as a leaving group. Loss of water yields a carbocation stabilized by resonance, which need only lose a proton to give the L-tryptophan methyl esters **30**, and regenerate the acid catalyst. Water is driven off under this non-equilibrium conditions until the final ester percentage reached. The residual carboxylic acid could be isolated and separated from the corresponding ester by treatment with aqueous 5% $NaHCO_3$. Yield was estimated on the basis of initial weight of the raw materials taken and the final weight of the ester obtained after the complete drying.

In this esterification reaction, o-methylation of L-tryptophan where carboxylic acid moiety was heated with alcohol in the presence of concentrated sulfuric acid is both slow and reversible routes. Compared to the previous report, the reaction affords lower yield however with comparably fast reaction time [23]. The detailed reaction mechanism of the synthesis of L-tryptophan methyl ester is shown in scheme 28.

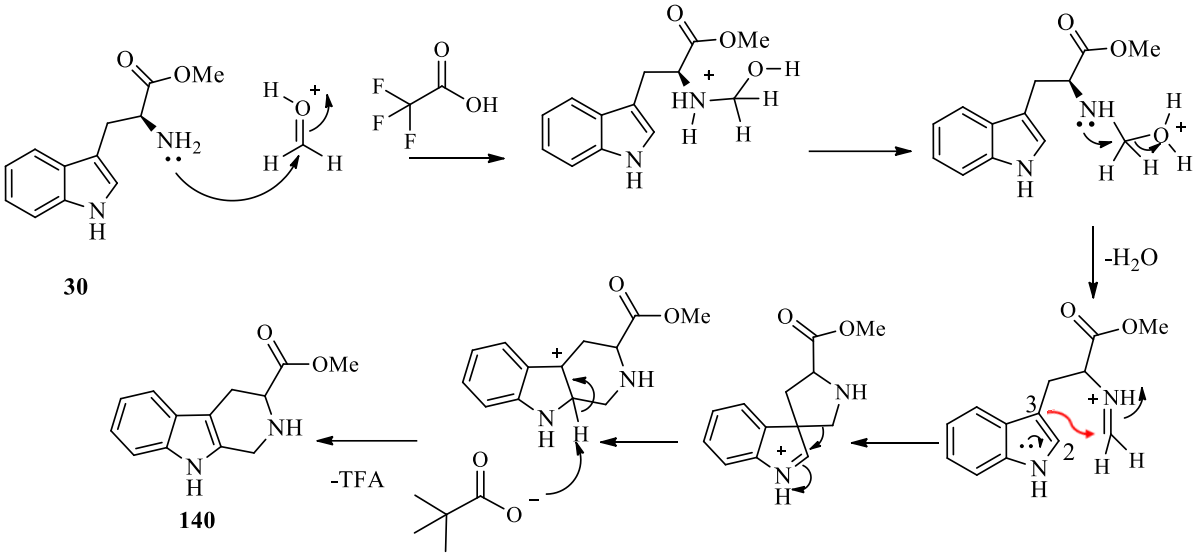


Scheme 28: Mechanism for synthesis of L-tryptophan methyl ester.

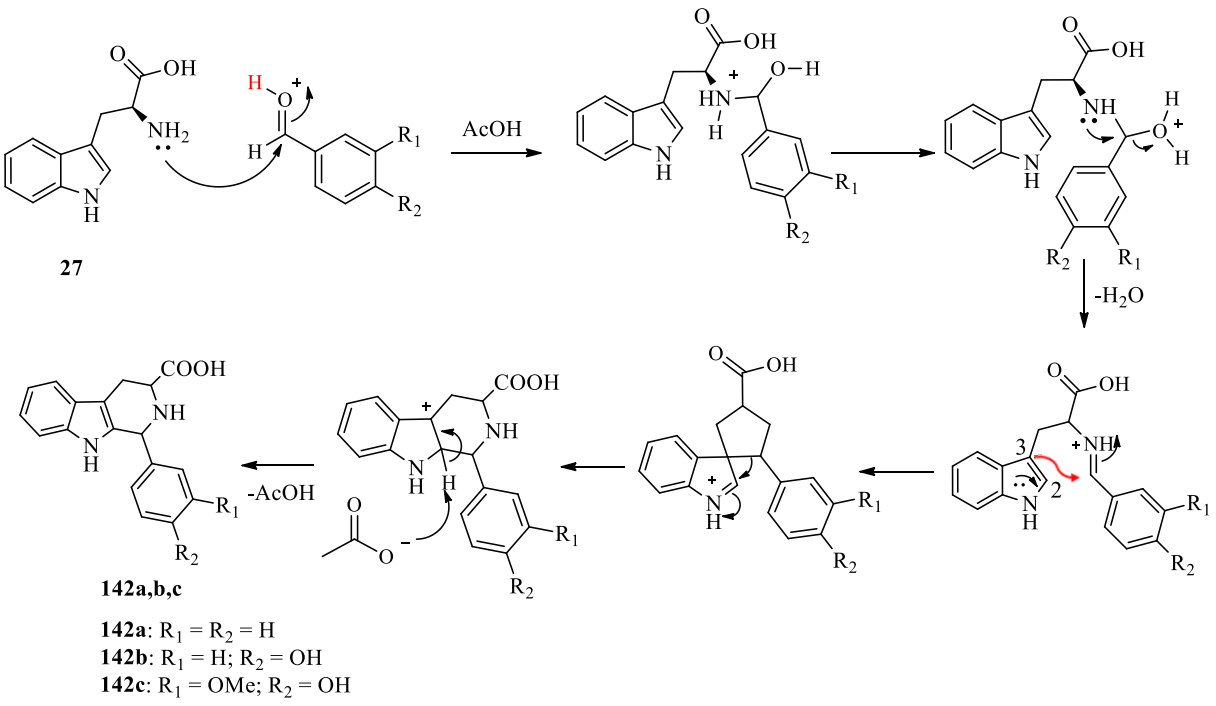
Compound **140** was synthesized after compound **30** was prepared and dried well. The synthetic strategy began with the Pictet-Spengler condensation of L-tryptophan methyl ester **30** with formaldehyde under acidic condition which affords tetrahydro-*β*-carbolines **140**. Here, the method of reaction produced higher product yield compared to the previously reported [137]. Even though this system was fruitful in terms of short reaction times and good product yields, there still is a need for the development of more efficient and ecofriendly methodologies.

In the synthesis of **140** and **142a-142c** Pictet-Spengler reaction was applied. The Pictet-Spengler reaction is essentially a two-step reaction in which the reaction involved the participation of the indole ring as a result of its π-excessive nature. First, an electron-rich aromatic amine (tryptophane **27** and tryptophane methyl ester **30**) and an aldehyde condense to form an iminium species (scheme 29 and scheme 30). Regardless of which path occurs, it is the electrophilic nature of the imine double bond which is the driving force of the cyclization [144, 145, 146]. Then, an electrophilic aromatic substitution reaction occurs in which the aryl amine attacks the electrophilic iminium to yield a positively charged intermediate which is then deprotonated to yield the *β*-carboline product. Notably, both carbons 2 and 3 of tryptophane and/or tryptophane methyl ester are nucleophilic. Therefore, after iminium formation, the reaction proceeds either by attack of carbon 2 to directly yield the six-membered ring intermediate or by attack of carbon

3 to yield a spiroindolenine intermediate that would undergo a 1,2-alkyl shift to form the product **140**, **142a**, **142b**, and **142c**.



Scheme 29: Mechanism for the synthesis of 1,2,3,4-tetrahydro-*β*-carboline-3-carboxylic acid methyl ester.



Scheme 30: Mechanism for the synthesis of tetrahydro-*β*-carboline-3-carboxylic acid derivatives.

In this regard, three tetrahydro-*β*-carboline were synthesized using L-tryptophan as a substrate and aromatic aldehydes (benzaldehyde, *p*-hydroxybenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde) in the presence of acetic acid as a catalyst and solvent **(**Scheme 30). These tetrahydro-*β*-carboline were 1-phenyl-1,2,3,4-tetrahydro-*β*-carboline-3-carboxylic acid (**142a)**, 1-(4-hydroxy) phenyl-1,2,3,4-tetrahydro-*β*-carboline-3-carboxylic acid (**142b)**, and 1-(4-hydroxy-3 methoxy) phenyl-1,2,3,4-tetrahydro-*β*-carboline-3-carboxylic acid (**142c)**. The overall reaction involving the formation of tetrahydro-*β*-carboline and the proposed mechanism is indicated (scheme 30).

With sensible choice of tryptophan derivatives (**142b** and **142c**) good yields for the Pictet–Spengler products was obtained under the given condition compared to the previous reports [138, 147]. Another interesting feature of our studies was that aryl aldehydes bearing electron-donating groups successfully underwent the Pictet–Spengler reaction with ease.

4.2. Characterization of tetrahydro-*β*-carboline derivatives

4.2.1. Physical properties of the synthesized compounds

Physical constants and percent yield of the synthesized compounds are presented in each respective section. Progress of the reactions were monitored using TLC. The R_f values for each of the synthesized compounds were calculated. The spots on the TLC plate were visualized using UV lamp at 254 nm. The % yield was estimated on the basis of initial weight of the raw materials taken and the final weight of the dry product obtained after purification from column chromatography.

4.2.2. Characterization of the synthesized compounds

NMR spectra were recorded on a 'Bruker-400' instrument with tetramethylsilane (TMS) as the internal standard and Methanol-d_6 and $CDCl_3$ as a solvent. The summarized characteristic of ^1H and ^{13}C-NMR chemical shifts for each compound are discussed below.

4.2.2.1. Characterization of compound 30

Compound **30**, $C_{12}H_{15}N_2O_2$, was obtained as brown crystalline needle with melting point of 154-156 ºC, R_f 0.67/EtOAc:MeOH: NH_3 (3:2:1) solvent system and 46 % yield. The UV-Vis

spectrum (200-800 nm, Chloroform) showed maximum absorption at 273 nm and 223 nm, attributable to n→π* and π→π* transitions, respectively (**Appendix 1**).

4.2.2.2. Characterization of compound 140

Compound **140**, $C_{12}H_{14}N_2O_2$, was synthesized as brown crystalline needle with 89.0% yield and a melting point of 130-135 ºC. The R_f value was recorded as 0.77 with EtOAc: MeOH (5:2:1) solvent system.

The ^{1}H -NMR (400 MHz, $CDCl_3$) spectrum (**Table 1, Appendix 2**) showed the presence of four proton signals in the aromatic region and five proton signals in aliphatic region. The presence of aromatic ring A of the *β*-carboline was confirmed on basis of doublet peaks observed at δ_H 7.53 (d, J = 7.6 Hz, 1H, H-5), and 7.78 (d, J = 8.1 Hz, 1H, H-6), while the remaining peaks were observed at δ_H 7.18 (t, J = 9.1 Hz, 1H, H-7), and 7.46–7.38 (dd, 1H, H-8). The signals observed at δ_H 3.86 – 3.94 (m, 2H) is accounted for methylene proton of (H-1) and δ_H 3.68 (dd, J = 5.7, 3.6 Hz, 1H) is due to sp3 methine (H-3) connected to carboxyl moiety. The two diasterotopic methylene protons (H-4) appeared at δ_H 3.15-3.18 (dd, J = 7.9, 3.0 Hz, 2H) and a broad singlet peak at δ_H 2.10 (brs, 1H) is accounted to 2-NH. A characteristic peak at δ_H 3.62 (s, 3H) is attributed to mehyl ester protons.

The ^{13}C-NMR (100 MHz, $CDCl_3$) spectral data of compound **140** (Table 1, **Appendix 3**) with the support of DEPT-135 revealed the presence of well resolved thirteen carbon signals of which one is due to carbonyl carbons with the signal at δC 173.5. The spectrum also exhibited the presence of four aromatic sp^2 quaternary signals at δ_C 107.2(4a), 127.1(4b), 131.6(9a), 137.1(8a) which were suppressed in DEPT-135 spectrum. The signals at δ_C 43.6 and 24.7 were ascribed to C-1 and C-4 respectively which are accounted for methylenes also pointing down in DEPT-135 spectrum. The peaks at δc 55.7 suggest methyl ester preak whereas peak at δ 59.5 suggest methine (-CH-) of C-3. The aromatic sp^2 methines appeared at δ_C 121.8, 119.6, 117.9, and 109.3 are accounted for C-7, C-6, C-5, C-8 respectively.

The DEPT-135 (101 MHz, $CDCl_3$) spectrum showed signal at δ 55.7 for methyl ester peak and aromatic methines appeared at δ 121.77, 119.55, 117.90, 109.32, 24.66 for CH carbons and 59.5, and 43.6 for CH and CH_2 carbons respectively (**Appendix 4**). Thus, based on the above spectral

data generated and comparison with literature, the structure of compound was suggested as follows.

Table 1: ^{1}H, ^{13}C and DEPT-135 NMR spectral data of compound **140**.

Position	**142a**			**Ref** [148]	
	^{1}H	**^{13}C**	**DEPT-135**	**^{1}H**	**^{13}C**
1	3.94 – 3.86 (m, 2H)	43.6	43.58	4.30-4.08(m,2H)	40.7
NH	2.10 (brs, NH)	-	-	2.05(s, NH)	-
3	3.68 (dd, *J* = 5.7, 3.6 Hz, 1H)	59.1	59.1	3.81-3.85(m,1H)	54.7
4	3.15-3.18 (dd, *J* = 7.9, 3.0 Hz, 2H)	24.7	24.66	2.89-3.14(2H)	24.2
4a	-	107.2	q	-	105.5
4b	-	127.2	q	-	126.0
5	7.53 (d, *J* = 7.6 Hz, 1H)	117.9	117.9	7.15(1H)	116.6
6	7.78 (d, *J* = 8.1 Hz, 1H)	119.6	119.6	7.48(d, 1H)	118.2
7	7.18 (t, *J* = 9.1 Hz, 1H)	121.9	121.9	7.10(1H)	120.5
8	7.46 – 7.38 (dd, 1H)	109.3	109.3	7.30(d, 1H)	119.8
8a	-	137.1	q	-	135.0
9a	-	131.6	q	-	130.6
OCH_3	3.62 (s, 3H)	55.7	55.7	3.79 (s, 3H)	51.1
CO	-	173.5	q	-	173.2

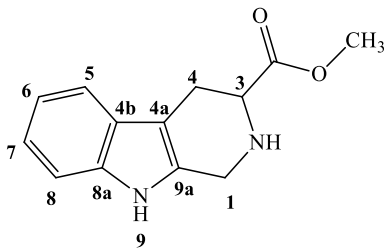


Figure 8: The structure of 1,2,3,4-tetrahydro-*β*-carboline-3-carboxylic acid methyl ester (**140)**.

4.2.2.3. Characterization of compound 140a

Compound **140a**, $C_{18}H_{16}N_2O_2$, was synthesized as white solid with 64.0 % yield melted at 150-153 ^oC which agreed with previously reported value for the same compound [148]. The R_f value was recorded as 0.66 with EtOAc: MeOH: NH_3 (4:2:1) solvent system.

The ^1H NMR (400 MHz, Methanol-d_4) spectrum (**Table 2, Appendix 5**) showed the presence of seven proton signals in the aromatic region and five proton signals in aliphatic region. The presence of aromatic ring of the β-carboline was confirmed on the basis of peaks observed at δ_H 8.23 (d, J = 8.0 Hz, 1H, H-5, 7.62–7.59 (m, 1H, H-6), 7.63 (dd, J=7.0, 10.7 Hz, 1H, H-7), 8.12–8.03 (m, 1H, H-8). The most downfield proton signals observed at δ_H 8.43 (brs, 1H), is accounted to the acidic proton, COO<u>H</u>. Mono substituted aromatic protons are clearly evident at δ_H 7.13 (d, J = 8.0 Hz, 2H, H-2'6'), 6.93 (dd, J = 11.1, 7.5 Hz, 1H, H-3'5') and δ_H 6.81–6.74 (m, 1H, H-4'). The signals observed at δ_H 5.21 (s, 1H) was accounted for methine (H-1) whereas peak at 4.20–4.05 (m, 1H) is attributed to methine proton (H-3) connected to carboxyl moiety. The two diastereotopic protons at δ_H 3.46–3.45 (m, 2H) accounted to H-4 whereas NH-pipieridine signal was observed at δ_H 2.12 (brs, 1H).

The ^{13}C-NMR (100 MHz, Methanol-d_4) spectral data (Table x, **Appendix 6**) with the aid of DEPT-135 revealed the presence of well resolved sixteen carbon signals of which one is due to carbonyl carbon at δ_C 173.96. The carbon signals due to sp^2 quaternary carbons were observed at δ_C 108.3(4a), 127.5(4b), 131.5(9a), 137.9(8a), and 132.9(C-1') as their signal's intensity were suppressed in DEPT-135. The signal at δ_C 22.3 belong to methylene (C-4), also confirmed by DEPT-135 pointing down. The signals at δ_C 58.5 and 55.5 belong to methines C-1 and C-3 respectively which are accounted for methine (-CH-). The aromatic sp^2 methines appeared at δ_C 118.1(5), 119.7, 122.9, 111.7, 130.0, 130.3, and 129.2 that are assigned to C-5, C-6, C-7, C-8, (C-2'6'), (C-3'5'), and (C-4'), respectively.

The DEPT-135 (101 MHz, Methanol-d_4) spectrum showed signal at δc 118.1, 119.7, 122.9, 111.7, 130.0, 130.3, and 129.2 for CH carbons and δc 22.3 for CH_2 carbon (**Table 2**, **Appendix 7**). The NMR spectral data reported herein is in good agreement with the NMR spectral data previously reported for the same compound.

Table 2: ^{1}H, ^{13}C and DEPT-135 NMR spectral data of compound **142a**.

Position	**142a**			
	^{1}H-NMR δ in ppm and J in Hz	^{13}C-NMR δ in ppm	NMR (DEPT-135) (δ,ppm)	Multiplicity
1	5.21 (s, 1H)	58.5	58.5	CH
2	2.12 (s, 1H, NH)	-	-	
3	4.20 – 4.05 (m, 1H, H-3	55.5	55.5	CH
4	3.46 – 3.45 (m, 2H)	22.3	22.3	CH_2
4a	-	108.3	q	q
4b	-	127.5	q	q
5	8.23 (d, *J* = 8.0 Hz, 1H, H-5	118.1	118.1	CH
6	7.62 – 7.59 (m, 1H, H-6)	119.7	119.7	CH
7	7.63 (dd, *J* = 7.0, 10.7 Hz, 1H)	122.9	122.9	CH
8	8.12 – 8.03 (m, 1H, H-8)	111.7	111.7	CH
8a	-	136.9	q	q
9a	-	137.5	q	q
1'	-	132.9	q	q
2'6'	7.13 (d, *J* = 8.0 Hz, 2H, H-2'6')	130.0	130.0	CH
3'5'	6.93 (dd, *J* = 11.1, 7.5 Hz, 1H)	130.3	130.3	CH
4'	6.81 – 6.74 (m, 1H)	129.2	129.2	CH
COOH	8.43 (s, 1H)	173.9	q	q

Therefore, according to the characterized spectral data given above, the given compound best fit with 1-phenyl-1,2,3,4-tetrahydro-*beta*-carboline-3-carboxylic acid (**142a, Figure 9**).

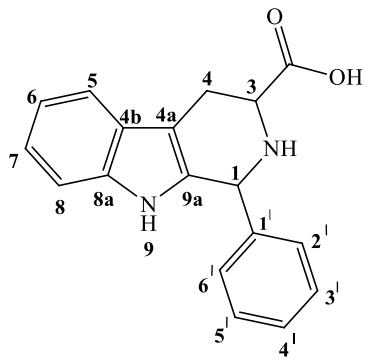


Figure 9: 1-phenyl-1,2,3,4-tetrahydro-*β*-carboline-3-carboxylic acid (**142a**).

4.2.2.4. Characterization of compound 142b

Compound **142b**, $C_{18}H_{16}N_2O_3$, was synthesized as white solid with 83.0% yield and a melting point (278-283 ºC). The R_f value was recorded as 0.45 with EtOAc: MeOH: NH_3 (3:2:1) solvent system.

The ^{1}H NMR (400 MHz, Methanol-d_4) spectrum (**Table 3, Appendix 8**) showed the presence of six proton signals in the aromatic region and six proton signals in aliphatic region. A broad singlet at δ_H 10.27 is due to carboxylic proton. The most downfield proton signals observed at δ_H 10.74 (brs, 1H) is accounted to H-N of indole. From the four-aromatic proton of *β*-carboline ring, two of the protons appeared at 7.85 (d, J = 7.9 Hz, H, H-5), 7.24 (d, J = 8.2 Hz, 1H, H-8) as doublet while one aromatic at δ_H 7.41 – 7.37 (m, 1H, H-6) is multiplate and the remaining fourth aromatic proton triplet appeared at 7.46 (t, J = 6.6 Hz, 1H, H-7). The presence of AA'XX' spin system aromatic ring was observed at δ_H 6.27 (J = 7.0 Hz, 2H, H-2', 6') and 7.65 (J = 9.0, 8.1 Hz, 2H, H-3', 5') suggesting a para substituted phenyl ring. The signals observed at δ_H 4.91 (s, 1H) accounted for alicyclic proton of H-1 and 3.91-3.81 (m, 1H) is due to H-3. Methylene (H-4) and NH-pipieridine signals were observed at δ_H 3.61 (d, J = 5.7 Hz, 2H) and 1.60 (s, 1H), respectively.

The proton decoupled ^{13}C-NMR (101 MHz, Methanol-d_4) spectrum (**Table 3**, **Appendix 9**) with aid of DEPT-135 (**Appendix 10**) showed the presence sixteen well resolved carbon resonances of which eight are due to methine, seven are quaternary and the other is due to carbonyl carbon. The carbon signals due to quaternary carbons were observed at δ_C 105.3, 124.1, 129.7, 138.8, and 167.9 (carbonyl carbon) which are ascribed to 4a, 4b, 8a, 9a, and CO respectively as their signal's intensity were suppressed in DEPT-135. The presence of methine alpha to carbonyl (C-

3), methine connected to nitrogen (C-1), and methylene carbon was confirmed at δ 54.4, 56.7, and 20.6, respectively. The presence of six aromatic peaks were observed between δ 109.6-135.7 of which two of them are quaternary at δ 135.7 (C-1') and 157.7(C-4'). The remaining peaks were observed at δ 116.1 (C-5), 117.7(C-6), 120.7 (C-7), 109.6 (C-8), and 126.5(C-2'6'), 114.0 (C-3'5') suggest the presence of aromatic ring.

The DEPT-135 (101 MHz, Methanol-d_4) spectrum showed signal at δc 56.7(C-1), 54.4(C-3), 116.1 (C-5), 117.7(C-6), 120.7 (C-7), 109.6 (C-8), 126.5(C-2'6'), and 114.0 (C-3'5') for CH carbons and δc 20.6 for CH_2 carbon (**Table 3**, **Appendix 10**). Therefore, according to the characterized spectral data given, the compound best fit with1-(4-hydroxy) phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**142b**).

Table 3: ^{1}H, ^{13}C and DEPT-135 NMR spectral data of compound **142b**.

Position	**142b**			
	^{1}H-NMR δ in ppm and J in Hz	^{13}C-NMR δ in ppm	^{13}C NMR (DEPT-135) (δ,ppm)	**Multiplicity**
1	4.91 (s, 1H)	56.7	56.7	CH
2	1.60 (s, 1H, Piperidine)		-	NH
3	3.91 – 3.81 (m, 1H)	54.4	54.4	CH
4	3.61 (d, *J* = 5.7 Hz, 2H)	20.6	20.6	CH_2
4a	-	105.3	-	q
4b	-	124.1	-	q
5	7.85 (d, *J* = 7.9 Hz, H)	116.1	116.1	CH
6	7.41 – 7.37 (m, 1H)	117.7	117.7	CH
7	7.46 (t, *J* = 6.6 Hz, 1H)	120.7	120.7	CH
8	7.24 (d, *J* = 8.2 Hz, 1H)	109.6	109.6	CH
8a		138.76	-	q
9a	-	129.7	-	q
1'	-	135.7	-	q
2'6'	6.27 (d, J=7.0 Hz, 2H)	126.5	126.5	CH

3'5'	7.65 (d, *J* = 8.1 Hz, 2H, H-3'5')	114.0	114.0	CH
4'	-	157.7	-	q
COOH	10.27 (brs, 1H, COOH)	167.9	-	COO<u>H</u>
9	10.74 (s, 1H, indole)	-	-	N<u>H</u>

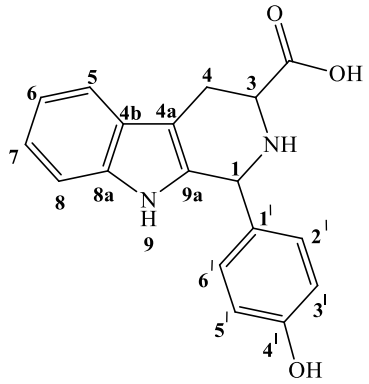


Figure 10: 1-(4-hydroxy) phenyl-1,2,3,4-tetrahydro-*β*-carboline-3-carboxylic acid (**142b**).

4.2.2.5. Characterization of compound 142c

Compound **142c**, $C_{19}H_{18}N_2O_4$, was synthesized as white solid with 85.0 % yield and a melting point of 238-242 ºC which agreed with previously reported for the same compound [147]. The R_f value was recorded as 0.71 with EtOAc: P. Ether (4:1) solvent system.

^{1}H NMR (400 MHz, Methanol-d_4) spectrum (**Table 4, Appendix 11**) showed the presence of seven proton signals in the aromatic region and seven proton signals in aliphatic region. The proton signals observed at δ_H 9.84 (brs, 1H) is accounted to COOH. From the four-aromatic proton of *β*-carboline ring, two of the protons appeared at δ_H 8.10 (d, *J* = 8.0 Hz, 1H, H-5) and 7.96 (d, *J* = 12.8 Hz, 1H, H-8) as doublet while one aromatic proton at δ_H 7.53 (dd, *J* = 7.8, 1.9 Hz, 1H, H-6) appeared as doublet of doublet and the fourth aromatic proton appeared at 7.21 – 7.05 (m, 1H, H-7) as multiplate. ABX spin system aromatic ring was observed at δ_H 7.29 (d, *J* = 6.5 Hz, 1H, ArH-2'), 7.01 (d, *J* = 6.9 Hz, 1H, H-5') and δ_H 6.81 (dd, *J* = 11.1, 7.5 Hz, 1H, H-6'). The signals observed at δ_H 5.09 (s, 1H) and 4.08 (dd, 1H) accounted for methine protons of H-1 and H-3, respectively. Methylene protons and NH-pipieridine signals were observed at δ_H 3.36-3.29 (m, 2H) and at δ_H 2.00 (s, 1H), respectively. A characteristic δ_H 3.78 (s, 3H) belong to methoxy proton.

The ^{13}C-NMR (101 MHz, Methanol-d_4) spectrum (**Table 4**, **Appendix 12**) with aid of DEPT-135 (**Appendix 13**) showed the presence nineteen well resolved carbon resonances of which nine are due to methine, eight are quaternary and the other is due to carbonyl carbon and methyl carbon. The carbon signals due to quaternary carbons were observed at δ_C 108.0, 127.8, 134.6, 137.0, and 171.8 which are ascribed to 4a, 4b, 8a, 9a, and CO (carbonyl carbon) respectively as their signal's intensity were suppressed in DEPT-135. The presence of methine alpha to carbonyl (C-3), methine connected to nitrogen (C-1), and methylene carbon (C-4) were confirmed at δ_C 56.2, 56.7, and 29.6 respectively. The presence of ten aromatic peaks were observed between δ_C 111.4-149.7 of which three of them are quaternary at δ_C 130.0 (C-1'), 149.7(C-3'), and 147.2(C-4'). The remaining peaks were observed at δ_C 118.0(C-5), 119.3(C-6), 121.6(C-7), 112.9(C-8), 113.9(C-2'), 111.4(C-5'), and 120.5(C-6') suggest the presence of aromatic ring. The presence of peaks at δ 54.2 ppm suggests the presence of methoxyl carbon signal.

The DEPT-135 (101 MHz, Methanol-d_4) spectrum showed signal at δc 56.7, 56.2, 118.0, 119.3, 121.6, 112.9, 113.9, 111.4, 120.5 for CH carbons and δc 29.6 for CH_2 carbon δc 54.24 for -OCH_3 (**Appendix 13**). Thus, based on the above NMR spectral data reported herein and comparison with literature [147, 149], the structure of compound was suggested as follows here below

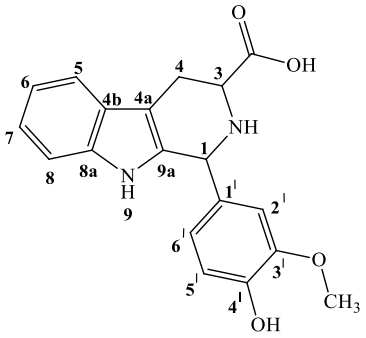


Figure 11: 1-(4-hydroxy-3-methoxy) phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**142c**).

Table 4: ^{1}H, ^{13}C and DEPT-135 NMR spectral data of compound **142c**.

Position	**Compound 142c**			**Ref** [147, 149]	
	^{1}H-NMR δ in ppm and J in Hz	**^{13}C**NMR δ in ppm	^{13}C NMR (DEPT-135) (δ,ppm)	**^{1}H**	**^{13}C**
1	5.09(s, 1H)	56.7	56.7	5.48(s, 1H)	58.9
2	2.00 (s, 1H)	-	-	1.90 (s, 1H)	-
3	4.08 (dd, *J* = 7.1 Hz, 1H)	56.2	56.2	3.64(1H)	57.2
4	3.36 – 3.29 (m, 2H)	29.6	29.6	2.94-3.13(2H, dd)	25.8
4a	q	108.0	-		108.9
4b	q	127.8	-		127.4
5	8.10 (d, *J* = 8.0 Hz, 1H)	118.0	118.0	7.42(d, 1H)	118.4
6	7.53 (dd, *J* = 7.8, 1.9 Hz, 1H)	119.3	119.3	7.04(1H)	119.8
7	7.21 – 7.05 (m, 1H)	121.6	121.6	6.97(1H)	122.1
8	7.96 (d, *J* = 12.8 Hz, 1H)	112.2	112.2	7.24(d, 1H)	111.2
8a	q	134.6	-		135.2
9a	q	137.0	-		136.3
1'	q	131.0	-		132.7
2'	7.29 (d, *J* = 6.5 Hz, 1H)	113.9	113.9	7.02(1H, d)	114.5
3'	q	149.7	-	-	147.3
4'	q	147.2	-	-	146.2
5'	7.01 (d, *J* = 6.9 Hz, 1H)	111.4	111.4	6.71(1H, d)	110.9
6'	6.81 (dd, *J* =11.1,7.5 Hz, 1H)	120.5	120.5	6.48(dd,1H)	121.8
COOH	9.84 (s, 1H)	171.8	-	-	173.5
4'-OH	8.30 (s, 1H)	-	-	8.9-9.4(s, OH)	-
OCH_3	3.78 (s, 3H)	54.2	54.2	3.76(s, 3H)	56.2

4.3. Antibacterial Activity

The antibacterial activity of the synthesized tetrahydro-*β*-carboline derivatives were determined by screening against two Gram-negative strains (*E. coli, and P.aeruginosa*) and two Gram-positive strains (*S. aureus and S. pyogenes)* using the disc diffusion method. Ciprofloxacin was used as positive control. The zone of inhibition values indicated that all the compounds exhibited a varied range of 7.3-15.0 mm of antibacterial activities against all the tested bacterial strains. In comparison, the antibacterial activity of compounds at 0.5 mg/mL was weaker than at 1.0 mg/mL suggesting that the activity displayed has a dose-dependent manner (Table 5).

The results obtained in this study revealed that all the studied compounds presented antibacterial activity. Compound **142c** showed strong activity with a maximum zone of inhibition of 15±1mm, 14.67±1.5mm, 13.3±1.5mm, and 13±2mm against *P. aeruginosa, E. coli, and S. aureus and S. pyogenes,* respectively. Compound **142b** showed the second most strong activity against *E. coli* (14.3±2.1mm), *P. aeroginosa* (14.3±1.53mm), *S. aureus* (13±2.0mm), *S. pyogenes* (14.3±1.53mm). Compound **142a** showed weak activity comparably, with zone of inhibition against *E. coli* (10.67±1.53mm), *P. aeroginosa* (12.67±0.58mm), *S. aurous* (8.67±0.58mm), and *S. pyogenes* (12.3±2.1mm).

Table 5: Zone of bacterial growth inhibition diameter (mm). SD = standard deviation.

		Inhibition diameter (mm) ±SD			
Samples	Conc.[n] (mg/mL)	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. pyogenes*
Compound **140**	0.5	9.67±0.58	8±1	7.3±0.58	10±1
	1.0	10.67±1.53	12.67±0.58	8.67±0.58	12.3±2.1
Compound **142a**	0.5	12±1	11.3±1.5	10.3±0.58	11.67±0.58
	1.0	14±1.0	14.67±0.5	10.3±0.58	14.67±0.58
Compound **142b**	0.5	11.3±1.2	11.0±0.0	8.3±0.58	11.3±0.58
	1.0	14.3±2.1	14.3±1.53	13±2.0	14.3±1.53
Compound **142c**	0.5	12±1	12.67±0.58	9.3±0.58	11±1
	1.0	14.67±1.5	15±1	13.3±1.5	13±2
Ciprofloxacin	0.5	15.3±0.57	14.67±0.57	15.67±1.53	16.3±1.15
	1.0	21±1	20±2	19.3±1.13	19±1

Further comparison was also made with literature reported for the compounds at different concentrations (0.1mg/mL, 0.05mg/mL, 0.025 mg/mL) against pathogenic bacterial strains such as, *S. aureus*, and *E. coli* [22]. The literature report revealed zone inhibition diameter of compounds **142c and 142b;** substituted with 3-methoxy-4-hydroxy and 4-hydroxy groups respectively are found to effectively inhibit the growth of microbial cultures of *E. coli, S. aureus* in good comparison to the standard drugs.

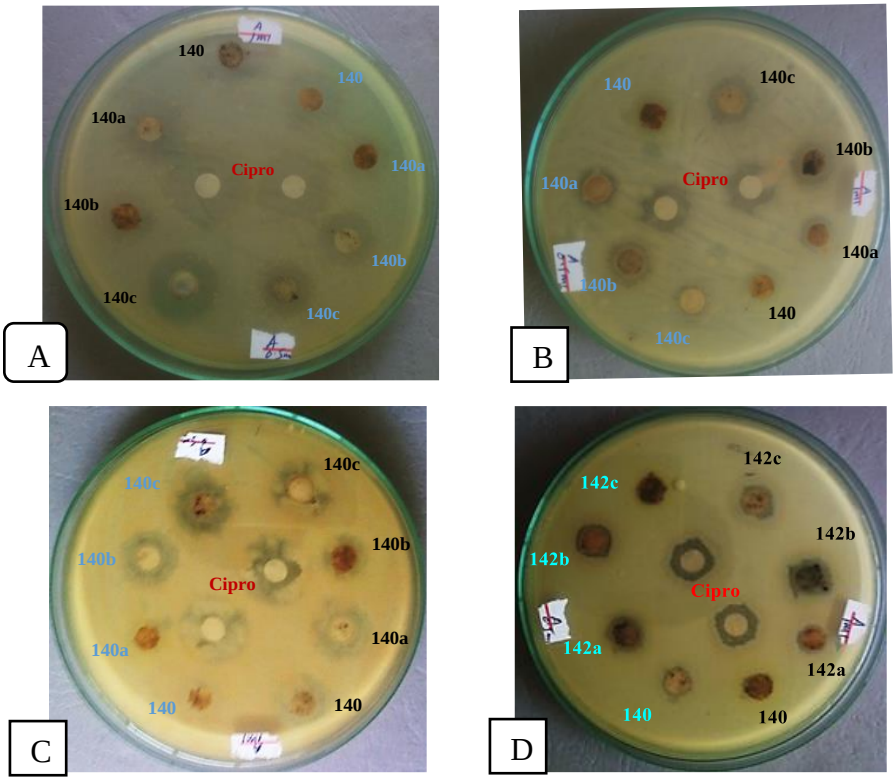


Figure 12: Disc diffusion antibacterial activity evaluation of the synthesized compounds against *P. aeruginosa* (A), *S. pyogenes* (B), *E. coli* (C), and *S. aureus* (D).

4.4. DPPH radical scavenging assay

The DPPH assay was used to assess the free radical scavenging activity of the synthesized compounds. DPPH antioxidant assay is based on the ability of 1,1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. A decrease in the absorbance of the reaction mixture indicates significant free

radical scavenging activity of the compound under test. *In vitro* antioxidant assay of tetrahydro-*β*-carboline revealed the presence of antioxidant potential. The percentage of inhibition was observed in all the antioxidant models that free radicals were scavenged by the synthesized compounds in a concentration dependent manner up to the given concentration. The data are compared to those obtained with reference compound ascorbic acid. The relationship between different analogues of the synthesized compounds and their scavenging activity for the DPPH radical is shown in **table 6**.

All of the investigated substances showed moderate to high radical scavenging activities compared with absorbance of the control (1.06). The DPPH radical-scavenging activities at 100µg/mL were 90.0%, 92.0%, 91.0% and 95.0% for **140**, **142a**, **142b** and **142c**, respectively. The order of inhibition was as follows: **142c**>**142a**>**142b**>**140**. Compound **142c** showed a significantly higher percentage of inhibition and compound **142b** scored the second most potent percentage of inhibition whilst compound **142a** displayed a lower percentage inhibition of DPPH. The lowest DPPH radical scavenging rate was shown by **142a** at a concentration of 12.5 µg/mL and 25 µg/mL, which is only 74% and 75%. The scavenging activity of tetrahydro-*β*-carboline could be attributed to its hydrogen-donating ability.

Table 6: % Radical scavenging activity of tetrahydro-*β*-carboline.

Conc. (µg/mL)	**Compound 140**		**Compound 142a**		**Compound 142b**		**Compound 142c**		**Standard**	
	A	%RSA	A	%RSA	A	%RSA	A	%RSA	A	%RSA
12.5	0.182	83	0.280	74	0.190	82	0.134	87	0.062	91
25	0.154	86	0.273	75	0.148	86	0.110	90	0.009	94
50	0.129	88	0.151	86	0.113	90	0.062	94	0.007	95
100	0.111	90	0.086	92	0.092	91	0.045	95	0.005	98

A= Absorbance; Conc. = Concentration**;** %RSA= Radical scavenging activity.

The synthesized compounds showed a good ability as radical scavengers in investigations using the DPPH method supported by different moieties which could be a significant contributor to antioxidant activity of TH*β*C. The introduction of phenolic hydroxyl and methoxy groups promoted the antioxidant activities of the lead structure. In comparison to Nicole *et al*., report, from the seven investigated C-1 aryls substituted TH*β*C derivatives, including compound **140**,

exhibited radical scavenging activities ranging from 50% to 74% compared to those of α-tocopherol which is lower than the antioxidant activity reported here. [111].

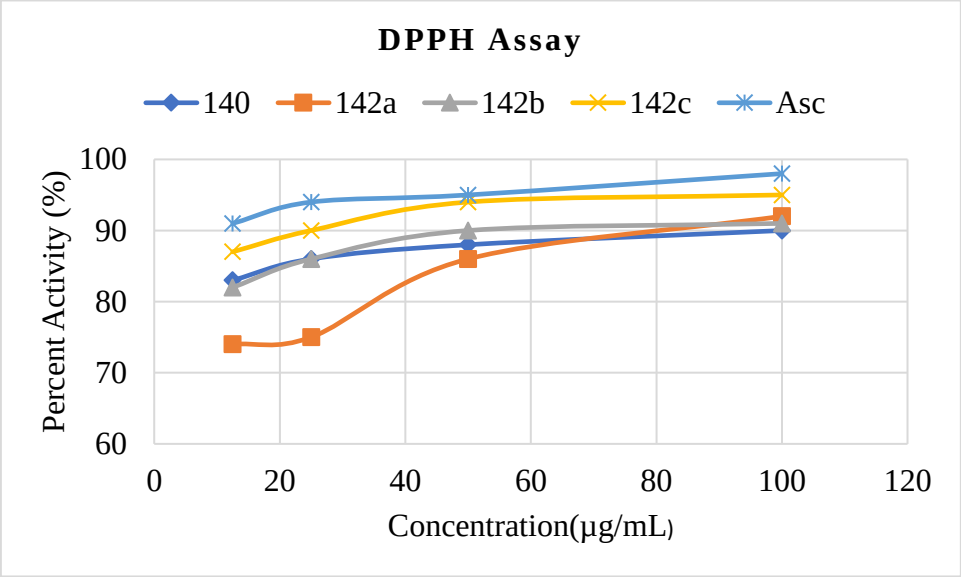


Figure 13: DPPH scavenging activity (%) of the synthesized compound and the positive reference (L-Ascorbic acid).

4.5. Docking Study

In the help of *in vitro* bacterial and activity studies, *in silico* study was conducted to predict the orientation and binding affinity at the active site of the receptor in the prediction of synthetic inhibitors as antibacterial drug candidate. In this regard, the molecular docking analysis for the synthesized compounds was performed to elucidate their binding interactions with bacterial (*E. coli*) enzyme DNA gyrase. The binding affinity, H-bond, and residual interaction of the synthesized compounds and ciprofloxacin is summarized in **Table 7**.

In the docking study, all the compounds were found to have minimum binding energy ranging from -6.1 to -6.9 kcal/mol. From the synthesized compounds, the best result was achieved using compound **142c** (-6.9 kcal/mol) compared with standard clinical drug ciprofloxacin (-7.2 kcal/mol). On comparative basis, compounds **140** scored minimum binding energy of -6.1 while both compound **142a** and **142b** scored better activity of -6.4 kcal/mol. All the compounds have showed hydrogen bonding interaction with amino acid Thr-165 in common. Compounds **140**

(Glu-50, Asn-46), **142a** (Gly-77), and **142c** (Asp-73) have additional hydrogen bonding interaction with amino acid residues. Compound **142c** (Asp-73, Asn-46, Thr-165) docking results were partially matching the clinical drug ciprofloxacin (Asp-73, Arg-76, Thr-165) interactions with amino acid residues. Compared to ciprofloxacin, the synthesized compounds showed similar residual hydrophobic interactions profile with amino acid residues Ile-78, Asn-46, Glu-50. Besides, all these docked compounds exhibited more residual Van der Walls interaction than the standard drug ciprofloxacin. The *in-silico* interaction results are a best fit with *in vitro* antimicrobial analysis of the synthesized compounds against *E. coli*.

Table 7: Molecular docking value of synthetic compounds against *E. coli* DNA gyrase B(PDB ID: 6F86).

Compounds	**Affinity (kcal/mol)**	**H-bond**	**Residual Amino acid Interactions**	
			Hydrophobic/Pi-Cation/Pi-Anion/ Pi-Alkyl interactions	**Van-der Walls interactions**
140	-6.1	Glu-50, Thr-165, Asn-46	Pro-79, Ile-78	Asp-73, Ala-47, Gly-77, Val-43
142a	-6.4	Gly-77, Thr-165	Ala-47, Asn-46, Ile-78, Ile-94, Val-167	Asp-73, Val-43, Gly-75, Pro-79
142b	-6.4	Thr-165	Asn-46, Ile-78, Ala-53, Glu-50, Arg-76	Asp-73, Asp-49, Ala-47, Pro-79, Gly-77
142c	-6.9	Asp-73, Asn-46, Thr-165	Ile-78, Pro-79, Glu-50	Ala-47, Ile-94, Gly-75
Ciprofloxacin	-7.2	Asp-73, Arg-76, Thr-165	Glu-50, Gly-77, Ile-78, Asn-46	Ala-47

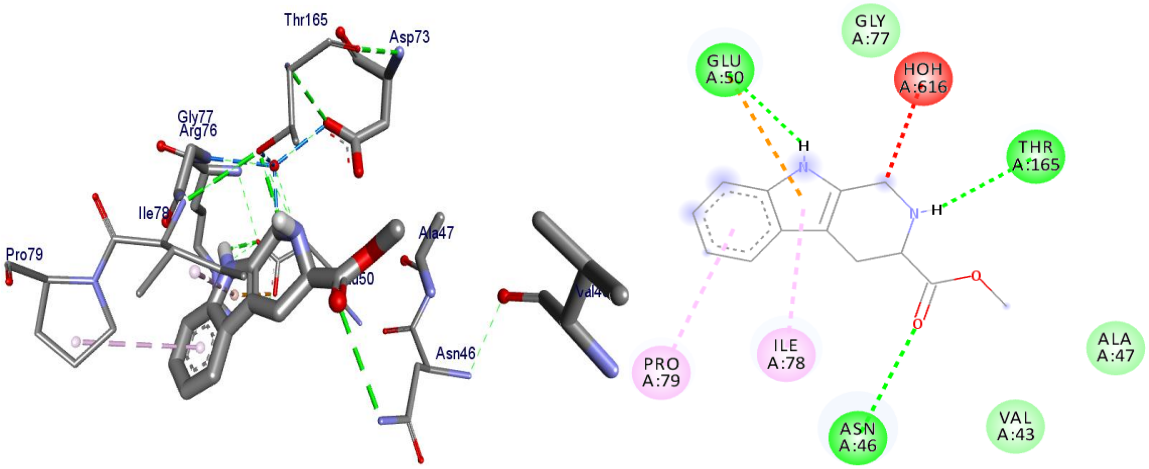


Figure 14: The binding interactions of **140** against *E. coli* DNA gyrase B (PDB ID: 6F86).

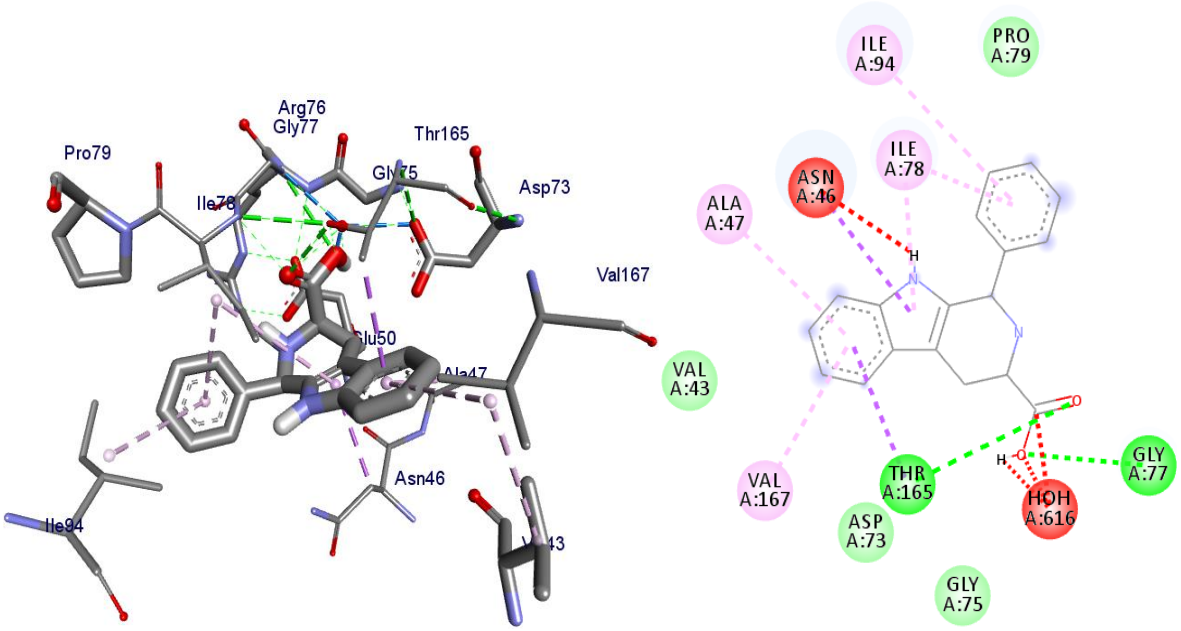


Figure 15: The binding interactions of **142a** against *E. coli* DNA gyrase B (PDB ID: 6F86).

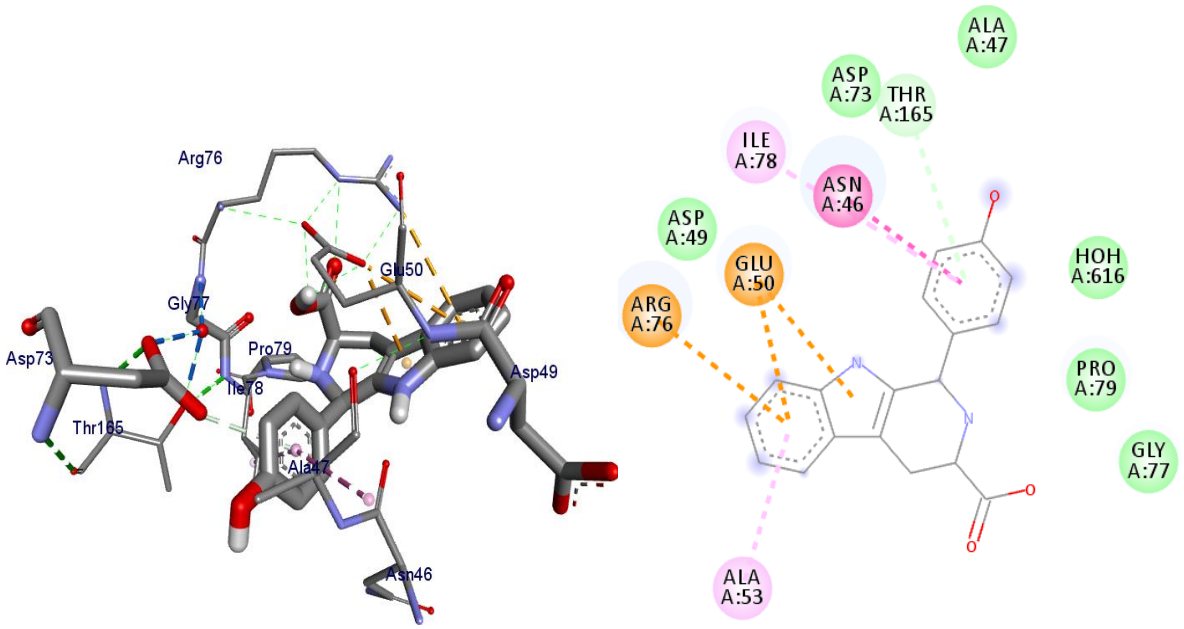


Figure 16: The binding interactions of **142b** against *E. coli* DNA gyrase B (PDB ID: 6F86).

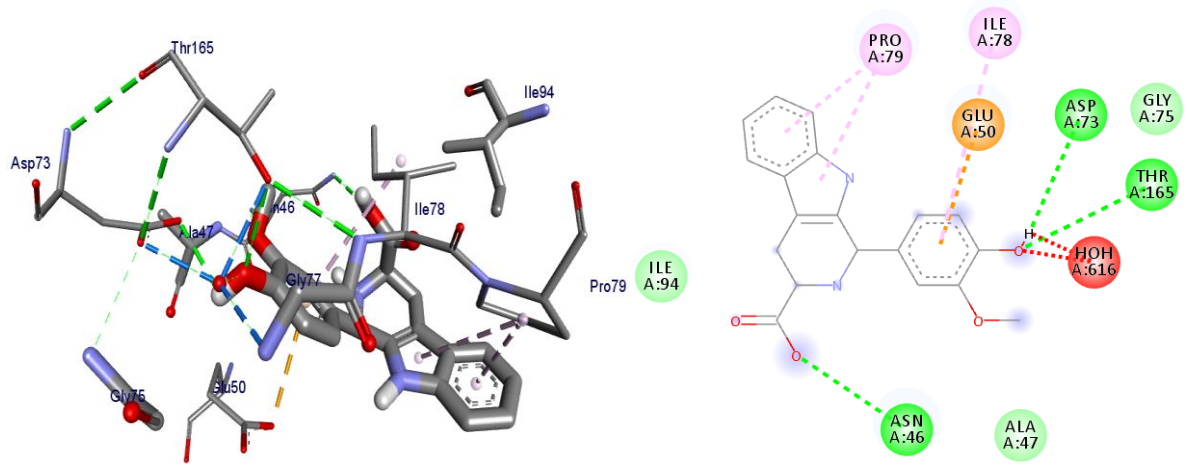


Figure 17: The binding interactions of **142c** against *E. coli* DNA gyrase B (PDB ID: 6F86).

In order to support the experimental data and understand the possible mechanism behind the antioxidant activity, molecular docking study was examined using human peroxiredoxin 5 enzyme (PDB: 1HD2), which has a broader activity against ROS and was mostly involved in stress protection mechanisms and in cell differentiation. Since compound **142c** showed a promising *in vitro* antibacterial and antioxidant activity, in the help of *in vitro* antioxidant activity studies, *in silico* study was conducted to predict the orientation and binding affinity. In

this regard, the molecular docking analysis for the compound **142c** was performed to elucidate its binding interactions with human peroxidoxin 5 (PDB ID: 1HD2) as antioxidant drug candidate. The binding affinity, H-bond, and residual interaction of compound **142c** in comparison with the natural antioxidant ascorbic acid is summarized in **Table 8**.

In the docking study, compound **142c** scored a minimum binding energy of -5.4 kcal/mol compared with standard antioxidant ascorbic acid (-4.9 kcal/mol). From the molecular docking results, it was found that compound **142c** was identified as potent inhibitor of the enzyme, with human peroxiredoxin 5 interacting favorably with its catalytic site *via* one H-bonding interaction with Thr-147; five van der Waals interactions with Gly-46, Phe-120, Leu-116, Gly-148, and Leu-149. Both compound **142c** and the standard ascorbic acid showed residual interactions profile with two amino acid residues Pro-40 and Arg-127 in which ascorbic acid having additional residual interaction with Pro-45, Phe-120, and Leu-149.

Table 8: Molecular docking value of compound **142c** against human peroxidoxin 5 (PDB ID: 1HD2).

Compounds	**Affinity (kcal/mol)**	**H-bond**	**Residual Amino acid Interactions**	
			Hydrophobic/Pi-Cation/Pi-Anion/ Pi-Alkyl interactions	Van-der Walls interactions
142c	-5.4	Thr-147	Pro-40, Arg-127	Gly-46, Phe-120, Leu-116, Gly-148, Leu-149
Ascorbic Acid	-4.9	Cys-47, Thr-44, Gly-46, Thr-147	Pro-40, Pro-45, Phe-120, Arg-127, Leu-149	--

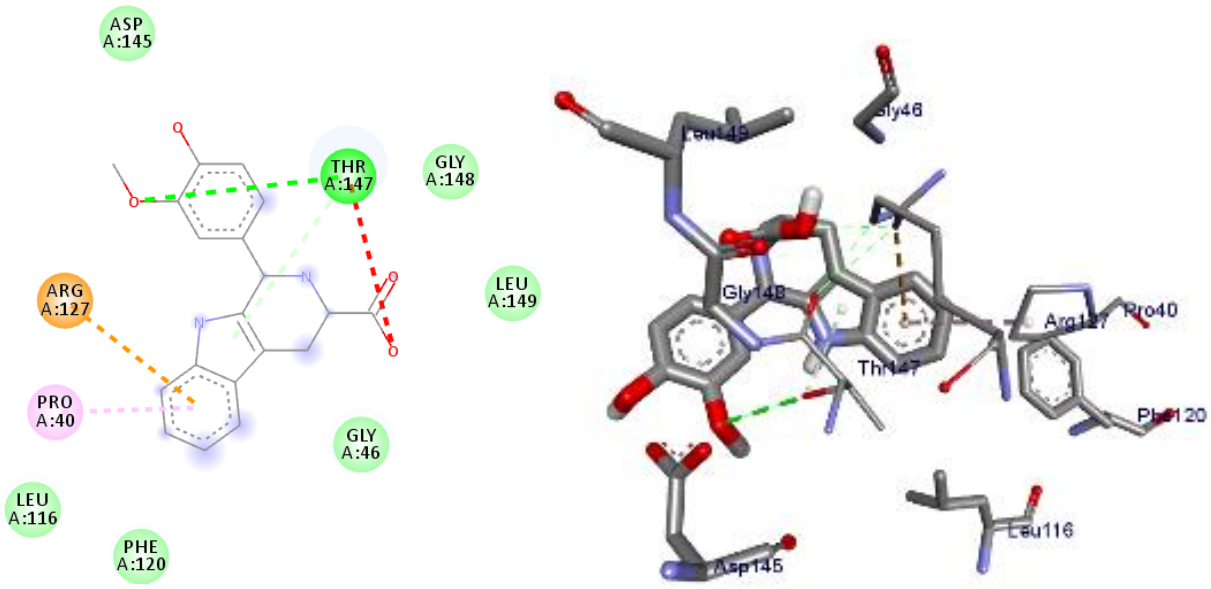


Figure 18: The 2D and 3D binding interactions of compound **142c** against human peroxiredoxin 5 (PDB ID: 1HD2).

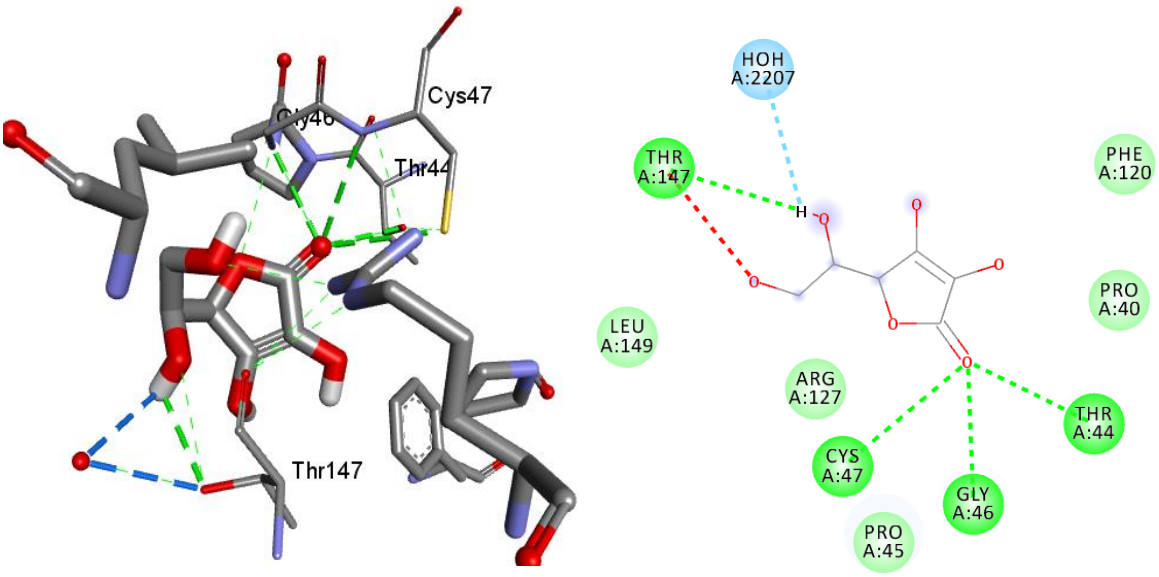


Figure 19: The 2D and 3D binding interactions of ascorbic acid against human peroxiredoxin 5 (PDB ID: 1HD2).

CHAPTER 5

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

In this research work, four C-1 substituted tetrahydro-*β*-carboline derivatives were synthesized using Pictet-Spengler reaction under acidic condition. Chemical structures of the synthesized compounds were characterized using melting point, UV-Vis, and ^{1}H and ^{13}C NMR spectral analysis. The antibacterial activities of the synthesized compounds were screened against *S. aureus, S. pyogenes, E. coli* and *P. aeruginosa* using disc diffusion method and resulted potentially high antibacterial activity. The antioxidant activities displayed by the tetrahydro-*β*-carboline derivatives were significant compared with ascorbic acid demonstrating the potential antioxidant activity. Among the synthesized compounds, compound **142c** displayed the highest activity against *P. aeruginosa, E. coli*, and *S. aureus* with mean inhibition zone of 15.0 ± 1mm, 14.67 ± 1.5 mm, and 13.3 ± 1.5mm mm at 1.0 mg/mL, respectively whereas compound **142b** showed higher activity against *E.coli* (14.3±2.1mm), *P.aeroginosa* (14.3±1.53mm), *S. aureus* (13±2.0mm), and *S. pyogenes* (14.3±1.53mm). DPPH radical scavenging activity displayed 90%, 92%, 91%, and 95% inhibition for compounds **140**, **142a**, **142b**, **142c**, respectively, at 100 μg/mL. *In silico* molecular docking analysis revealed that compound **142c** exhibited better docking efficiency with DNA gyrase displaying binding affinity of -6.9 kcal/mol close to ciprofloxacin (−7.2 kcal/mol). From the *in-silico* antioxidant molecular docking study, compound **142c** scored a minimum binding energy of -5.4 kcal/mol compared with standard antioxidant ascorbic acid (-4.9 kcal/mol) against human peroxiredoxin 5 (PDB ID: 1HD2). The results of *in silico* molecular docking study of the compounds against *E. coli* DNA gyrase B were also in good agreement with *in vitro* antibacterial analysis. The *in vitro* antibacterial activity of compound **142b** and antioxidant activity of compound **142c** suggest the potential use of these compounds as potential drug lead candidates.

5.2. Recommendations

Based on the results of this investigation, we recommend;

- ✓ Since the tested compounds displayed potent antibacterial and antioxidant activity, it is necessary to evaluate other activities including antifungal, anticancer, and other activity.
- ✓ Synthesis of a series of C-3 and C-1 substituted tetrahydro-β-carboline derivatives, substitution at phenyl ring of indole moiety and conduct structure-activity relationship.
- ✓ Synthesis of a series of N^2, N^9-substituted tetrahydro-β-carboline derivatives at indole and pipieridine moiety and conduct structure-activity relationship.

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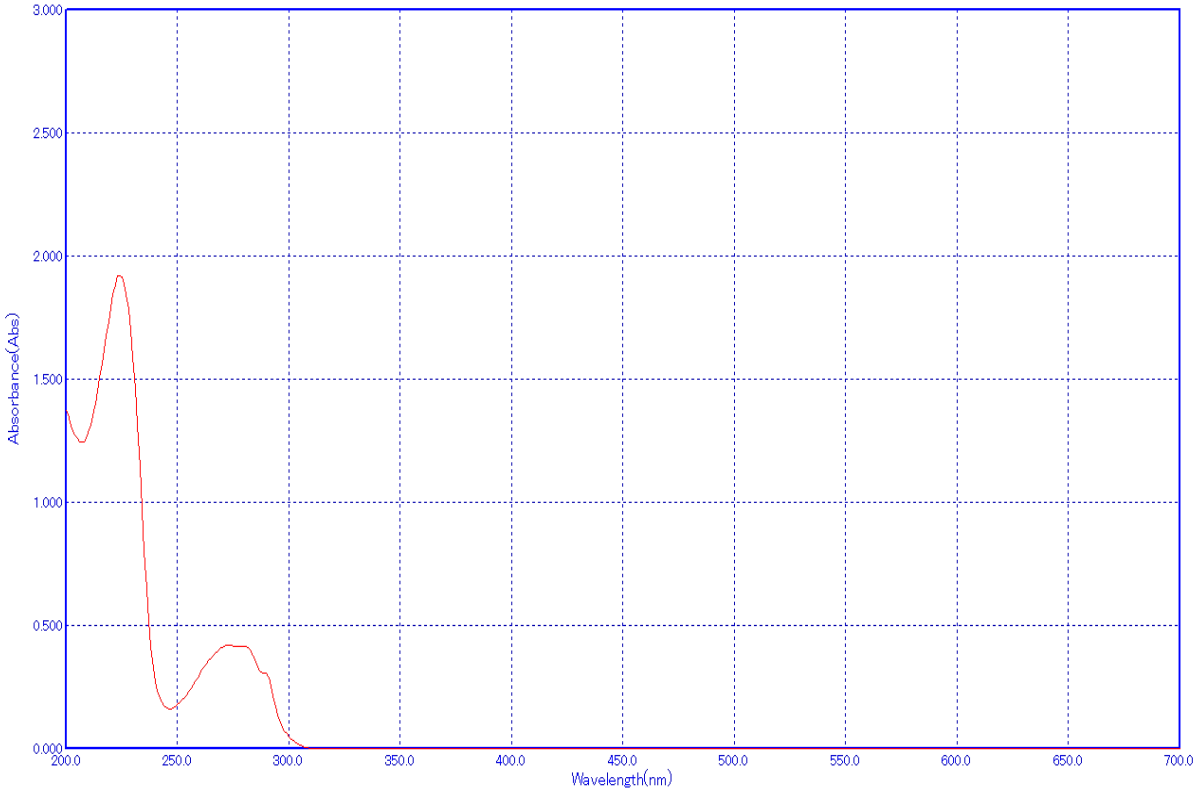
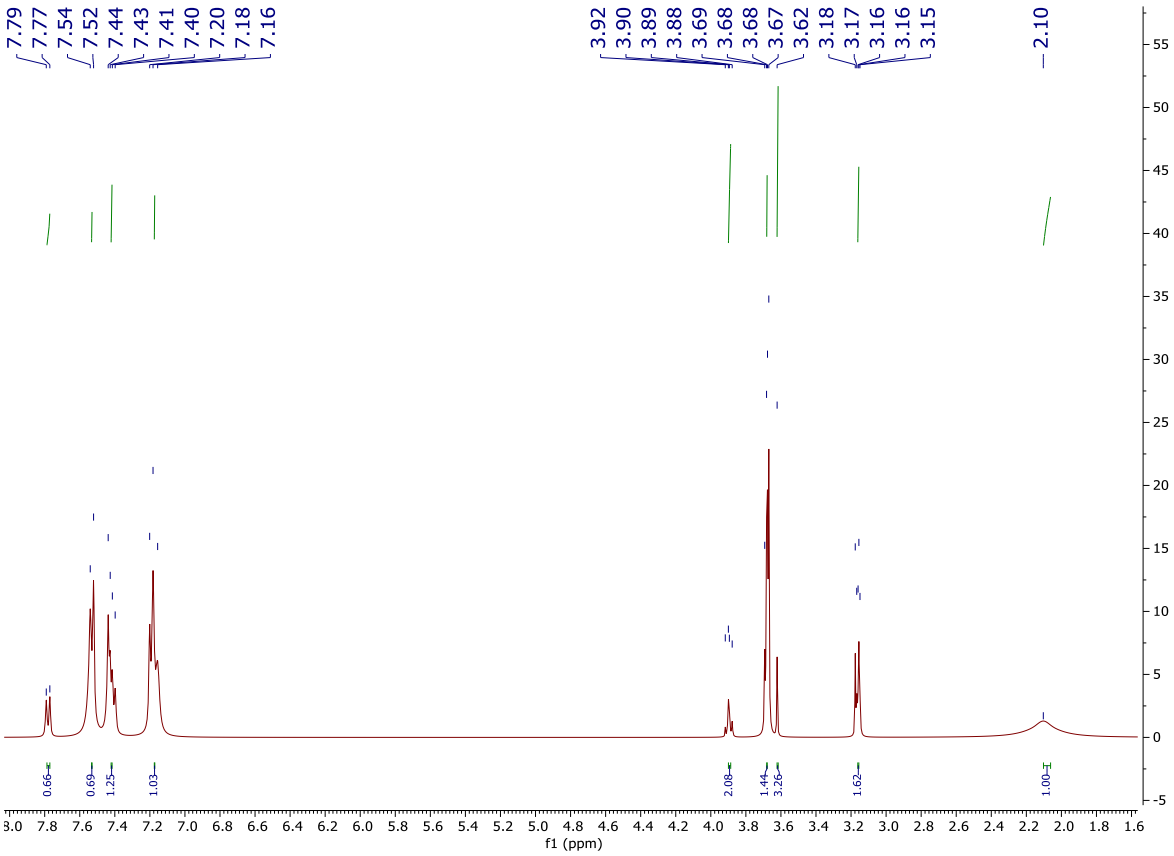
APPENDICES

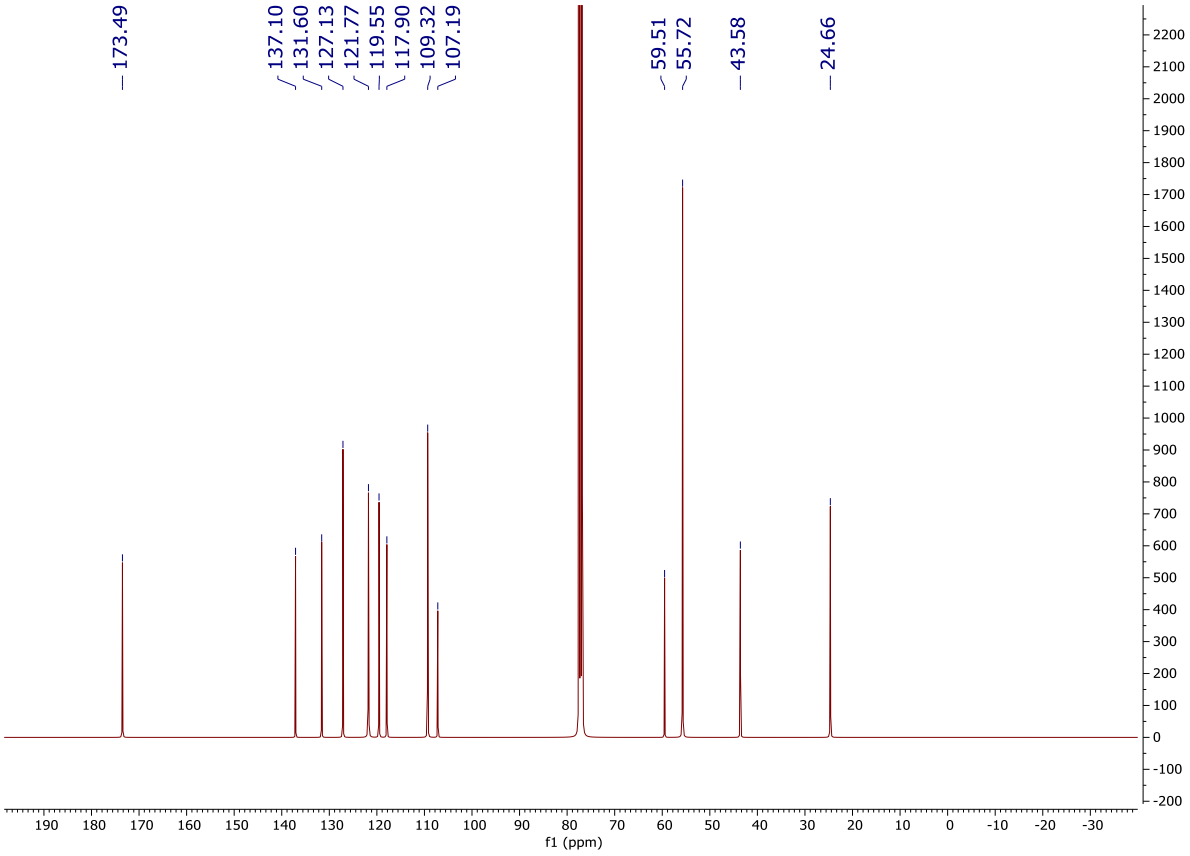
Appendix 1: UV-Vis (200-700 nm) spectrum of compound **30**.



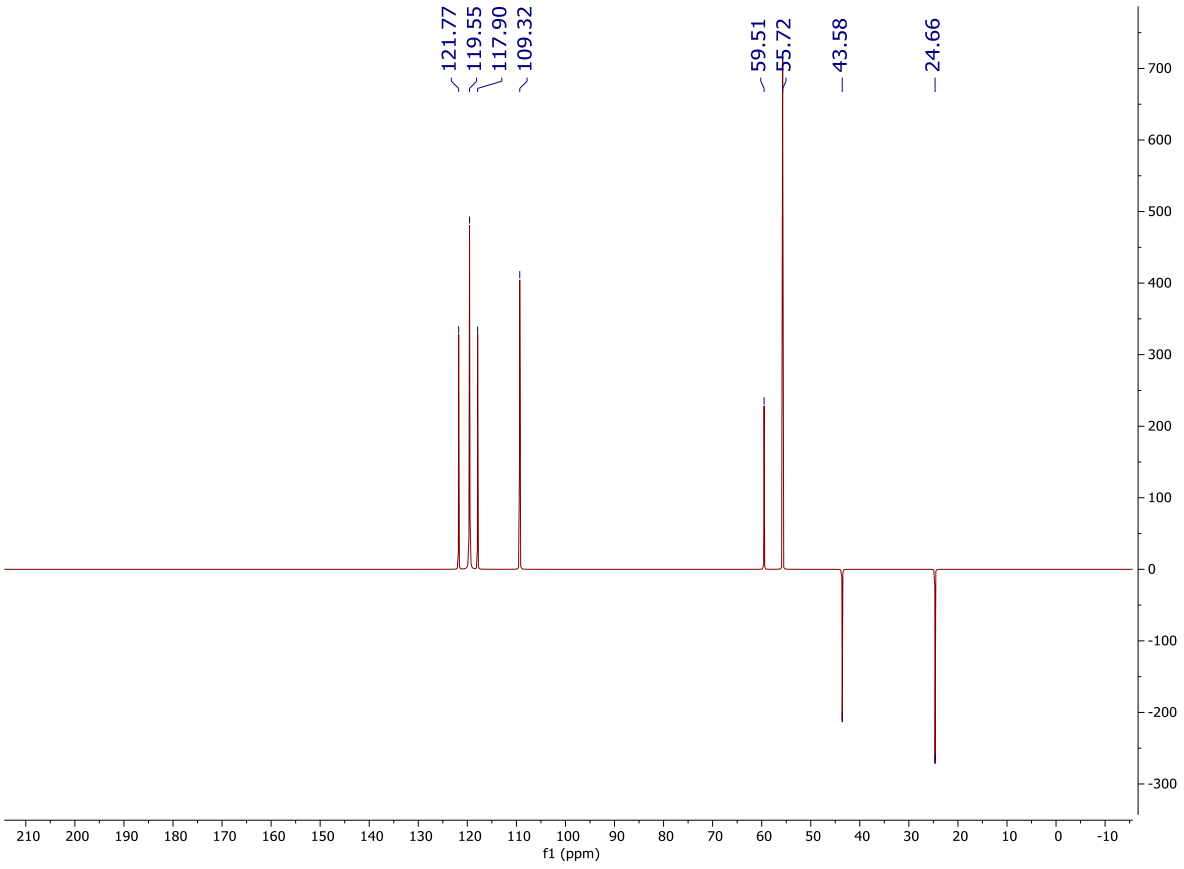
Appendix 2: The ^{1}H-NMR spectrum (400 MHz, Chloroform-*d*) of compound **140**.



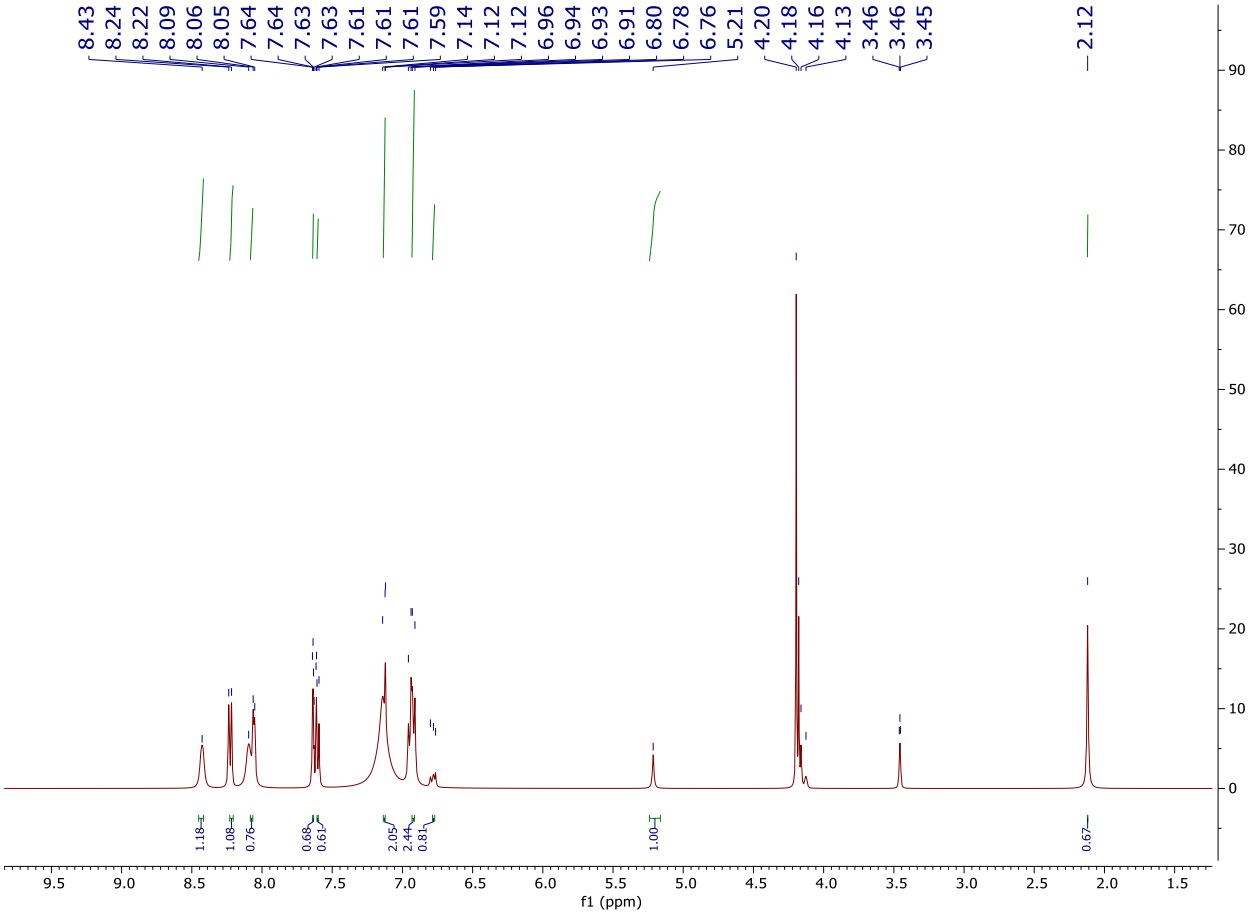
Appendix 3: The ^{13}C-NMR (101 MHz, Chloroform-*d*) spectrum of compound **140**.



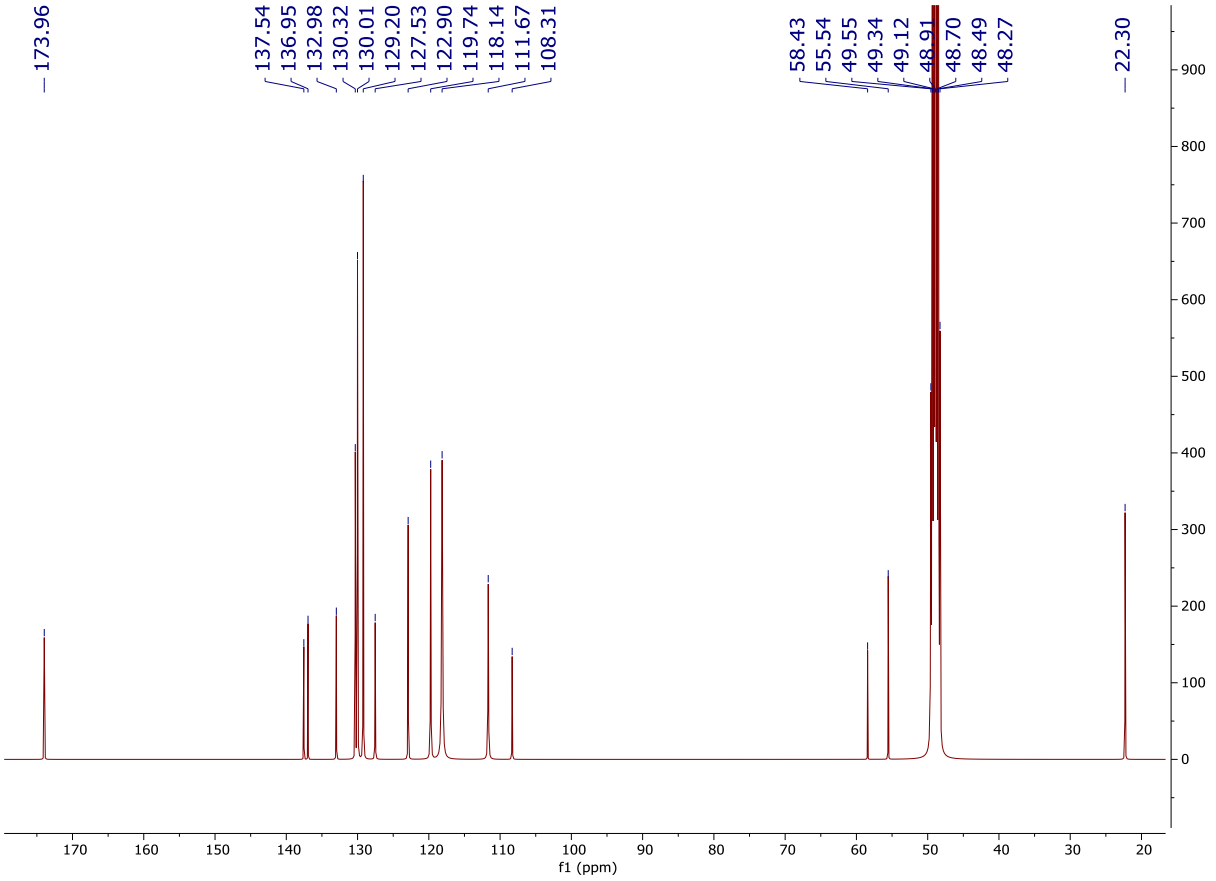
Appendix 4: The DEPT-135(101 MHz, Chloroform-*d*) spectrum of compound **140**.



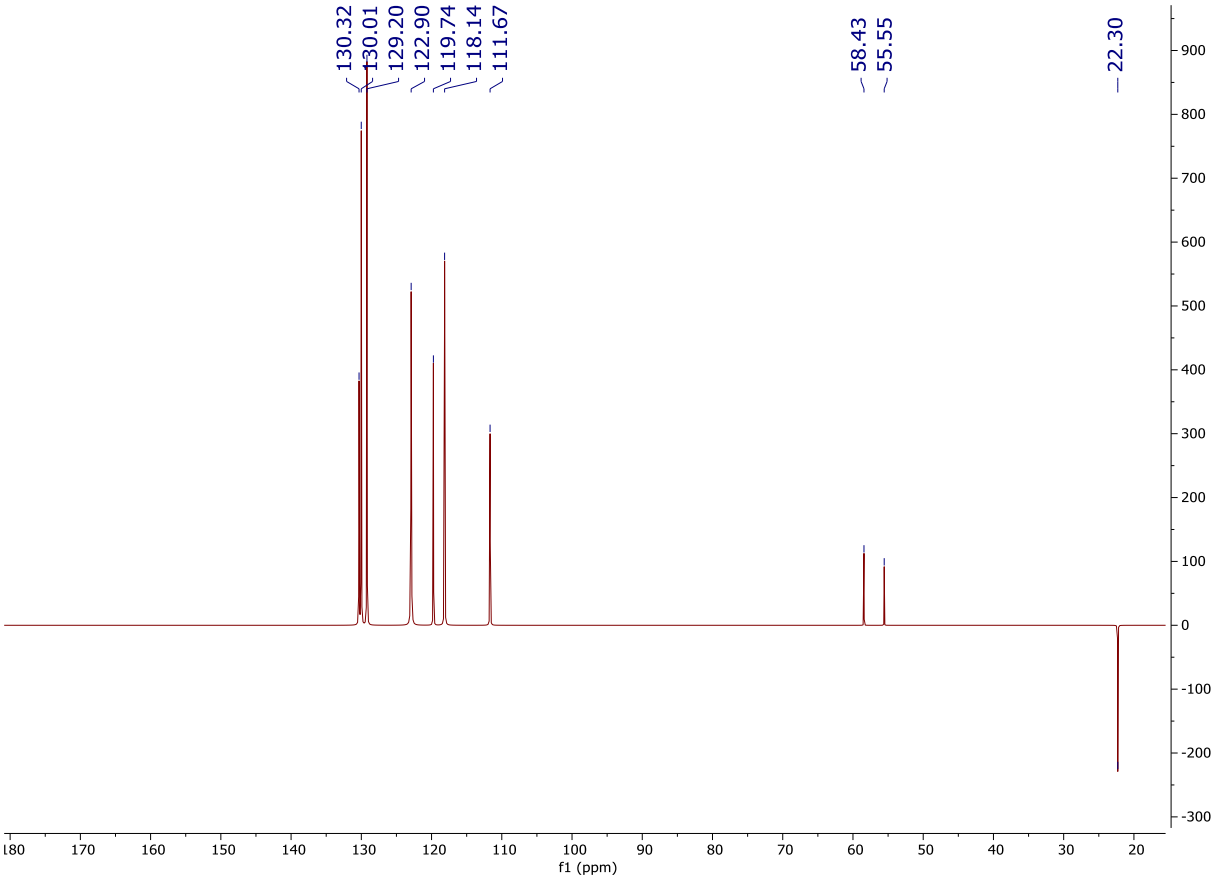
Appendix 5: The ^{1}H-NMR (400 MHz, Methanol-d_4) spectrum of compound **142a**.



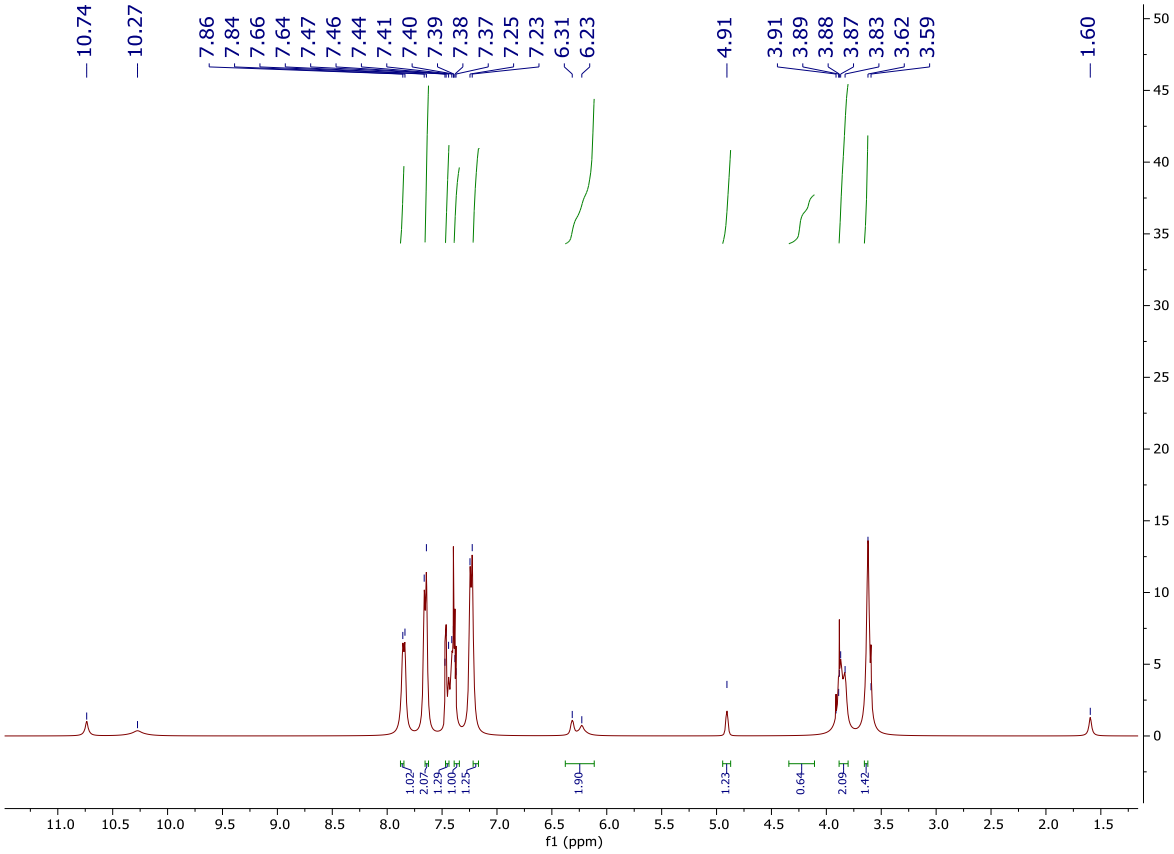
Appendix 6: The ^{13}C-NMR (100 MHz, Methanol-d_4) spectrum of compound **142a**.



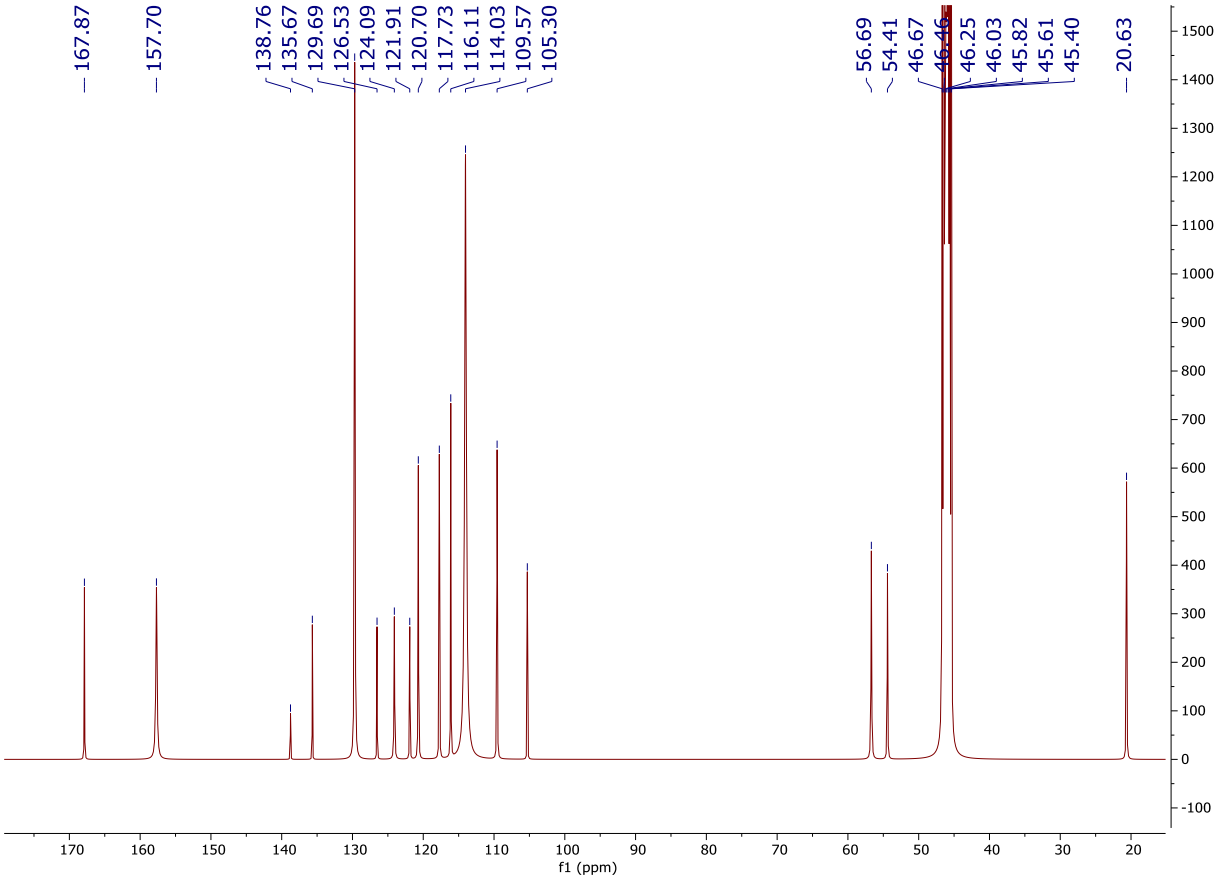
Appendix 7: The DEPT-135 (100 Hz, Methanol-d_4) spectrum of compound **142a**.



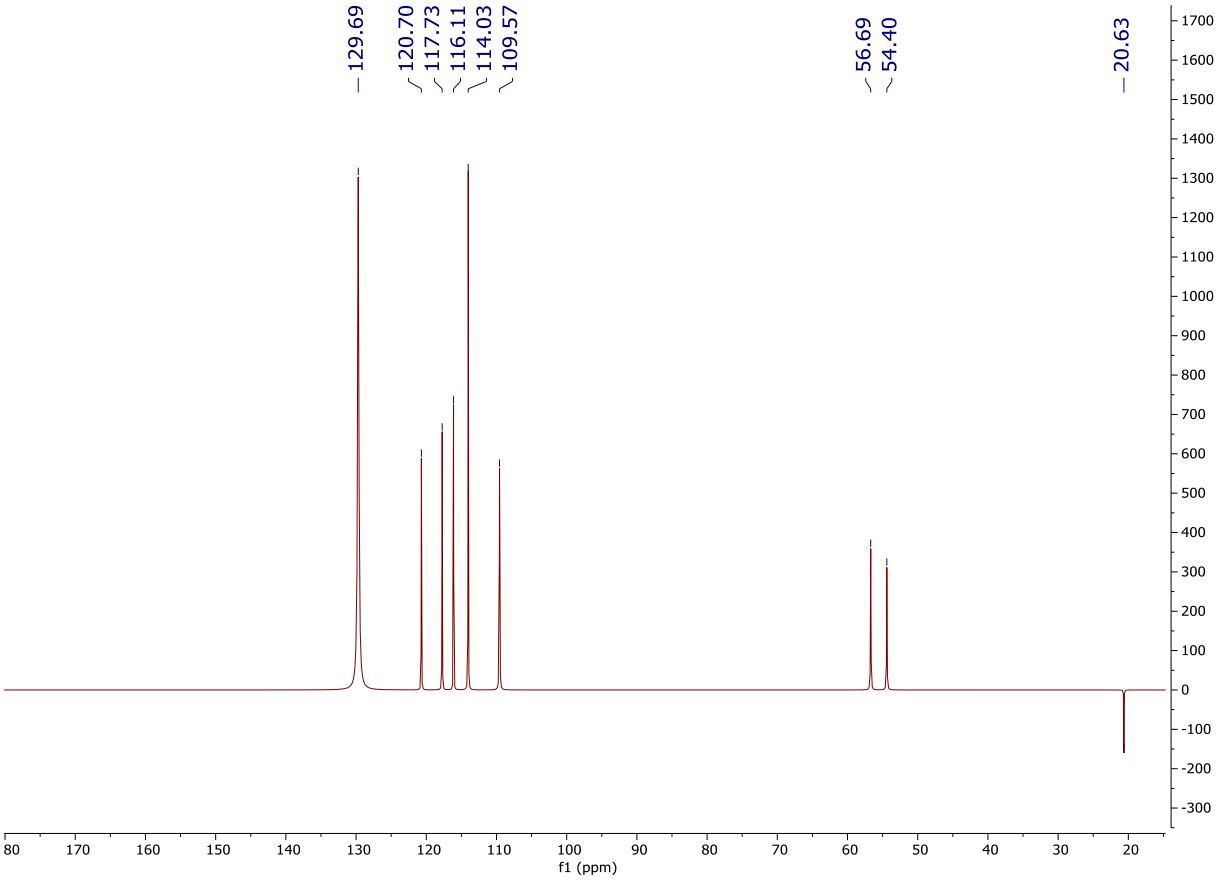
Appendix 8: The ^{1}H-NMR (400 MHz, Methanol-d_4) spectrums of compound **142b**.



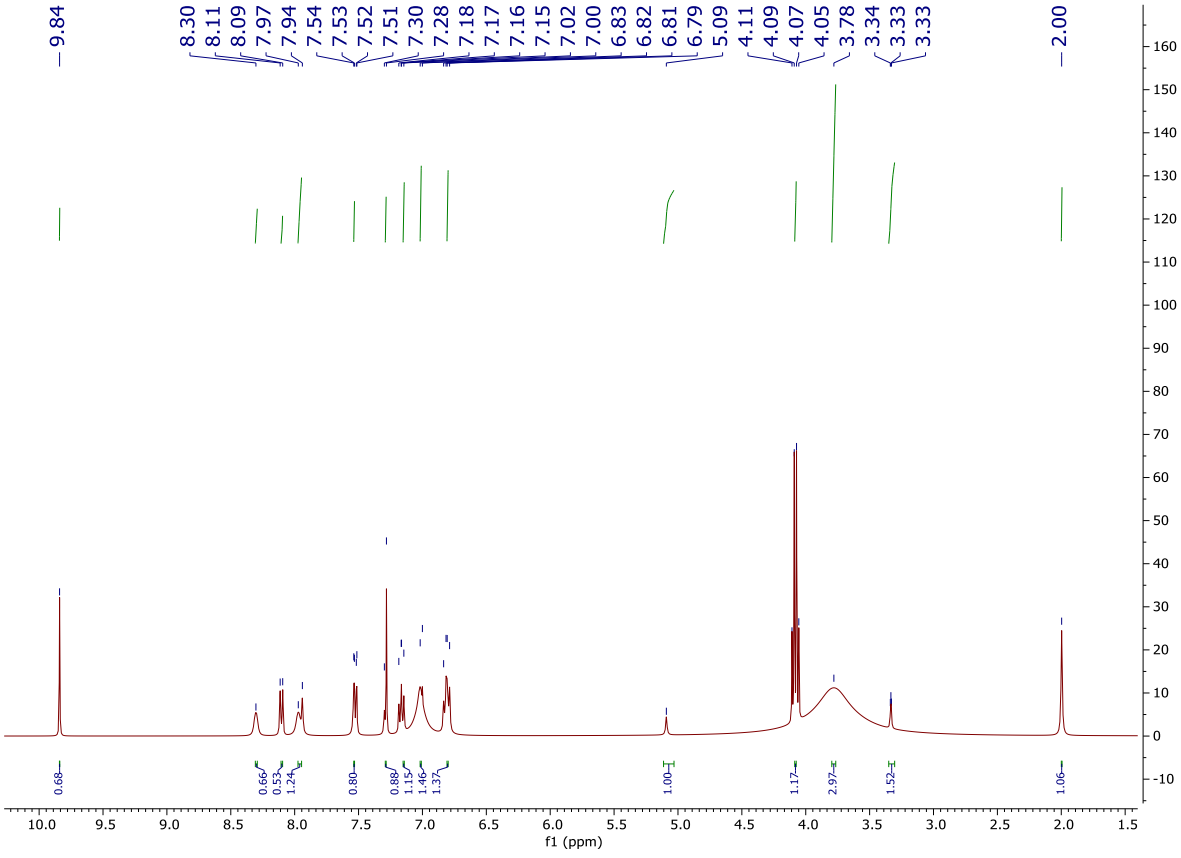
Appendix 9: The ^{13}C-NMR (100 Hz, Methanol-d_4) spectrum of compound **142b**.



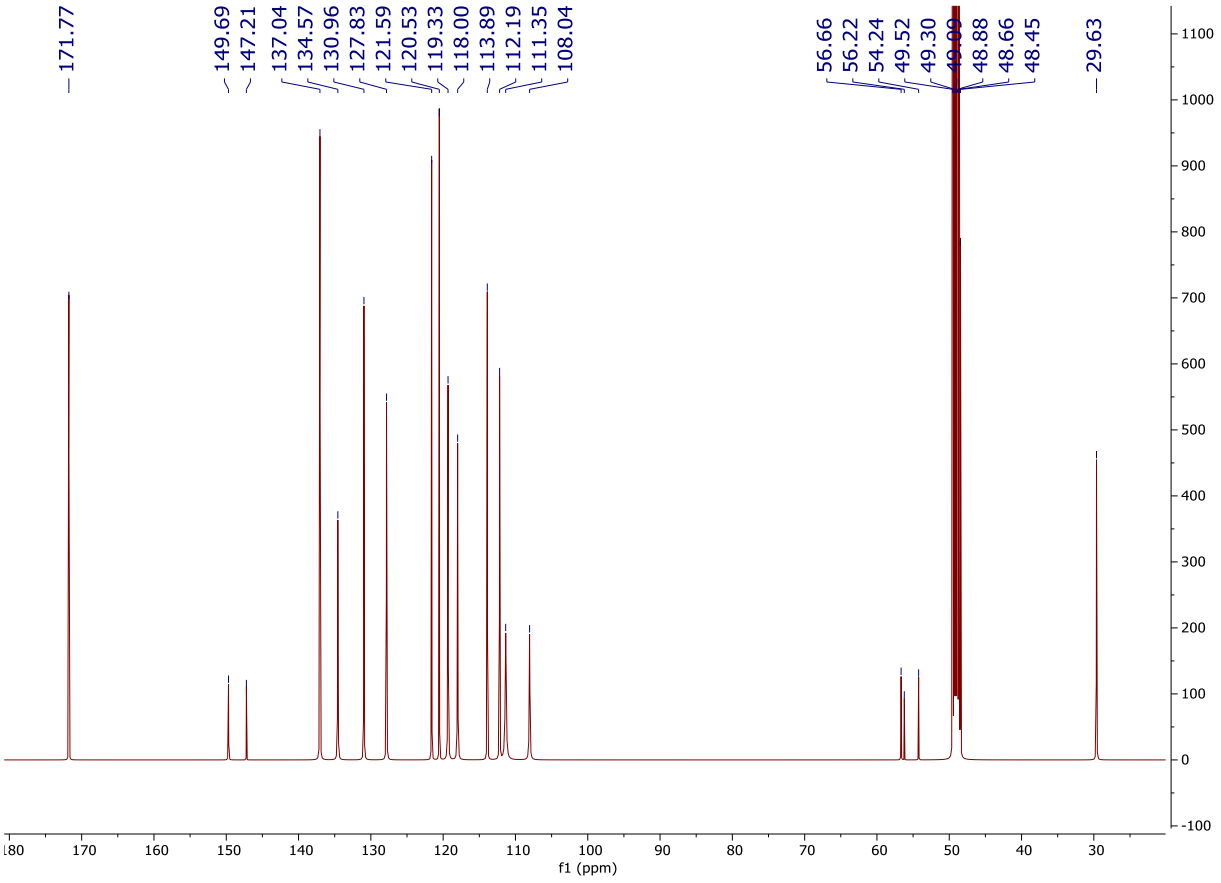
Appendix 10: The DEPT-135 (101 Hz, Methanol-d_4) spectrum of compound **142b**.



Appendix 11: The ^{1}H-NMR (400 MHz, Mthanol-d_4) spectrums of compound **142c.**



Appendix 12: The ^{13}C-NMR (100 Hz, Methanol-d_4) spectrum of compound **142c**.



Appendix 13: The DEPT-135 (101 Hz, Methanol-d_4) spectrum of compound **142c**.