THE DESIGN, SYNTHESIS, AND ANTITUBERCULAR PROPERTIES OF THE TRI-SUBSTITUTED BENZOFURAN DERIVATIVES

MASTER OF SCIENCE

MOJAPELO S.K

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THE DESIGN, SYNTHESIS, AND ANTITUBERCULAR PROPERTIES OF THE TRI-SUBSTITUTED BENZOFURAN DERIVATIVES

By

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DISSERTATION

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SUPERVISOR: Dr T.C. Leboho

Co-SUPERVISOR: Prof W. Nxumalo

2025

DEDICATION

To my grandmother Nelly Manhlwa Mojapelo, My mother Melita Mamonare Mojapelo, My aunt Mankome Rosina Mojapelo and Saliminah Ngwanahlapa Nhlane, Siblings Nelly , Maria,Tumisho, Sekgale, Minah, Morokgadi, and Masebe.

DECLARATION

I declare that the project titled “The design, synthesis, and antitubercular properties of the tri-substituted benzofuran derivatives” hereby submitted to the University of Limpopo, for the degree of Master of Science (Chemistry) has not previously been submitted by me for degree at this or any other university: that it is my original work in design and in execution, and that all the material contained herein has been duly acknowledged.

……

Mr Mojapelo S.K

21 August 2025

……

Date

ACKNOWLEDGEMENTS

My sincerest and utmost gratitude goes to my supervisors, Prof T.C. Leboho and Prof W. Nxumalo, for their support, guidance and unrivalled patience during this work. I would further like to extend much thanks to the following individuals and institutions for all the help offered during this academic journey, without whom successful completion would have been near impossible:

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- My family for their support, encouragement and prayers.
- Lastly, to the almighty God of Mount Zion, for the strength to complete this degree.

LIST OF ABBREVIATIONS

ACN: Acetonitrile

ADC: Albumin-dextrose-catalase

ADMET: Absorption, distribution, metabolism, excretion and toxicity

AAs Amino acids

CAS: Casitone

C: Charcoal

CHO: Chinese hamster ovarian cell

Cu: Copper

CuI: Copper Iodide

^{13}C-NMR: ^{13}Carbon Nuclear Magnet Resonance

$CDCL_3$: Deuterated chloroform

Cs_2CO_3: Cesium carbonate

CYP2D6: Cytochrome P450

DCM: Dichloromethane

DMF: N, N-Dimethylformamide

DMSO: Dimethyl sulfoxide

DNA: Deoxyribonucleic acid

EtOH: Ethanol

Et_3N: Triethylamine

FT-IR: Fourier transform infrared

Fraction V: Bovine albumin fraction

GSK: GlaxoSmithKline

Glu: Glucose

H_2O: Water

H3D: The Holistic drug discovery and development centre at UCT

HIV: Human immunodeficiency virus

HRMS(ES): High resolution mass spectrometry electrospray Ionization

$H_{37}Rv$: Mycobacterium tuberculosis strain

^{1}H-NMR: Proton nuclear magnetic resonance

7H9: Middlebrook 7H9 broth

H_2SO_4: Sulphuric acid

IC_{50}: 50 % Inhibitory concentration

I_2: Iodine

K_2CO_3: Potassium carbonate

LC-MS: Liquid chromatography-mass spectrometry

LHMDS: Lithium hexamethyl disilazide

MABA: Microplate Alamar blue assay

MDR-TB: Multi drug resistant tuberculosis

MHz: Megahertz

MIC_{90}: 90 % Minimum Inhibitory Concentration

MP: Melting point

MS: Mass spectrometry

Mtb: Mycobacterium tuberculosis

MW: Molecular weight

m/z: Mass to charge ratio

µM: Micromolar

N_2: Nitrogen gas

$NaBH_4$: Sodium borohydride

NaOH: Sodium hydroxide

NaI: Sodium iodide

$Na_2S_2O_3$: Sodium thiosulfate

mM: Micromolar

NMR: Nuclear magnetic resonance

PBS: Phosphate buffered saline

Pd: Palladium

PK: Pharmacokinetics

$PdCl_2(PPh_3)_2$: Palladium (II)bis(triphenylphosphine) dichloride

PPB: Plasma Protein Binding

PPh_3: Triphenylphosphine

ppm: Parts per million

RFU: Relative fluorescence units

SAR: Structure-activity relationship

SI: Selectivity index

SMP30: Senescence marker protein 0

TB: Tuberculosis

TLC: Thin layer chromatography

TPSA: Topological polar surface area

Tw: Tween-80

Tx: Tyloxapol

UCT: University of Cape Town

UVA: Ultraviolet A light

WHO: World Health Organization

XDR-TB: Extensively drug-resistant tuberculosis

ABSTRACT

Tri-Substituted benzofuran derivatives exhibit greater biological activities influenced by natural compounds with highest potency, selectivity, and multifunctionality. Naturally occurring benzofuran with hydroxyl, methoxy, phenyl modification in different position showed antimicrobial, anticancer, antitubercular, and neuroprotective activities. These modification groups can influence lipophilicity, binding affinity and membrane permeability of *mycobacterium* tuberculosis which impact bioactivity. The aim of the study was to design, synthesise, and evaluate the anti-tubercular properties of the tri-substituted benzofuran derivatives. 5-iodovanillin **1B** was made by iodinating commercially available vanillin **1A** in a 92% yield. 2-(substituted)-7-methoxybenzofuran-5-carbaldehydes **2A-2E** were produced by a Sonogashira cross-coupling reaction with palladium using a variety of acetylenes in a 60–80% yield. 2-(substituted)-7-methoxybenzofuran-5-carbaldehydes (**2A-2E**) were reduced with sodium borohydride in ethanol at room temperature, producing 2-substituted-7-methoxybenzofuran-5-ylmethanols (**3A-3E**) in a 60–82% yield. The esterification of the 2-substituted-7-methoxybenzofuran-5-carbaldehydes **3A-3E** was catalysed by a Mukaiyama catalyst with several carboxylic acids in dichloromethane at room temperature, producing esters **3A1-3E5** in 40–80% yields. FTIR and NMR spectroscopy were used to characterise each of the synthesised substances. The online platform ADMET3.0 was used to perform the ADMET characteristics of esters **3A1-3E5**. The antimycobacterial, cytotoxic, and solubility properties of every synthesised compound were assessed biologically. Several trisubstituted benzofuran derivatives that were synthesised demonstrated antimycobacterium tuberculosis (Mtb $H_{37}R_v$) activity. For example, compound **3B3** showed good activity in all three media used with less toxicity. Additionally, six compounds (**2A**, **3A**, **3A2**, **2B**, **3B**, and **3B1**) showed solubility results ranging between 5-195 µM. Compound **3B** showed the best solubility of 195 µM but poor antitubercular activity in all three media used. On the contrary, compound **2A** showed promising antimycobacterial activity of 2.02-18.08 µM in two media but had poor solubility of less than 5 µM. Additionally, compound **3A2** demonstrated activity in one medium (7H9/CAS/Glu/Tx), whereas compound **3B1** demonstrated activity in two media (7H9/ADC/Glu/Tw and 7H9/ADC/Glu/Tx). Both

compounds displayed comparable outcomes, including good solubility at 50 μM and cytotoxicity at or above 50 μM, respectively.

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

Tuberculosis (TB) is an infectious airborne disease[1] that is usually present in the lungs as pulmonary tuberculosis, and it is caused by the bacteria called *Mycobacterium tuberculosis (Mtb)*[2]*.* Tuberculosis symptoms include chest pain, weight loss, blood in the mucus, and nonspecific causes such as cardiac arrest, heart failure, or shock[1–6]. Tuberculosis, also known as the captain of all these men of death, remains a major public health challenge in developing countries, whereby *Mycobacterium tuberculosis* is the leading cause of mortality and morbidity. During the 1980s, approximately two billion people (33% of the world population) were infected with tuberculosis[5]. According to the World Health Organisation (2022), 10600000 people became ill with tuberculosis in 2021 as compared to 1600000 in 2020. Additionally, about 1600000 individuals died from tuberculosis in 2021 which included 187000 infected with HIV, compared to 1500000 in 2020 (including two hundred fourteen thousand individuals living with HIV)[1,6–8]. The incidence of tuberculosis increased by 3.6% in 2021[7]. In 2022, South African data showed that tuberculosis caused roughly 54000 cases of death and predicted that 280000 persons developed the disease[3,4]. Although several drugs have been developed for the treatment of tuberculosis such as isoniazid, rifampicin, pyrazinamide, and ethambutol, the emergence of drug-resistant tuberculosis strains has negatively affected the use of these drugs[9–11]. Additionally, the severe side effects associated with some of the approved tuberculosis medications such as *isoniazid* (vomiting), *rifampicin* (dizziness), *pyrazinamide* (fever), and *ethambutol* (color blindness), require careful use of these drugs[1,2,9,11]. Thus, there is an urgent need to develop drugs with improved safety profiles that are tuberculosis-specific and contain fewer to no side effects.

1.2 Treatment of tuberculosis

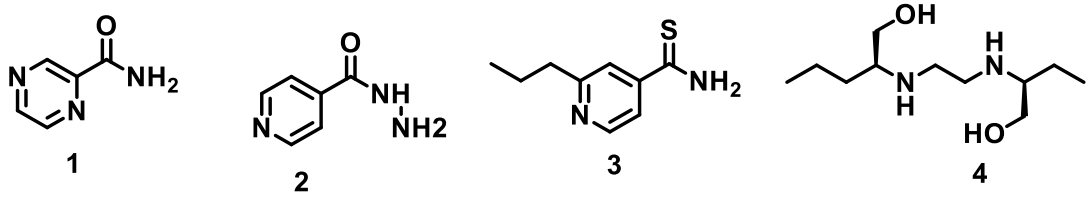
To treat tuberculosis, a well-organised treatment strategy and a combination of drugs are needed. The patient's recovery, relapse prevention, and drug resistance risk reduction are the main objectives. First-line and second-line treatments are the two categories for tuberculosis treatment[12–14].

1.2.1 First-line drugs for the treatment of tuberculosis

Two phases are often included in the treatment plan for newly diagnosed cases of pulmonary tuberculosis: an intense phase and a continuation phase. The four medications used in the intense phase are isoniazid **2**, rifampicin **10**, pyrazinamide **1**, and ethambutol **4** (**Figure 1**) are usually administered for two months. Thereafter, the use of isoniazid and rifampicin continues to be administered as part of the continuation phase, which usually lasts four to six months[2,9,14,15]. Tuberculosis is only treated with second-line therapy as a backup when first-line therapy is ineffective because of medication resistance, intolerance, or other factors. The main objectives of second-line therapy are to cure the patient and stop drug-resistant strains from spreading.

1.2.2 Second-line drugs for tuberculosis treatment

The second-line medications for tuberculosis treatment include amikacin **7**, capreomycin **9**, levofloxacin **6**, ethionamide **11**, prothionamid **3**, bedaquiline **8**, and delamanid **5** (**Figure 1**). Drug-resistant tuberculosis is treated with a few of the second-line medications. Multidrug-Resistant Tuberculosis (MDR-TB) is treated with delamanid/amikacin combination for 4 months, followed by levofloxacin/ delamanid, ethionamide/prothionamide, and prothionamide/capreomycin[1,9,11,13]. The treatment for extensively drug-resistant tuberculosis (XDR-TB) lasts 6 months, depending on the patient's drug susceptibility test results [16]



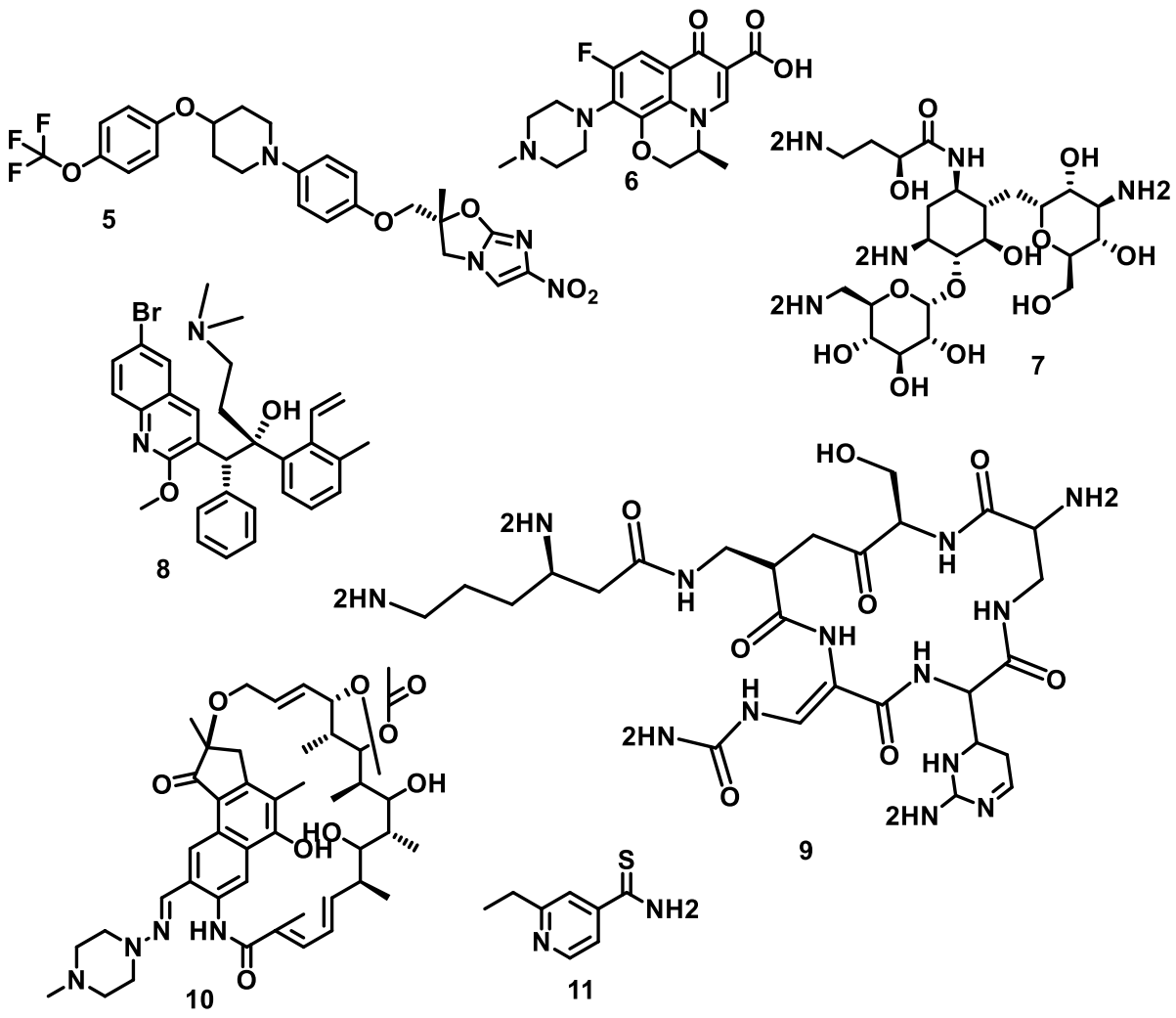


Figure 1: Structures of drugs used to treat tuberculosis[1,9,11,13]

1.3 Benzofuran and its derivatives

Benzofuran **12** (**Figure 2**) is composed of furan and benzene rings fused together and its derivatives are found imbedded in natural products[17]. It has a bicyclic structure, and it is one of the most important nuclei, especially in medicinal chemistry[18,19]. The benzofuran structure serves as a core in various organic and pharmaceutical compounds. Benzofuran derivatives are essential compounds that hold important biological activities [20]. They are adaptable biodynamic agents that can be employed to design and develop new, potentially useful pharmacophore drugs[18,21]. There are valuable benzofuran scaffolds that are used for anti-microbial[8], anti-cancer[22], anti-tubercular[20], and anti-HIV treatment [1].

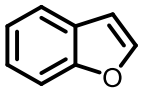


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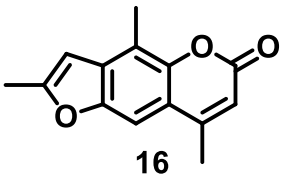
Figure 2: Structure of benzofuran nucleus[23]

1.3.1 Approved benzofuran-containing drugs

Benzofuran is also present in pharmaceuticals that have been licensed for medical use. For example, trioxsalen **16** and prosalen **17** are used as photosensitisers to improve pigmentation and skin resistance to sunshine. Additionally, isoprosalen **18** is used to cure skin disorders including psoriasis and vitiligo[21,24]. Methoxsalen (also called xanthotoxin or 8-methoxypsoralen) **19** is sold under the Oxsoralen name, among others. It is used to treat psoriasis, vitiligo, eczema, and some cutaneous lymphomas when combined with UVA light exposure from lamps or the sun. One antiarrhythmic drug used to treat irregular heartbeats is amiodarone **20**. Moreover, benzbromarone **21** is a uricosuric drug and a non-competitive xanthine oxidase inhibitor that is used to treat gout, particularly when allopurinol, the first-line treatment, is ineffective or has unacceptable side effects and it shares structural similarity with amiodarone, an antiarrhythmic drug. Furthermore, bufuralol **22** is a powerful beta-adrenoceptor antagonist with partial agonist activity. It is metabolised by CYP2D6. Most beta blockers are aryloxypropanolamine-based. In this unusual exception, the benzofuran oxygen is part of a ring rather than obtained from the epichlorohydrin precursor. The structures of benzofuran-containing drugs (**16-22**) are shown in **Figure 3**.



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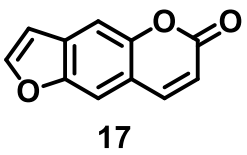

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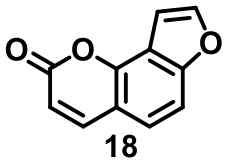

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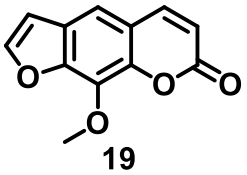

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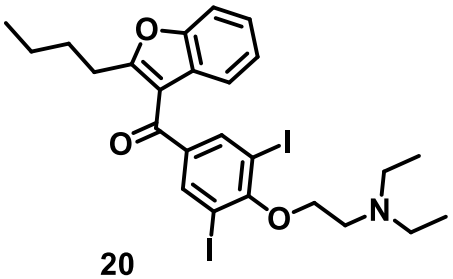

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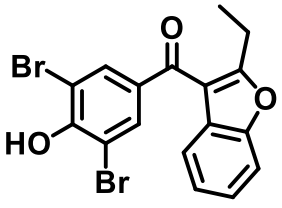

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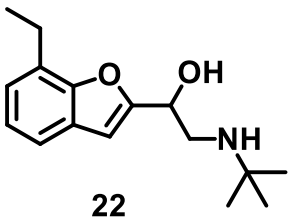

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Figure 3: Benzofuran derivatives drugs with varying biological activity

1.3.2 Natural occurring benzofuran derivatives

Benzofurans' presence in natural products from plants and microorganisms has spurred interest in natural product chemistry and drug discovery. For many years, scientists have been studying this class of chemical compounds due to their wide range of properties. Several benzofuran moieties have been identified and isolated from plant and animal sources. For instance, it was discovered that Krameria *ramosissima* [25] contained natural products with benzofuran nucleus that had anti-inflammatory and antioxidant qualities. For example, (5-(3-hydroxyprop-1-en-1-yl)) (E)-4-(6)methoxybenzofuran-2-yl-7-2-methoxyphenol **23**[26,27] isolated from Krameria *ramosissima* possessed anticancer activities. Apoptosis was triggered by 5-(6-hydroxy-5-(3-methylbut-2-en-1-yl) benzofuran-2-yl) benzene-1,3-diol **24** (**Figure 4**) because it inhibited cell growth[28]. In medicinal chemistry, benzofuran derivatives have drawn interest because of their wide range of biological properties.

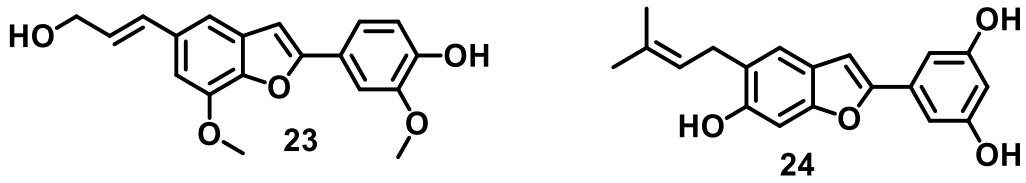


Figure 4**:** Natural isolated benzofuran derivatives[21,24]

1.4 Medicinal importance of benzofuran derivatives

1.4.1 Benzofuran derivatives as antitubercular agent

Benzofuran derivatives have been investigated for their potential use in treating tuberculosis (TB). Tuberculosis, caused by *Mycobacterium tuberculosis*, remains a significant global health challenge, and the rise of drug-resistant strains has intensified the need for new therapeutic agents. Benzofuran derivatives exhibit anti-tubercular activity. Studies have shown that synthetic benzofuran derivatives with modifications at specific positions on the benzofuran ring possess significant anti-tubercular activity. **Figure 5** shows example of benzofuran derivatives **25-28** that displayed potent activity against *Mycobacterium* tuberculosis strains [22,29]. The use of benzofuran derivatives in

combination with existing anti-tubercular drugs can potentially enhance the treatment efficacy and overcome drug resistance [21,30].

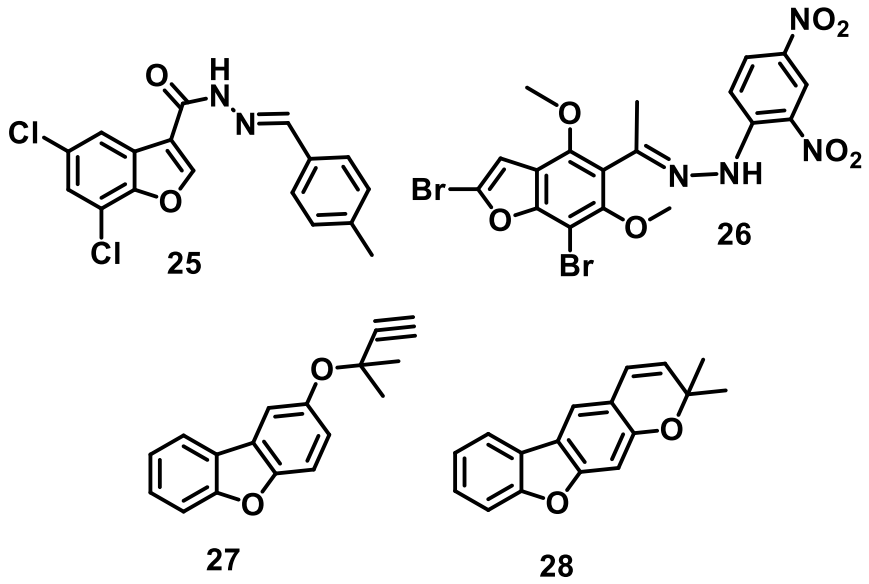


Figure 5: Benzofuran derivatives with anti-tubercular properties[7]

Structure-activity relationship (SAR) studies have identified key structural features that enhanced the anti-tubercular activity of benzofuran derivatives[31]. For example, 2-(pyrrolidin-1-yloxy)naphtho[2,3-b]benzofuran-6,11-dione **31**[32] (**Figure 6**) was synthesised and it exhibited strong activity against *Mycobacterium tuberculosis* by inhibiting crucial enzymes involved in mycolic acid synthesis. Additionally, benzofuran-3-carbohydrazide derivative **32** showed *in vitro* inhibitory activity against *Mycobacterium tuberculosis* ($H_{37}Rv$) strains[33]. Moreover, benzofuran-based hydrazone derivatives **29 and 30** exhibited anti-tubercular activity by targeting multiple pathways within the mycobacterial cell[1,34]. Thus, benzofuran derivatives hold promise as novel anti-tubercular agents due to their diverse action mechanisms and potential to overcome drug resistance[11].

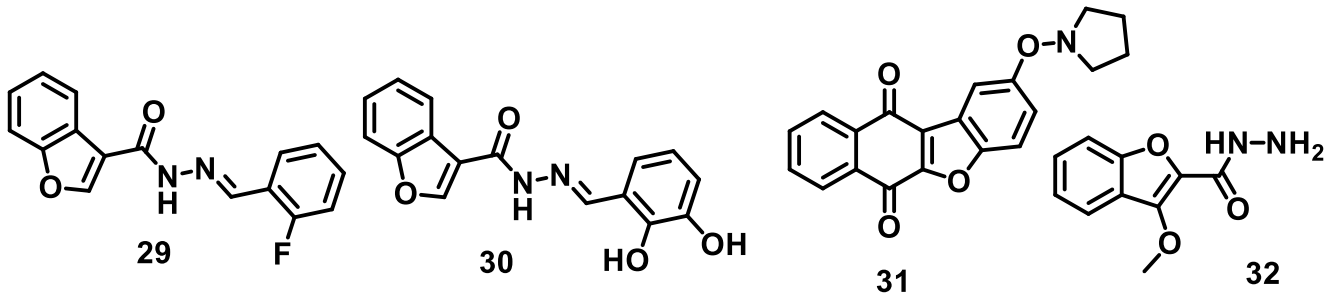


Figure 6: Anti-tuberculosis benzofuran derivatives[34]

1.4.2 Benzofuran derivatives as antimalaria agents

Benzofuran derivatives have potential application in the treatment of malaria, a disease that is caused by Plasmodium parasites and transmitted by anopheles' mosquitoes. Benzofuran derivatives targeted malaria parasites through several mechanisms, such as inhibition of parasite enzymes involved in the parasite's life cycle[21,35,36]. Benzofuran derivatives may also disrupt the synthesis of DNA in the malaria parasite, inhibiting its replication and growth[35]. Several synthetic benzofuran derivatives have been evaluated for their antimalarial activity[18,23]. The modification to the benzofuran core with various substituents enhanced potency against malaria parasites[17,19]. For example, 3-amino-1-benzofuran-2-carboxamide **35** (**Figure 7**) is an example of benzofuran derivatives with antimalarial activity[37], particularly against drug-resistant strains of *Plasmodium falciparum*. Additionally, benzofuran derivatives **33** and **34** hold promise as antimalarial agents due to their diverse action mechanisms and potential to combat drug-resistant malaria strains[38–40].

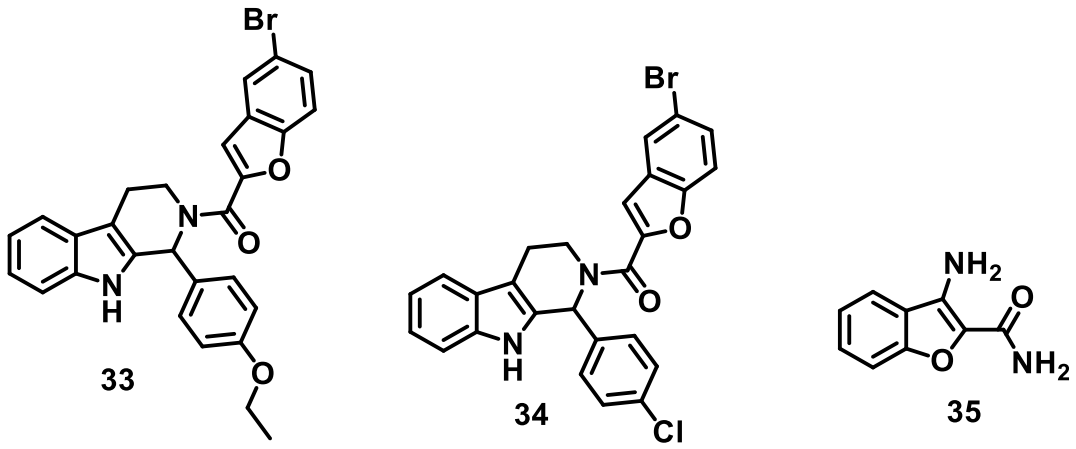


Figure 7: Structures of anti-malarial agents containing a benzofuran nucleus[23,41]

1.4.3 Benzofuran derivatives as anticancer agents

Derivatives of benzofurans demonstrate a wide range of biological and pharmacological activities, including anticancer. Furthermore, benzofuran derivatives have gained interest in oncology due to their potential anticancer properties [42]. The mechanisms by which anticancer compounds induce cancer cell death vary, with

apoptosis being a critical cellular event that contributes to the effectiveness of anticancer drugs [43]. Benzofuran compounds inhibit the proliferation of cancer cells by disrupting key signalling pathways involved in cell cycle regulation [44,45]. Benzofuran compounds with their inhibitory concentration such as **36** (IC_{50} = 4.866 µg/ml) and **37** (IC_{50} = 4.569 µg/ml) (**Figure 8**) inhibited the metastatic spread of cancer cells by targeting matrix metalloproteinases and other factors involved in cell migration and invasion[46,47].

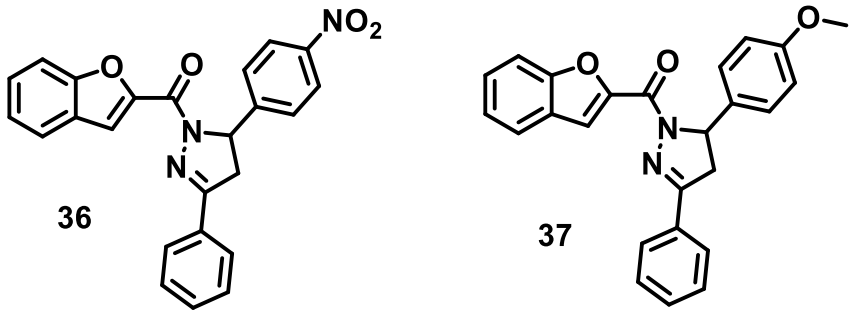


Figure 8: Benzofuran derivatives with anti-cancer activity[44,45]

A notable subgroup of benzofurans with anticancer potential includes compounds containing halogens like amiodarone known as pacerone **38** (**Figure 9**), as their addition to the benzofuran ring has shown to significantly enhance anticancer activity. This improvement is likely due to halogen bonds, which strengthen the binding affinity between the halogen's electrophilic region and nucleophilic sites in molecules [48]. Naturally occurring benzofuran derivatives known as psoralens **16** (**Figure 3**) have been studied for their ability to induce apoptosis and inhibit cell proliferation in various cancer cell lines [49]. Coumarin-benzofuran hybrid molecules combine the structural features of coumarins and benzofurans, exhibiting potent anticancer activity through multiple mechanisms, including DNA intercalation and inhibition of topoisomerases[50,51]. Benzofuran-imidazole conjugates **39-41** (**Figure 9**) have demonstrated significant anticancer activity by targeting multiple signalling pathways involved in cancer cell survival and proliferation[28]. Benzofuran derivatives offer promising potential as anticancer agents due to their diverse action mechanisms and ability to target various aspects of cancer cell biology [42].

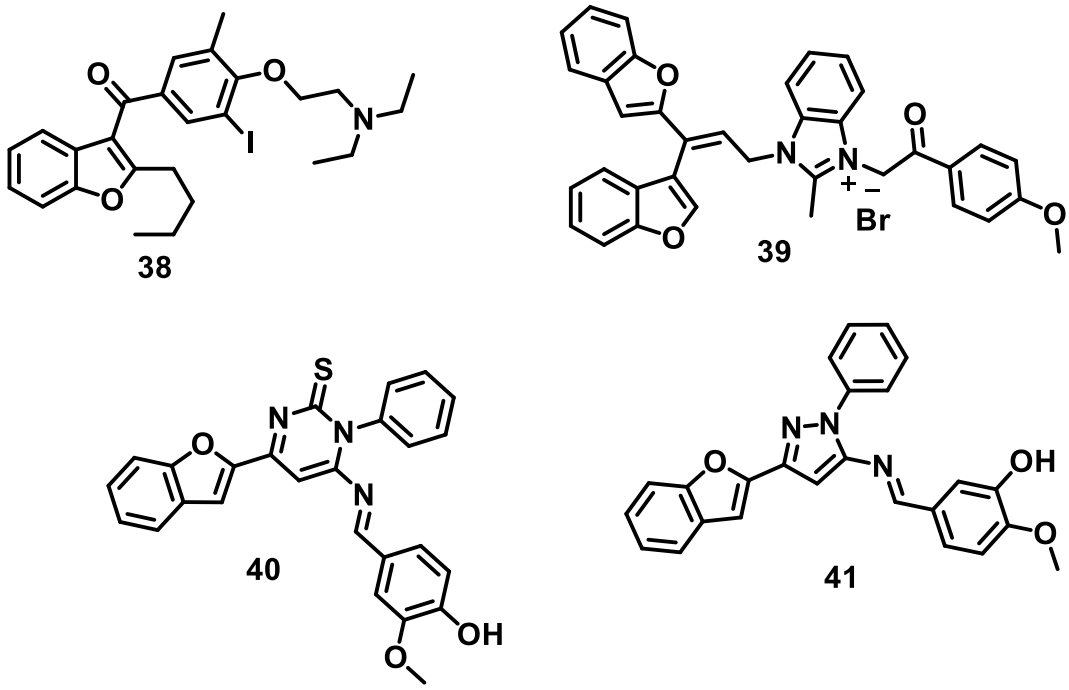
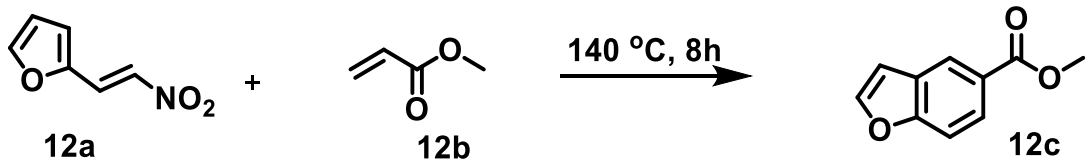


Figure 9: Structures of benzofuran derivatives with anticancer activities[28]

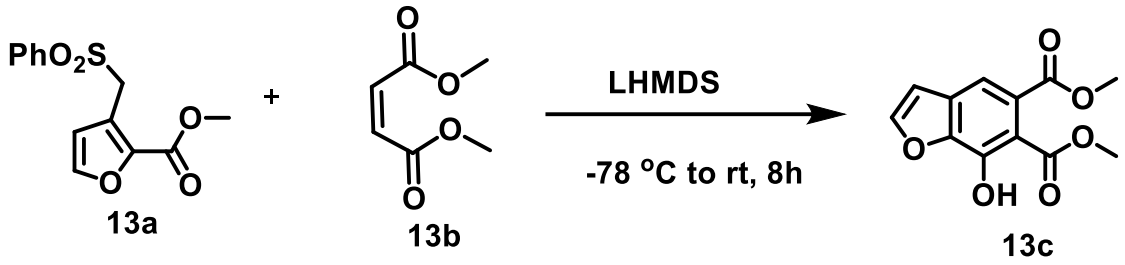
1.4.4 Synthesis of benzofuran derivatives using different methods and reagents

The synthesis of benzofuran from a furan precursor has received far less attention up to this point. The Diels-Alder reaction of nitro vinyl furan **12a** with several dienophiles was used by R. S. Kusurkar *et al*. to disclose a unique synthetic technique to access benzofuran **12c** in 61% (**Scheme 1**)[23,52].



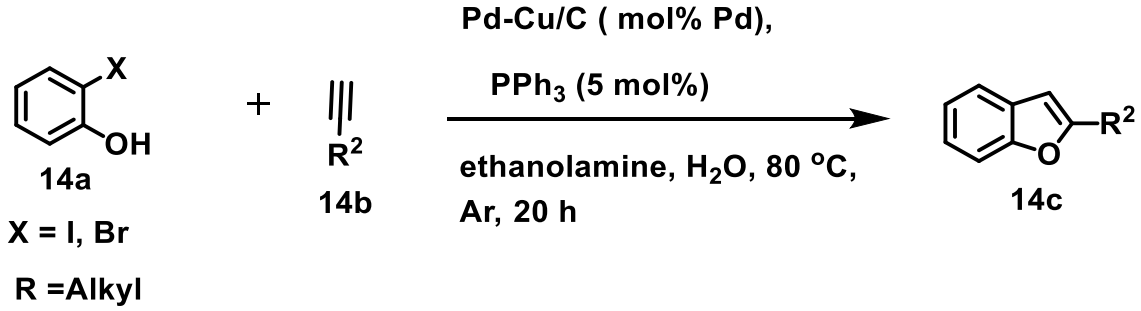
Scheme 1: Synthesis of 5-carbomethoxy benzofuran **12c**[23,52]

Using an anionic annulation technique, D. Mal and colleagues disclosed a simple pathway to 7-hydroxybenzofuran derivatives[23,49]. In the presence of lithium hexamethyl ldisilazide (LHMDS), furoate **13a** was annulated with dimethyl maleate (**13b**), yielding 7-hydroxybenzofuran **13c** in 75% yield (**Scheme 2**).



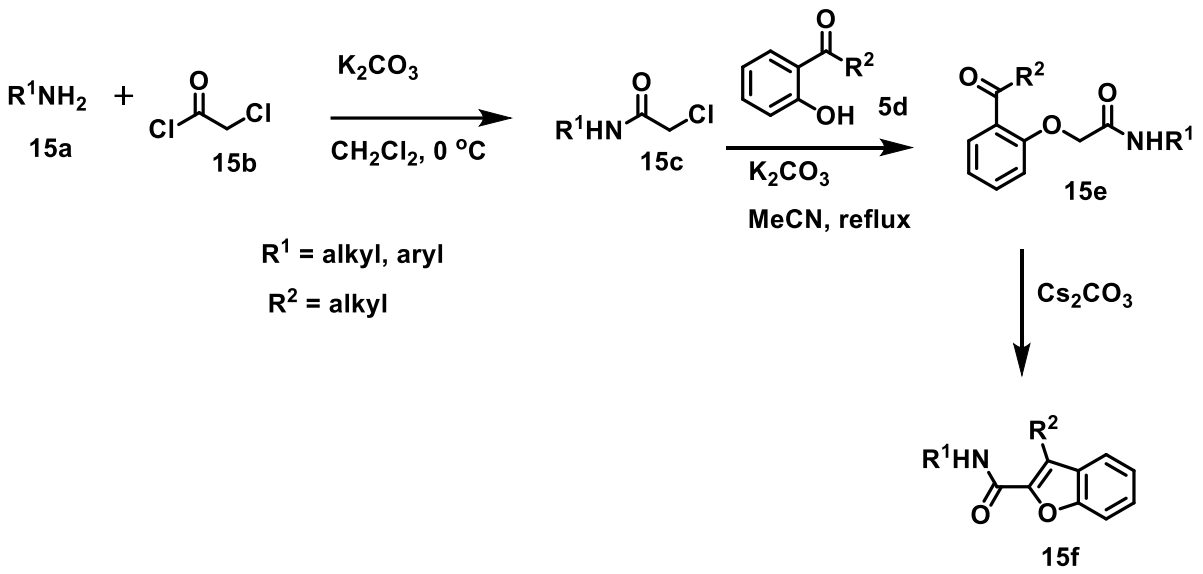
Scheme 2: Simple pathway to 7-hydroxybenzofuran **13c** derivative using anionic annulation strategy[23]

In addition to the use of furan to access benzofuran derivatives, 2-halophenols were used to synthesise benzofurans. For example, benzofurans were prepared in water utilising a novel heterogeneous Pd-Cu/C catalyst, as reported by F-X Felpin *et al*[53]. Benzofurans **14c** were produced in good yields (64–98%) via Sonogashira alkynylation of 2-iodophenol **14a** with alkynes **14b** (**Scheme 3**). This method can be used to prepare different heterocycles in high yields and has tolerance to a broad range of functional groups.



Scheme 3:Synthesis of benzofuran **14c** by Sonogashira cross coupling[53,54]

Moreover, a recent work by D-S Shin and colleagues described an effective method for synthesising a new class of benzofuran compounds[26]. In the presence of K_2CO_3, chloroacetyl chloride was treated with primary amines **15a** to yield N-substituted-2-chloroacetamide **15c.** Compounds **15c** were used to O-alkylate the easily accessible o-hydroxy aryl ketones **5d** in the presence of K_2CO_3 to produce substituted acetamides **15e** in fair yields. Substituted benzofurans **15f** were finally produced by cyclizing compounds **15e** in the presence of cesium carbonate (**Scheme 4**).



Scheme 4: The preparation of **15f** from primary amines **15a**[37,55]

Designing benzofuran derivatives with ester functional groups[56] instead of amide functionalities can be motivated by several factors related to their chemical properties and biological activities such as, enhanced membrane permeability, prodrug potential, and metabolic stability. Ester groups are generally more lipophilic than amides, facilitating better penetration through biological membranes and increased in permeability can lead to improved bioavailability of the compounds. Esters can serve as prodrugs, being hydrolyzed in vivo to release the active parent compound while this strategy can enhance the delivery and efficacy of drugs that may otherwise have poor [18]pharmacokinetic profiles[24]. In certain contexts, esters may offer favourable metabolic stability compared to amides, depending on the specific enzymatic environment and the presence of esterases[57] or amidases[58]. The substitution strategy where R1 is primary cyclic and aromatic while R2 is linear, cyclic, and aromatic. The setup reflects a targeted medicinal chemistry approach, aiming at enzyme inhibition, anticancer, or antimicrobial activities[23].

1.5 Aims and Objectives

1.5.1 Aim

The study aims to design, synthesise, and evaluate the anti-tubercular properties of the tri-substituted benzofuran derivatives.

1.5.2 Objectives

The objectives of the research project were to:

i. Synthesise benzofuran derivatives using Sonogashira cross-coupling.
ii. Functionalise the synthesised benzofuran derivatives to form esterified benzofuran derivatives.
iii. Characterise the resulting compounds using Nuclear Magnetic Resonance (NMR), Fourier Transform Infrared (FT-IR), and Mass Spectrometry (MS).
iv. Biologically evaluate the synthesised compounds against *Mycobacterium tuberculosis*.

1.6 Dissertation structure

This dissertation consists of four chapters. Chapter 1 gives the background and literature review, aim and objectives related to this work. Chapter 2 presents and discusses the results obtained in this research. Chapter 3 gives the conclusion and recommendations for future work. Chapter 4, the details methodology and experimental procedures for the preparation of benzofuran (**2A-3E5**) from iodinated vanillin **1B**. Each chapter has its own references to avoid confusion and limit repetition.

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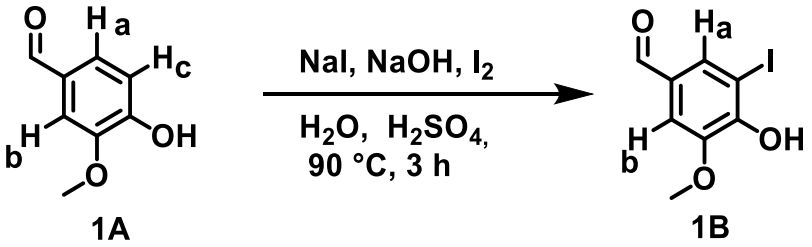
CHAPTER 2: RESULTS AND DISCUSSION

2.1 Synthesis of tri-substituted benzofuran derivatives

2.1.1 Synthesis of 4-hydroxy-3-iodo-5-methoxybenzaldehyde 1B

Adopting the literature procedure [1],commercially available 4-hydroxy-3-methoxybenzaldehyde **1A** was iodinated with the use of sodium iodide, iodine, sulfuric acid, and sodium hydroxide. The mixture was refluxed in water for 3 hours 4-hydroxy-3-iodo-5-methoxybenzaldehyde **1B** in **92%** yield (**Scheme 5**). The prepared 4-hydroxy-3-iodo-5-methoxybenzaldehyde **1B** was confirmed using ^{1}H-NMR and ^{13}C-NMR spectroscopy (**Figure 10**). The replacement of proton **c** by the attachment of iodine (**Figure 10**) was observed and **1B**'s spectrum displayed the presence of two protons in the aromatic region. The ^{1}H-NMR spectrum revealed two equal sharp peaks for protons **a** and **b** appearing at 7.36 and 7.81 ppm respectively as singlets. Additionally, a broad peak corresponding to an OH peak at 6.78 ppm and a singlet for methoxy (-OCH_3) was observed at 3.96 ppm. The ^{13}C-NMR spectrum (**Figure 10**), revealed the appearance of a peak for a carbon that is attached to iodine at 80.50 ppm and -OCH_3 peak at 56.54 ppm, and the aldehyde peak at 189.95 ppm.



Scheme 5: Iodination of 4-hydroxy-3-methoxybenzaldehyde **1A**[1]

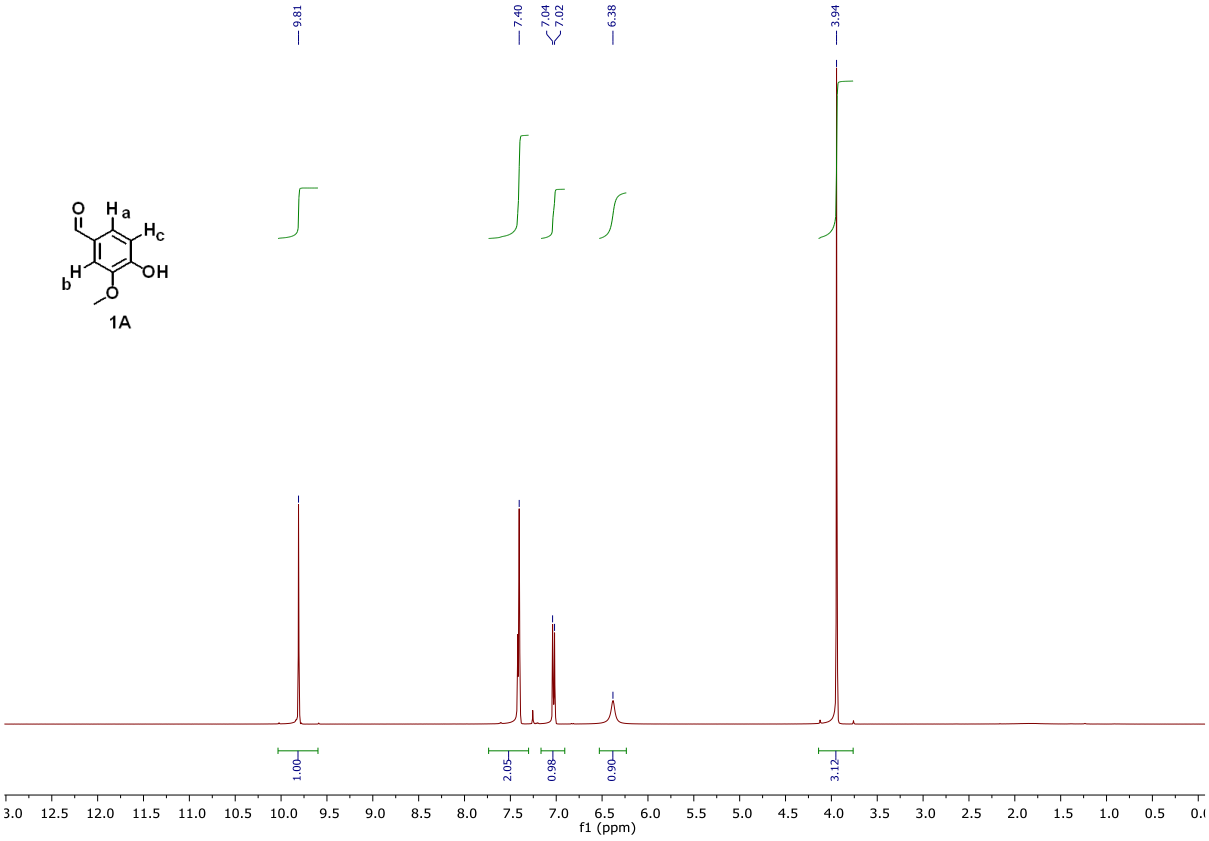
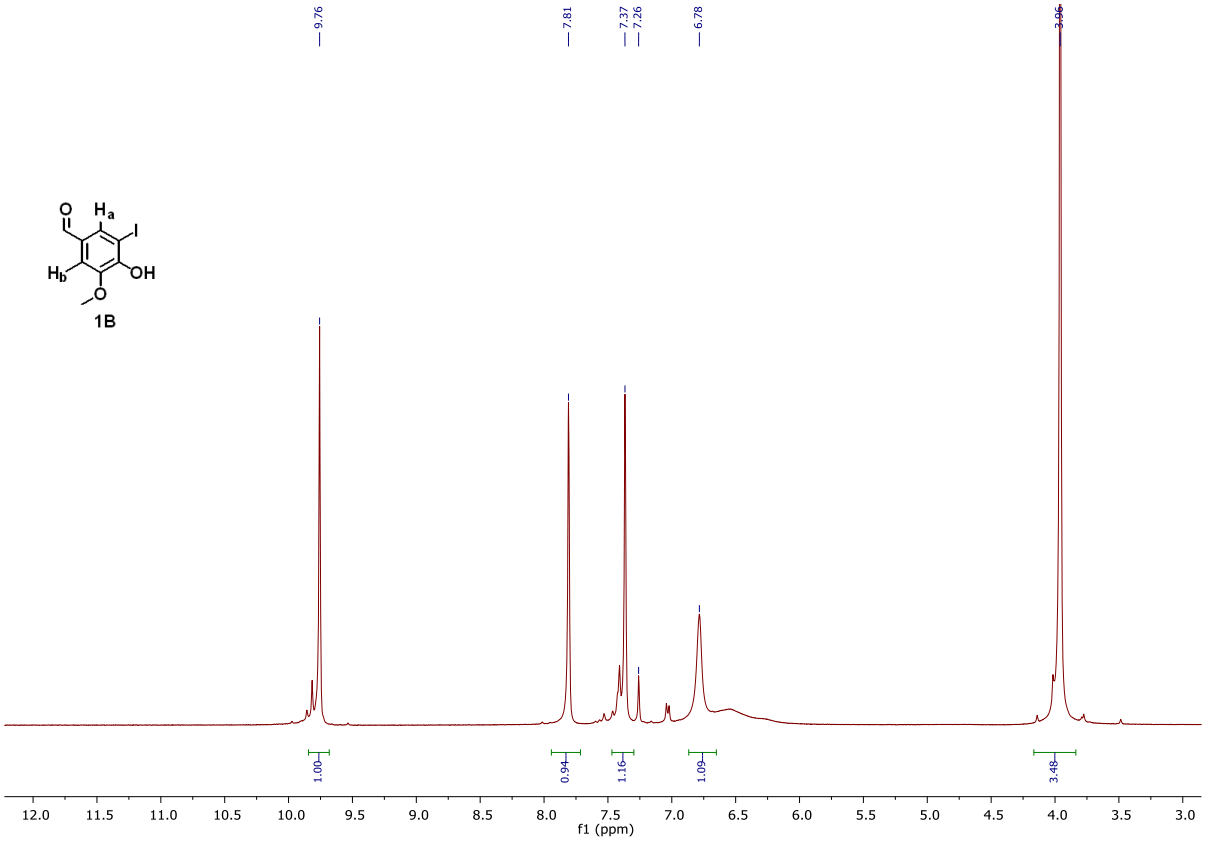


Figure 10: ^{1}H NMR spectra of 4-hydroxy-3-methoxybenzaldehyde **1A**



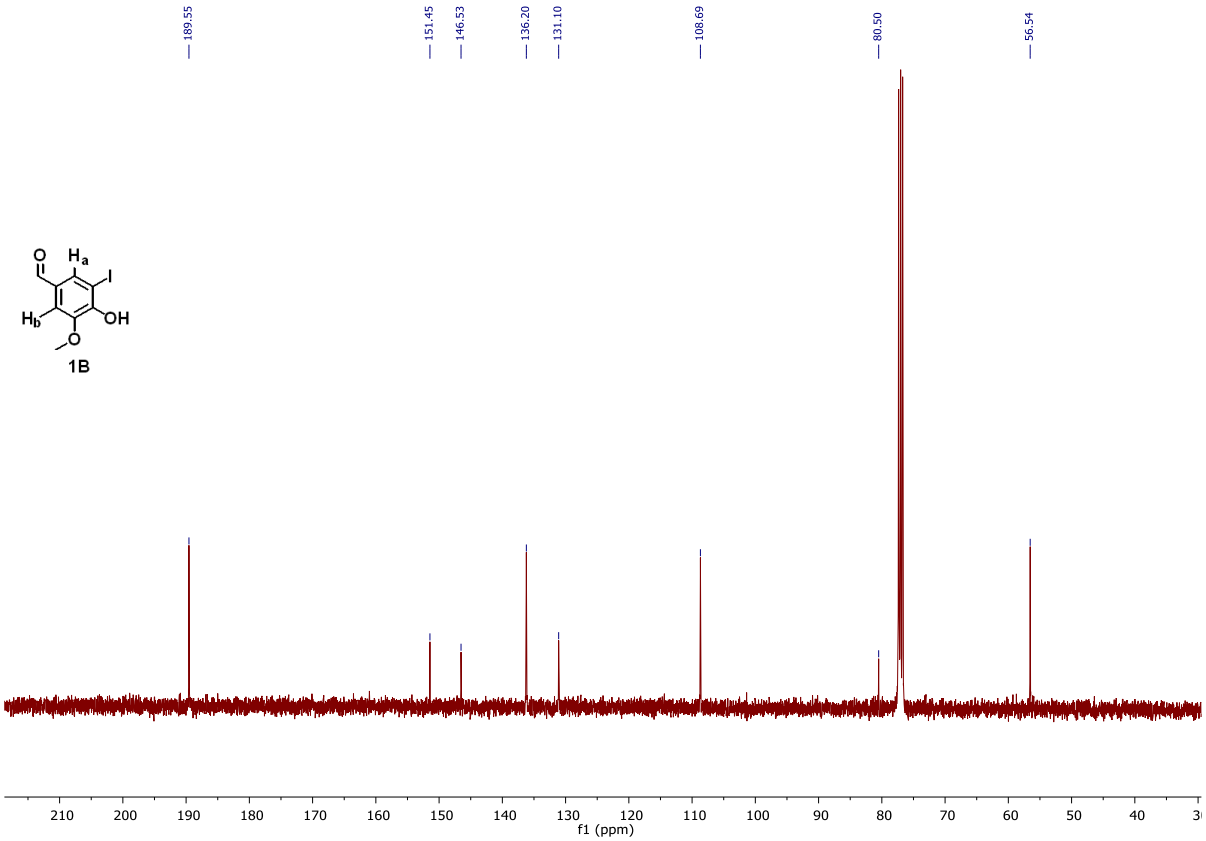
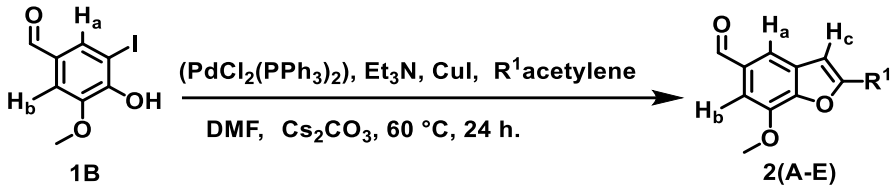


Figure 11: ^{1}H and ^{13}C-NMR spectra of 4-hydroxy-3-iodo-5-methoxybenzaldehyde **1B**.

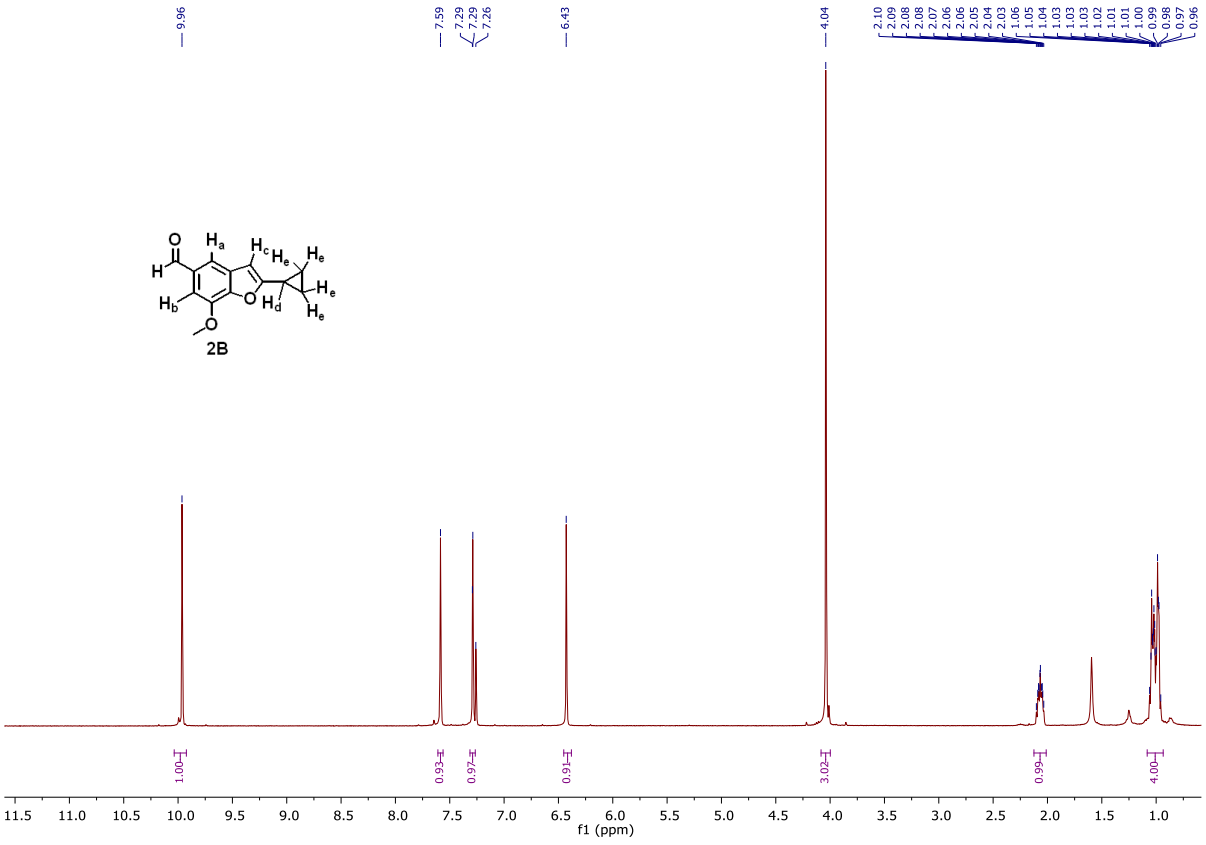
2.1.2 . Synthesis of benzofuran derivatives using Sonogashira cross coupling reaction.

Palladium (II)bis(triphenylphosphine) dichloride ($PdCl_2(PPh_3)_2$) catalysed Sonogashira cross coupling reaction of compound **1B** carried out in the presence of triethylamine (Et_3N), copper iodide (CuI), appropriate acetylene derivative, cesium carbonate (Cs_2CO_3) in dimethylformamide (DMF) at 60 °C under nitrogen atmosphere (N_2) for 24 hours afforded the desired 2-substituted-7-methoxybenzofuran-5-carbaldehyde **2B-2E** in 60-80% yield (**Scheme 6**) upon column chromatography purification. All the prepared compounds isolated in this reaction step were characterised using of both ^{1}H-NMR and ^{13}C-NMR spectroscopy. As expected, the ^{1}H-NMR spectra of the prepared compounds revealed the presence of an additional peak in the aromatic region corresponding to proton **c** as a singlet at around 6.43 ppm. The presence of additional peaks in the aliphatic and aromatic regions is included in **Table 1**. The ^{13}C-NMR spectra of all compounds showed the disappearance of the iodine carbon signal at 80.50 ppm and the appearance of extra aromatic carbons due to the 2-substituted benzofuran nucleus. For example, the spectra of **2B** (**Figure 11**) show the presence of an additional aromatic peak at 6.43 ppm (singlet) and additional

aliphatic peaks appearing between 0.97 and 2.10 ppm. The ^{1}H-NMR multiplicity of **2B** is as follows: multiplets (0.97-1.04 ppm, CH_2 and 2.03-2.10 ppm, CH), confirming the presence of the cyclopropyl substituent.



Scheme 6: Sonogashira cross coupling of various benzofuran derivatives from **1B**[2–4]



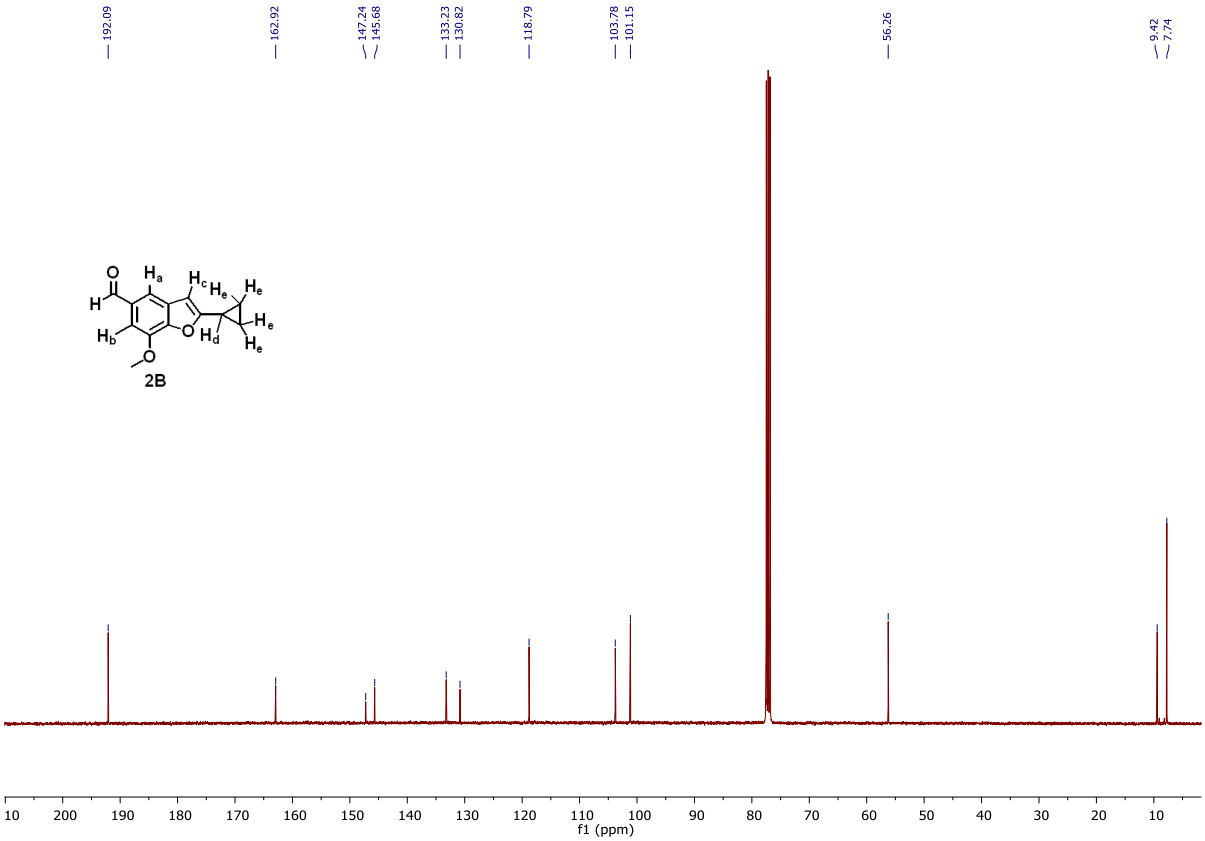
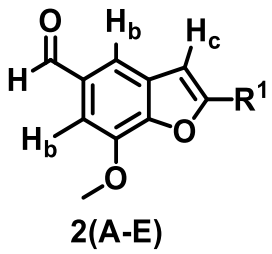
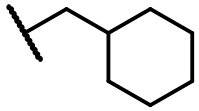


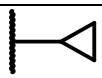
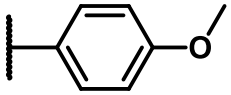
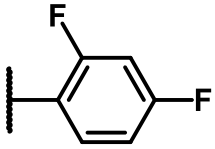
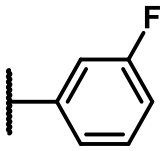
Figure 12: ^{1}H-NMR and ^{13}C-NMR spectra of 2-(cyclopropyl)-7-methoxybenzofuran-5-carbaldehyde **2B**.

Table 1: Substitution pattern, percentage yield and melting point of 2 (A- E)



2(A-E)

Compound	R^1	^{1}H-NMR	% Yield	Melting point (°C)
2A		6.48 ppm (1H, s), 2.68-2.67 ppm (2H, d), 1.80-1.62 ppm (7H, m), 1.29-1.13 ppm (4H, m)	69	74.6-76.2

2B		6.43 ppm (1H, s), 2.10-2.03 ppm (1H, m), 1.04-0.97 ppm (4H, m)	69	101-103
2C		7.83-7.80 ppm (2H, d), 6.97-6.95 ppm (2H, d), 6.85 ppm (1H, s), 4.05 ppm (3H, s)	60	158-160
2D		8.10-8.04 ppm (1H, m), 7.39 ppm (1H, s), 7.04-6.93 ppm (2H, m)	70	145-147.5
2E		7.24 ppm (1H, s), 7.22-7.15 ppm (1H, m), 7.06-6.99 ppm (1H, m), 6.90 ppm (1H, s)	80	81.2-84.6

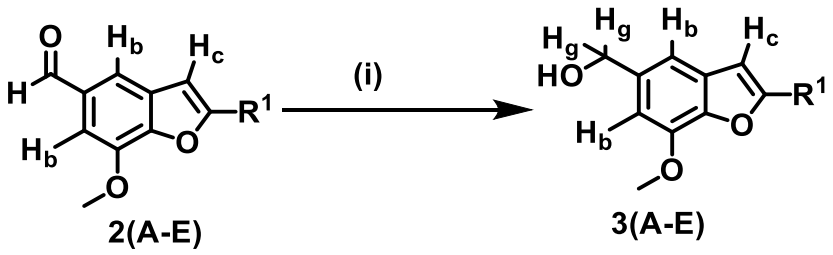
Singlet (s), doublet (d), multiplet (m), triplet (t)

The synthesised benzofuran derivatives **2A-2E** were obtained in yields ranging from 60 to 80 percent. The 3-Fluorophenyl group (**2E**) had the highest percentage yield among all other substituents. However, **2E** possessed the lowest melting point (around 74.6 °C) while the highest melting point belonged to **2C** (around 158 °C).

2.1.3 Reduction of benzofuran derivatives

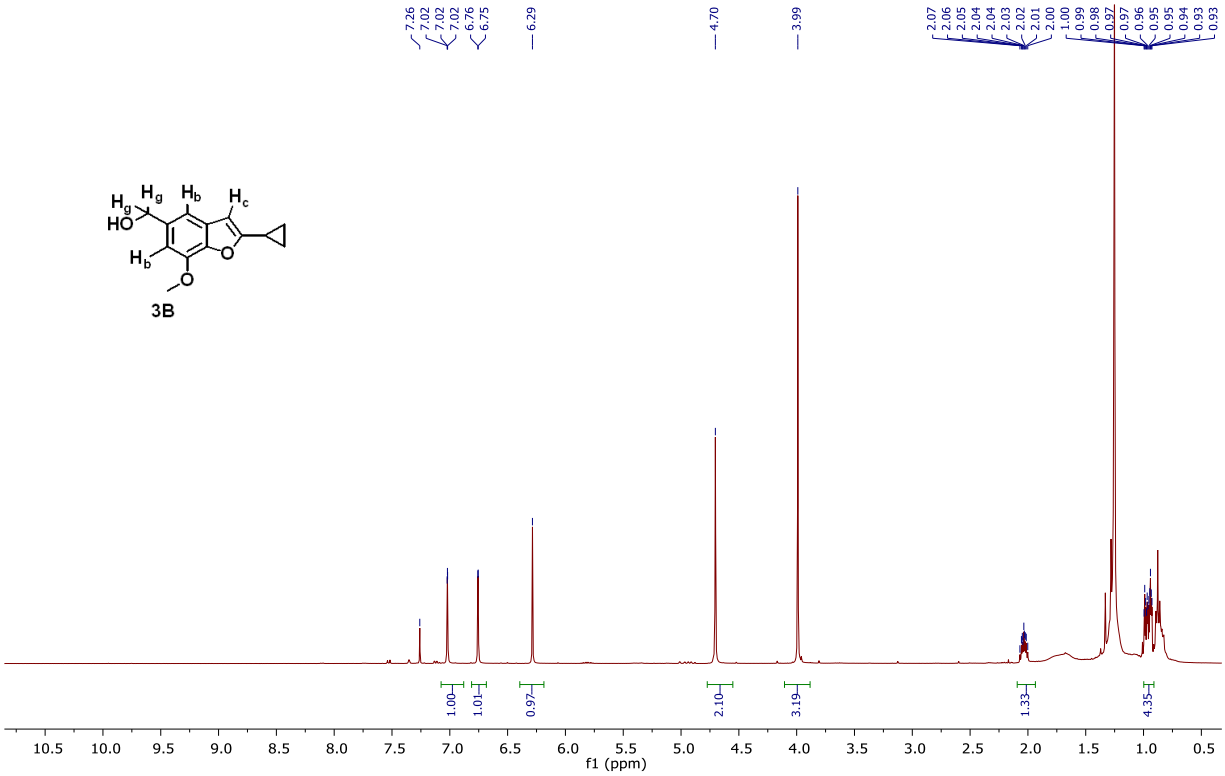
The reduction of 2-(substituted)-7-methoxybenzofuran-5-carbaldehydes **2B-2E** in the presence of sodium borohydride ($NaBH_4$) and ethanol (EtOH) for 3 hours at 25 °C to afford 2-substituted-7-methoxybenzofuran-5-yl methanol **3A-3E** in 60-82 % yields (**Scheme 7**) after column chromatography purification. All the prepared compounds isolated in this reaction step were characterised using both ^{1}H NMR and ^{13}C NMR spectroscopy. As expected, the ^{1}H-NMR spectra of the prepared compounds revealed the disappearance of C-H aldehyde peak at 9.96 ppm and the appearance of -CH_2- peaks ranging from 4.70-4.76 ppm was observed. The presence of an additional peak

in the aliphatic region due to the reduction of aldehyde to alcohol is included in **Table 2**. The (^{1}H and ^{13}C) NMR spectra for all compounds show the disappearance of the C-H aldehyde at 9.96 ppm and 192.40 ppm, respectively. For example, the spectra of **3B** (**Figure 12**) show the presence of the additional aliphatic peak at 4.70 ppm (singlet) (-CH_2-) confirming the disappearance of the aldehyde proton. The ^{13}C-NMR spectra, on the other hand, revealed the disappearance of the -C=O aldehyde carbon peak at 192.40 ppm and the appearance of a -CH_2- carbon signal observed at 66.12 ppm with respect to the reduction of aldehyde.



Scheme 7: Reduction of 2-(substituted)-7-methoxybenzofuran-5-carbaldehyde **2(A-E)**[2]

Reagents and conditions: (i) EtOH, $NaBH_4$, 0-25 °C, 3 h.



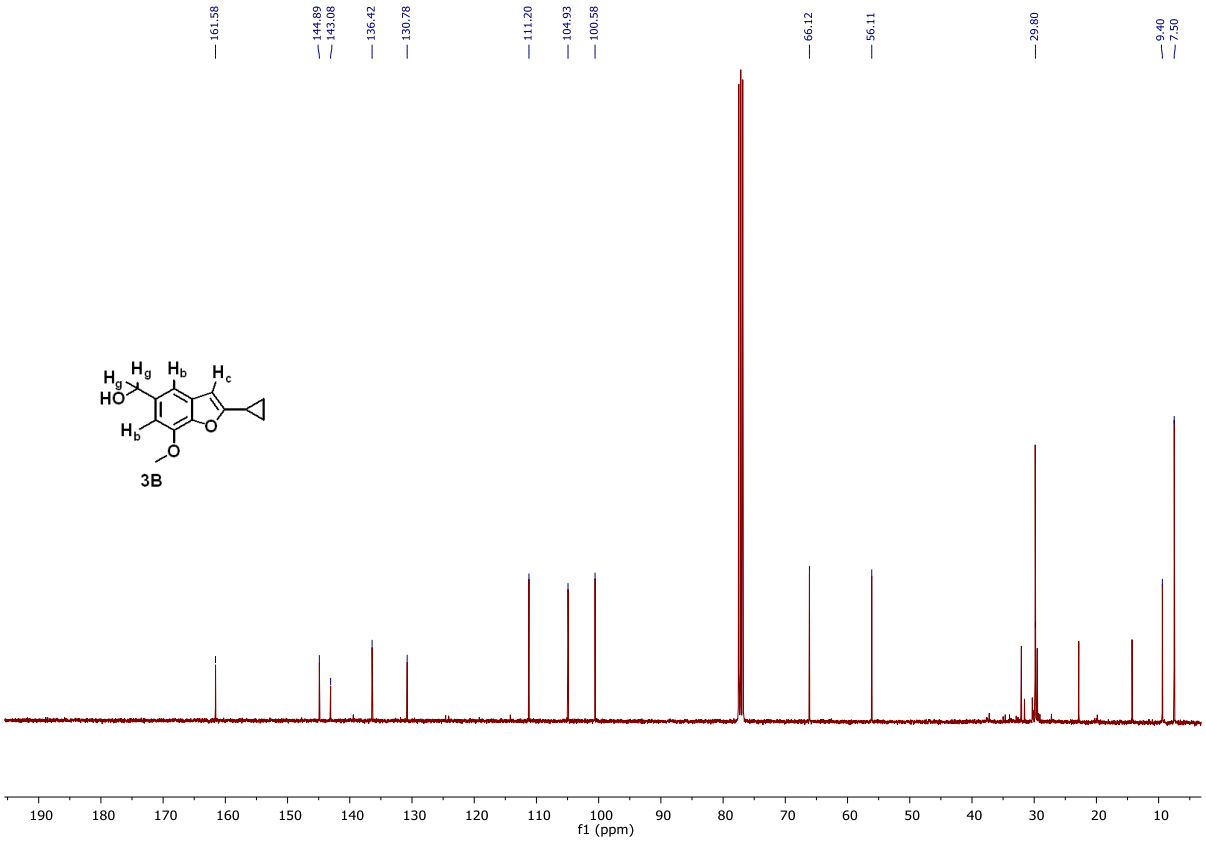
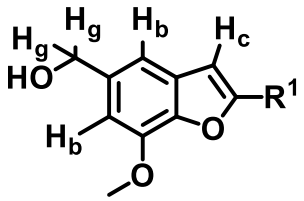
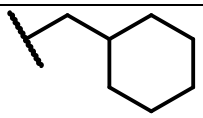
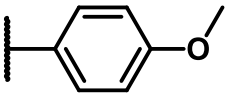
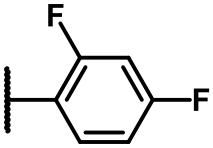
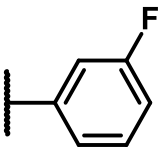


Figure 13: ^{1}H-NMR and ^{13}C-NMR spectra of (2-cyclopropyl-7-methoxybenzofuran-5-yl) methanol **3B** CDCl3 at 400 and 100 MHz respectively.

Table 2: Substitution pattern, percentage yield and melting point of 3 (A- E)



3A-3E)

Compound	**R^1**	**^{1}H-NMR of the new Peak**	**% Yield**	**Melting point (°C)**
3A		4.72 ppm (2H, s)	82	Oil
3B		4.70 ppm (2H, s)	82	Oil
3C		4.75 ppm (2H, s)	78	130-131.6
3D		4.76 ppm (2H, s)	82	145-147.2
3E		4.75 ppm (2H, s)	60	81.2-84.1

Singlet (s), doublet (d), multiplet (m), triplet (t)

The synthesised benzofuran derivatives of **3A-3E** have promising yields ranging from 60-82%. The melting points range from 81.2 to 147.2 °C for all the synthesized benzofuran compounds (**Table 2**).

2.1.4 Esterification of benzofuran derivatives

Esterification of 2-substituted-7-methoxybenzofuran-5-yl methanol **(3A-3E)** in the presence of different desired carboxylic acids, 2-chloro-methylpyridinium iodide, triethylamine and dichloromethane (DCM) for 24 hours at 25 °C afforded (2-substituted)-7-methoxybenzofuran-5-yl) methyl 6-(substituted) esters (**3A1-3E5)** in 40-80 % yields upon column chromatography purification. All the prepared compounds isolated in this reaction step were characterized using a combination of ^{1}H NMR and ^{13}C-NMR. The additional peaks in the aliphatic and aromatic regions are included in **Table 3**. The (^{1}H and ^{13}C) NMR spectra of all compounds show the presence of additional peaks in the aliphatic and aromatic regions due to the formation of different esters. The ^{13}C-NMR spectra for all compounds showed an ester carbonyl peak in the range of 145.32-172.1 ppm, indicating a successful esterification reaction. For example, the ^{1}H-NMR spectrum of **3B1** (**Figure 13**) revealed three additional protons in the aromatic region belonging to the 6-chloronicotinic moiety, which added up to a total of six (6) aromatic protons at 6.31-9.01 ppm. The ^{1}H-NMR multiplicity of **3B1** is as follows: doublets at 8.23 ppm CH, 7.41-7.39 ppm CH, and a singlet (9.01 ppm CH) confirming the presence of the 6-chloronicotinic substituent. The ^{13}C-NMR spectra (**Figure 13**) revealed the presence of an ester carbonyl peak at 164.31 ppm for successful esterification.

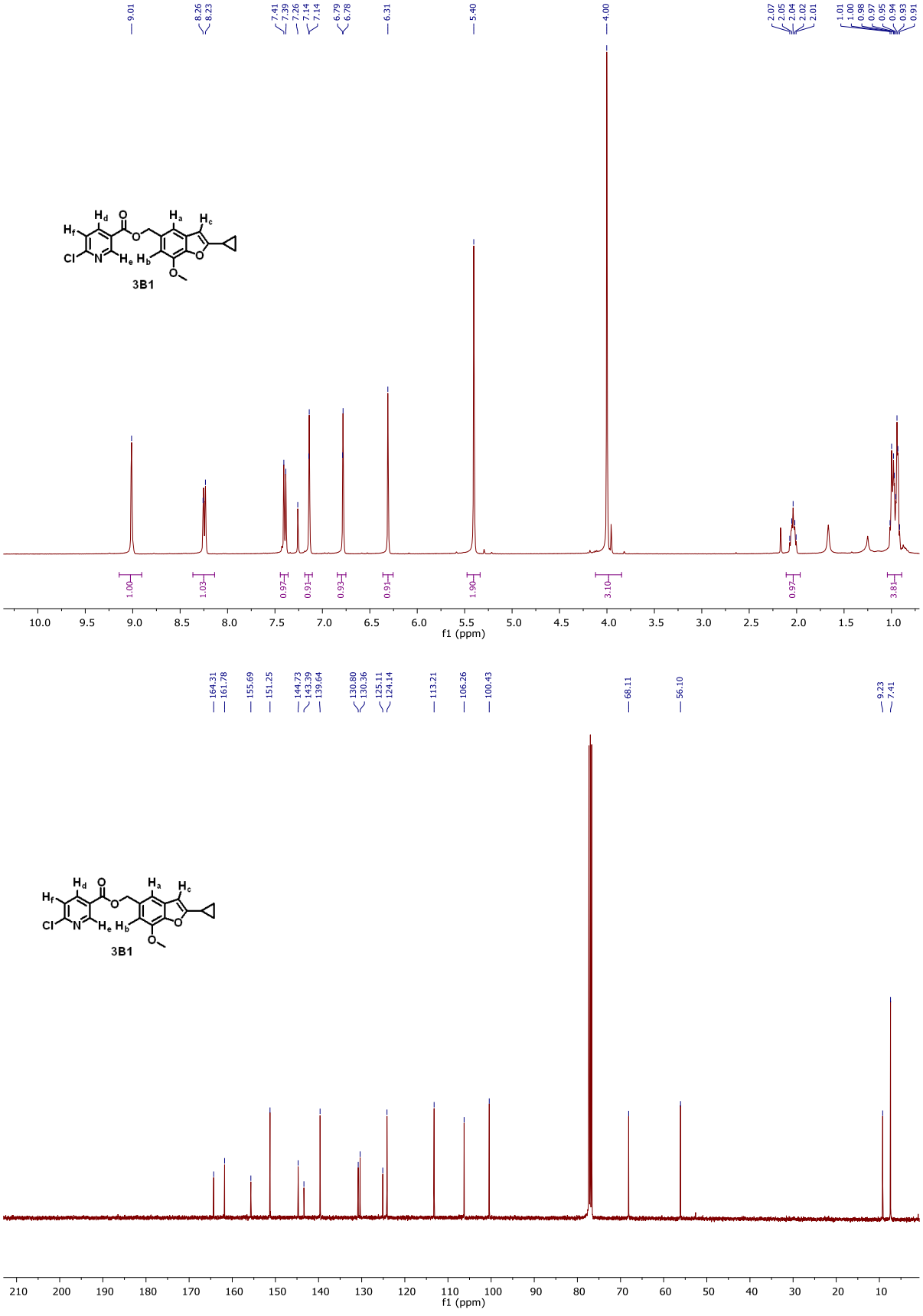
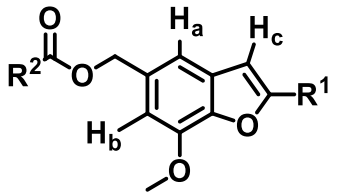
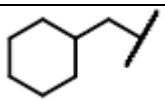
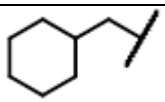
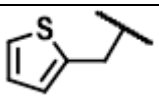
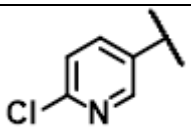
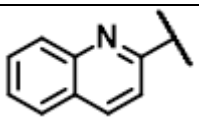


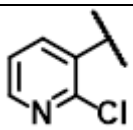
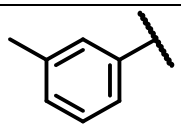
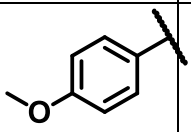
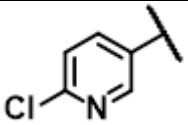
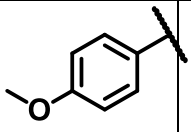
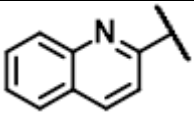
Figure 14: ^{1}H-NMR and ^{13}C-NMR spectrum of (2-cyclopropyl-7-methoxybenzofuran-5-yl) methyl 6-chloronicotinate **3B1**

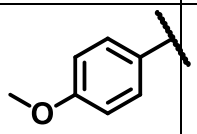
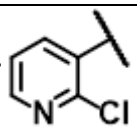
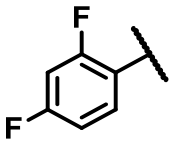
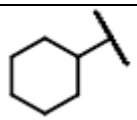
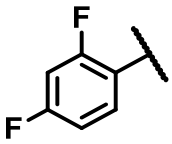
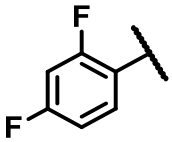
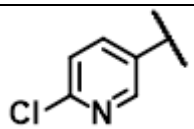
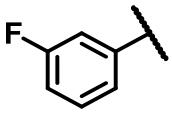
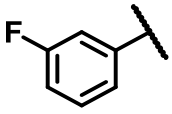
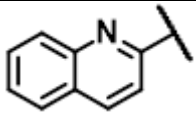
Table 3: Substitution pattern, percentage yield and melting point of 3 (A1- E4)

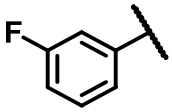
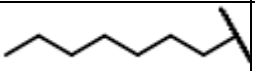
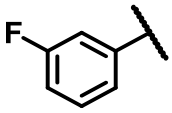
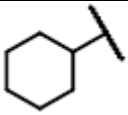
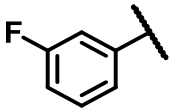
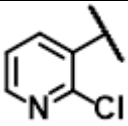


3A1-3E1

Compound	**R^1**	**R^2**	**^{1}H-NMR of the new peaks**	**^{13}C-NMR**	**% Yield**	**Melting point (°C)**
3A1			4.01 ppm (s, 3H)	171.02 ppm	60	Oil
3A2			8.28 ppm (d, 1H), 8.18 ppm (s, 2H), 7.05-6.98 ppm (m, 1H), 6.54 ppm (d, 1H)	171.36 ppm	60	Oil
3B1			9.01 ppm (s, 1H), 8.25 ppm (d, 1H), 7.40 ppm (d, 1H)	164.31 ppm	61	61-63
3B2			8.32 ppm (dd, 2H), 8.17 ppm (d, 1H), 7.87 ppm (d, 1H), 7.83-7.76 ppm (m, 1H), 7.72-	164.92 ppm	61	128-130

			7.57 ppm (m, 1H),			
3B3			8.50 ppm (dd, 1H), 8.22-8.13 ppm (m, 1H), 6.82 ppm (d, 1H)	164.79 ppm	61	Oil
3B4			2.06 ppm (s, 3H)	170.7 ppm	43	Oil
3B5			7.95 ppm (dd, 1H), 7.87 ppm (dd, 1H), 7.50 ppm (dd, 1H), 7.37 ppm (m, 1H), 2.43 ppm (s, 3H)	164.3 ppm	30	Oil
3C1			9.04 ppm (s, 1H), 8.27 ppm (dd, 1H), 7.41 ppm (d, 1H)	164.52 ppm	40	164-166
3C2			8.31 ppm (dd, 2H), 8.18 ppm (d, 1H), 7.87 ppm (d, 1H),	165.25 ppm	63	158-161

			7.68-7.60 ppm (m, 1H), 6.86 ppm (d, 1H)			
3C3			8.52 ppm (dd, 1H), 8.19 ppm (dd, 1H), 7.33 ppm (dd, 1H)	164.48 ppm	56	Oil
3D1			2.40 ppm (ddq, 7H), 0.92-0.80 ppm (m, 4H)	172.05 ppm	50	Oil
3D2			2.16 ppm (s, 3H)	171.11 ppm	72	122-125.7
3D3			9.04 ppm (s, 1H), 8.31-8.24 ppm (m, 1H), 7.16 ppm (d, 1H),	164.50 ppm	40	Oil
3E1			2.12 ppm (s, 3H)	170.95 ppm	60	Oil
3E2			8.40 ppm (d, 1H), 8.33 ppm (d, 1H), 8.21 ppm (d, 1H), 7.89 ppm (d, 1H),	147.67 ppm	80	166-169

			7.67 ppm (dd, 2H)			
3E3			2.36 ppm (t, 2H), 1.76-1.56 ppm (m, 3H), 0.89 ppm (q,7H),	173.85 ppm	50	Oil
3E4			2.41-2.36 ppm (m, 1H), 1.81-1.55 ppm (m, 10H)	145.32 ppm	40	74-76
3E5			9.04 ppm (d, 1H), 8.27 ppm (d, 1H), 7.65 ppm (d, 1H)	164.48 ppm	55	99.2-101.4

Singlet (s), doublet (d), doublet of doublet (dd), multiplets (m), triplet (t), quartet (q),

The synthesised benzofuran derivatives of **3A1-3E4** have promising yields, which ranged from 40 to 72% for esters. The melting point ranged from 61 to 187.9 °C for all synthesized benzofuran (**Table 3**).

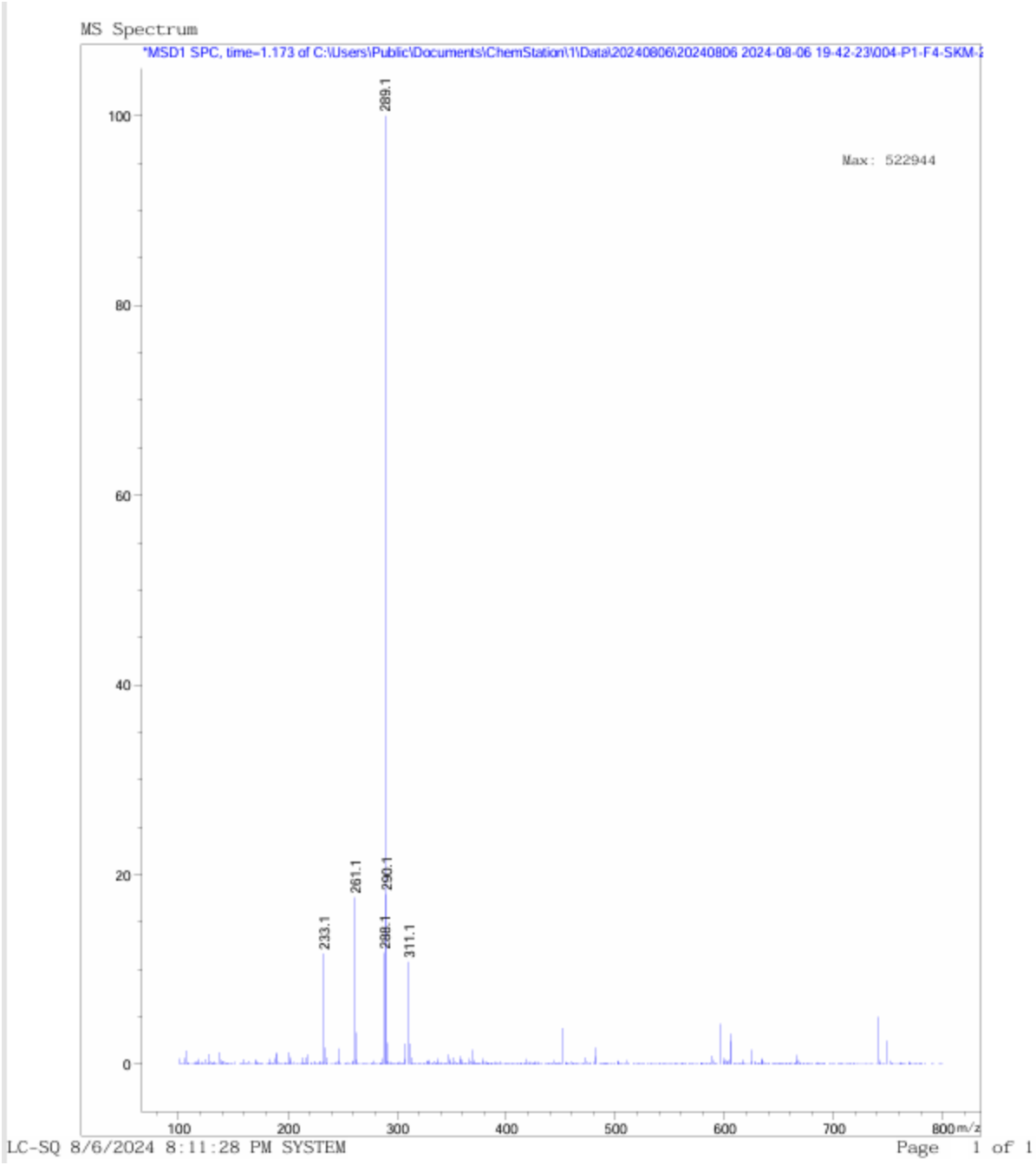


Figure 15: Mass spectra of the 2-(2,4-difluorophenyl)-7-methoxybenzofuran-5-carbaldehyde **2D**

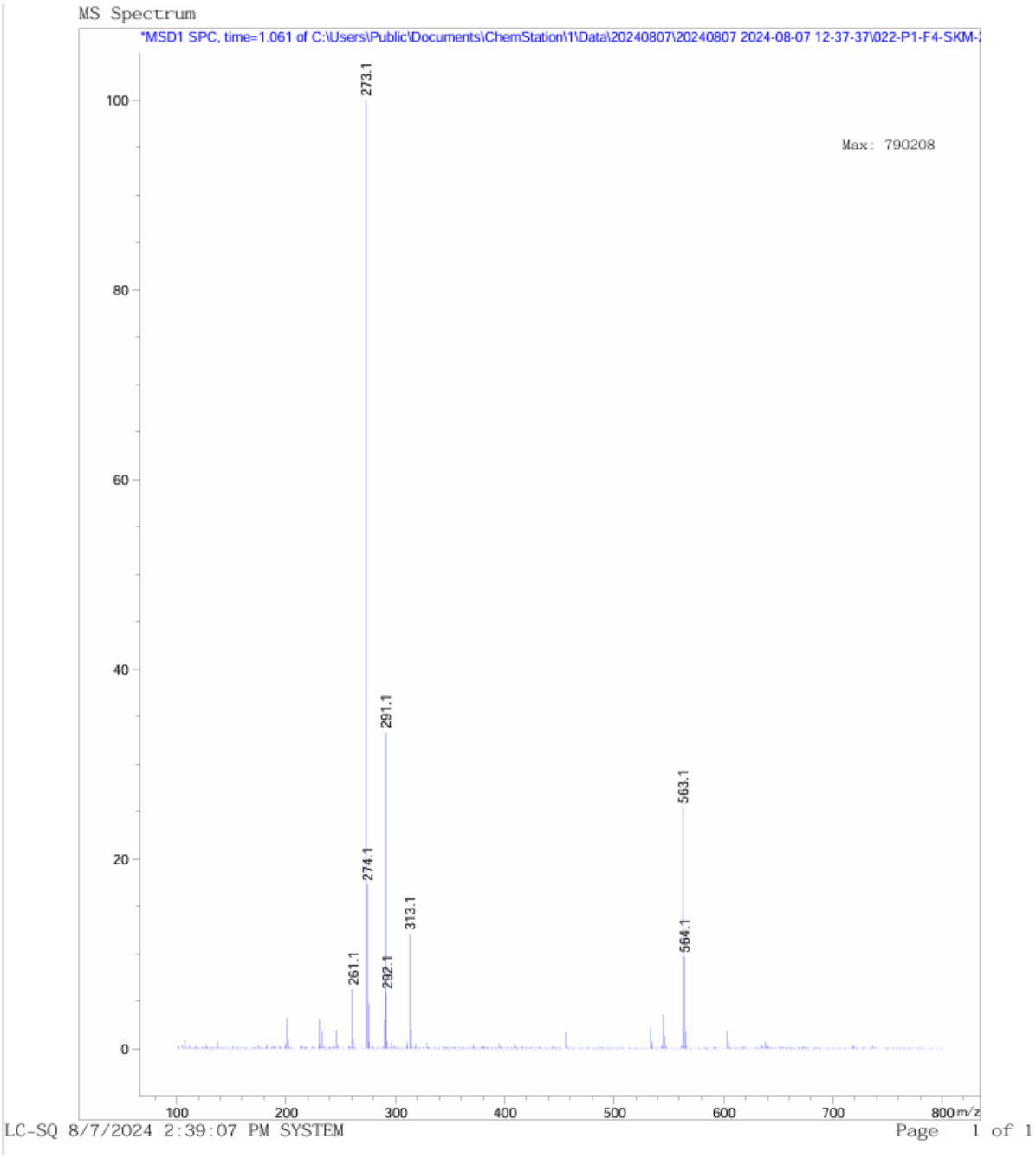


Figure 16: Mass spectra of the 2-(2,4-difluorophenyl)-7-methoxybenzofuran-5-yl) methanol **3D**

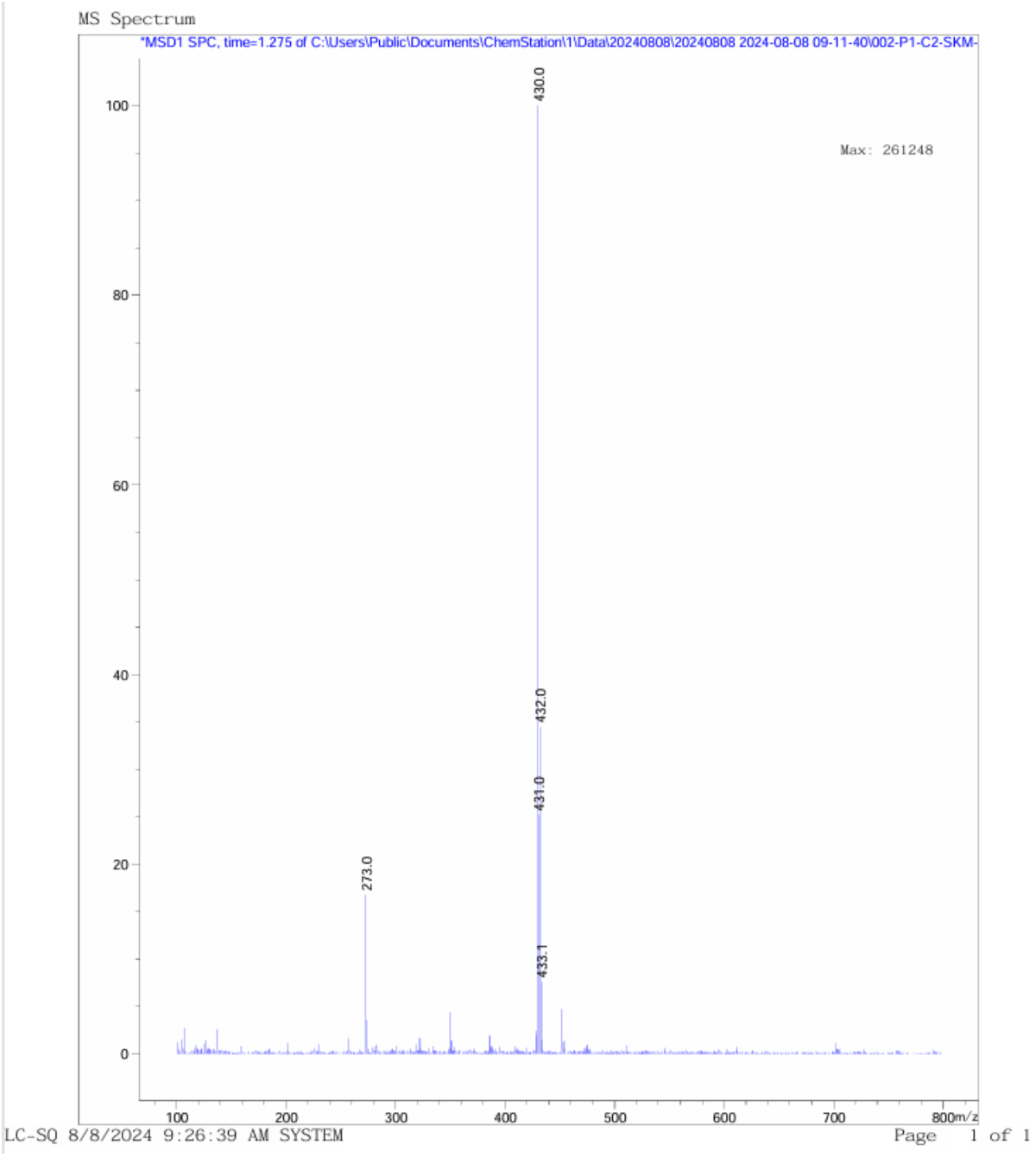


Figure 17: Mass spectra of the 2-(2,4-difluorophenyl)-7-methoxybenzofuran-5-yl) methyl-6-chloronicotinate **3D3**

2.2 Physicochemical properties

Physicochemical [3], (Drug metabolism and Pharmacokinetics), and safety profiles are presented in **Table 4**. The physicochemical properties include topological polar surface area (TPSA), Log S (solubility of the molecule), Log P (measure of

lipophilicity), Caco-2 permeability, plasma protein binding (PPB), human hepatotoxicity and A549 cytotoxicity (human pneumocyte tissue culture model).

A key field in drug discovery and development that benefits the pharmaceutical business and aids in determining which medicine is a good contender is drug metabolism and pharmacokinetics[4]. The chemical alteration of pharmaceutical chemicals in the body, mostly by means of specialised enzyme systems, is known as drug metabolism. Understanding how the body will break down and digest (metabolise) a drug is crucial for determining its safety profile and effectiveness. Pharmacokinetics (PK) is the study of how the body reacts to medications and includes the following: absorption, distribution, metabolism, and excretion (ADME) of drugs[4]. PK tests reveal how rapidly and effectively a medication is taken in, transported throughout the body, metabolised, and eliminated. This aids in establishing a drug's dosage and frequency.

2.2.1 Screening for biopharmaceutical attributes

The screening of the benzofuran derivatives' biopharmaceutical attributes was generated with the help of online open access software (ADMET.3.0)[5]

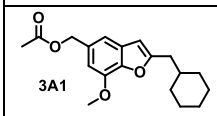
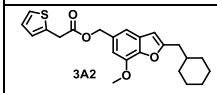
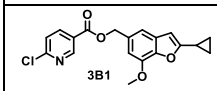
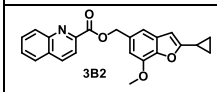
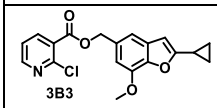
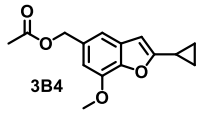
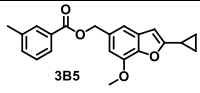
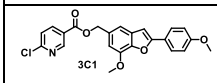
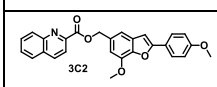
2.2.2 Drug likeliness property

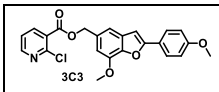
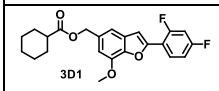
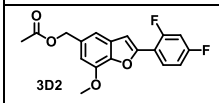
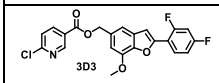
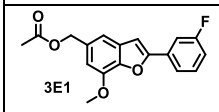
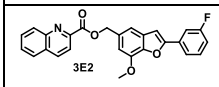
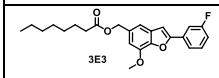
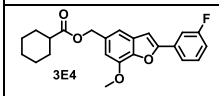
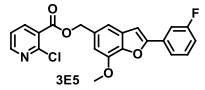
The analysis was done for all ester derivatives. To find the best compound, it must follow or obey Lipinski rule of 5, GSK and Pfizer rules were selected Log P <5 M, Log S >-4 M, PPB <90% (drug with high protein-bound may have low therapeutic index), optimal TPSA <90 Å, Caco-2 permeability (related to toxicity) higher than -5.15/g unit, A549 <1, and hepatotoxicity of 0.10-0.30 considered (nontoxic) and >0.31 as poor (toxic). Five (5) compounds (**3B1, 3B5, 3C1, 3C3**, and **3D3**) show good physicochemical properties with all the parameters illustrated in (**Table 4**). Additionally, their hepatotoxicity ranged from 0.106-0.262 for all ester benzofuran derivatives. One compound, 3B4, had the highest TPSA of 94.42. All the compounds have PPB values >95%, and they are considered to have high protein-bound properties and leading to a low therapeutic index.

3B1, 3C1, 3C3, and 3D3 share a common structural feature. R2 appears to be that common element chlorine atom substituted at different position on the pyridine moeity.

Both 2-chloronicotonic ester derivatives possess features favourable for drug-like behaviour, but:2-chloronicotinic ester (3C3) might offer better metabolic stability but slightly lower solubility/permeability due to electron withdrawing group chlorine being ortho director to both of pyridine nitrogen atom and ester group where 6-chloronicotinic ester (3B1, 3C1, and 3D3), generally exhibits better solubility and permeability, possibly at the cost of slightly lower metabolic stability caused by electron withdrawing chlorine been para to ester group and meta to pyridine nitrogen atom.

Table 4: In Vitro ADMET (absorption, distribution, metabolism, excretion and toxicity) properties of the ester benzofuran derivatives

Compound	TPSA (Å)	Log S (M)	Log P (M)	PPB %	A549 cytotoxicity	Human hepatotoxicity	Caco-2 Permeability
 3A1	48.67	-5.049	4.461	98.5	0.129	0.579	-4.722
 3A2	48.67	-6.005	5.433	98.8	0.082	0.745	-4.787
 3B1	61.56	-5.718	4.514	97.5	0.106	0.178	-4.930
 3B2	61.56	-5.239	4.071	97.6	0.126	0.572	-4.841
 3B3	61.56	-5.239	4.071	97.6	0.120	0.391	-4.930
 3B4	94.42	-4.611	3.773	98.9	0.133	0.635	-4.940
 3B5	48.67	-5.907	5.364	98.7	0.117	0.137	-4.827
 3C1	70.79	-6.185	4.433	96.4	0.127	0.106	-4.958
 3C2	70.79	-5.843	4.709	98.7	0.150	0.424	-4.789

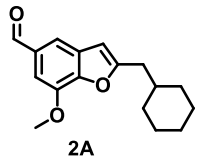
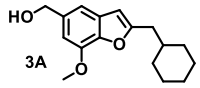
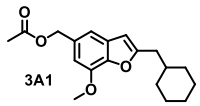
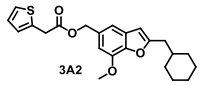
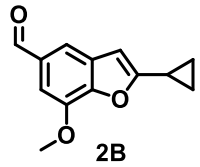
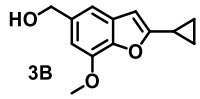
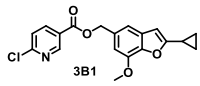
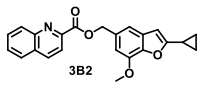
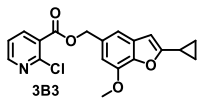
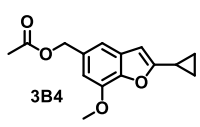
	70.79	-5.456	3.844	95.3	0.143	0.262	-5.040
	48.67	-6.749	5.592	98.9	0.108	0.677	-4.781
	48.67	-4.489	3.551	98.2	0.056	0.565	-4.964
	61.56	-6.135	4.948	97.2	0.145	0.204	-5.053
	48.67	-4.257	3.613	98.7	0.060	0.460	-4.907
	61.56	-4.661	5.196	98.8	0.184	0.511	-4.849
	48.67	-6.176	6.400	99.1	0.138	0.484	-4.846
	48.67	-6.481	5.549	98.9	0117	0.579	-4.742
	61.56	-5.406	4.299	98.2	0.176	0.335	-5.097

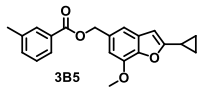
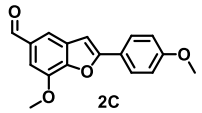
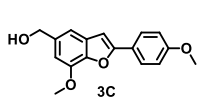
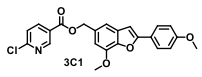
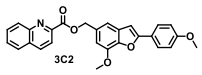
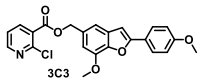
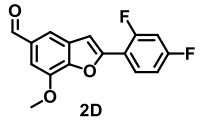
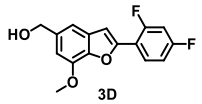
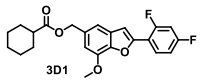
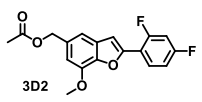
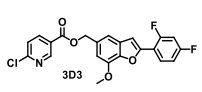
2.3 Bioassay Analysis

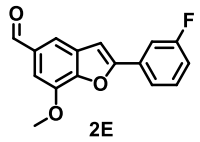
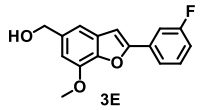
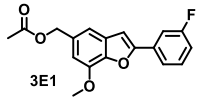
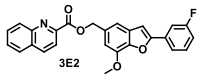
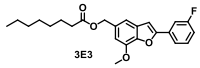
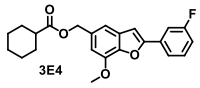
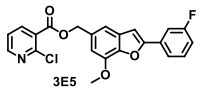
All the synthesised compounds were biologically evaluated for antimycobacterial, cytotoxicity, and solubility and the results are presented in **Table 5**.

Table 5: Minimum inhibitory concentrations (MICs) of compounds tested against M. tuberculosis H37Rv together with their solubility and cytotoxicity.

Compound	**MIC_{90} CAS TX result (µM)**	**MIC_{90} ADC TW result (µM)**	**MIC_{90} ADC TX result (µM)**	**Cytotoxicity (IC_{50} µM)**	**Solubility (µM, pH 7.4)**

 2A	**2.02**	>62.5	**18.08**	-	5
 3A	**18.92**	>62.5	>62.5	-	20
 3A1	-	-	-	-	-
 3A2	**7.8**	>62.5	>62.5	>50	50
 2B	56.17	>62.5	>62.5	-	30
 3B	>62.5	>62.5	>62.5	-	195
 3B1	**30.27**	**6.56**	**7.6**	>50	50
 3B2	**7.85**	>62.5	**32.23**	>50	<5
 3B3	**3.93**	**11.47**	**2.73**	>50	<5
 3B4	**15.6**	>62.5	>62.5	>50	-

 3B5	>62.5	>62.5	>62.5	>50	<5
 2C	>62.5	>62.5	>62.5	>50	<5
 3C	>62.5	>62.5	>62.5	>50	<5
 3C1	>62.5	>62.5	**2.91**	>50	<5
 3C2	>62.5	>62.5	>62.5	>50	<5
 3C3	>62.5	**12.79**	**8.22**	>50	-
 2D	>62.5	>62.5	>62.5	>50	<5
 3D	32.78	>62.5	>62.5	>50	<5
 3D1	>62.5	>62.5	>62.5	>50	-
 3D2	>62.5	>62.5	>62.5	>50	<5
 3D3	>62.5	>62.5	>62.5	>50	-

 2E	-	-	-	-	-
 3E	>62.5	>62.5	>62.5	>50	10
 3E1	>62.5	>62.5	>62.5	>50	<5
 3E2	>62.5	>62.5	>62.5	>50	<5
 3E3	>62.5	>62.5	>62.5	>50	-
 3E4	>62.5	>62.5	>62.5	>50	<5
 3E5	>62.5	>62.5	>62.5	>50	-
Controls[6,7]					
Moxifloxacin	0.2	0.2	0.4	-	-
Rifampicin	1.9	1.9	3.9	-	-

Chinese hamster ovary-derived epithelial cell line (CHO); minimum inhibitory concentration against *mtb* $H_{37}R_v$; ADC (albumin-dextrose-catalase) supplement; CAS (casitone); IC_{50}-50% inhibitory concentration; solubility-aqueous solubility evaluated at pH 7.4.

2.3.1 Antimycobacterial Assay

The antimycobacterial evaluation of compounds (**2A-3E5**) against *Mtb* $H_{37}R_v$ showed that the media in which the biological evaluations were performed affected the potency/activity of the tested synthesised benzofuran derivatives (**Table 5**). It should be mentioned that Middlebrook 7H9, the normal broth base, and the glucose (Glu) supplement were shared among all media. The distinction is that one medium contains casitone (CAS) and tyloxapol (Tx), whereas the other media contains albumin-dextrose-catalase (ADC) and Tween-80 (Tw). Surfactants Tx and Tw prevent mycobacteria from clumping together. Whereas CAS is a source of amino acids (AAs), ADC contains albumin (bovine albumin fraction (V)), which is thought to be a helpful proxy for the investigated benzofuran derivatives' possible propensity to bind serum protein. The protein binding may be the cause of the lower activity in 7H9/ADC/Glu/Tw medium. Additionally, there can be additional factors that contributed to the low quality of the activity [8].

As observed, compound **3B3** showed good activities in all three media 7H9/CAS/Glu/Tx, 7H9/ADC/Glu/Tx, and 7H9/ADC/Glu/Tw, from 7-8 days [9]. Compounds (**3A**, **3A2**, **2B**, **3B1**, **3B2**, **3B4**, **3D**) with moderate activity in only one media 7H9/ADC/Glu/Tx with the MIC_{90} (7.8-56.17 µM). Compounds (**3B1**, **3C3**) were active in two media 7H9/ADC/Glu/Tx and Tw with MIC_{90} (6.56-12.79 µM), while compounds (**2A** and **3B2**) were active in two media 7H9/CAS/Glu/Tx and 7H9/ADC/Glu/Tx with MIC_{90} (2.02-32-23 µM). The following compounds (**3B**, **3B5**, **2C**, **3C**, **3C2**, **2D**, **3D1-3D3**, **3E-3E5**) showed poor activity in all the three media with MIC_{90} >62.5 µM. Compound **2A** was the only compound from various 2-(substituted)-7-methoxybenzofuran-5-carbaldehyde (**2A-2E)** which showed a promising activity in two media 7H9/CAS/Glu/Tx and 7H9/ADC/Glu/Tx MIC_{90} 2.02 µM and 18.08 µM form the 2-substituted-7-methoxybenzofuran-5-yl methanol derivatives **3A-3E**, only two compounds **3A** and **3D** showed promising activity in 7H9/CAS/Glu/Tx medium with MIC_{90} (18.92-32.78 µM). On the other hand, the (2-substituted)-7-methoxybenzofuran-5-yl) methyl 6-(substituted) ester derivatives **3A1-3E5** only compounds **3A2**, **3B2**,**3B3**, and **3B4** showed good activity in 7H9/CAS/Glu/Tx medium with MIC_{90} 3.93-15.6 µM. Moreover, compounds **3B1**, **3B3**, and **3C3** showed good activity in7H9/ADC/Glu/Tx and 7H9/ADC/Glu/Tw media with MIC_{90} (6.56-12.79 µM) and (2.73-8.22 µM) respectively. Furthermore, compound **3B2** has good activity in 7H9/CAS/Glu/Tx with

MIC_{90} value of 7.8 µM and poor activity in other media while compound **3C1** showed activity in 7H9/ADC/Glu/Tx medium with MIC_{90} value of 2.91 µM.

Cyclopropyl(R1) in 3B1, 3B2, and 3B3 act as a good substituent like cyclohexane methyl(R1) used in 2A, 3A, and 3A2 which also shows potency in casitone protein. The problem of this cyclohexane propyl acetylene is now expensive unlike cyclopropyl acetylene which can be used in the future designs for medicinal chemistry used for drug discovery since it showed good activity against mycobacterium tuberculosis. Aromatic acetylene attached with electron withdrawing groups such as (Fluoro (3D1-3D3, 3E1-3E5 having a same R1, Methoxy (3C1-3C3 having a similar R1) shows poor potency as from cyclisation via sonogashira cross coupling, reduction to alcohol and ester but still shows poor potency with all the three-medium used **i**n **table 5**.

2.3.2 Cytotoxicity Assay

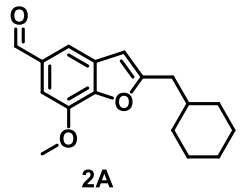
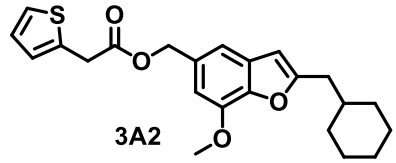
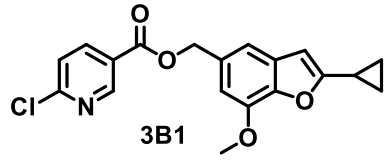
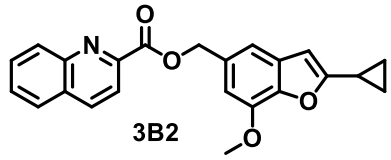
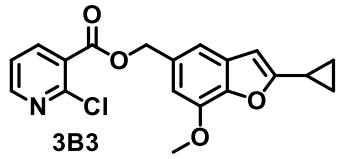
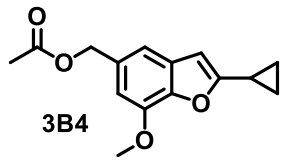
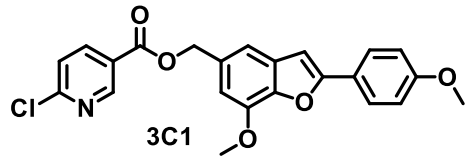
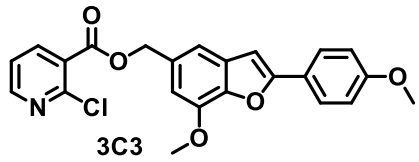
The synthesised trisubstituted benzofuran derivatives were tested for their cytotoxicity against CHO (Chinese hamster ovarian cell line using emetine as reference compound. The IC_{50} values of all the compounds tested are greater than fifty micromolar (>50 µM) (**Table 5**).

2.3.3 Selectivity index

Selectivity index (SI) is a ratio that is measured between cytotoxicity and anti-TB activity by dividing the given IC_{50} value by the MIC_{90} value (IC_{50} µM/ MIC_{90} µM). The values are shown in **Table 6**. The following compounds show the best activity with the MIC_{90} of 2.02-15.6 µM against the *mycobacterium* tuberculosis and have a good selective index (>24.75 and <3.9).

Table 6: Compounds (2-substituted)-7-methoxybenzofuran-5-yl) methyl 6-(substituted) ester with at least good activity with one medium.

Compound	**MIC_{90} µM**	**IC_{50} µM**	**SI**

 2A	2.02	>50	>24.75
 3A2	7.8	>50	>6.41
 3B1	6.56	>50	>7.62
 3B2	7.85	>50	>6.37
 3B3	2.73	>50	>18.32
 3B4	15.6	>50	>3.21
 3C1	2.91	>50	>17.18
 3C3	8.22	>50	>6.08

2.3.4 The solubility of the synthesised benzofuran compounds (2A-3E5).

The solubility of a compound is influenced by the presence of hydrogen bonding from groups such as OH, NH and SH. Out of the twenty-eight synthesised benzofuran derivatives, only six compounds showed acceptable solubility results. The solubility

value of ≤ 50 μM is considered poorly soluble, while the value of ≥ 50 μM is considered soluble. Compound **2A** has showed promising activity with two media ranging from 2.02-18.08 μM but poor solubility of 5 μM, while compounds **2B** and **3A** showed promising activity with one media each in a range of 18.92-56.17 μM and improved solubility values of 20 and 30 μM respectively. Additionally, compound **3A2** showed promising activity in one media with 7.8 μM and better solubility of 50 μM. Furthermore, compound **3B** showed greater solubility of 195 μM, but it was inactive (>62.5 μM) in all the three media mentioned in **Table 5**. Lastly, compound **3B1** showed good solubility of 50 μM in two media with good activity ranging from 6.56-7.6 μM. In summary, from all the synthesised benzofuran compounds tested for biological evaluation (solubility and potency/activity), there is no clear correlation between solubility and the potency of the compounds.

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CHAPTER 3: CONCLUSION AND RECOMMENDATIONS

3.1. Summary and conclusions

The study aimed to design, synthesise, and evaluate anti-tubercular properties of the tri-substituted benzofuran derivatives. The first objective of the research project was to successfully synthesise benzofuran derivatives using Sonogashira cross-coupling. The second objective was the successful functionalisation of all the synthesised benzofuran derivatives to esters. The third objective was the characterisation of the synthesised compounds using spectroscopic techniques. The fourth objective was the biological evaluation of all the synthesised benzofuran derivatives against *Mycobacterium tuberculosis*.

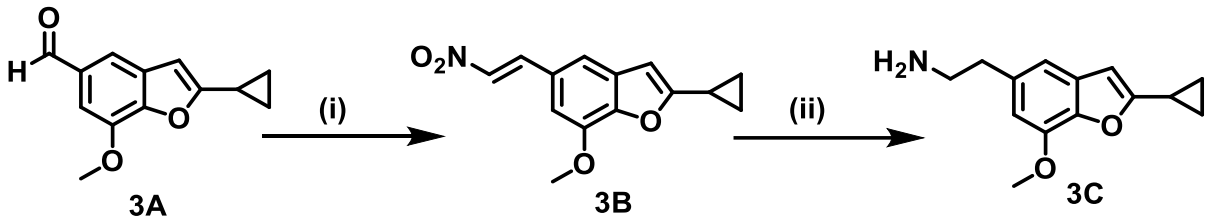
In summary, iodination of commercially available vanillin **1A** at high temperature afforded the known 5-iodovanillin **1B** has been demonstrated. One pot sequential palladium catalysed Sonogashira cross coupling of 5-iodovanillin **1B** and terminal acetylenes using cesium carbonate-promoted cyclisation in DMF and afforded a series of 2-(substituted)-7-methoxybenzofuran-5-carbaldehydes (**2A-2E**). Sodium borohydride promoted the reduction of 2-(substituted)-7-methoxybenzofuran-5-carbaldehyde (**2A-2E**) in ethanol at room temperature to afford 2-substituted-7-methoxybenzofuran-5-yl methanols (**3A-3E**). Moreover, the esterification reaction was catalysed by Mukaiyama catalyst to afford the corresponding ester derivatives (**3A1-3E5**).

All the synthesised compounds were biologically evaluated for antimycobacterial, cytotoxicity, and solubility. One from seven compounds was found to be active in all three media mentioned in **Table 5,** while the remaining six compounds are active in one or two media. There is no further exploration which were done for this project's aims. Structure activity relationships (SAR) for benzofuran formation from Sonogashira using R1 as a cyclic with terminal alkyne such as cyclopropyl and methane cyclohexyl show great activity and enhanced antitubercular potency with low MIC_{90} and nontoxic trends. The esters with cyclopropyl (R1) and nicotinic ester (R2) with chlorine at position 2 and 6 also showed excellent activity on mycobacterium tuberculosis as shown in **Table 5**.

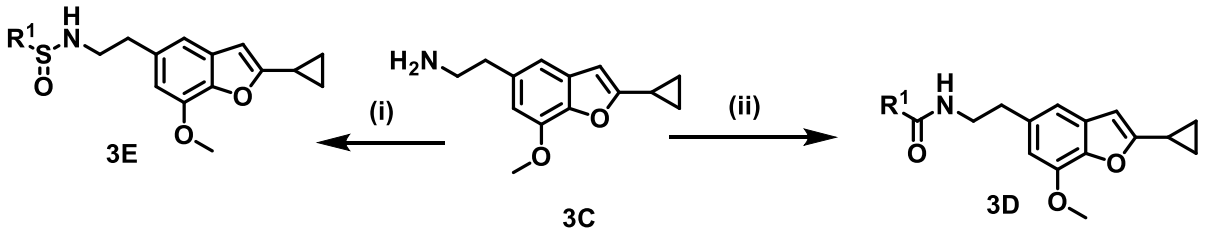
3.1 Recommendation

The following recommendations are suggested for future work

Henry aldol condensation reaction 2-(substituted)-7-methoxybenzofuran-5-carbaldehyde (**1A-3E**) followed by the reduction of the nitrovinyl functional group to an amine functional group. Focusing only on aldehyde benzofuran with R1(cyclopropyl) and only changing functional group of aldehydes to amide and sulfonyl ester with different R2 group that would provide good activity without having solubility issues



1. The resulting amines can be amidated using carboxylic acids and sulfonyl chlorides.



2. Biological evaluation of the synthesised benzofuran with nitro, amino, and ester derivatives. Characterise synthesised benzofuran further with Mass spectrometry (MS) and FT-IR for all the synthesised benzofuran derivatives.

CHAPTER 4: EXPERIMENTAL

4.1 GENERAL INFORMATION

Commercially available reagents and solvents were purchased from Sigma Aldrich and Merck (South Africa) and used without further purification. All reactions involving moisture-sensitive reagents were carried out in oven-dried glassware under a nitrogen (N_2) gas atmosphere. Reactions that needed hot or cold temperatures were carried out at the appropriate temperatures in an oil bath and an ice bath, respectively. All the measurements were carried out at room temperature with fluctuations ranging from 25 ℃ to 27 ℃. Glassware was thoroughly washed with distilled water, followed by rinsing with acetone, and oven-dried at 80 °C the day before use. Thin layer chromatography (TLC) on aluminum-baked Merck silica 60 F_{254} was visualized under Ultra-Violent light with a wavelength of 254 nm to monitor the reaction progress. Lasec Cole-Parmer Stuart SMP30 was used to record the melting points. Biological assays were performed at the Holistic Drug Discovery and Development (H3D) Centre at the University of Cape Town, South Africa.

4.2 ANALYSIS AND CHARACTERIZATION TECHNIQUES

The ^{1}H NMR and ^{13}C NMR spectra were obtained from Nuclear Magnetic Resonance (NMR) (Bruker Ascend 400 MHz Topspin 3.2). The NMR spectra were referenced internally using solvent signals. For ^{1}H NMR, the signal was at 7.25 ppm for $CDCl_3$, and for ^{13}C NMR, the signal was 77.0 ppm for $CDCl_3$. The ^{1}H NMR spectra were presented as follows: (I) chemical shift (δ) in ppm, (II) Multiplicity (s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet), (III) Coupling constant (J) in Hz. The structural properties of the compounds were recorded and confirmed by using LC-MS XS00R QTOF High-Resolution Mass Spectrometry (HRMS). The purity of the compounds was obtained using LC-UV with solvents that were soluble to the compounds like Methanol, acetonitrile, and Dimethyl sulfoxide.

BIOLOGICAL ASSAYS

The minimum inhibitory concentration (MIC) of each chemical was ascertained using the microplate alamar blue assay (MABA), with a few minor adjustments[1].The MIC is the lowest concentration at which 90% of growth is inhibited in this context. Certain

chemicals' behaviour has been demonstrated to be influenced by the medium composition used for MIC testing. In particular, the action of substances has been demonstrated to be impacted by the presence of serum (Albumin) and the detergent Tween 80[2]. To determine whether the supplements indicated potentially alter compound activity, we evaluate each compound in three media compositions as part of our normal in-house screening methodology. Both qualitative and quantitative measures of growth inhibition were identified. A bacterial pellet's visual detection served as the basis for the qualitative assessment, and the visual MIC is the lowest concentration at which no bacterial pellet was seen. Alamar blue, a fluorescent, redox-responsive dye based on resazurin, is a commercially available reagent that gives a readout on cell metabolism. After adding the resazurin dye and measuring the fluorescence, the quantitative/calculated MIC value was determined[3,4].

The stock concentration for the test samples was 10 mM, and they were prepared in 100 % DMSO (Sigma Aldrich). 96-well round-bottom plates were used to prepare additional 2-fold serial dilutions in growth medium on the day of the experiment. Each sample in this experiment was tested within the concentration range of 62.5 to 0.122 µM. All experiments used Moxifloxacin and rifampicin as reference medications, with concentrations ranging from 62.5 to 0.122 µM. Both a minimum inhibition control (0.625% DMSO) and a maximum inhibition control (Rifampicin at 0.15 µM) were present on every test plate.

In 7H9_ADC_GLU_TW, a culture of M. tuberculosis *$H_{37}R_vMA$* was cultivated to an optical density (OD600) of 0.5–0.7 and diluted to an OD600 of 0.001 in each medium. For a total volume of 100 µl per well, 50 µl of the diluted culture was applied to each test plate. Every chemical was put through two tests.

Each assay plate well received a one-tenth of the assay volume of resazurin (0.2 mg/ml prepared in PBS) and was re-incubated for 24 hours. On day 8, the relative fluorescence units (RFU) (excitation 540 nm; emission 590 nm) of each well were measured using a SpectraMaxi 3x Plate reader. The assay was scored visually on day 7, and the visual MIC was noted as the lowest concentration at which no bacterial pellet was observed.

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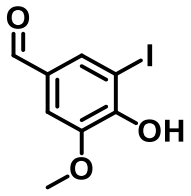
Dotmatics software was used to analyse the data (https://www.dotmatics.com/solutions/data-intelligence-lab-software). The Levenberg-Marquardt damped least-squares method was used to create a dose response curve (% inhibition) from raw RFU data that had been normalised to the minimum and maximum inhibition controls. The 4-parameter curve fit protocol was then used to determine the MIC. The computed minimum inhibitory concentration (MIC) is the lowest drug concentration that prevents 90% of the bacterial population from growing.

Media used:

ADC_GLU_TW 7H9: Middlebrook albumin-dextrose-catalase (ADC) enrichment (Difco), 0.2% glucose, and 0.05% Tween added to 7H9 medium 80. ADC_GLU_TX 7H9: Middlebrook 7H9 medium (Difco) enhanced with 0.05% Tyloxapol, 0.2% glucose, and Middlebrook albumin-dextrose-catalase (ADC) enrichment (Difco). 7H9_CAS_GLU_TX: Middlebrook 7H9 medium (Difco) enhanced with 0.05% Tyloxapol, 0.4% Glucose, and 0.03% Casitone (Gibco Bacto). Every process that involved working with pathogenic mycobacterial strains was carried out in a facility that was accredited and complied with Biosafety Level III.

4.3 SYNTHESIS

4.3.1 Synthesis of 5-iodo-vanillin



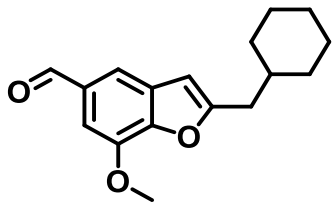
1B

Vanillin **1A** (10.0 g, 65.77 mmol) was added to distilled water (100 mL) in a 250 ml round bottom flask followed by the addition of sodium iodide (NaI) (19.72 g, 131.54 mmol), sodium hydroxide (NaOH) (2.63 g, 65.77 mmol) and iodine (I_2) (8.35 g, 65.77 mmol) over 10 min (minutes) at 90 °C followed by the addition of sulphuric acid (H_2SO_4) (3.55 M, 10 mL) over 3 hours. The reaction was quenched with ice water, followed by addition of sodium thiosulfate ($Na_2S_2O_3$) (1.04 g, 6.577 mmol) to remove excess iodine present in the reaction. The filtered product was further purified by flash chromatography using 60% dichloromethane (DCM) in 40% hexane to afford compound 5-iodo-vanillin (**1B**)**.** White solid (16.824 g, 92%), R_f(40% hexane:60% DCM) 0.55, mp.180 °C - 183 °C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.77 (s, 1H), 7.82 (d, *J* = 1.7 Hz, 1H), 7.37 (d, *J* = 1.7 Hz, 1H), 6.73 (s, 1H), 3.97 (s, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 189.69, 151.58, 146.67, 136.34, 131.23, 108.82, 80.64, 56.68. HRMS(EI):m/z calcd for $C_8H_7IO_3$ $[M+H]^+$277.944, found 278.9474.

General procedure for the synthesis of compounds 2A -2E.

A mixture of 5-iodo-vanillin **1** (1.20 g, 4.32 mmol), appropriate terminal alkyne (0.60 g, 8.64 mmol), Bis(triphenylphosphine)palladium(ii) dichloride ($PdCl_2(PPh_3)_2$ (0.05 g, 0.072 mmol), copper iodide (CuI) (0.084 g, 4.32 mmol) and triethylamine (Et_3N) (0.654 g, 6.06 mmol) in dimethylformamide (DMF) (6 mL) was stirred at 25 °C under nitrogen atmosphere (N_2) for 3 hours. After 3 hours, cesium carbonate (Cs_2CO_3) (1.68 g, 5.184 mmol) was added to the mixture and stirred at 60 °C overnight. At completion, the reaction was quenched with water, extracted into dichloromethane, and the organic layer washed with brine. Column chromatography of the residue with (20%:80%) dichloromethane-hexane as eluent afforded compounds **2A to 2E**.

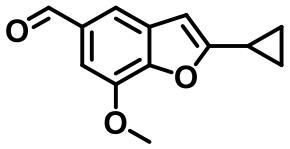
2-(Cyclohexylmethyl)-7-methoxybenzofuran-5-carbaldehyde 2A



2A

Prop-2-yn-1-ylcyclohexane (1.06 g, 8.64 mmol), white solid (0.81 g, 69%), R_f (20% DCM:80% Hexane) 0.56, mp.133 °C -135.7 °C ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.97 (s, 1H), 7.62 (d, *J* = 1.4 Hz, 1H), 7.31 (d, *J* = 1.4 Hz, 1H), 6.48 (s, 1H), 4.05 (s, 3H), 2.67 (d, *J* = 6.8 Hz, 2H), 1.78 – 1.55 (m, 7H), 1.33 – 1.12 (m, 4H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 192.09, 160.70, 147.60, 145.78, 133.14, 130.68, 119.08, 103.91, 103.67, 56.25, 51.01, 36.93, 36.37, 33.22, 26.42, 26.20. HRMS(EI):m/z calcd for $C_{17}H_{20}O_3$ [M+H] $^+$ 272.1412, found 273.1446.

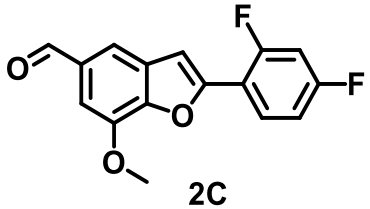
2-(Cyclopropyl)-7-methoxybenzofuran-5-carbaldehyde 2B



2B

Cyclopropyl acetylene (0.571 g, 8.64 mmol), a yellow solid (0.644g, 69%), R_f (20% DCM:80% Hexane) 0.58, mp.101.5 °C -103 °C: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.96 (s, 1H), 7.59 (s, 1H), 7.29 (s, 1H), 6.43 (s, 1H), 4.04 (s, 3H), 2.19 – 1.93 (m, 1H), 1.13 – 0.95 (m, 4H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 192.40, 163.24, 147.56, 145.99, 133.54, 131.13, 119.11, 104.10, 101.47, 56.58, 9.73, 8.06. HRMS(EI):m/z calcd for $C_{13}H_{12}O_3$ [M+H] $^+$216.0786, found 217.0820.

2-(2,4-Difluorophenyl)-7-methoxybenzofuran-5-carbaldehyde 2C

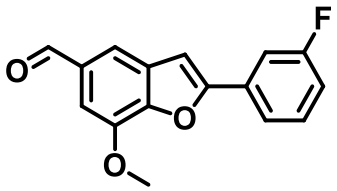


2C

2,4-Difluorophenyl acetylene (1.35 g, 8.64 mmol), brown solid (0.868 g, 70%), R_f (20% DCM:80% Hexane) 0.55, mp.145 °C -147.5 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ

10.01 (s, 1H), 8.07 (td, *J* = 8.6, 6.6 Hz, 1H), 7.73 (d, *J* = 1.2 Hz, 1H), 7.39 (s, 1H), 7.25 (d, *J* = 3.4 Hz, 1H), 7.06 – 6.91 (m, 2H), 4.09 (s, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 192.14, 151.42, 147.44, 146.49, 134.12, 131.33, 128.88, 128.74, 120.19, 112.59, 112.34, 106.95, 106.82, 105.48, 105.23, 104.97, 56.68. HRMS(EI):m/z calcd for $C_{16}H_{10}F_2O_3$: $[M+H]^+$ 288.0598, found 289.0632.

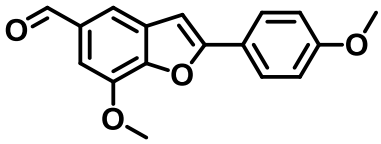
2-(3-Fluorophenyl)-7-methoxybenzofuran-5-yl) methanol 2D



2D

3-Fluorophenyl acetylene (1.037 g, 8.64 mmol), yellow solid (0.934 g, 80%), R_f (20% DCM:80% Hexane) 0.52, mp.81.2 °C -84.6 °C; ^{1}H NMR (400 MHz^{1}H NMR ,Chloroform-*d*) δ 10.00 (s, 1H), 7.71 (d, *J* = 1.5 Hz, 1H), 7.69 – 7.62 (m, 1H), 7.58 (dt, *J* = 9.7, 2.2 Hz, 1H), 7.49 (dd, *J* = 7.3, 2.0 Hz, 1H), 7.37 (d, *J* = 1.4 Hz, 1H), 7.11 (s, 1H), 7.07 (m, *J* = 8.3, 2.5 Hz, 1H), 4.08 (s, 3H).^{13}C NMR (100 MHz, Chloroform-*d*) δ 191.65, 146.08, 132.29, 132.19, 128.55, 128.43, 120.84, 120.82, 119.39, 116.13, 115.92, 112.20, 111.97, 104.84, 102.90, 56.20. HRMS(EI):m/z calcd for $C_{16}H_{11}FO_3$: $[M+H]^+$ 270.0692, found 271.0726.

7-methoxy-2-(4-methoxyphenyl) benzofuran-5-carbaldehyde 2E



2E

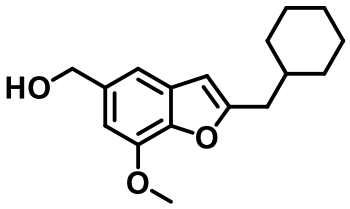
4-Methoxyphenyl acetylene (1.436 g, 8.64 mmol), white solid (0.731 g, 60%), R_f (20% DCM:80% Hexane) 0.58, mp.158 °C -160 °C.^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.00 (s, *J* = 4.7 Hz, 1H), 7.83 (d, *J* = 8.5 Hz, 2H), 7.69 (s, 1H), 7.34 (s, 1H), 7.04 – 6.92 (m, 3H), 4.09 (s, 3H), 3.87 (s, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 192.31, 160.90, 158.45, 147.89, 146.36, 133.83, 131.59, 127.20, 122.80, 121.33, 119.88,

119.51, 114.76, 104.73, 100.67, 56.66, 55.85. HRMS(EI):m/z calcd for $C_{17}H_{14}O_4$: $[M+H]^+$ 282.0892, found 283.0926.

4.3.2 General method for the synthesis of alcohols 3A – 3E

To a solution of appropriate7-methoxybenzofuran-5-carbaldehyde (1.00 g, 4.6 mmol) in ethanol (EtOH) (10.00 mL) in an ice water bath sodium borohydride ($NaBH_4$ (0.035 g, 0.920 mmol) was added, and the reaction mixture was left to stir for two hours at room temperature (25 °C). The product was purified by column chromatography using 40% ethyl acetate and 60% hexane as an eluent to afford compounds **3A** to **3E**

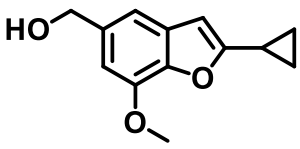
(2-(Cyclohexylmethyl)-7-methoxybenzofuran-5-yl) methanol 3A



3A

2-(Cyclohexylmethyl)-7-methoxybenzofuran-5-carbaldehyde (1.00 g, 3.675 mmol), brown oil (0.826 g, 82%), R_f (40% EtOAc:60% Hexane) 0.61.^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.06 (d, J= 1.2 Hz 1H), 6.78 (d, *J* = 1.5 Hz, 1H), 6.34 (s, 1H), 4.72 (s, 2H), 4.01 (s, 3H), 2.64 (d, *J* = 6.7 Hz, 2H), 1.77 – 1.64 (m, 8H), 1.23 (d, *J* = 14.3 Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 159.37, 145.05, 143.45, 136.36, 130.75, 111.35, 104.92, 103.35, 66.16, 56.12, 37.01, 36.43, 33.26, 29.84, 26.50, 26.26, 14.26. HRMS(EI):m/z calcd for $C_{17}H_{22}O_3$: $[M+H]^+$ 274.1569, found 275.1603.

(2-Cyclopropyl-7-methoxybenzofuran-5-yl) methanol 3B

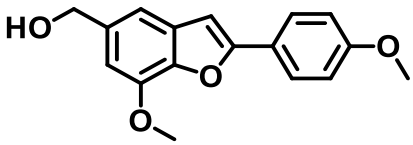


3B

A solution of 2-(cyclopropyl)-7-methoxybenzofuran-5-carbaldehyde (1 g, 4.63 mmol), light brown oil (0.83 g, 82%), R_f (40% EtOAc:60% Hexane) 0.60,^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.02 (d, *J* = 1.4 Hz, 1H), 6.76 (d, *J* = 1.4 Hz, 1H), 6.29 (s, 1H), 4.70

(s, 2H), 3.99 (s, 3H), 2.04 (tt, *J* = 8.4, 5.2 Hz, 1H), 1.00 – 0.92 (m, 4H). HRMS(EI):m/z calcd for $C_{13}H_{14}O_3$: $[M+H]^+$ 218.0943, found 219.0977.

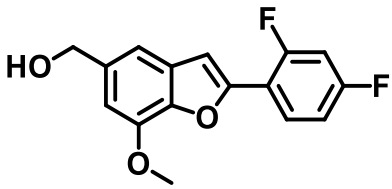
2-(4-Methoxyphenyl)-7-methoxybenzofuran-5-yl) methanol 3C



3C

2-(4-Methoxyphenyl)-7-methoxybenzofuran-5-carbaldehyde (1.00 g, 3.545 mmol) white solid (0.790 g, 78%), R_f (40% EtOAc:60% Hexane) 0.60, mp.130 °C -131.6 °C.^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.81 (d, *J* = 8.8 Hz, 2H), 7.14 (s, 1H), 6.96 (d, *J* = 8.8 Hz, 2H), 6.84 (d, *J* = 10.3 Hz, 2H), 4.75 (s, 2H), 4.05 (s, 3H), 3.86 (s, 3H).^{13}C NMR (100 MHz, Chloroform-*d*) δ 206.85, 159.87, 156.57, 145.00, 143.24, 136.51, 130.98, 126.37, 122.91, 114.01, 111.35, 105.51, 99.83, 65.80, 55.98, 55.20, 30.77. HRMS(EI):m/z calcd for $C_{17}H_{18}O_4$: $[M+H]^+$ 286.1205, found 287.1268.

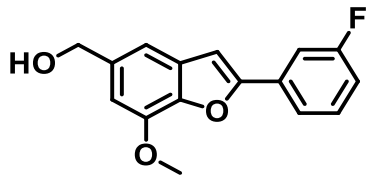
2-(2,4-Difluorophenyl)-7-methoxybenzofuran-5-yl) methanol 3D



3D

2-(2,4-Difluorophenyl)-7-methoxybenzofuran-5-carbaldehyde (1.00 g, 3.472 mmol), yellow solid (0.826 g, 82%), R_f (40% EtOAc:60% Hexane) 0.65, mp.145 °C -147.2 °C; 1H NMR (400 MHz, Chloroform-*d*) δ 8.06 (m, *J* = 8.6, 6.7 Hz, 1H), 7.18 (s, 1H), 7.13 (d, *J* = 3.6 Hz, 1H), 7.02 – 6.95 (m, 2H), 6.94 – 6.89 (m, 1H), 6.87 (s, 1H), 4.76 (s, 2H), 4.05 (s, 3H).^{13}C NMR (100 MHz, Chloroform-*d*) δ 149.69, 149.65, 145.24, 142.99, 136.99, 130.86, 128.30, 128.25, 128.20, 128.16, 112.01, 111.91, 111.88, 111.70, 111.67, 106.33, 106.27, 106.26, 106.15, 106.13, 104.85, 104.60, 104.34, 65.86, 56.12. HRMS(EI):m/z calcd for $C_{16}H_{12}F_2O_3$: $[M+H]^+$ 290.0755, found 291.0788.

2-(3-Fluorophenyl)-7-methoxybenzofuran-5-yl) methanol 3E



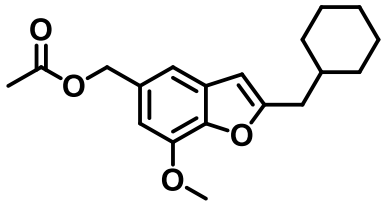
3E

2-(3-Fluorophenyl)-7-methoxybenzofuran-5-carbaldehyde (1.00 g, 3.703 mmol), yellow solid (0.605 g, 60%), R_f (40% EtOAc:60% Hexane) 0.65, mp.98.9 °C -101.4 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.67 – 7.62 (m, 1H), 7.57 (m, *J* = 10.0, 2.1 Hz, 1H), 7.39 (m, *J* = 8.0, 5.8 Hz, 1H), 7.16 (s, 1H), 7.04 (dd, *J* = 8.5, 2.6 Hz, 1H), 7.00 (s, 1H), 6.86 (d, *J* = 1.5 Hz, 1H), 4.75 (s, 2H), 4.05 (s, 3H) ^{13}C NMR (100 MHz, Chloroform-*d*) δ 164.29, 155.20, 145.33, 143.75, 136.97, 132.32, 132.24, 130.54, 130.38, 130.29, 120.67, 120.64, 115.53, 115.32, 112.01, 111.78, 106.34, 102.64, 65.83, 56.13. HRMS(EI):m/z calcd for $C_{16}H_{13}FO_3$: $[M+H]^+$ 272.0849 found 273.0882

. **General method of the synthesis of ester 3A1 to 3E4**

Carboxylic acid or acid chloride (1.2 eq.) was dissolved in dichloromethane (10 mL) and 2-chloro-methyl pyridinium iodide (281.16 mg,1.100 mmol), triethyl amine (1 mL, 139.196 mmol) were added followed by the addition of appropriate alcohol (60 mg, 0.275 mmol) and the reaction mixture was left stirring at 25 °C for overnight. The reaction was quenched with water (2 mL), washed with dichloromethane (10 mL). The product was purified by column chromatography using dichloromethane-hexane (90%:10%) as an eluent to afford compound **3A1 to 3E4.**

(2-(cyclohexyl methyl)-7-methoxybenzofuran-5-yl) methyl acetate

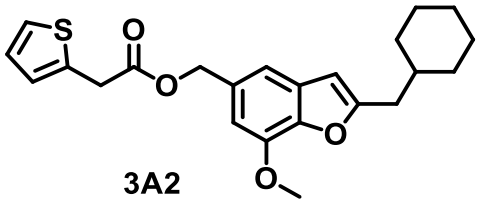


3A1

Acetyl chloride (20.48 mg, 0.263 mmol), yellow oil (41.52 mg, 60%), R_f (90% DCM:10% Hexane) 0.48, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.10 (d, *J* = 1.6 Hz, 1H), 6.74 (d, *J* = 1.6 Hz, 1H), 6.34 (s, 1H), 5.14 (s, 2H), 4.01 (s, 3H), 2.64 (d, *J* = 6.7 Hz, 2H), 2.10 (s, 3H), 1.77 – 1.60 (m, 6H), 1.26 (s, 5H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 171.02, 159.44, 144.89, 143.72, 131.12, 130.76, 113.25, 106.23, 103.33, 67.13,

56.11, 36.99, 36.35, 33.20, 29.80, 26.45, 26.21, 21.19, 14.21. HRMS(EI):m/z calcd for $C_{19}H_{24}O_4$: $[M+H]^+$ 316.1675, found 317.1737.

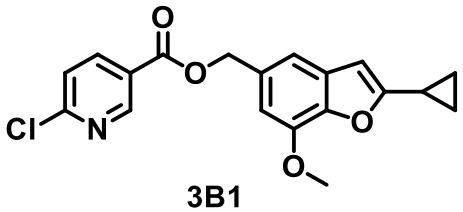
(2-(cyclohexylmethyl)-7-methoxybenzofuran-5-yl)-methyl-2-(thiophen-2-yl) acetate



3A2

2-(thiophen-2-yl) acetyl chloride (42.01 mg, 0.263 mmol), brown oil (52.3 mg, 60%), R_f (90% DCM:10% Hexane) 0.44. 1H NMR (400 MHz, Chloroform-d) δ 8.28 (d, J = 7.2 Hz, 1H), 8.18 (s, 2H), 7.68 (m, J = 45.4, 7.4 Hz, 2H), 7.05 – 6.98 (m, 1H), 6.54 (d, J = 5.4 Hz, 1H), 5.51 (s, 1H), 4.91 (s, 2H), 4.18 (d, J = 7.7 Hz, 3H), 2.83 (d, J = 6.6 Hz, 2H), 2.01 – 1.81 (m, 6H), 1.45 (d, J = 22.5 Hz, 5H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 171.36, 159.37, 145.04, 136.29, 133.87, 130.73, 130.32, 128.63, 111.38, 104.90, 103.35, 66.18, 56.11, 37.00, 36.43, 33.25, 29.84, 26.50, 26.25. HRMS(EI):m/z calcd for $C_{23}H_{26}O_4S$: $[M+H]^+$ 398.1552, found 399.1546.

(2-cyclopropyl-7-methoxybenzofuran-5-yl) methyl 6-chloronicotinate

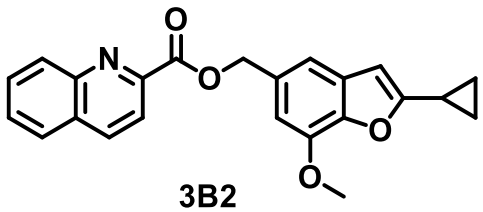


3B1

6-chloronicotonic acid (52.0 mg, 0.330 mmol), white solid (59.9 mg, 61%), R_f (90% DCM:10% Hexane) 0.49, mp.164.8 °C -166 °C) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.01 (s, 1H), 8.25 (d, *J* = 10.2 Hz, 1H), 7.40 (d, *J* = 8.3 Hz, 1H), 7.14 (s, 1H), 6.78 (s, 1H), 6.31 (s, 1H), 5.40 (s, 2H), 4.00 (s, 3H), 2.11 – 1.96 (m, 1H), 1.04 – 0.91 (m, 4H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 164.31, 161.78, 155.69, 151.25, 144.73, 143.39, 139.64, 130.80, 130.36, 125.11, 124.14, 113.21, 106.26, 100.43, 68.11, 56.10, 52.59,

9.23, 7.41. Calculated for HRMS(EI):m/z calcd for $C_{19}H_{16}ClNO_4$: $[M+H]^+$ 357.0768, found 359.0738.

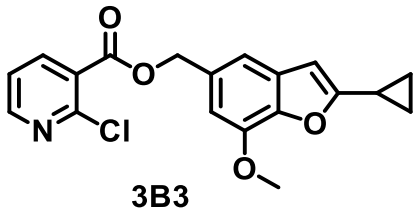
(2-cyclopropyl-7-methoxybenzofuran-5-yl) methyl quinoline-2-carboxylate



3B2

2-quinoline carbonyl chloride (57.1 mg, 0.330 mmol), brown solid (80.0 mg, 78%), R_f (90% DCM:10% Hexane) 0.48, mp.128 °C -130 °C). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.32 (dd, *J* = 19.8, 8.6 Hz, 2H), 8.17 (d, *J* = 8.5 Hz, 1H), 7.87 (d, *J* = 8.2 Hz, 1H), 7.83 – 7.76 (m, 1H), 7.72 – 7.57 (m, 1H), 7.24 (d, *J* = 1.5 Hz, 1H), 6.92 (d, *J* = 1.5 Hz, 1H), 6.31 (s, 1H), 5.57 (s, 2H), 4.01 (s, 3H), 2.03 (q, *J* = 12.0, 6.5, 4.1 Hz, 1H), 1.05 – 0.89 (m, 4H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 164.92, 161.59, 147.20, 144.67, 137.70, 130.76, 130.68, 130.53, 130.47, 129.36, 128.75, 127.52, 121.12, 113.49, 106.57, 100.50, 68.55, 56.11, 30.93, 9.24, 7.38. HRMS(EI):m/z calcd for $C_{23}H_{19}NO_4$: $[M+H]^+$ 373.1314, found 374.1284.

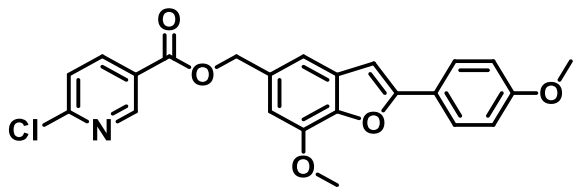
(2-cyclopropyl-7-methoxybenzofuran-5-yl) methyl 2-chloronicotinate



3B3

2-chloronicotonic acid (52.0 mg, 0.330 mmol), yellow oil (49.1 mg, 61%), R_f (90% DCM:10% Hexane) 0.49.^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.50 (dd, *J* = 4.8, 2.0 Hz, 1H), 8.22 – 8.13 (m, 1H), 7.30 (dd, *J* = 7.7, 4.7 Hz, 1H), 7.15 (d, *J* = 1.5 Hz, 1H), 6.82 (d, *J* = 1.5 Hz, 1H), 6.31 (s, 1H), 5.41 (s, 2H), 4.00 (s, 3H), 2.09 – 1.98 (m, 1H), 1.04 – 0.91 (m, 4H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 164.79, 162.18, 152.27, 150.47, 145.17, 143.83, 140.92, 131.20, 130.64, 127.40, 122.56, 113.70, 106.73, 100.91, 69.02, 56.53, 30.13, 9.70, 7.87. HRMS(EI):m/z calcd for $C_{19}H_{16}ClNO_4$: $[M+H]^+$ 357.0768, found 359.0738.

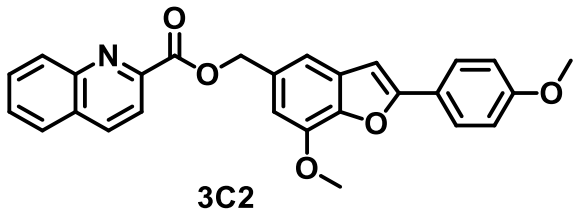
(7-methoxy-2-(4-methoxyphenyl) benzofuran-5-yl) methyl 6-chloronicotinate



3C1

6-chloronicotonic acid (33.27 mg, 0.253 mmol),yellow solid (30.2 mg, 40%), R_f (90% DCM:10% Hexane) 0.48, mp.74.6 °C -76.2 °C) ^{1}H NMR (400 MHz, Chloroform-d) δ 9.04 (s, 1H), 8.27 (dd, J = 8.3, 2.3 Hz, 1H), 7.82 (d, J = 8.8 Hz, 2H), 7.41 (d, J = 8.3 Hz, 1H), 7.25 (s, 1H), 6.97 (d, J = 8.8 Hz, 2H), 6.86 (d, J = 8.7 Hz, 2H), 5.44 (s, 2H), 4.07 (s, 3H), 3.86 (s, 3H).^{13}C NMR (100 MHz, Chloroform-d) δ 164.52, 157.21, 155.91, 151.46, 145.68, 145.30, 144.19, 143.99, 139.84, 131.45, 130.93, 126.77, 125.27, 124.34, 123.03, 114.37, 113.83, 107.24, 100.08, 68.22, 56.43, 55. 53. HRMS(EI):m/z calcd for $C_{23}H_{18}ClNO_5$: $[M+H]^+$ 423.0874, found 425.0844.

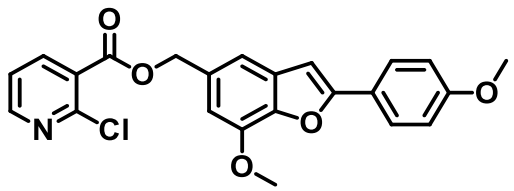
(7-methoxy-2-(4-methoxyphenyl) benzofuran-5-yl) methyl quinoline-2-carboxylate



3C2

2-quinoline carbonyl chloride (43.86 mg, 0.253 mmol), (49.6 mg, 63%), R_f (90% DCM:10% Hexane) 0.50, mp.158 °C -161 °C ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.31 (dd, *J* = 17.8, 8.5 Hz, 2H), 8.18 (d, *J* = 8.5 Hz, 1H), 7.87 (d, *J* = 8.2 Hz, 1H), 7.84 – 7.75 (m, 3H), 7.68 – 7.60 (m, 1H), 7.34 (s, 1H), 7.00 – 6.92 (m, 3H), 6.86 (d, *J* = 1.3 Hz, 1H), 5.59 (s, 2H), 4.06 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 165.25, 160.11, 156.89, 148.02, 147.59, 145.09, 143.83, 137.38, 131.25, 130.78, 130.34, 129.36, 128.67, 127.54, 126.61, 123.02, 121.17, 114.21, 113.92, 107.36, 100.06, 68.41, 56.27, 55.39. HRMS(EI):m/z calcd for $C_{23}H_{19}NO_4$: $[M+H]^+$ 373.1314, found 374.1377.

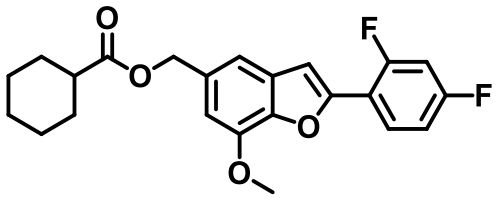
(7-methoxy-2-(4-methoxyphenyl) benzofuran-5-yl) methyl 2-chloronicotinate



3C3

2-chloronicotonic acid (33.27 mg, 0.253 mmol), brown oil (42.3 mg, 56%), R_f (90% DCM:10% Hexane) 0.48.^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.52 (dd, *J* = 4.8, 2.0 Hz, 1H), 8.19 (dd, *J* = 7.8, 2.0 Hz, 1H), 7.82 (d, *J* = 8.9 Hz, 1H), 7.33 (dd, *J* = 7.7, 4.8 Hz, 1H), 6.97 (d, *J* = 8.9 Hz, 2H), 6.88 (d, *J* = 5.0 Hz, 2H), 5.45 (s, 2H), 4.07 (s, 3H), 3.86 (s, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 164.48, 160.25, 157.10, 152.00, 151.98, 145.24, 143.92, 140.63, 131.35, 130.72, 126.71, 122.99, 122.27, 114.32, 113.82, 107.21, 100.07, 68.63, 56.35, 55.48, 29.81. HRMS(EI):m/z calcd for $C_{23}H_{18}ClNO_5$: $[M+H]^+$ 423.0874, found 425.0844.

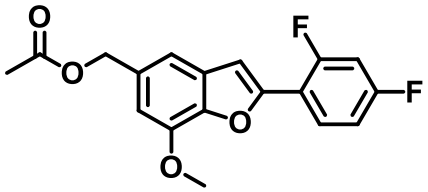
(2-(2,4-difluorophenyl)-7-methoxybenzofuran-5-yl) methyl cyclohexane carboxylate



3D1

Cyclohexane carbonyl chloride (31.79 mg, 0.248 mmol), yellow oil (41.4 mg, 50%), R_f (90% DCM:10% Hexane) 0.52. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.06 (m, *J* = 8.7, 6.8 Hz, 1H), 7.20 (s, 1H), 7.14 (d, *J* = 3.6 Hz, 1H), 7.03 – 6.88 (m, 2H), 6.80 (s, 1H), 5.17 (s, 2H), 4.05 (s, 3H), 2.40 (ddq, *J* = 15.3, 11.5, 3.9, 3.1 Hz, 7H), 0.92 – 0.80 (m, 4H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 215.16, 172.05, 113.69, 107.45, 74.39, 66.50, 56.29, 44.11, 43.37, 29.84, 29.17, 29.00, 28.54, 25.87, 25.72, 25.57, 25.31, 25.25, 22.12. HRMS(EI):m/z calcd for $C_{23}H_{22}F_2O_4$: $[M+H]^+$ 400.1486, found 401.1549.

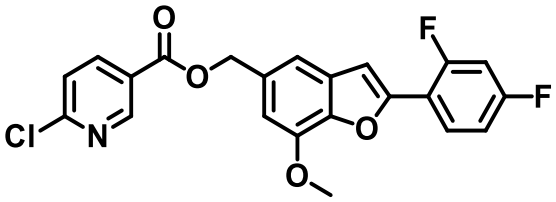
(2-(2,4-difluorophenyl)-7-methoxybenzofuran-5-yl) methyl acetate



3D2

Methyl carbonyl chloride (19.6 mg, 0.248 mmol), yellow solid (49.5 mg, 72%), R_f (90% DCM:10% Hexane) 0.49, mp.122 °C -125.7 °C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.10 (dd, *J* = 8.6 Hz, 1H), 7.30 (s, 1H), 7.18 (d, *J* = 3.5 Hz, 1H), 7.06 – 7.02 (m, 1H), 7.02 – 6.94 (m, 1H), 6.87 (s, 1H), 5.21 (s, 2H), 4.09 (s, 3H), 2.16 (s, 3H).^{13}C NMR (100 MHz, Chloroform-*d*) δ 171.11, 145.27, 143.40, 131.97, 131.04, 128.42, 114.07, 112.07, 112.03, 111.82, 107.72, 106.37, 106.23, 104.76, 66.99, 56.31, 29.85, 21.27. HRMS(EI):m/z calcd for $C_{18}H_{14}F_2O_4$: $[M+H]^+$ 332.0860, found 333.0894.

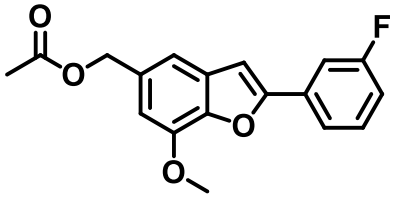
(2-(2,4-difluorophenyl)-7-methoxybenzofuran-5-yl) methyl 6-chloronicotinate



3D3

6-chloronicotonic acid (33.27 mg, 0.248 mmol), brown oil (30.2 mg, 40%), R_f (90% DCM:10% Hexane) 0.56. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.04 (s, 1H), 8.31 – 8.24 (m, 1H), 8.06 (m, *J* = 8.7, 6.4 Hz, 1H), 7.42 (d, *J* = 8.3 Hz, 2H), 7.16 (d, *J* = 3.6 Hz, 1H), 7.02 – 6.91 (m, 3H), 5.45 (s, 2H), 4.06 (s, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 164.50, 155.95, 151.44, 145.38, 139.84, 131.28, 131.15, 128.38, 125.21, 124.36, 114.34, 112.07, 111.86, 107.81, 106.32, 106.20, 105.05, 104.79, 68.09, 56.37. HRMS(EI):m/z calcd for $C_{22}H_{14}ClF_2NO_4$: $[M+H]^+$ 429.0579, found 431.0550.

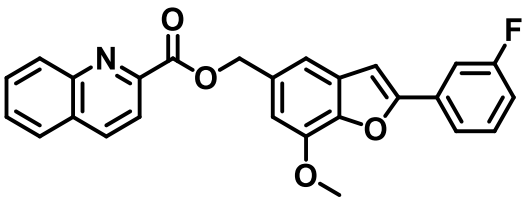
(2-(3-fluorophenyl)-7-methoxybenzofuran-5-yl) methyl acetate



3E1

Methyl carbonyl chloride (20.64 mg, 0.265 mmol), yellow oil (41.56 mg, 60%), R_f (90% DCM:10% Hexane) 0.48. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.65 (d, *J* = 7.6 Hz, 1H), 7.58 (d, *J* = 9.7 Hz, 1H), 7.40 (dd, *J* = 7.6 Hz, 1H), 7.21 (s, 1H), 7.05 (d, *J* = 7.9 Hz, 1H), 7.01 (s, 1H), 6.83 (s, 1H), 5.17 (s, 2H), 4.06 (s, 3H), 2.12 (s, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 170.95, 145.22, 144.03, 131.83, 130.58, 130.40, 130.32, 120.68, 115.61, 115.40, 113.73, 112.06, 111.82, 107.63, 102.59, 66.82, 56.19, 29.69. HRMS(EI):m/z calcd for $C_{18}H_{15}FO_4$: $[M+H]^+$ 314.0954, found 315.1017.

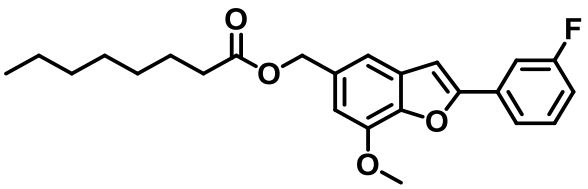
(2-(3-fluorophenyl)-7-methoxybenzofuran-5-yl) methylquinoline-2-carboxylate



3E2

2-quinoline carbonyl chloride (42.95 mg, 0.248 mmol), yellow solid (70.7 mg, 80%), R_f (90 % DCM:10% Hexane) 0.50, mp.166 °C -169 °C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.40 (d, *J* = 8.9 Hz, 1H), 8.33 (d, *J* = 8.5 Hz, 1H), 8.21 (d, *J* = 8.5 Hz, 1H), 7.89 (d, *J* = 8.3 Hz, 1H), 7.81 (t, *J* = 7.7 Hz, 1H), 7.67 (dd, *J* = 11.2, 7.6 Hz, 2H), 7.58 (d, *J* = 9.8 Hz, 1H), 7.40 (dd, *J* = 7.9 Hz, 2H), 7.04 (d, *J* = 2.8 Hz, 3H), 5.61 (s, 2H), 4.08 (s, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 147.67, 145.27, 144.18, 137.78, 132.26, 131.54, 130.60, 130.48, 130.32, 128.83, 127.55, 121.15, 120.73, 115.40, 114.25, 112.08, 108.07, 102.69, 95.59, 68.35, 56.28. HRMS(EI):m/z calcd for $C_{26}H_{15}FNO_4$: $[M+H]^+$ 424.0985, found 425.1048.

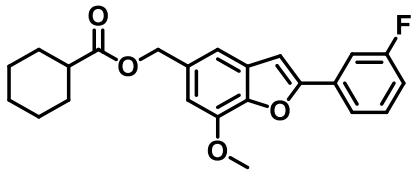
(2-(3-fluorophenyl)-7-methoxybenzofuran-5-yl) methyl octanoate



3E3

Heptyl carbonyl chloride (40.2 mg, 0.248 mmol), yellow oil (41.2 mg, 50%), R_f (90% DCM:10% Hexane) 0.42. ^{1}HNMR (400 MHz, Chloroform-*d*) δ 7.63 (d, *J* = 7.7 Hz, 1H), 7.56 (d, *J* = 9.8 Hz, 1H), 7.42 – 7.34 (m, 1H), 7.18 (s, 1H), 7.07 – 7.00 (m, 1H), 6.99 (s, 1H), 6.81 (d, *J* = 1.6 Hz, 1H), 5.17 (s, 2H), 4.04 (s, 3H), 2.36 (t, *J* = 7.5 Hz, 2H), 2.16 (s, 2H), 1.76 – 1.56 (m, 3H), 0.87 (q, *J* = 6.9, 6.3 Hz, 7H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 173.85, 145.30, 132.20, 130.67, 130.50, 130.42, 120.80, 120.77, 115.68, 115.47, 113.72, 112.13, 111.90, 107.65, 102.73, 66.63, 56.25, 34.49, 31.76, 29.03, 29.03, 25.09, 22.69, 14.16. HRMS(EI):m/z calcd for $C_{24}H_{27}FO_4$: $[M+H]^+$ 398.1893, found 399.1927.

(2-(3-fluorophenyl)-7-methoxybenzofuran-5-yl) methyl cyclohexane carboxylate



3E4

Cyclohexane carbonyl chloride (33.90 mg, 0.265 mmol), yellow solid (33.7 mg, 40%), R_f (90% DCM:10% Hexane) 0.45, mp. 74 °C -76 °C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.65 (d, *J* = 7.8 Hz, 1H), 7.57 (d, *J* = 9.9 Hz, 1H), 7.40 (dd, *J* = 8.0 Hz, 1H), 7.19 (s, 1H), 7.08 – 7.02 (m, 1H), 7.01 (s, 1H), 6.80 (s, 1H), 5.17 (s, 2H), 4.05 (s, 3H), 2.41 – 2.36 (m, 1H), 1.81 – 1.55 (m, 10H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 145.32, 132.42, 130.71, 120.84, 115.51, 113.52, 111.95, 107.53, 102.78, 66.50, 56.32, 44.10, 43.37, 29.84, 29.17, 29.00, 28.54, 25.87, 25.72, 25.56, 25.31, 22.12. HRMS(EI):m/z calcd for $C_{23}H_{23}FO_4$: $[M+H]^+$ 382.1580, found 383.1643.

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