SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL POTENTIAL ANTI-DIABETIC DRUGS

A thesis submitted as the minimum requirements for the fulfilment for the Degree **Doctor of Philosophy in Chemistry (Ph.D.)** to the Faculty of Science, Engineering and Agriculture.

Department of Chemistry

University of Venda, Private bag X5050

Thohoyandou 0950

by

Ndivhuwo Raymond Tshiluka

11641072

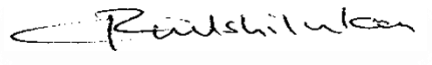
Supervisor : Dr. S.S. Mnyakeni-Moleele

Co-supervisor : Dr. M.V. Bvumbi

Declaration

I ***Ndivhuwo Raymond Tshiluka*** hereby declare that the work presented in this thesis titled ***'Synthesis and biological evaluation of novel potential anti-diabetic drugs'*** 'is my own, unaided work carried out exclusively by me under the promotion of ***Dr Simon Mnyakeni-Moleele*** and the co-promotion of ***Dr Victoria Bvumbi***. It is being submitted for the Degree **Doctor of Philosophy in Chemistry** in the University of Venda, Faculty of science, engineering and agriculture at the department of chemistry. It has not been submitted before for any degree or examination in this or any other University.

Ndivhuwo Raymond Tshiluka



02 th day of February 2022

Abstract

Glitazones are derivatives of thiazolidine with two carbonyls at 2- and 4-positions. Replacing the thio group with amino group gives rise to hydantoins while replacing carbonyl group at positions 2 with the thio group produces rhodanines. In this study, three class: glitazones, hydantoins and rhodanines were successfully synthesized using known conventional methods and evaluated for their anti-diabetic activity. The structures of synthesized compounds **103a-o, 104a-v** and **105a-j** were elucidated by a combination of ^{1}H NMR, ^{13}C NMR, HRMS and IR spectroscopic analysis.

The project began by utilizing a four-step synthesis of 5-(4-arylidine)-2,4-thiazolidinedione butanoates, valinates and norvalinates **103a-o**. The initial synthetic step involved conversion of 1,3-thiazolidine-2,4-dione into its potassium salt, which was then treated with ethyl (2-chloroacetamido) butanoates, valinates and norvalinates, respectively, to obtain the penultimate products. These products were then subjected to a Knoevenagel condensation reaction with different aldehydes to obtain the desired products in low to excellent yields (6-65%). Cytotoxicity results of the synthesized esters **103a-o** revealed that only compound **103d** and **103h** were toxic exhibiting cells lives of 1374.556±168.976 and 1782.722±157.3676 µM respectively. The results of the α-glucosidase inhibitory of the newly synthesized compounds **103a-o** indicated that they had no activities at 10, 50 and 100 µM. Only the butanoate **103a** (33.38±5.65%), **103d** (37.69±0.39%) together with valinate **103f** (32.66±4.31%), **103h** (29.67±3.09%) and norvalinate **103m** (31.83±2.85%) and **103o** (51.49 ±5.65%) were found to be moderately active against α-glucosidase at 200 µM.

The second part of this study describes the synthesis of 5-(4-benzylidine)-2,4-hydantoin esters **104a-v**, which were successfully synthesized over four reaction steps using conventional methods. Their synthesis began by subjecting hydantoin to Knoevenagel condensation reaction conditions with different aldehydes to obtain penultimate products which were further reacted with ethyl or methyl ethyl 2-(2-bromoacetamido) esters in order to obtain the desired products as esters in low to moderate yields (24-63%). *In vitro* cytotoxicity results of the synthesized intermediates showed that compounds **110c** exhibiting 464±78 µM and compound **110d** with live cell of 1997±80 µM were found to be toxic. Among the newly synthesized ethyl or methyl esters **104a-v**, no α-glucosidase activities was observed at 10, 50 and 100 µM.

At the highest concertation of 200 µM, alaninate **104a** (51.65±2.92%), valinate (45.23±3.60%) norvalinate (42±76.3.60%) butanoate **104f** (52.05±2.83 %), **104g** (48.47±2.33%), **104o** (57.77±2.79%) and **104p** (57.41±6.38%) showed moderate α-glucosidase inhibition.

The last part of this study was an attempt to design and synthesize a new series of novel 5-arylidene-2,4-rhodhanine conjugates with improved anti-diabetic biological properties. To this end some fused 5-(4-benzylidine)-2,4-rhodanine esters **105a-j** was prepared by known conventional methods from readily available starting materials. The synthesis began by subjecting rhodhanine in Knoevenagel condensation with various aldehydes to obtain 5-(4-arylidne)-2,4-rhodhanines as intermediates. Finally, nucleophilic substitution of 5-(4-arylidne)-2,4-rhodhanines with ethyl 2-(bromoacetamido) esters gave the desired compounds **105a-j** in good to excellent yields (52-94%). *In vitro* cytotoxicity results showed that unsubstituted phenyl **111a**, piperonyl **111e**, 3-hydro-4-methoxyphenyl **111f** and furanyl **111g** with live cells of 2716±289, 2372±172, 2464±132 and 2868±132 µM respectively were nontoxic among the synthesized intermediates **111a-g**. *In vitro* toxicity results of the target compounds **105a-j** showed that only the *para* fluorophenyl alaninate **105b** exhibiting live cell of 2982±112, *para* nitrophenyl butanoate **105f** with live cell of 2551±158 and *para* fluoro butanoate **105g** exhibiting 2551±186 µM were found to be nontoxic. *An in vitro* antidiabetic screening results showed that all the synthesized compounds **111a-g** and **105a-**j were not activity against the α-glucosidase at 10, 50 and 100 µM. Only the unsubstituted phenyl derivative **111a** among the synthesized intermediate **111a-g** was the most active exhibiting moderate α-glucosidase activity of 50.44±1.31% at 200 µM. With the final synthesized compound **105a-j**, *para* nitrophenyl butanoate **105f** was the most active followed by, *para* fluorophenyl alaninate **105b** and *para* nitrophenyl alaninate **105c**, exhibiting α-glucoside inhibition of 51.32±3.62%, 42.88±4.33% and 40.20±1.65% respectively.

Dedication

This Ph.D. thesis is dedicated to my late mother ***Miss Marumo Jane Mashapha****. You have been an amazing mother who raised us from nothing, your parenting skills were so excellent in a such a way that it produced a responsible young man like me. Your support, sacrifices and patience cannot be measured and will never be forgotten. Rest in peace my dearest mother, my guardian angel. II COR 7V9*

Acknowledgements

I would like to thank the ***almighty God*** himself and the following ***people*** for their **personal contributions** towards the successful completion of this thesis:

- To my promoter ***Dr Sana Simon Mnyakeni-Moleele***, thank you for being the best supervisor, academic father, an adviser, and a motivator. Thank you so much for giving me an opportunity to undertake my postgraduate studies under your supervision, you gave me all the reason not to quit. Your assistance towards the completion of this thesis will never be measured. Thank you so much Simon.
- To my co-promoter ***Dr Mpelegeng Victoria Bvumbi*****,** I have no words to express my greatest gratitude for all your contributions toward the successful completion of my studies. You have been the best co-supervisor and academic mother one would ever ask for; your advices were like angel sent from heaven. Thank you so much for all your assistance contributions and career wise. Thank you, Victoria!
- To our NMR operator ***Mr Mswazi Patrick Pandelani,*** thank you so much for making sure that we received our results in time and for making sure that NMR is always running smoothly. Thank you, Patrick!
- To ***Prof. Ntakadzeni Edwin Madala***, thank you so much for running our mass spectral analysis (HRMS) of all our synthesized compounds, thank you very much once again sir and all your efforts were much appreciated.
- To our Lab technicians ***Mr Fhumulani Baldwin Mutshaeni*** and ***Madam Nontlantla Regina Maseko***, thank you so much for keeping the labs in a much safer conditions and for your assistance in providing the much-needed resources to carry this study.
- To ***Univen Drug synthesis and isolation (DSI) and Chemistry department*** in general, thank you so much for all your assistance and for the opportunity to work with you guys, it was a pleasure.
- To all my close friends ***Miss Neani Tshilande, Miss Dakalo Sandra Mugwena, Miss Bafedile Dorcas Thlapi, Mr Thembani Silas Vukeya, Mr Nyiko Samuel Mabasa, Mr Allen Tauya Gordon, Mr Matodzi Vhahangwele, and Mr Isaac Mulaudzi****,* I thank dear God for giving me friends like you guys,

you always had my back and supported me unconditionally, no amount of word will be enough to thank you guys.

- To my advisors ***Adv. Ndivhuwo Emmanuel Phuthu***, ***Mr Simphiwe Baloyi*** and ***Adv. Mboneni Bernard Mashamba,*** thank you so much for your support and assistance.
- To the ***University of Venda United African Apostolic Church (Univen UAAC)*** led by ***Mr Rotondwa Ramutsheli***, thank you so much for connecting to our father in heaven in order to make this study a success, all the intersession prayer and chain prayer we had praying for this study will never be forgotten.
- To the ***Orphans of Laudecia Apostolic Church*** *(****TOLAC****)* under ***Bishop Marubini Joseph Nonyana****, and* ***Mulima Zion Christian Church (ZCC) s.t enganasi (Ha-Maphiri)*** under ***Pastor Malange Muraga Alex,*** Thank you very much for your all prayers.
- To the ***Prayer and fasting group***, I have no doubt that your prayers towards this study and my future, God has been with us. Thank you very much!!
- To my Father ***Mr Shumani Isaac Tshiluka***, no number of words is enough to thank you for bringing me to this world.
- To my sisters ***Miss Rebbeca Tshiluka***, ***Miss Muneiwa Beauty Tshiluka*** and ***Miss Tshimangadza Sheron Mudau*** thank you much for your support and for being best sisters in my life.
- To my little brother ***Mr Mashudu Evans Tshiluka***, thank you so much for being there for me little brother.
- To my aunts ***Miss Gladys Mashamba****,* ***Mrs Naledzani Tshiluka*** *and* ***Miss Matshivha Tshiluka*** thank you so much for the support you have shown during my studies in worst and happy moments.
- To my partner in love, ***Miss Lufuno Tshikonwane*** and my son ***Mr Tshiluka Mulanga Khanombuya*** thank you so much for all the experiences, advices, support, and assistance.
- To my nephew ***Miss Hulisani Theodorah Nedzamba***, and my close friends ***Miss Lindelani Masikhwa, Miss Musiiwa Khorombi****,* ***Mr Gundo Melton Ramuthaga and Mr Mugari Dzivhuluwani***, thank you so much for the support and for being there for me whenever I needed any kind of assistance.

- To ***Prof Maryna van de Venter*** from the Nelson Mandela Metropolitan University Bioassaix, thank you so much for screening our compounds for their anti-diabetic activities.

I would like to thank the following **organisations** for their **financial contributions** towards the successful completion of this thesis:

- To the ***National Research Foundation of South Africa (NRF)***, thank so much for your financial assistance.
- To the ***Sasol Foundation***, Only God is a witness that without your financial assistance I would have quitted long time ago, Thank you so much for your financial support.
- To the ***Thuthuka research funds***, thank you so much for sponsoring this study with the commercially available reagents.

Glossary of Abbreviations/Acronyms

A.D: Anno Domini

ARIs: Aldose Reductase Inhibitors

APE1: Apurinic Apyrimidinic endonuclease 1

AcOH: Acetic Acid

AcONa: Sodium Acetate

Ac_2O: Acetic Anhydride

AIBN: Azobisisobutyronitrile

BCE: Before Common Era

BTZD: Benzylidene Thiazolidin 2,4-dione Derivatives

BOP: (Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate)

Bu: Butyl

CuI: Copper iodide

CoCl: Cobalt Chloride

Con HCl: Concetrated Hydrochloric Acid

ACN: Acetonitrile

COX-1/2: Half Cyclooxygenase

DM: Diabetes Mellitus

DPP IV: Dipeptidyl Peptidase IV

DMSO: Dimethylsulfoxide

DCM: Dichloromethane

DMF: *N,N*-Dimethylformamide

DMA: Dimethylacetamide

DIEA: *N,N*-Diisopropylethylamine

DCC: Dicyclohexylcarbodiimide

EMA: European Medicines Agency

EC_{50}: Half Maximal Effective Concentration

EDDA: Ethylenediammonium Diacetate

EGCG: Epigallocatechin gallate

Et: Ethyl

EC: Eastern Cape province

FS: Free State province

EDC: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

GDM: Gestational Diabetes Mellitus

GHS: General Household Survey

GLP-1: Glucagon like Peptide-1

GIP: Glucose-Dependent Insulinotropic Polypeptide

GP: Gauteng province

HbA1c: Haemoglobin A1c Test

HIV: Human Immunodeficiency Virus

HOBT: Hydroxy Benzotriazole

HEP: 2-hydroxyethylpiperazine

H: Hour (s)

iPr: Isopropyl

IC50: Half Maximal Inhibitory Concentration

IDF: International Diabetes Federation

IDDM: Insulin-Dependent Diabetes Mellitus

IFN-γ: Interferon Gamma

IL-6, 17A, 22: Interleukin 6, 17a and 22

KZN: Kwazulu Natal province

LP: Limpopo province

MGAM: Maltase-Glucoamylase

MENA: Middle East and North Africa

Me: Methyl

MIC: Minimum Inhibitory Concentration

MW: Microwave

MP: Mpumalanga province

M.p; Melting point

MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

NDDM: Non-Insulin-Dependent Diabetes mellitus

NADPH: Nicotinamide Adenine Dinucleotide-2-Phosphate

NAH: Non-Acting Insulin

NPH: Neutral Protamine Hagedorn

$NH_2NH_2.H_2O$: hydrazine hydrate

NaHMDS: Sodium bis(trimethylsilyl)amide

NW*:* North West province

NC: Northern Cape province

PfENR: Plasmodium falciparum enoyl ACP reductase

Pt1,2: Patient 1 and 2

PPAR-γ: Peroxisome Proliferator- Activated Receptor

PPE: Polyphosphoric ester

$Pd(PPh)_3Cl_2$: Bis(triphenylphosphine)palladium(II) dichloride

Ph: Phenyl

RT: Reverse Transcriptase

Rt: Room temperature

SSA*:* Statistics South Africa

SADHS*:* South Africa Demographic and Health Survey

T1D*:* Type1 Diabetes

T2D: Type 2 Diabetes

TZDS**:** Thiazolidine-2,4-diones

TMP: 2,2,6,6-Tetramethylpiperidine

THF: Tetrahydrofuran

TZD: Thiazolidinedione

TCICA: Trichloroisocyanuric acid

US FDA: United States Food and Drug Administration

WC: Western Cape province

Table of Content

Quote

''When something is important enough, you do it even if the odds are not in your favour''

~Elon Musk

Chapter I

1. Introduction and literature review

This chapter looks at introducing diabetes, its different types, and its prevalence accross the world and more specifically in South Africa. It also describes the current treatment of diabetes, and pharmacological properties of glitazone, hydantoin and rhodanine containing compounds.

1.1. Origin of diabetes

Diabetes mellitus is one of the oldest diseases and has been known for over 3000 years.[1] Diabetes was first described in 1500 BCE by an Egyptian manuscript calling it ‘too great emptying of the urine’.[2] Around the same time interval, diabetes was also identified by an Indian physician and classified it as madhumeha[3] or "honey urine."[4] Around 230 BCE, the meaning of the word diabetes was described as "to pass through" by the Greek Appollonius of Memphis.[5] Around 150 A.D, Greek physician Arateus modified the meaning of the word diabetes to mean ‘’to flow through’’. The word Mellitus was also derived from Greece in 1675 meaning honey and it was combined with diabetes for the diesease to be called Diabetes mellitus.[6]

1.2. Definition of diabetes

Diabetes mellitus (DM) often simply referred to as diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin (a hormone that regulates the amount of glucose in the blood) or because cells do not respond to the insulin that is produced, or both.[7] Generally, in diabetes the body either does not produce enough insulin or does not use the insulin properly, thus causing insulin deficiency.[8] This deficiency adversely affects the crucial body organs responsible for maintaining and supplying glucose to the body cells.[9]

1.3. Classification of diabetes

Based on the 16th International Diabetes Federation (IDF) conference in 1997,[10] diabetes can be divided into three broad categories: (i) Type 1 diabetes which is also referred to as insulin-dependent diabetes mellitus (IDDM), (ii) Type 2 diabetes which is also referred as non-insulin-dependent diabetes mellitus (NIDDM) and (iii) Gestational diabetes

1.3.1. Type 1 diabetes (T1D)

The pancreas of the patient who has type 1 diabetes progressively fails to produce insulin resulting in a lack of insulin, or no insulin production (**Figure 1**).[11] This insulin deficiency results from the autoimmune destruction of β-cells of the islets of Langerhans.[12] Type 1 diabetes is also called insulin-dependent diabetes mellitus (IDDM) and accounts for only 5% of all diabetes cases.[13] This type can be further classified as immune-mediated or idiopathic diabetes.[14] People with type 1 diabetes rely on insulin injections or a continuous infusion of insulin via an insulin pump.[15]

1.3.2. Type 2 diabetes (T2D)

T2D is the most common form of diabetes prevalent in 90-95% of diabetics. T2D is regarded as adult-onset diabetes, obesity-related diabetes, and noninsulin-dependent diabetes mellitus (NIDDM).[16] It is identified by insulin resistance which may be combined with relatively reduced insulin secretion.[17] In this type the pancreas produces enough insulin, but the body does not recognize it or simply rejects it (**Figure 1**). The pancreas tries to overcome this resistance by secreting more and more insulin.[18] The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor, however the specific defects are not known. It is however associated with microvascular complications such as blindness, neuropathy and nephropathy and macrovascular complications like atherosclerosis and limb amputation.[19]

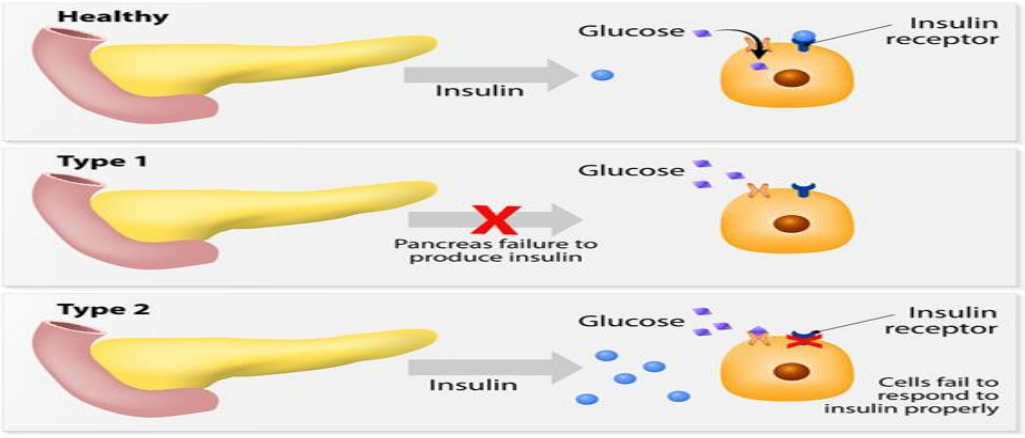


Figure 1: *Type 1 vs Type 2 diabetes*[20]

1.3.3. Gestational diabetes mellitus (GDM)

GDM is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. It affects 7% of pregnant women worldwide [21] During pregnancy, the placenta produces hormones that help the baby to grow and develop. Some of these hormones induce insulin resistance by blocking the action of the mother's insulin.[22] To keep the blood glucose levels normal, pregnant women need to make 2 to 3 times the normal amount of insulin due to this type of resistance. If the body is unable to produce the extra insulin or become more resistant, gestational diabetes develops.[23] Patients who are diagnosed with gestational diabetes are at higher risk for macrosomia, birth trauma, and shoulder dystocia. After delivery, infants have a higher risk of developing hypoglycaemia, hypercalcemia, hyperbilirubinemia, respiratory distress etc.[24]

1.4. Prevalence of diabetes

1.4.1. Global prevalence of diabetes

Diabetes remains the seventh leading cause of death globally.[25] In 2013 alone 74.9 million people died as a result of diabetes. The International Diabetes Federation (IDF) estimated that globally there were approximately 285 million people who had diabetes in 2009 (T1D and T2D combined)[26] increasing to 366 million in 2011, 382 million in 2013,[27] 415 million in 2015,[28] 425 million in 2017[29] and 463 million in 2019. This number was expected to increase to 578 million (10.2%) in 2030 and 700 million (10.9%) in 2045.[30] It was also estimated that globally 174.8 million cases (45.8%) are estimated to be living with undiagnosed diabetes mellitus.[31]

1.4.2. Diabetes per continent

Figure 2 represents people infected with diabetes and the world-age standardised prevalence of diabetes in all IDF continental regions in the periods 2019, 2030 and 2045. In 2019,[32] the IDF continental region with the highest world-age standardised diabetes prevalence was the Middle East and North Africa (MENA), where 12.2% of the population was estimated to have diabetes.[33]. By 2030 and 2045, world-age standardised diabetes prevalence is projected to increase to 13.3% and 13.9% in the MENA region and 5.1% and 5.2% in the African region.[34,35]

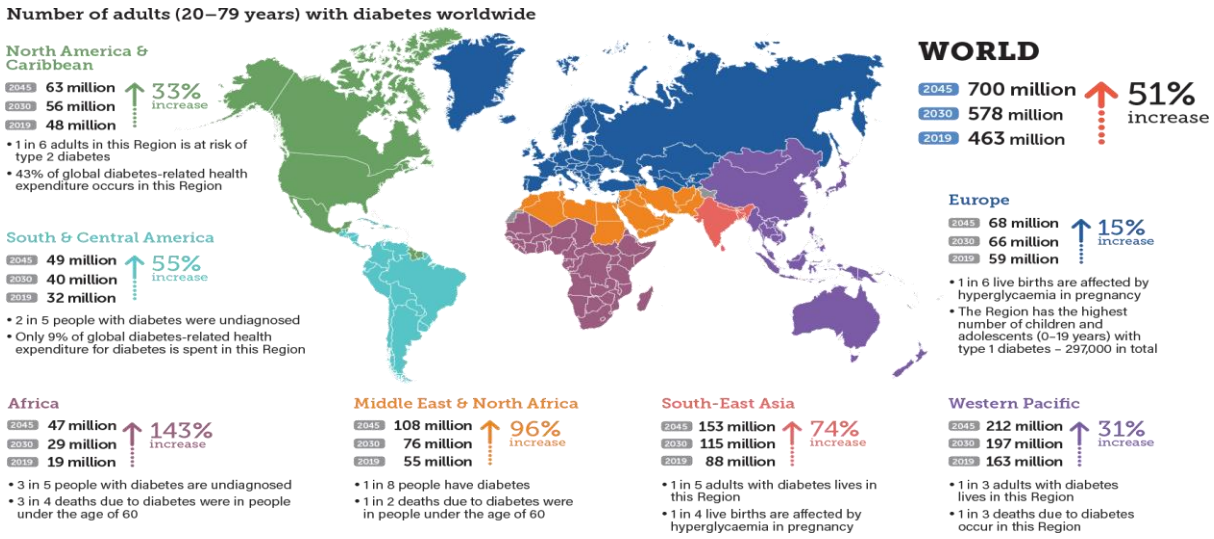


***Figure 2**: IDF Continental region diabetes prevalence*[20]

1.4.3. Diabetes in South Africa

The prevalence of diabetes is rapidly increasing in South Africa. In 2009, approximately 2 million (9%) people aged around 30 years and older had diabetes. [36] According to the recent Diabetes Atlas report by IDF in 2019, there was 12.7% of adults in SA with diabetes, which was a 41.2% increase on the 2017 figure of 5.4%.[37] This means South Africa has the highest proportion of adult diabetics on the African continent and the greatest number of deaths due to the disease.[38] In a study conducted in South Africa it was found that diabetes mellitus impacts negatively on the disability status and quality of life in older adults due to trends in obesity, poor diet, high fasting blood glucose levels and low physical activities levels.[39]

According to a 2019 report by Statistics South Africa (SSA),[40] Provincial diffferences, as shown in **Figure 3** indicate that the proportions of elderly communicated by a health professional that they had diabetes mellitus varied from 4,7% in North West to 22,0% in KwaZulu-Natal. During the South African Demographic and Health Survey (SADHS) of 2016 it was reported that in three provinces, namely Kwazulu-Natal which has 22%, Western Cape amounting to 20.1% and Eastern Cape (19.1%) had approximately one in five elderly people reported that they were told that they had diabetes. Elderly men in Western Cape (25,1%) and Eastern Cape (23,0%) had the highest proportions of ever being told by a health professional that they had diabetes mellitus. KwaZulu-Natal (22,7%) was the only province where more than 20% of women were ever told that they had diabetes mellitus during this 2016 SADHS. The 2016 General Household

Survey (GHS) revealed very similar patterns concerning the elderly reporting that they had ever been told by a health professional that they had diabetes mellitus as with the 2016 SADHS. There were not bigger variations on a provincial level between the 2016 SADHS and the 2016 GHS; for example, in KwaZulu-Natal, 22,0% versus 22,3% of the elderly were told that they had diabetes. In Eastern Cape, a small percentage of more elderly in the GHS (19,7%) were told that they are diabetic than in the SADHS (19,1%).

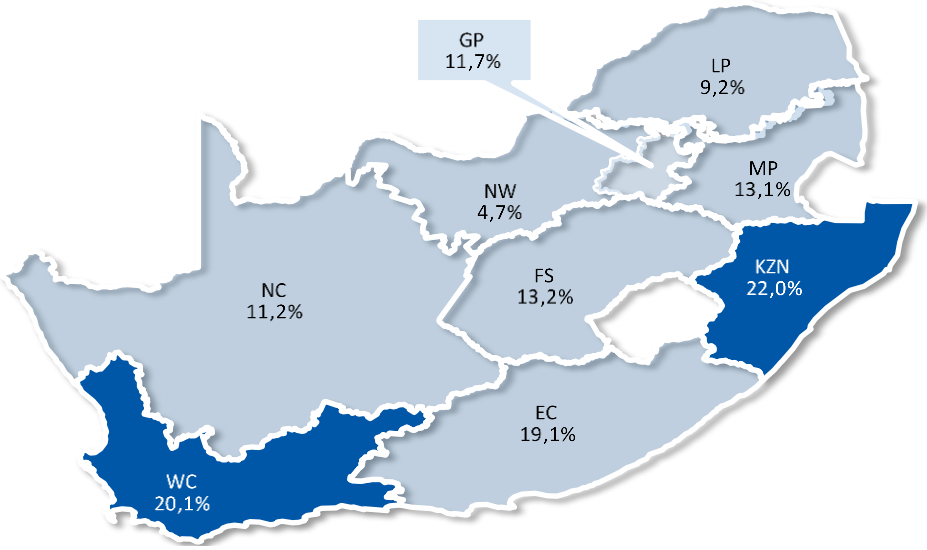


Figure 3: *Percentage of the elderly reporting being diagnosed with diabetes mellitus by province, SADHS 2016*[40]

1.5. History and current therapeutic treatment of diabetes

Many measures have been tried in order to develop an effective treatment for diabetes. In the early 20th century, insulin was developed by Canadians Frederick G. Banting, Charles H. Best and JJR MacLeod from the University of Toronto in 1921 and 1922 respectively.[41] After this discovery, a long-lasting insulin known as neutral protamine Hagedorn (NPH) insulin was then developed at Nordisk Insulin laboratorium in the early 1940s by Hans Christian Hagedorn.[42] To date, the treatment for T1D almost always involves the daily injection of insulin, usually a combination of short-acting insulin lispro [Humalog] or aspart [NovoLog]) and longer-acting insulin (NPH), Lente, glargine [Lantus] and detemir [Levemir].[43]

Current therapeutic approaches for the management of T2D have been developed based on the understanding of molecular pathways of cell targets available. Diet,

physical exercise and antidiabetic drugs are the main treatment for type 2 diabetes. Mechanistically, all antidiabetic agents have been classified into six major classes.[44] (**Table 1)**

Table 1: Classification of anti-diabetic medication or drugs

Class of drug	Category of drug	Principal mode of action
Insulin Secretagogues	Sulfonylureas	Increase the exocytosis of insulin from β-cells
	Meglitinides	
Carbohydrate Modulators	Alpha-Glucosidase Inhibitors	Delay intestinal absorption of monosaccharide
Glucagon Suppressor	Amylin Analogue	Suppress glucagon secretion
Incretin Potentiators	Glucagon like Peptide-1 (GLP-1) 4-agonists	Stimulate insulin secretion
	Dipeptidyl Peptidase IV (DPP-IV) inhibitors	
Polyol Pathway Inhibitors	Aldose Reductase Inhibitors	Majorly for the prevention of diabetic complications
Insulin Sensitizer	Biguanides	Direct insulin sensitizing effects on peripheral insulin-responsive tissues
	Thiazolidinediones	

1.5.1. Insulin secretagogues

- **Sulfonylureas**

Sulphonylureas constitute a well-known class of compounds that exhibit a wide range of biological activities like antidiabetic and diuretic activities.[45] They are more specifically referred to as *N*-(*phenylsulphonyl*)-N_1-alkylureas and molecules differing mainly with respect to elaboration on the benzene ring at the *para*-position of the phenylsulphonyl moiety. (**Figure 4**)

Their mechanism is to act at the molecular level primarily as insulin secretagogues i.e., these compounds elicit insulin secretion from pancreatic *β*-islet cells. These were the first class of oral hypoglycaemic agents and have been in clinical use since the 1960s.[46]

First-generation sulfonylureas mainly include tolbutamide **(1)**, chlorpropamide **(2)**, tolazamide **(3)** and acetohexamide **(4)**. (See **Figure** 4) The second-generation of more potent sulfonylureas has been available since the 1990s, which are better tolerated and more effective. These include glyburide **(5)** and glipizide **(6)**. Glimepiride **(7)** belongs to third-generation sulfonylureas which are less toxic and exhibit strong hypoglycemic potency, as compared to second generation sulfonylureas **(Table 2)**.[47]

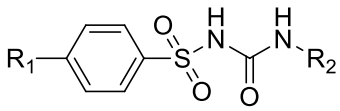
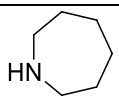
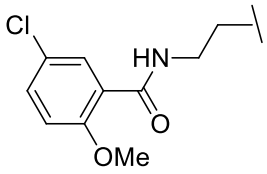
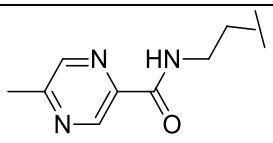
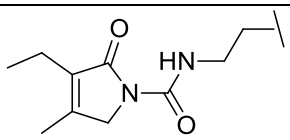
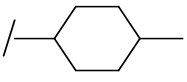


***Figure 4**: Phenylsulphonyl urea moiety*

Table 2: first, second and third generation sulfonylureas

Generic name	R_1	R_2
First-generation Tolbutamide **(1)**	$-CH_3$	$-CH_2-CH_2-CH_2-CH_3$
Chlorpropamide **(2)**	-Cl	$-CH_2-CH_2-CH_3$
Tolazamide **(3)**	$-CH_3$	
Acetohexamide **(4)**	CH_3CO-	
Second-generation Glyburide (Glibenclamide) **(5)**		
Glipizide **(6)**		
Third-generation Glimepiride **(7)**		

- **Meglitinides or Glinides**

Meglitinides are carboxylic acid derivatives belonging to the class of oral antidiabetic agents which increase insulin secretion in the pancreas. The phenylacetic acid

derivative mitiglinide **(8)** and the phenylalanine derivative nateglinide **(9) (Figure 5)** have been initially developed independently and belong to the same therapeutic class more because of their pharmacokinetic and their chemical structure.[48]

Figure 5**: Examples of Meglitinides drugs **8** and **9

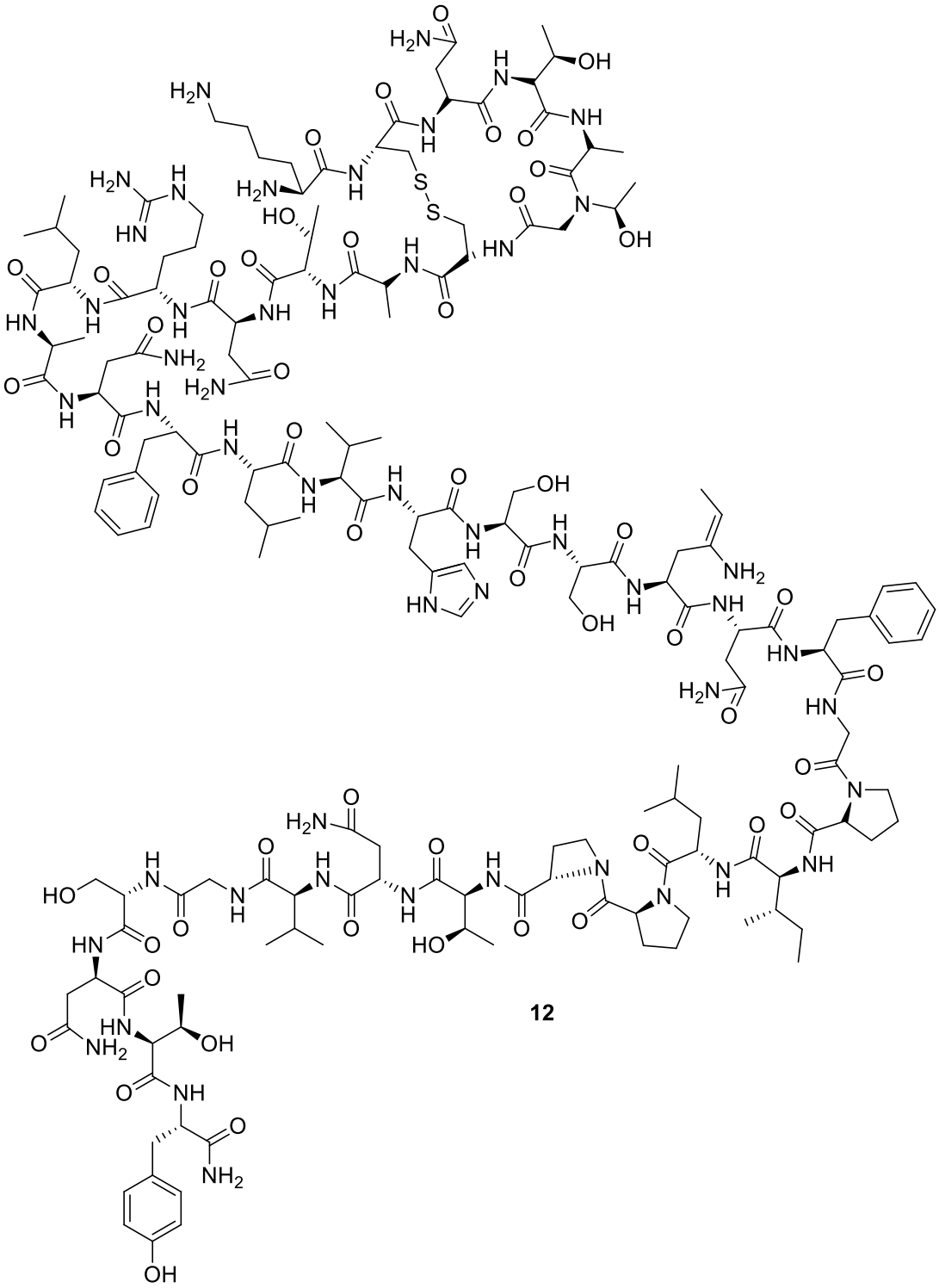
1.5.2. Carbohydrate modulators (Alpha-glucosidase inhibitors)

α-glucosidase inhibitors **(Table 1**) are known to inhibit the cleaving of di- and oligosaccharides to monosaccharides such as glucose prior to absorption. This delays the absorption of glucose and alters the release of glucose-dependent intestinal hormones.[49] α-Glucosidase inhibitors are not widely used in the treatment of T2D due to their high cost and limited efficacy.[50] Examples of these drugs are voglibose (**10**) and miglitol (**11**) (**Figure 6)**

Figure 6**: Examples of carbohydrate modulators drugs **10** and **11

1.5.3. Glucagon suppressors (Amylin analogues)

Glucagon suppressors are categorized or also known as amylin analogues **(Table 1)**. These are hormones that are responsible to release first-line counter hormones during hypoglycemia in a normal suppressed manner.[51] The main role of glucagon suppressors is to facilitate the increase of glucose production in patients suffering from type 2 diabetes. An example of amylin analogues is the peptide hormone called pramlintide **12** (**Figure 7)**, which is co-secreted with insulin from the pancreatic β-cells in response to glucose absorption.[52,53]



12

***Figure 7**: Example of amylin analogue **12** (glucagon suppressor)*

1.5.4. Incretin potentiators

- **GLP-1 receptor agonist**

Incretin hormones, namely glucose-dependent insulinotropic polypeptide (GIP), also known as gastric inhibitory polypeptide and glucagon-like peptide 1 (GLP-1), which

are released from the gut, play a significant role in glucose homeostasis in healthy subjects.[54] It has been estimated that the incretins are responsible for 50-70% of postprandial insulin release. Examples of GLP-1 receptor agonists are liraglutide (**13**) (**Figure 8**), which was approved by the U.S. Food and Drug Administration (US FDA) on 25 January 2010 to control high blood sugar, and exenatide (**14**) (**Figure 8**) which is used as adjunctive therapy to improve glycemic control in T2DM patients who are taking metformin, a thiazolidinedione (TZD), or a combination thereof, but have not achieved adequate glycemic control.[55]

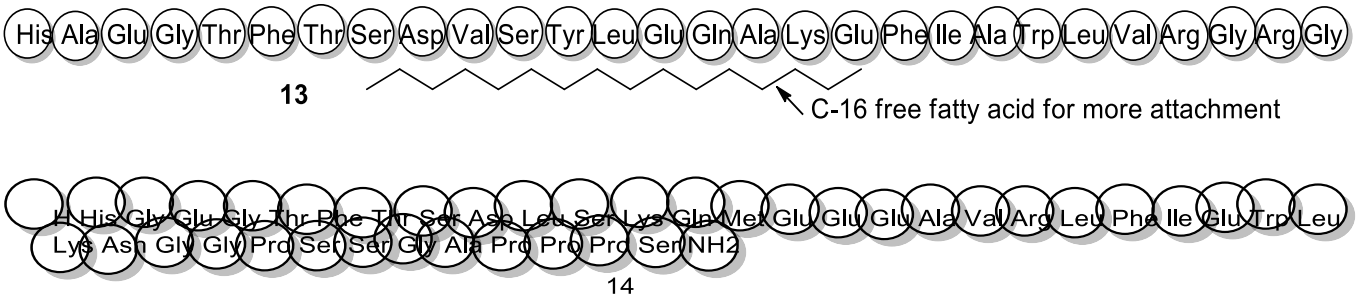


Figure 8**: Examples of GLP-1 receptor agonist enzymes **13** and **14

➢ Dipeptidyl Peptidase IV (DPP-IV) Inhibitors

Dipeptidyl peptidase IV (DPP-IV) inhibitors are a group of antihyperglycemic medications used in the management of type 2 diabetes mellitus.[56] Examples of dipeptidyl peptidase IV (DPP-IV) inhibitors include sitagliptin (**15,** trade name januvia) and vildagliptin **(16**, trade name galvus) **(Figure 9)**. Sitagliptin **(15)** was the first DPP-IV inhibitor approved by the FDA in 2006 which significantly lowers blood glucose and hemoglobin A1c Test (HbA1c) when used as monotherapy whereas vildagliptin **(16)** is a member of DPP-IV inhibitor class of drugs approved by The European Medicines Agency (EMA), but not by the FDA. It was later withdrawn due to skin lesions and kidney impairment.

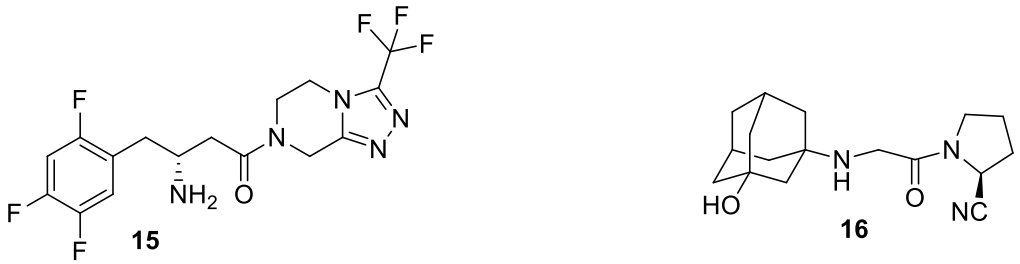
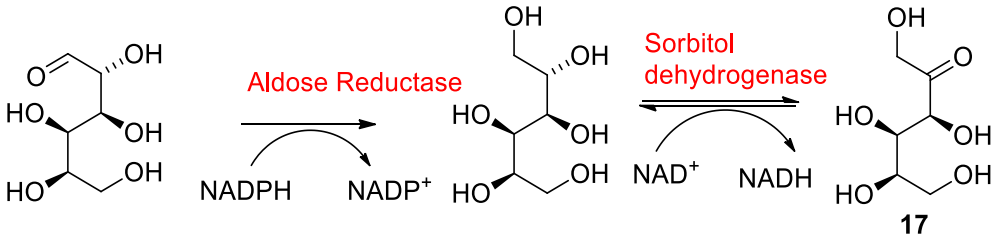


Figure 9:** Examples of dipeptidyl peptidase IV (DPP-IV) Inhibitor drugs **15** and **16

1.5.5. Polyol pathway inhibitors

Polyol pathway is a two-step metabolic process in which glucose is reduced to sorbitol, which is then converted into fructose **17**.[57] The main example of processes responsible in the elevation of polyol pathway inhibitors are the aldose reductase and the sorbitol dehydrogenase inhibitors. During the first process aldose reductase inhibitors reduces glucose to sorbitol using nicotinamide adenine dinucleotide-2-phosphate (NADPH) as a co-factor,[58] whereas sorbitol dehydrogenase metabolizes sorbitol to fructose **17** which is absorbed by blood cells to increase glucose levels.[59] (**Scheme 1**)



Scheme 1: *Polyol pathway metabolism process*

1.5.6. Insulin sensitizers

- **Biguanides**

Biguanides as a class were derived from traditional European herbal therapies of the 1920s involving *Galega officinalis* which was rich in glucose-lowering guanidine derivatives.[60] Several biguanides were also reported in the 1950s and include metformin **18**, phenformin **19**, and buformin **20 (Figure 10)**. Metformin **20** is the only biguanide still available in the market for clinical use in treating T2DM while phenformin **19** and buformin **20** were withdrawn due to adverse effects.[61]

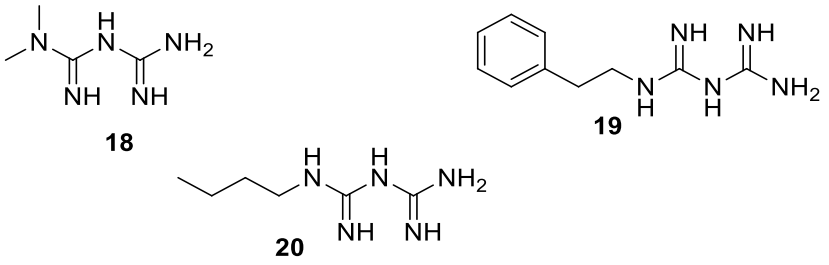


Figure 10: *Examples of biguanides drugs* ***18, 19 and 20***

1.6. Thiazolidinediones (TZD's) or glitazones

During the last decade, a new class of drugs called the 'glitazones' were approved by the FDA for the treatment of T2DM. These agents share a common molecular scaffold: namely that of the structure 2,4-thiazolidinediones (TZD).[62]

Mechanism of action

TZDs act through a mechanism that involves activation of the gamma isoform of peroxisome proliferator activated receptor (PPAR-γ), which stimulates certain transcriptional events of lipid and carbohydrate metabolism.[63] This ultimately results in transcription of PPAR-γ target genes. TZD's activate the nuclear receptor PPAR-γ in a variety of different tissues, thereby improving the insulin sensitivity of the whole body. Troglitazone **21** (trade name rezulin) with the potential for glucose lowering effect was the first glitazone to be launched in the market in the early 1990s by the Sankyo company, but was withdrawn in 1997 because of liver toxicity and related deaths associated with the drug.[64] Rosiglitazone **22 (Figure 11)** is the only TZD currently available in the market, approved by the FDA for the management of diabetes specifically for monotherapy and for use in combination therapy with metformin or sulphonylureas.[65]

Figure 11**: Examples of thiazolidinediones drugs **21** and **22

1.6.1. Chemistry of glitazones

The glitazone core **(23)** also known as 1,3-thiazolidine-2,4-dione (TZD), is a derivative of thiazolidine with two carbonyl groups at the 2- and 4-positions. **(Figure 12**). Substituents in the 3- and 5-positions may also be varied.

23

Figure 12: *Glitazone structure*

1.6.2. Clinically approved TZD containing drugs

In recent times, 2,4-thiazolidinediones have gained more importance as anti-diabetic agents and their mechanism of action has been thoroughly investigated. Rosiglitazone (**22)** (trade name avandia) is an insulin sensitizer which binds to the PPAR-γ in fat cells thus makes the cell more responsive to insulin. Pioglitazone **24** (**Figure 13**) (trade name actos) is used to restore one's body's proper response to insulin, thereby lowering blood sugar, whereas englitazone **25** prevents the excess glucose entering the body cells. Both rosiglitazone **22** and pioglitazone **24** were reported to be safe on the hepatic system.[66]

Prototypical 2,4-thiazolidinedione ciglitazone **26 (Figure 13)** was discovered by Takeda Chemical Industries Ltd in Japan to have antihyperglycemic activities in insulin-resistant animal models, KK-Ay mice and wistar fatty rats, but had no effect in insulin-deficient animal models of diabetes. Furthermore, ciglitazone **26** was never used as a medication, but it sparked interest with promising lipid and glucose lowering effects in animal models and was thus found to display significant anti-diabetic activities. It was later found to be associated with liver toxicity and discontinued.[67] Recently, netoglitazone **27** and KRP-297 **28** have been reported with PPAR-γ dual agonist activities. However, KRP-297 **28** has been withdrawn following instances of carcinogenecity.[68]

There was intense research that has led to the development of several TZDs PPARs based ligands. Among these were the prominent ones which has made it to market which include lobeglitazone **29** (trade name duvie) (**Figure 13**) that was developed by the Chong Kun Dang research group and was approved after phase III trials in Korea as an antidiabetic drug from the thiazolidinedione class of drugs.[69] As an agonist for both PPAR-α and PPAR-γ, this drug works as an insulin sensitizer by binding to the PPAR receptors in adipocytes and make the cells more responsive to insulin.[70]

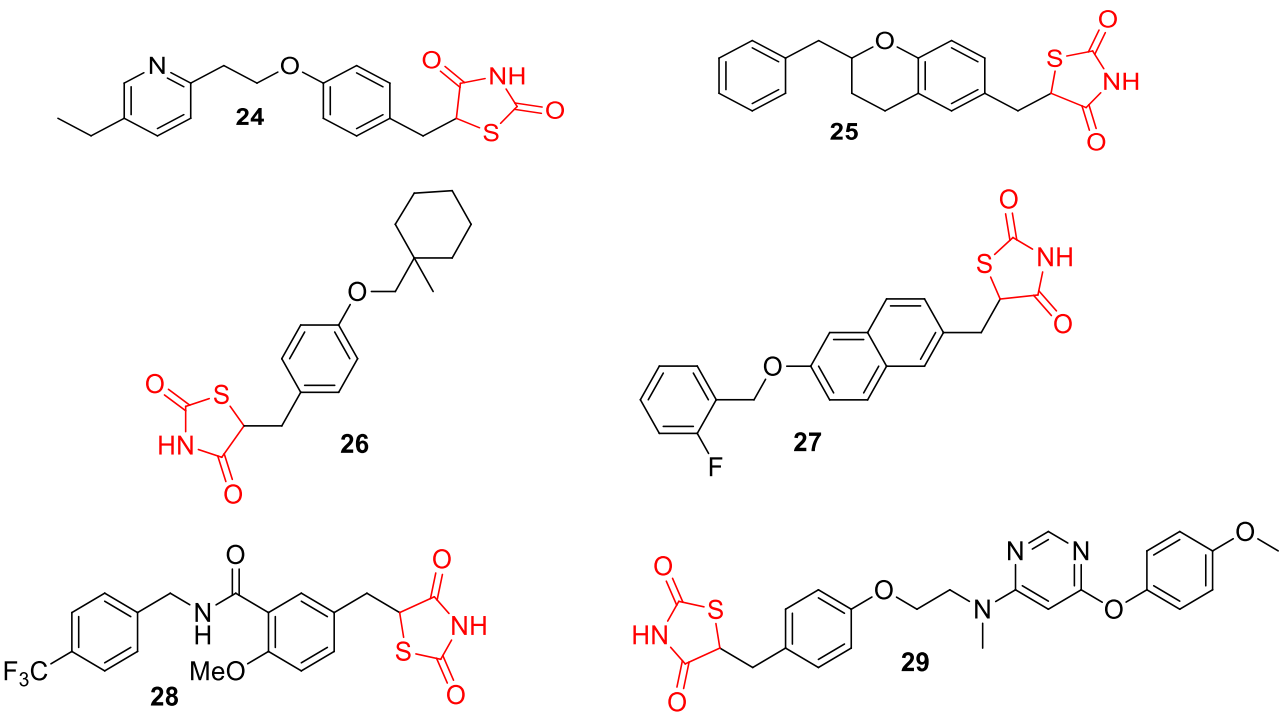


Figure 13**: Clinically approved TZDs Drugs **24-29

In early 2003s, Reddy's Research Foundation had successfully developed newer and improved glitazone analogues with promising and potential anti-diabetic activities, namely DRF-2189 **30** and balaglitazone **31** (**Figure 13**) for treatment of type 2 diabetes mellitus. DRF-2189 **30** was very promising in phase III clinical trials but unfortunately was associated with multiple unacceptable side effects because of the dose at which it was reproduced. This drug was therefore withdrawn and suspended in the late 2003. Balaglitazone **31** was another second generation PPAR-γ agonist with only partial agonistic properties which also failed in phase III clinical trials.[71]

Figure 14:** Clinically tested DRF-2189 **30** and balaglitazone **31

1.6.3. Glitazone, hydantoin and rhodanine containing compounds with different pharmacological activities

Due to their diverse and flexible nature, glitazone, hydantoin and rhodanine-containing compounds are found to exhibit a wide range of pharmacological activities which include: anti-diabetic, anti-oxidant/viral, anti-malarial, anti-inflammatory, anti-cancer, anti-tuberculosis, anti-convulsant, anti-microbial, anti-HIV, as well as aldose reductase inhibition[72] (**Figure 15**). Because of their wide pharmacological profile; glitazone, hydantoin and rhodanine containing compounds are included in various studies for better, safer and potential pharmacological agents.[73]

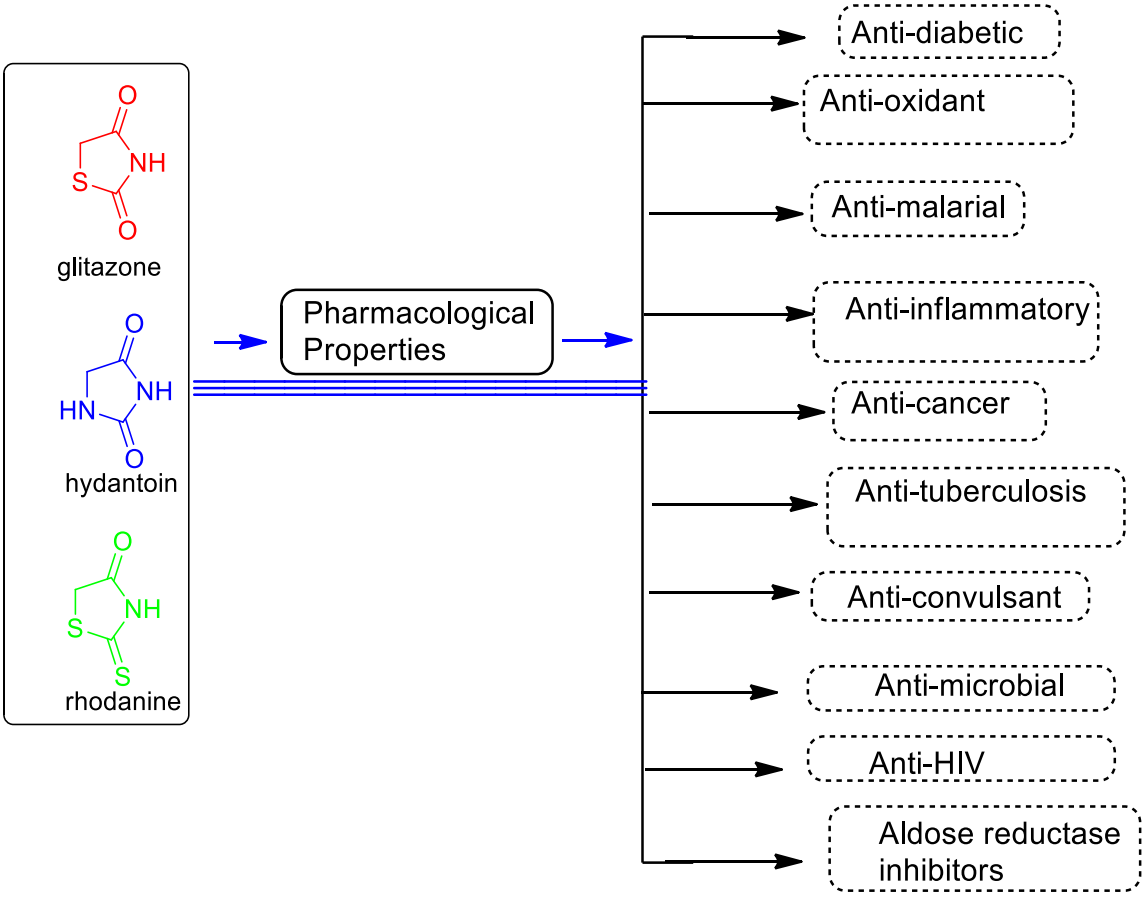
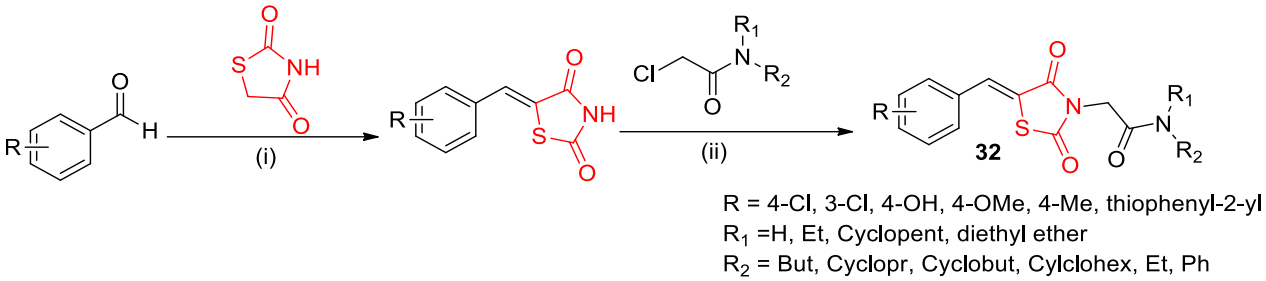


Figure 15: *Pharmacological properties of glitazone, hydantoin and rhodanine*

1.6.3.1. TZD derivatives as anti-diabetic agents

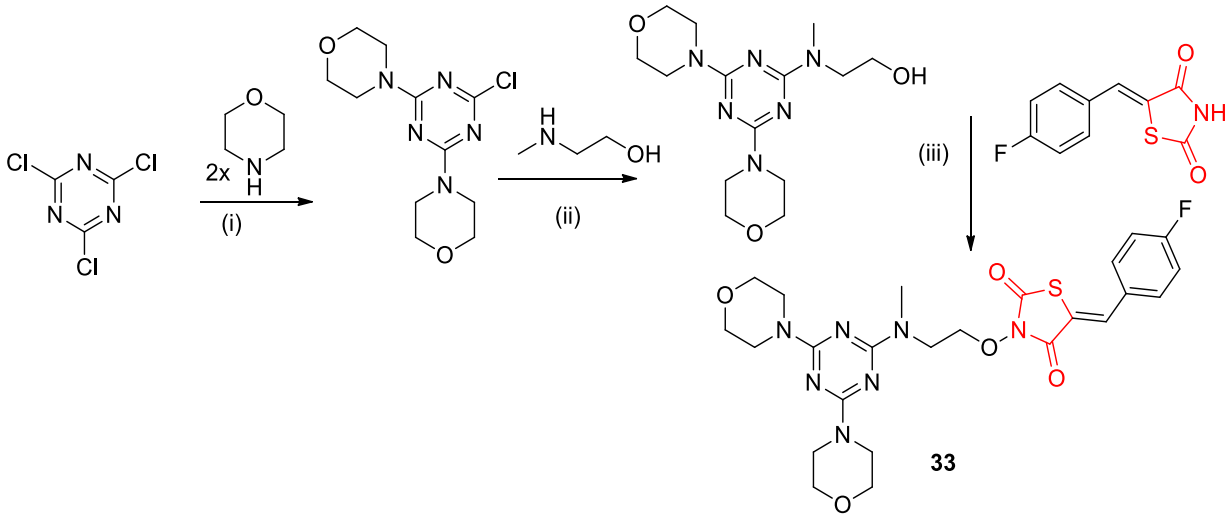
- After their synthesis of novel benzylidene thiazolidinedione derivatives (BTZD) **32** (**Scheme 2**) as partial Peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists, Yasmin *et. al.*[74] had evaluated derivatives of compound **32** for their anti-diabetic activities. Their results showed that only the ethyl, butyl and 4-

chloro phenyl derivatives exhibited selectivity towards PPAR-γ and these researchers concluded that these compounds showed weak to moderate partial agonists.



Scheme 2: Synthetis of novel BTZD derivatives **32**; **Reagents and Conditions:** (i) piperidine, reflux; (ii) CH_3CN, Et_3N, reflux

- Abbas Ahmadi *et. al.*[75] have successfully synthesized and evaluated a series of novel arylidene thiazolidine analogues **33** (**Scheme 3**) as anti-hypoglycaemic and hypolipidemic agents of type 2 diabetes model. Their anti-diabetic assay results showed that these compounds demonstrated good hypoglycaemic and hypolipidemic activities which were comparable to the control rosiglitazone.

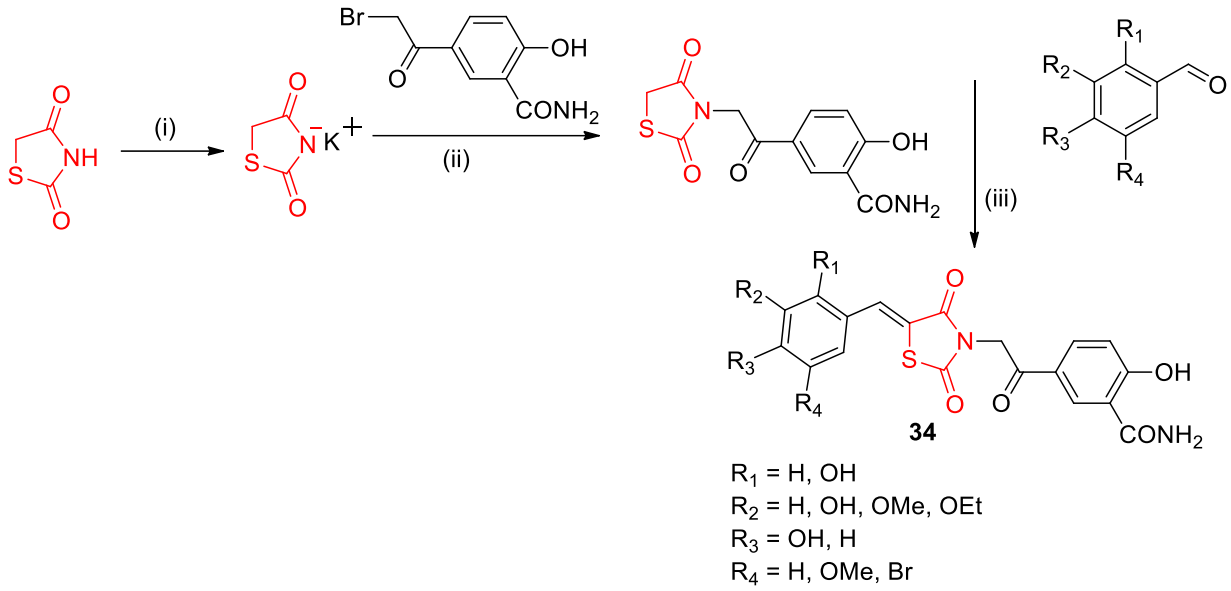


Scheme 3: *Synthesis of a series of novel arylidene thiazolidine analogues* ***33****;* ***Reagents and conditions****: (i)* Na_2CO_3*, THF, stirring at rt; (ii) Acetone, reflux; (iii) DMSO,* K_2CO_3*, 100*℃

1.6.3.2. TZD derivatives as anti-viral/ oxidizing agents

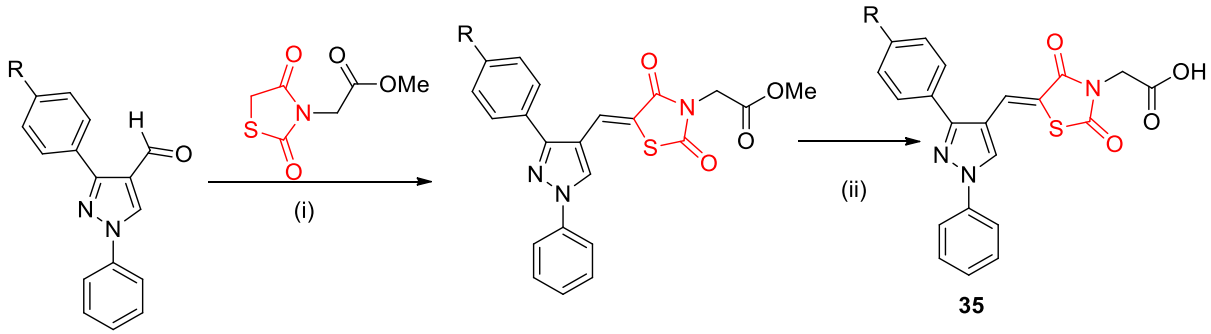
- Marc *et. al.*[76] successfully synthesized a series of new phenolic derivatives of thiazolidine-2,4-dione **34** (**Scheme 4**) and evaluated them for their anti-oxidant and anti-radical properties. They reported that 2,3-dihydroxy and 2-hydroxy-

4-ethoxy polyphenolic compounds acted as potent antiradical and electron donors, with activities comparable to the reference antioxidants used.



Scheme 4: *Synthesis of new phenolic derivatives of thiazolidine-2,4-dione* ***34****;* ***Reagents and conditions****: (i) K_2CO_3, DMF, reflux; (ii) K_2CO_3/DMF stirring at rt; (iii) piperidine, MeOH, reflux*

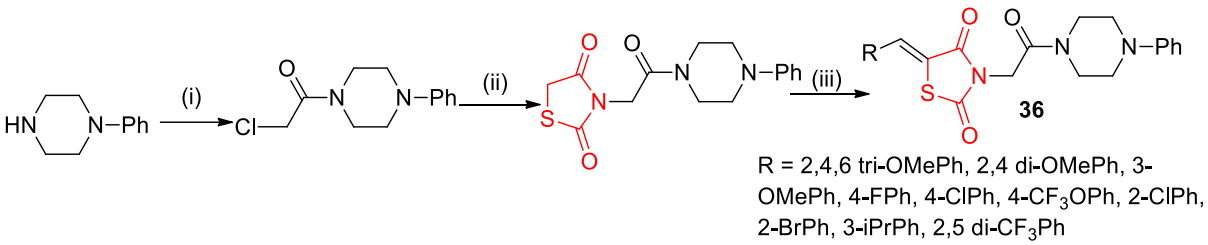
- Aneja *et. al*.[77] successfully synthesized new pyrazolyl-2,4-thiazolidinediones **35** (**Scheme 5**) and evaluated them for their anti-bacterial, anti-fungal as well as anti-oxidant activities. Their results showed that phenyl and *para*-hydroxy phenyl derivatives were associated with remarkable anti-fungal activity as well having good effectiveness against gram-positive bacteria.



Scheme 5*: Synthesis of pyrazolyl-2,4-thiazolidinediones* ***35****;* ***Reagents and conditions****: (i) MeOH, piperidine, reflux and cooling to rt; (ii) AcOH, H_2SO_4, Reflux*

1.6.3.3. TZD derivatives as anti-malarial agents

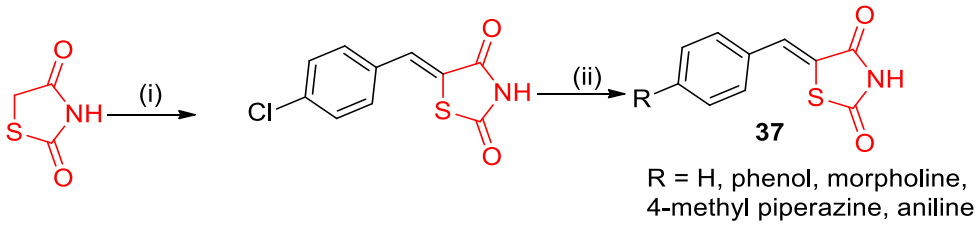
- Sharma and co-workers[78] have successfully synthesized thiazolidinediones **36** (**Scheme 6**) as anti-plasmodial inhibitors. Their results indicated that most of their compounds exhibited low micromolar antiplasmodial activities against the *P. falciparum* drug resistant *W2* strain.



Scheme 6: *Synthesis of thiazolidinediones as anti-plasmodial inhibitors* ***36; Reagents and conditions:*** *(i) Chloroacetyl chloride, Et_3N, DCM, 0 ℃; (ii) 2,4-thiazolidinedione, K_2CO_3, Ac_2O, 60-70 ℃; (iii) substituted benzaldehydes, NH_4OH, toluene, MW, 170 ℃*

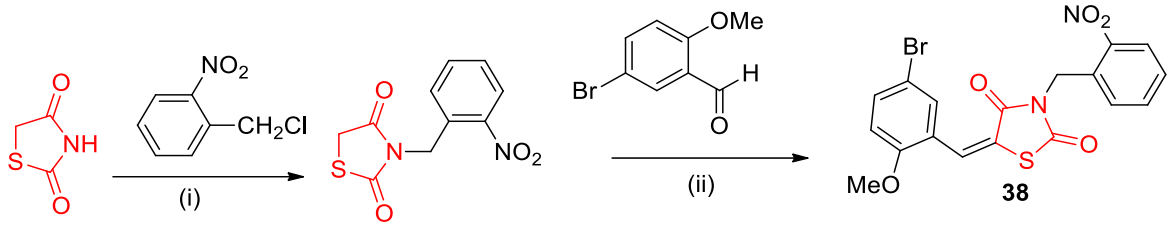
1.6.3.4. TZD derivatives as anti-inflammatory agents

- Rekha *et.al.*[79] synthesized and evaluated novel 2,4-thiazolidinediones **37** **(Scheme 7)** for their anti-inflammatory activities. Their results showed that all synthesized compounds were weakly active in a bovine serum denaturation assay.



Scheme 7: *Synthesis of novel 2,4-thiazolidinediones* ***37****;* ***Reagents and conditions****: (i) 4-chlorobenzaldehyde, toluene, piperidine, 80 ℃; (ii) N-methyl pyrrolidine, CuI, anhydrous K_2CO_3, reflux*

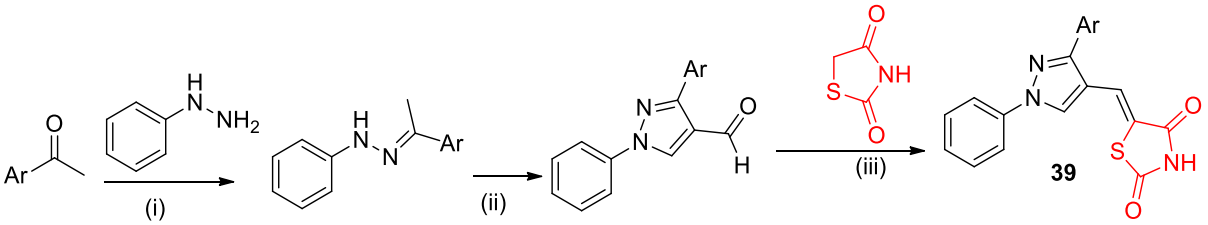
- Laurindo Rocha Junior *et. al.*[80] successfully synthesised a series of novel thiazolidinediones **38** (**Scheme 8**) and evaluated them for their modulatory effect on interferon gamma, Interleukin 6, 17a and 22 (IFN-γ, IL-6, IL-17A and IL-22) production in PBMCs from rheumatoid arthritis patients. Their results that the compounds presented a significant reduction in IL-17A, IL-22, and IFN-γ levels with no activity on IL-6 when compared with nontreated cells.



Scheme 8: *Synthesis of novel 2,4-thiazolidinediones* ***38****;* ***Reagents and conditions:*** *(i) Et_3N, benzene, 70℃; (ii) EtOH, piperidine, reflux*

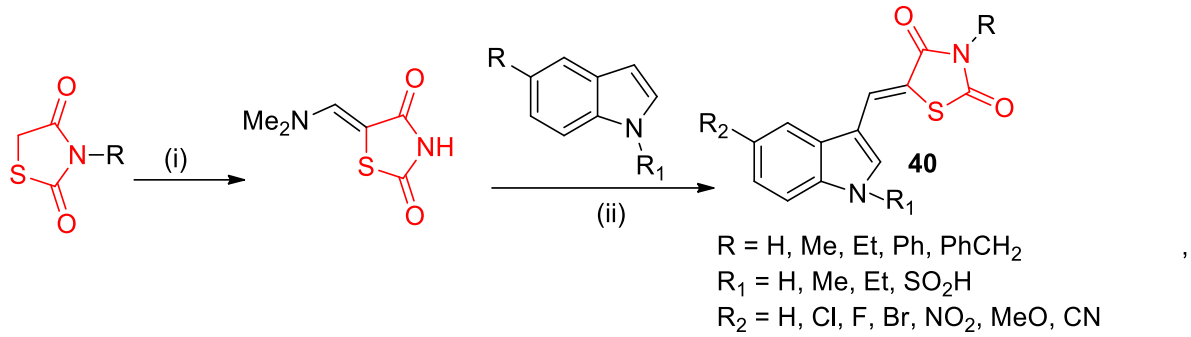
1.6.3.5. TZD derivatives as anti-cancer agents

- Kumar *et. al.*[81] have successfully synthesized some novel 2,4-thiazolidinedione-incorporated pyrazole derivatives **39** (**Scheme 9)** as anti-cancer agents**.** MTT assays of all their synthesized hybrids MTT assays showed promised and effective anti-cancer activities against the cell lines comparable to standard drug Doxil.



Scheme 9*: Synthesis of novel 2,4-thiazolidinedione incorporated pyrazole derivatives* ***39****;* ***Reagents and conditions****: (i) glacial AcOH, MeOH, reflux (ii) DMF-$POCl_3$, reflux 60-70 ℃ (iii) glacial AcOH, piperidine, reflux*

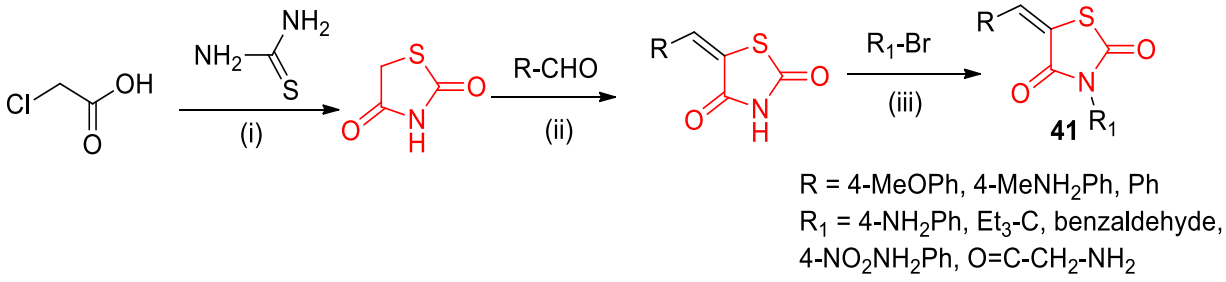
- Corigliano *et. al.*[82] have successfully synthesized indole and 2,4-thiazolidinedione conjugates **40 (Scheme 10)** as potential anticancer modulators and evaluated them for their anti-cancer activities. They reported that 6-methoxy indole, 6-fluoro indole and 1-ethyl-6-methoxy indole 2,4-thiazolidinedione derivatives showed promising anti-proliferative for the treatment of prostate and breast cancer.



Scheme 10: *Synthesis of indole and 2,4-thiazolidinedione conjugates* ***40****;* ***Reagents and conditions****: (i) DMF-DMA, 100 ℃ (ii) AcOH, 100℃*

1.6.3.6. TZD derivatives as anti-tuberculosis agents

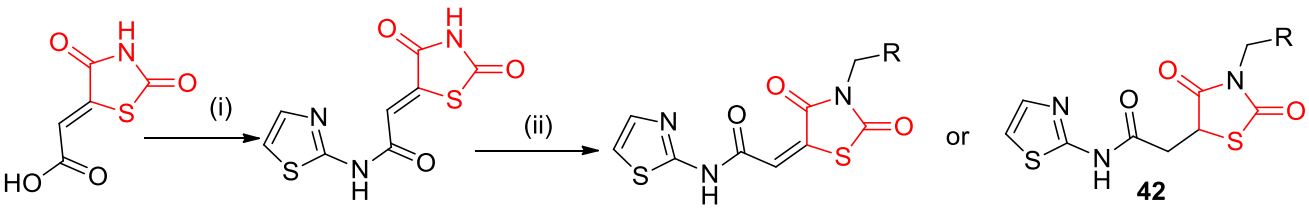
- Chilamakuru *et. al.*[83] have successfully synthesized a series of some 3,5-disubstituted 2,4-thiazolidinediones **41** (**Scheme 11**) as antituberculosis agents and found that among those synthesized derivatives, 4-amino-(5-(4-methoxy) phenyl, 2-amin-(5-(4-methoxy) phenyl, 5-(4-dimethyl amino)) phenyl and 5-(4-nitro amino) phenyl showed good antitubercular activity.



Scheme 11: *Synthesis of 3,5-disubstituted thiazolidinediones* ***41****;* ***Reagents and conditions:*** *(i) Conc HCl, H_2O, reflux; (ii) glacial AcOH, AcONa reflux; (iii) Ethanol or DMF, reflux*

1.6.3.7. TZD derivatives as anti-convulsant agents

- Mishchenko *et. al.*[84] have successfully synthesized thiazole-bearing hybrids based on 2-imino-4-thiazolidinone and 2,4-dioxothiazolidine-5-carboxylic acid cores **42** (**Scheme 12** and evaluated them for their anti-convulsant properties. They reported that carboxylic ester and 3-trifluoro phenyl derivatives showed excellent anticonvulsant activity in both pentylenetetrazole-induced seizures and maximal electroshock seizure models.

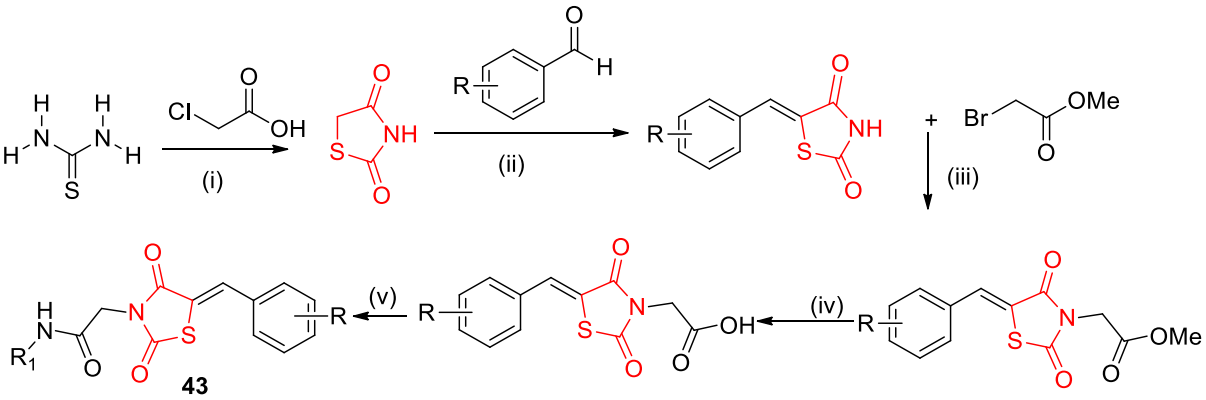


R = COOEt, 4-FPh, 4-MeOPh, 4-MeOPhNHCO, 3-CF_3PhNHCO, triazol-2-yl-NHCO

***Scheme 12**: Synthesis of 2,4-dioxothiazolidine-5-carboxylic acid amide cores **42**; **Reagents and conditions:** (i) 2,4-dioxothiazolidine-5-carboxylic acid, $SOCl_2$, dioxane, reflux, (ii) acid chloride, 2-aminothiazole, Et_3N, dioxane, 90 ℃ (iii) H_2, Pd, dioxane, reflux*

1.6.3.8. TZD derivatives as anti-microbial agents

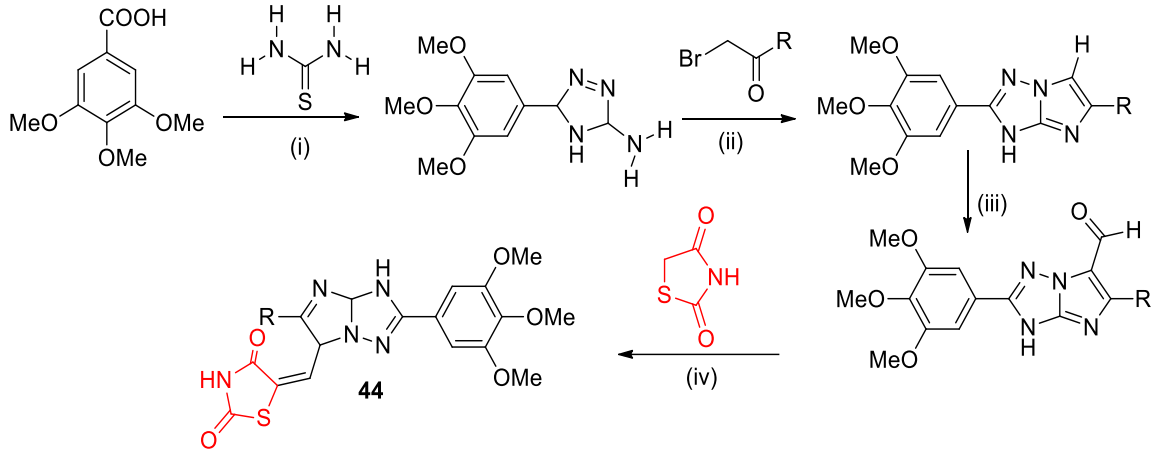
- Alhameed *et. al.*[85] have successfully synthesized a new series of thiazolidine-2,4-diones carboxamide and amino acid derivatives **43** (**Scheme 13**) and evaluated them for their anti-microbial activities. They reported that 2-(5-(3-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)acetic acid derivative was the most active against Gram-positive bacteria *S. aureus.*



R= H, 4-Br, 2-Cl, 4-Cl, 3-OMe
R_1 = gly-OMe, gly-Me, $(CH_3)_2$, val-OMe, ala-OMe, Ph-OMe

***Scheme 13**: Synthesis of thiazolidine-2,4-diones carboxamide and amino acid derivatives **43**; **Reagents and conditions**: (i) Conc HCl, water, reflux (ii) piperidine, Ethanol, Reflux, (iii) K_2CO_3, Acetone, reflux, (iv) HCl, AcOH, reflux, (v) NH_2-R_1, HCl, DIEA, 0 ℃-rt*

- Alagawadi *et. al.*[86] have successfully synthesized a series of new 2,4-thiazolidinediones bearing imidazo [2,1-*b*][1,3,4] thiadiazole moiety **44** (**Scheme 14**) and evaluated them against anti-microbial activities. Their results revealed that 4-bromo and 4-chloro phenyl derivatives showed moderate to high activity against the tested microorganisms.

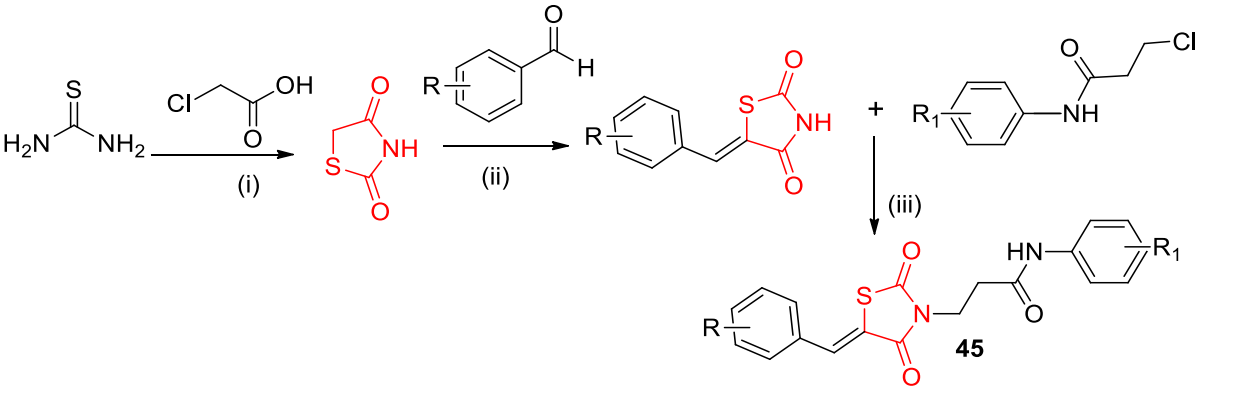


R = PH, 4-MePh, 4-MeOPh,4-NO_2Ph, 4-BrPh, 4-ClPh, 2,5-diMeOPh

Scheme 14: *Synthesis of 2,4-thiazolidinediones bearing imidazo[2,1-b][1,3,4]thiadiazole* ***44****;* ***Reagents and conditions****: (i) Conc HCl, H_2O, reflux; (ii) dry EtOH, reflux, (iii), Vilsmeir Haack formylation (iv) piperidine, AcOH, toluene*

1.6.3.9. TZD derivatives as anti-HIV agents

- Bahare *et. al*.[87] synthesized some novel *N*-substituted 5-benzylidine-2,4-thiazolidinediones **45** (**Scheme 15**) and evaluated them against HIV-1 RT inhibitory. They reported that among the synthesized compounds 2,3,5-(trihydroxy)-2-(thiol)-phenyl derivative showed significant HIV-1 RT inhibitory activity with 73% of inhibition with an IC_{50} value of 1.31 µM.

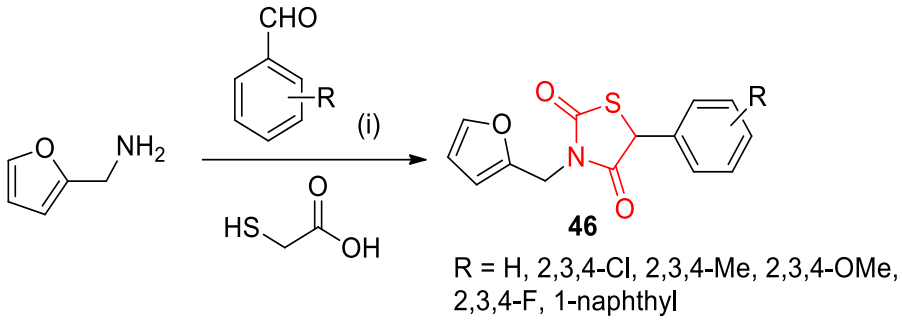


R = H, 2,3,4-OH, 2-F, 2,3,4,5-Me, 2,3,4-Cl,
R_1 = OH, H, 2,3-OH, 2,3,4-Me, 2,4-NO_2, 2-SH

Scheme 15*: Synthesis of novel N-substituted 5-benzylidine-2,4-thiazolidinediones* ***45****;* ***Reagents and conditions*** *(i) glacial AcOH, 0℃ to rt (ii) EtOH, piperidine, reflux, (iii) CH_3N, Et_3N, reflux, 12h*

- Rawal *et. al*.[88] have successfully synthesized a series of 2-(aryl)-furanyl-thiazolidin-2,4-dione derivatives **46** (**Scheme 16**) and evaluated them against HIV-1 Integrase and found that 2-(2,6-dichloro-phenyl)-3-furan-2-yl-methyl-

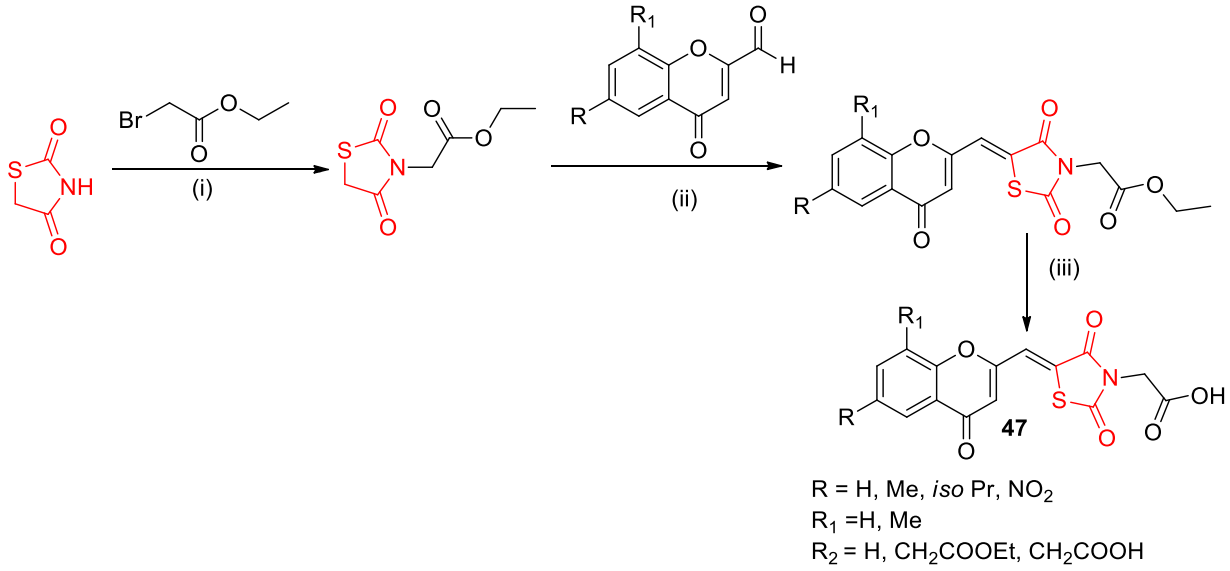
thiazolidin-4-one inhibited the enzyme at 0.204 μM concentration with minimal toxicity to MT-4 cells.



***Scheme 16**: Synthesis of 2-(Aryl)-3-furan-2-ylmethyl-thiazolidin-2,4-dione derivatives **46**; **Reagents and condition**: (i) DCC, THF, rt*

1.6.3.10. TZD derivatives as aldose reductase inhibitors

- Bozdag-Dundar *et. al.*[89] had successfully synthesized some new chromonyl-2,4-thiazolidinediones **47** (**Scheme 17**) and evaluated them for their aldose reductase inhibitory activity. They found that 2,4-dioxo-5-[(6-nitro-4-oxo-4H-chromen-3-yl)methylene]-1,3-thiazolidine-3yl acetic acid was the compound the highest aldose reductase inhibitory activity (82.43 ± 0.76%)



***Scheme 17**: Synthesis of new chromonyl-2,4-thiazolidinediones **47**; **Reagents and conditions** (i) NaH/THF, reflux (ii) AcONa, AcOH, reflux (iii) AcOH/HCl, reflux*

1.7. Hydantoin-containing compounds

1.7.1. Chemistry of hydantoins

Hydantoin or imidazolidine-2,4-dione **48** is a class of heterocyclic compounds with a five membered ring, reactive nucleus and having five possible points of diversity (**Figure 16)**. Furthermore, hydantoin **48** is stable in dilute acid but forms ureido acid salts in basic solution. Generally hydantoin with substituents at the N-1 and/or the N-3 positions are less reactive to hydrolysing or oxidising agents.[90]

48

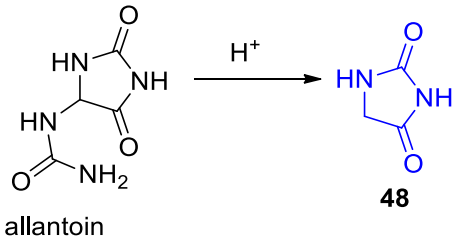
Figure 16: *Hydantoin structure* ***48***

1.7.2. Classical methods for synthesis of hydantoin

There are many different methods of synthesising hydantoins, depending on the choice of starting materials. However, there are a few classical methods that are still commonly employed.

1.7.2.1. Baeyer's synthesis

Hydantoin **48** was first isolated by Nobel laureate Adolph von Baeyer in 1861 from the hydrogenolysis of allantoin (**Scheme 18**).[91]



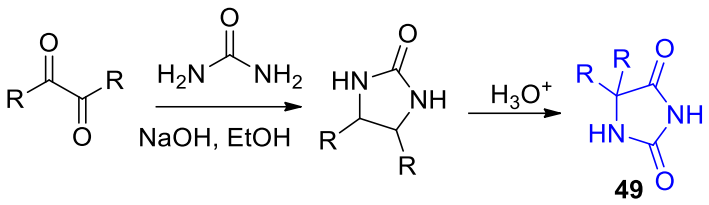
allantoin **48**

Scheme 18: *Baeyer's synthesis of hydantoin* ***48*** *from allantoin*

1.7.2.2. Biltz Synthesis

The most common synthetic route for the preparation of 5-substituted hydantoin analogues **49** is the Biltz synthesis. This reaction involves condensation of 1,2-diketo compounds with urea under basic conditions followed by benzylic rearrangement

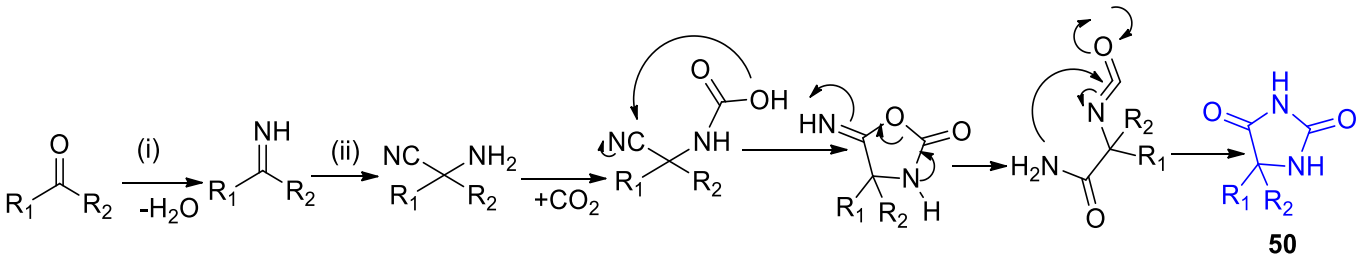
under acidic conditions, which involves conventional heating procedures for a long time at high temperatures. (**Scheme 19**).[92]



***Scheme 19**: Synthesis of hydantoin analogues **49** via Blitz condensation reaction*

1.7.2.3. Bucherer–Berg's reaction

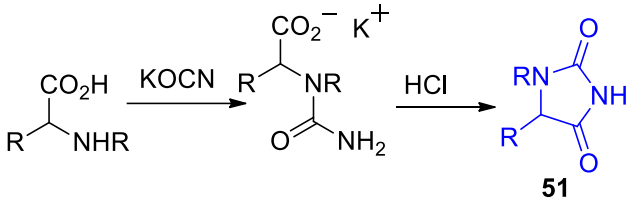
Bucherer–Berg's reaction is the second most common method which is used to synthesize 5-substituted hydantoin analogues **50** from aldehydes or ketones. In this reaction the carbonyl compound initially reacts with ammonium carbonate followed by the cyanide in order to give an anion which forms an α-aminonitrile. Nucleophilic addition of aminonitrile to CO_2 leads to cyano-carbamic acid, which undergoes an intramolecular ring closing to 5-imino-oxazolidin-2-one. The 5-imino-oxazolidin-2-one rearranges to form the hydantoin product via an isocyanate intermediate [93] (**Scheme 20**)



***Scheme 20:** Synthesis of hydantoin analogues **50** via Bucherer–Berg's reaction; **Reagents and Conditions:** (i) $(NH_4)_2CO_3$ (ii) KCN*

1.7.2.4. Urech reaction

In 1873 Urech reported the first general method for the synthesis of 5-monosubstituted hydantoins **51** where α-amino acids are reacted with potassium cyanate to give α-ureido acids. The intermediates are cyclised under acidic conditions to yield the desired hydantoins.[94] (**Scheme 21**)



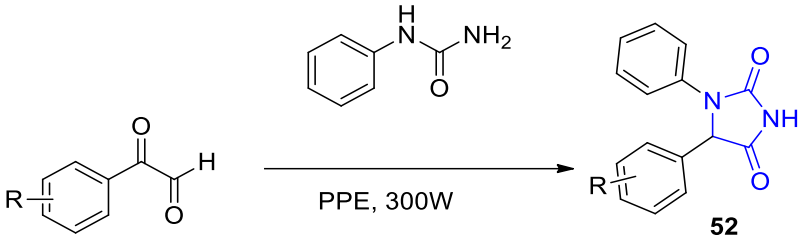
51

***Scheme 21**: Synthesis of Hydantoin **51** via Urech reaction; **Reagents and conditions**: (i)KOCN, (ii) HCl*

1.7.3. Microwave assisted synthesis of hydantoin

Microwave-assisted organic synthesis is an integral part of combinatorial synthesis and drug discovery processes. The use of this technology has been applied to the development of efficient methodologies for the production of hydantoins for drug discovery.[95]

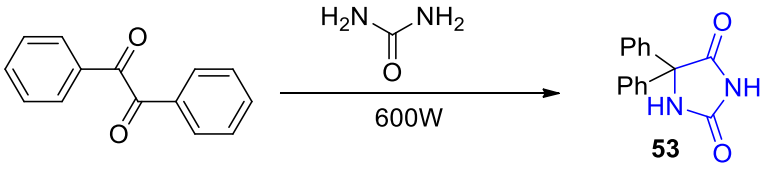
- Recently, several 1,5-diphenyl hydantoins analogues **52** (**Scheme 22)** have been prepared by microwave assisted synthesis by Paul and co-workers[96] employing solvent-free conditions, where a range of arylglyoxals are reacted with phenylureas using polyphosphoric ester (PPE) as a reaction mediator



52

***Scheme 22**: Solvent-free microwave-assisted synthesis of hydantoins **52** by Paul et al*

- In 2003 Muccioli *et. al.*[97] had applied microwave activation to the synthesis of N3-alkylated phenytoin **53** (**Scheme 23**). They discovered that microwave activation of the Biltz synthesis of phenytoin improves both yield and time reaction as compared to classical methods.



53

***Scheme 23**: Microwave-assisted synthesis of phenytoin **53** by Muccioli et al*

1.7.4. Naturally occurring hydantoin containing compounds

Several hydantoin containing compounds have been reported to be found in naturally occurring substances of marine organisms and in bacteria. (**Figure 17)**. Hydantoin containing compound such as 3'-deimino-3'-oxoaplysinopsin (**54**) was reported by Bialonska *et. al.*[98] from the isolation of several aplysinopsin-related compounds from the sponge *Ianthella CF. flabelliformis* with cytotoxic properties. This compound has been associated with the ability to inhibit neurotransmission.[90] Hydantocidin (**55**) is a relatively new phytotoxin discovered in the streptomyces hygroscopicus SANK 63584 which possesses herbicidal and plant growth regulatory activities due to the inhibition of adenyl succinate synthetase.[99]

Axinohydantoin (**56**) is an alkaloid which is isolated from the sponge *axinella sp* and *Monochora sp.* and has been shown to inhibit protein kinase C.[100] Naamidine A (**57**) is a dehydrohydantoin derivative from the *genus Leucettu* used as a selective inhibitor[101] of the epidermal growth factor whereas Mukanadin B (**58**) is isolated from *Agelus species* and is used as a neuroprotective agent.[101] Midpacamide (**59**) has been isolated from marine sponges of the *genus Agela* and has been used for therapeutic applications such as kinase inhibition or antiviral and antifungal activities.[102] Hemimycalins B (**60**) is isolated from the sponge *Hemimycale arabica* and has been reported to possess good anti-microbial activities.[103]

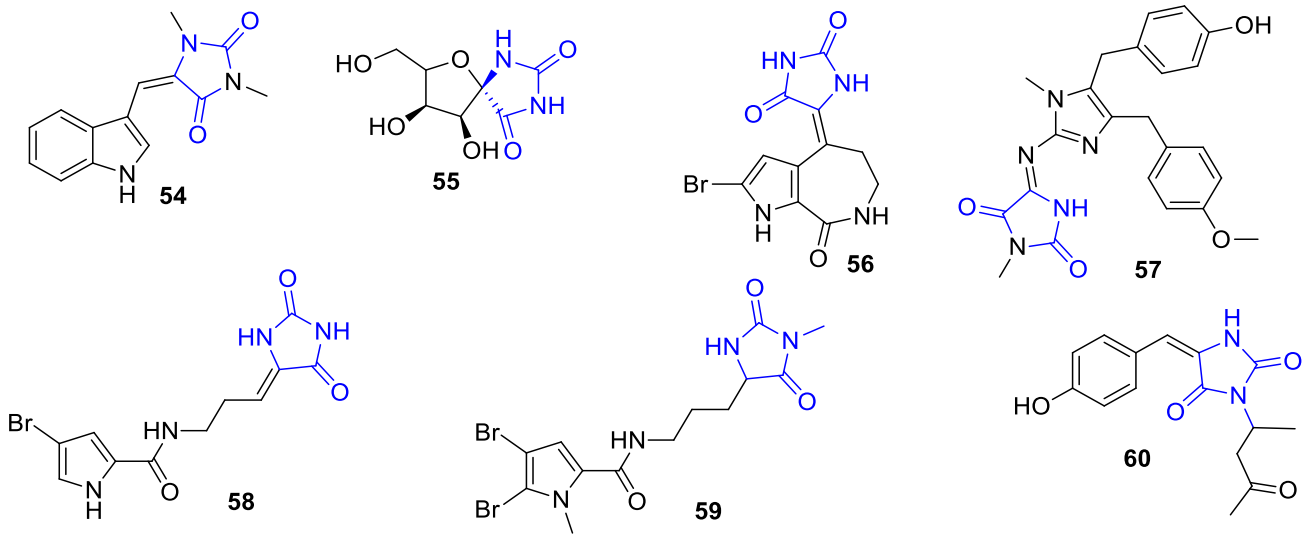


Figure 17: *Natural products containing hydantoin moiety* ***54-60***

1.7.5. Clinically approved hydantoin containing drugs

The importance of the hydantoin scaffold in drug discovery has been reinforced by several medicines in clinical use. Phenytoin (phenytek) (**53**) is a well-known therapeutic drug for the treatment of epileptic seizures and ethotoin (peganone) (**61**) is currently used as an anticonvulsant drug, nitrofurantoin (macrodantin) (**62**) and dantrium (ryanodex) (**63**) are used as muscle relaxants whereas nilutamide (nilandon) (**64**) and fosphenytoin (cerebyx) (**65**) are used as newer antiepileptic drugs.[104] (**Figure 18**)

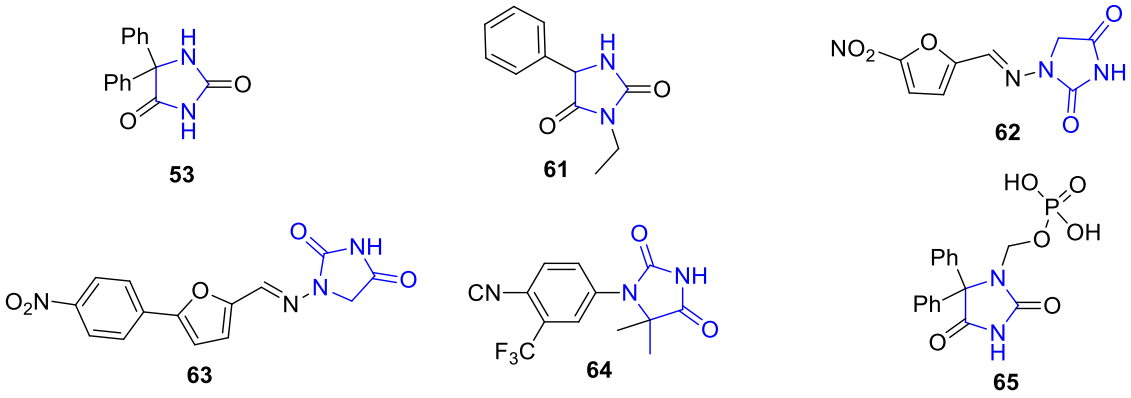


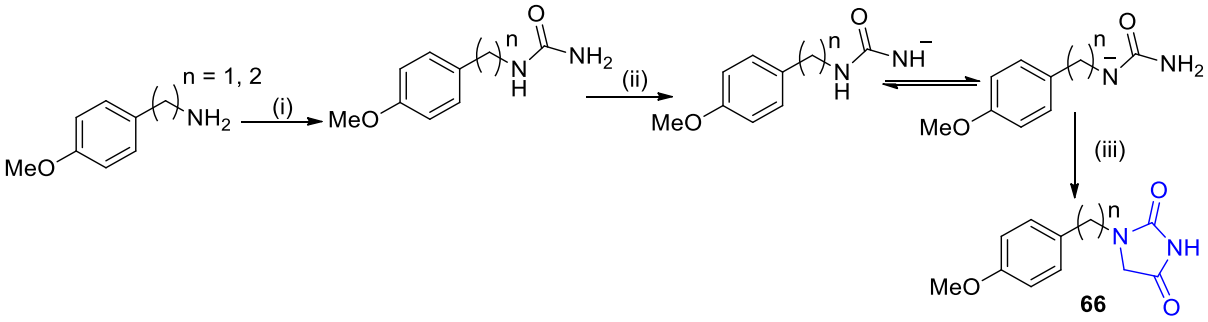
Figure 18**: Clinically approved hydantoin containing drugs **60-65

1.7.6. Hydantoin containing compounds with pharmacological properties

Hydantoin is a privileged structural motifs, widely used in medicinal chemistry as potent drug.[105] It has been reported to show different pharmacological properties reported above.[106] **(Figure 15)**

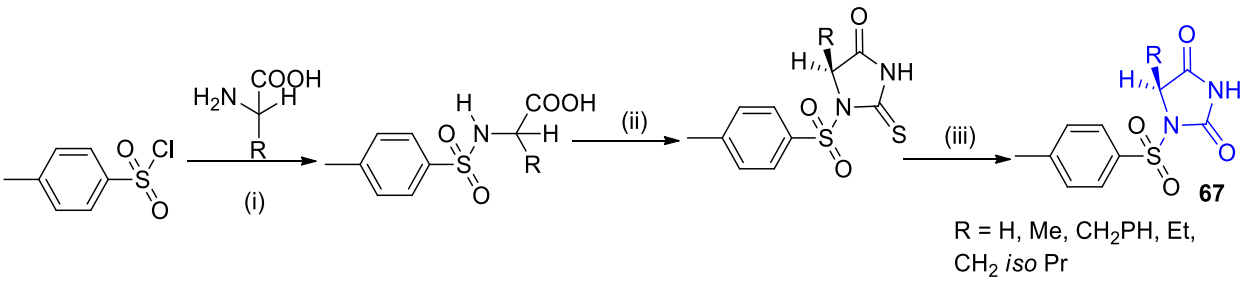
1.7.6.1. Hydantoin derivatives as anti-diabetic agents

- Cheng *et. al* [107] had successfully evaluated compounds **66** and evaluated them for their anti-diabetic activities. Only 5-(4-methoxybenzyl)-2,4-hydantoin (n=1 in **66**) showed good anti-diabetic activity.



Scheme 24: *Synthesis of novel imidazolidine-2,4-dione derivatives* ***66****;* ***Reagents and conditions:*** *(i) KCN, HOAc, H_2O, 35 ℃; (ii) NaH, DMF, rt-70 ℃; (iii) $ClCH_2CO_2CH_2CH_3$, rt-70 ℃*

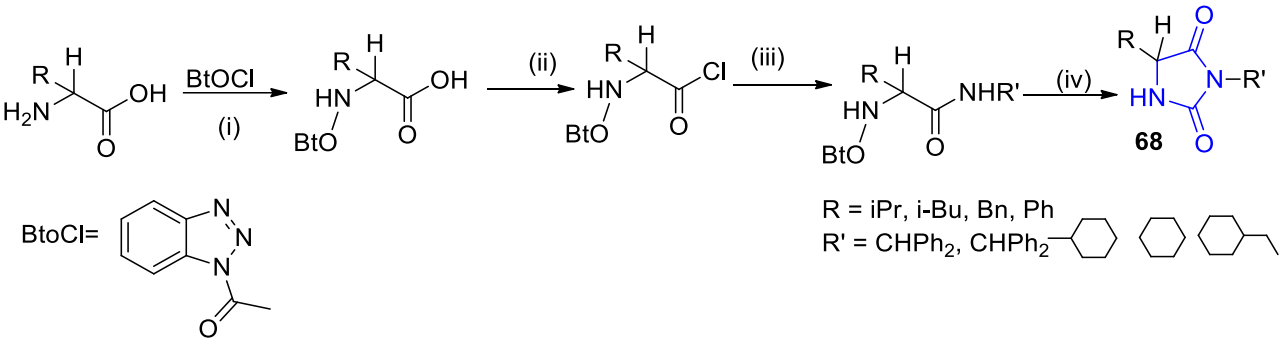
- Ahmad *et al.*[108] had successfully synthesized some chiral sulfonyl hydantoin derivatives **67 (Scheme 25)** and evaluated them for their anti-diabetic activities. They found that only methyl and isopropyl derivatives exhibited antidiabetic activity at lower concentrations of 10^{-4} or 10^{-3} M.



Scheme 25*: Synthesis of novel of some chiral sulfonyl hydantoin derivatives* ***67****;* ***Reagents and conditions****: (i) K_2CO_3, dioxane; (ii) NH_4SCN, Ac_2O/Py; (iii) HNO_3, H_2O, rt*

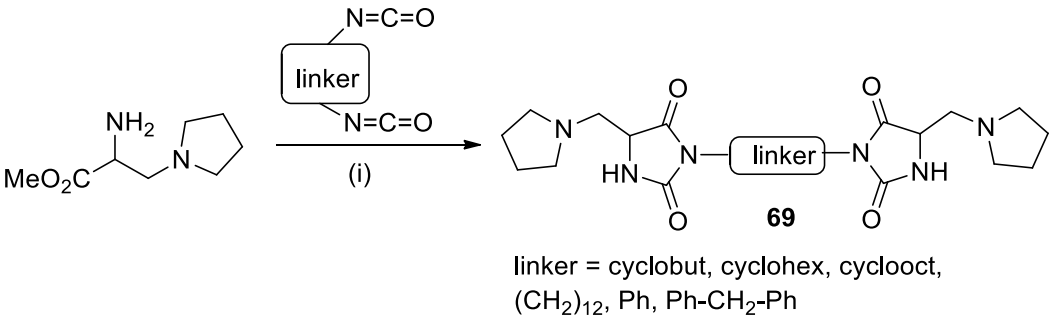
1.7.6.2. Hydantoin derivatives as anti-viral/antioxidant agents

- In their synthesis of hydantoin derivatives of L-and D-amino acids, Zrinka Rajic *et al*.[109] had successfully synthesized a series of 3,5-hydantoin derivatives **68 (Scheme 26)** and evaluated them for their antiviral and antitumoral activities and found that among all the compounds evaluated only 3-benzhydryl-5-isopropyl hydantoin derivative showed a weak but selective inhibitory effect against vaccinia virus (EC_{50} = 16 µg/mL).



***Scheme 26**: Synthesis of 3,5-hydantoin derivatives **68**; **Reagents and conditions:** (i) anhydrous dioxane, rt; (ii) $SOCl_2$, rt, 24 h or reflux; (iii) amines, Et_3N, anhydrous toluene, rt; (iv) Na_2CO_3, $(CH_3)_2CO$, rt.*

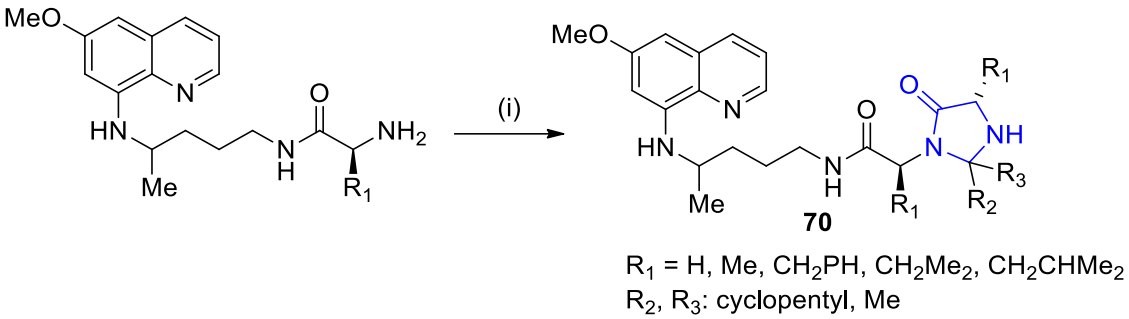
- Fujisaki *et. al.*[110] had successfully reported a series of new 5-substituted hydantoin **69** (**Scheme 27**) which was evaluated for their anti-bacterial activities. They found that substituted phenyl and diphenyl linker showed significant antibacterial activity against a gram-positive strain (Staphylococcus aureus) (MIC= 0.026 µM and 0.116 µM.



***Scheme 27**: Synthesis of new 5-substituted hydantoin **69**: **Reagents and conditions:** (i) Conc HCl, rt*

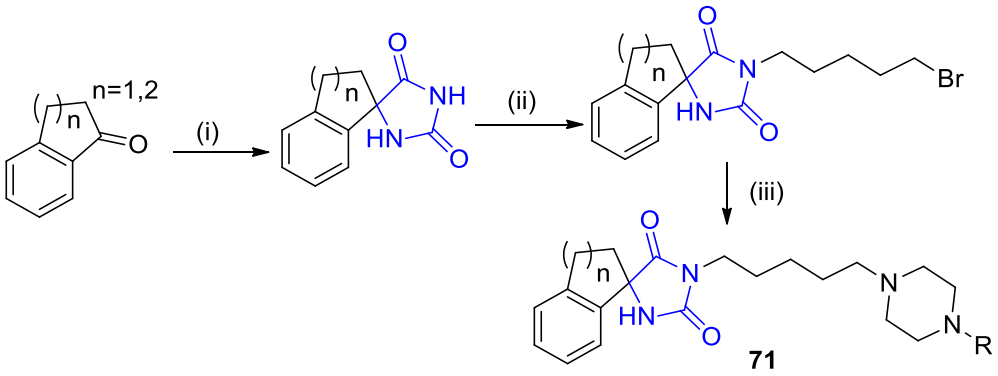
1.7.6.3. Hydantoin derivatives as anti-malarial agents

- In their synthesis of imidazolidin-4-one primaquine derivatives **70 (Scheme 28)** as novel transmission-blocking antimalarials, Maria Araújo *et. al.*[111] had evaluated compounds **70** for their anti-malarial activities. They reported that all their compounds were both active in human plasma and in pH 7.4 buffer.



***Scheme 28**: Synthesis of hydantoin derivatives of primaquine **70**: **Reagents and conditions:** (i) C5, C6 or C7 cyclic ketones in refluxing MeOH, Et_3N, molecular sieves.*

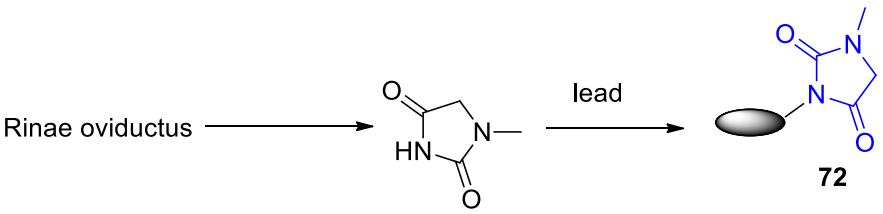
- Jaromin and co-workers[103] had successfully synthesized a series of novel bioinspired hydantoin **71 (Scheme 29)** derivatives and evaluated them for their antiplasmodial activities. Their results showed that the antiplasmodial effects were stronger against the *W2* strain (IC50 between 2424.15–5648.07 ng/mL (4.98–11.95 µM)) as compared to the D10 strain (6202.00–9659.70 ng/mL (12.75–19.85 µM)).



***Scheme 29**: Synthesis of novel bioinspired hydantoin derivatives **71: Reagents and conditions**: (i) $(NH_4)_2CO_3$, KCN, rt; (ii) 1,5- dibromopentane, K_2CO_3, CH_3CN, 80 ℃, (iii) arylpiperazine, Et_3N, CH_3CN, 100 ℃, 1 h, MW*

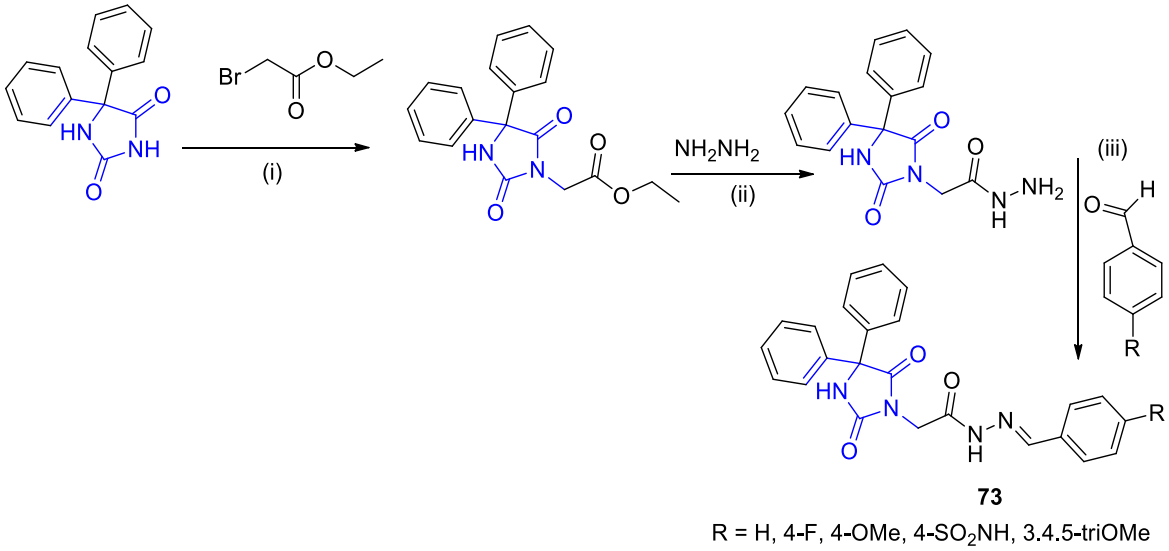
1.7.6.4. Hydantoin derivatives as anti-inflammatory agents

- Yang Xu and co-workers[112] had successfully isolated 1-methylhydantion **72 (Scheme 30)** as lead compound from the natural product ‘’rinae oviductus’’ and evaluated it for its anti-inflammatory activities. They found that at the same dosage (100 mg/Kg), the newly prepared agent had an inhibition rate 53.18% which was much higher as compared to the lead compound (22.69%).



Scheme 30**: Isolation of methyl hydantoin as lead compound **72

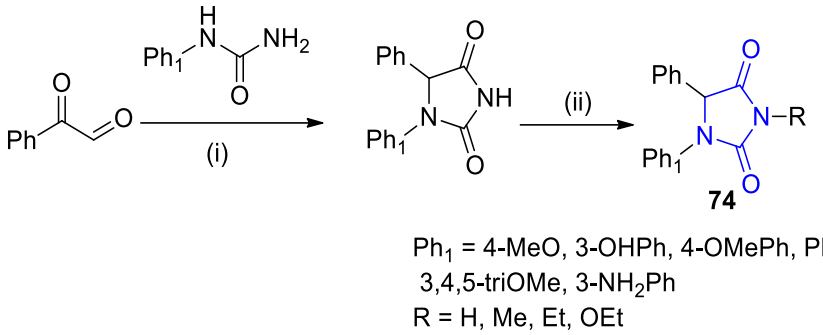
- Abdel-Aziz *et. al.*[113] had successfully synthesized a group of phenytoin derivatives **73 (Scheme 31)** and evaluated them for their anti-inflammatory activities. They reported that among the tested compounds 4-sulfanilamide, 3,4,5-trimethoxy-phenyl and 4-fluoro-phenyl derivatives showed significant potent anti-inflammatory and analgesic activities almost equivalent to reference drug celecoxib.



***Scheme 31**: Synthesis of 5,5 dipheylimidazolidinedine-2,4-dione derivatives **73**; **Reagents and conditions**; (i) Acetone, K_2CO_3, rt; (ii) MeOH, rt; (iii) MeOH, reflux*

1.7.6.5. Hydantoin derivatives as anti-cancer agents

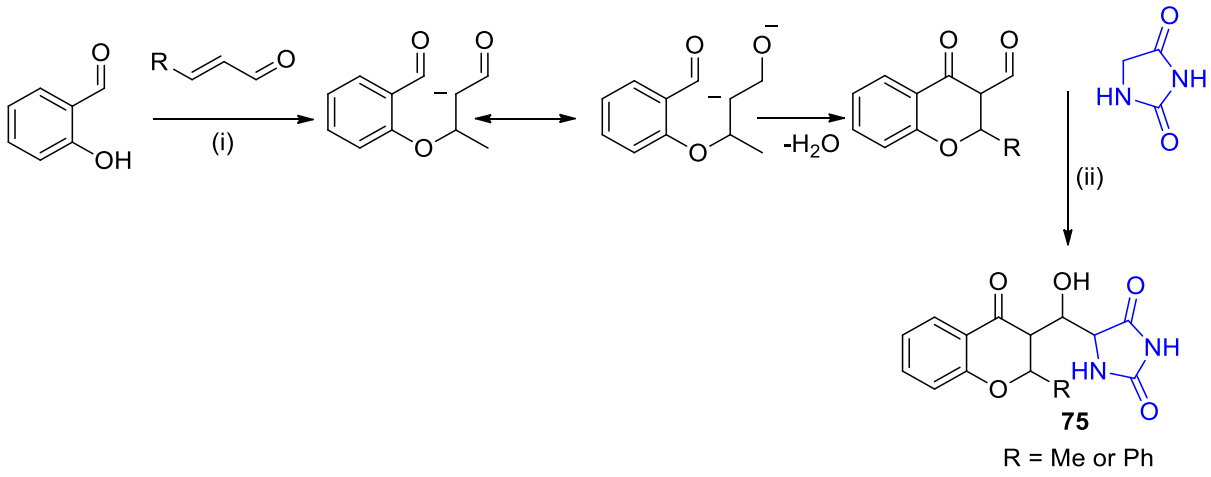
- In their design, synthesis and biological evaluation of hydantoin bridged analogues of combretastatin A-4 as potential anticancer agents, Mao Zhang *et. al.*[114] had successfully synthesized a series of novel hydantoin analogues **74** of combretastatin A-4 (**Scheme 31**) and evaluated them for their anti-cancer activities. They reported that only 1-(3-amino-4-methoxyphenyl)-3-methyl-5-(3,4,5-trimethoxyphenyl) hydantoin significantly inhibited the tumour growth and showed low toxicity.



***Scheme 32**: Synthesis of hydantoin bridged analogues of combretastatin A-4 **74**; **Reagents and conditions**: (i) AcOH/ conc. HCl, 50 ℃; (ii) R-X, K_2CO_3, Acetone, reflux*

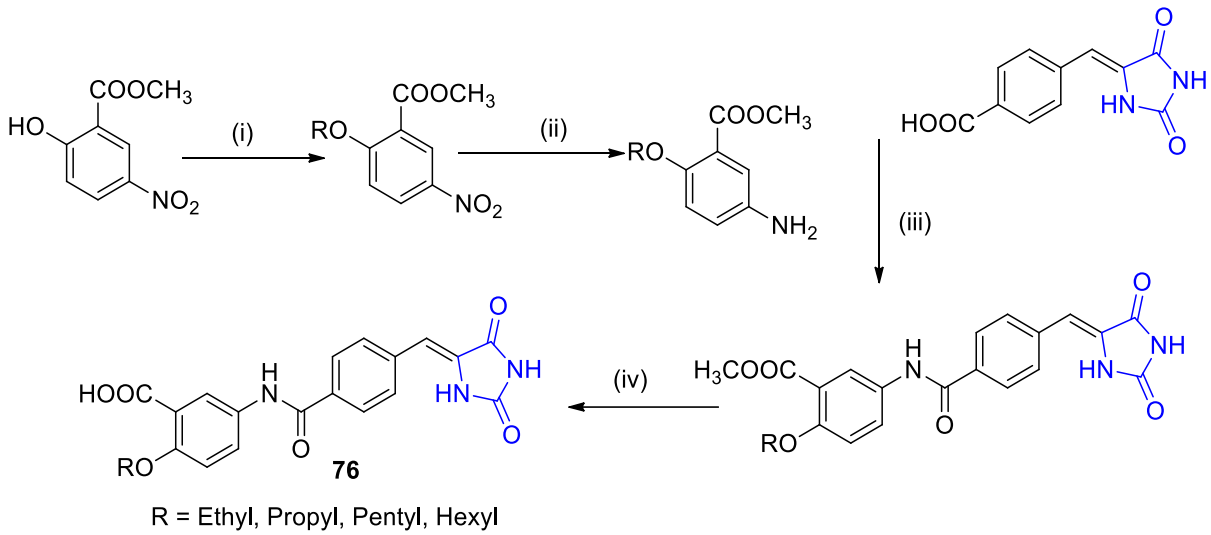
1.7.6.6. Hydantoin derivatives as anti-tuberculosis agent

- Angelova *et. al*.[115] had successfully synthesized a series of new 2H-chromene derivatives bearing hydantoin moieties **75 (Scheme 33)** and evaluated them for their antimycobacterial activities. They reported that both methyl and phenyl derivative showed significant antimycobacterial activity against mycobacterium tuberculosis H37Rv strain with MIC ranging from 0.29 to 0.36 µM.



***Scheme 33**: Synthesis of 2H-chromene derivatives bearing hydantoin moieties **75**; **Reagents and conditions**: (i) NaOH, 1,4-dioxane, reflux (ii) MeOH, piperidine, reflux*

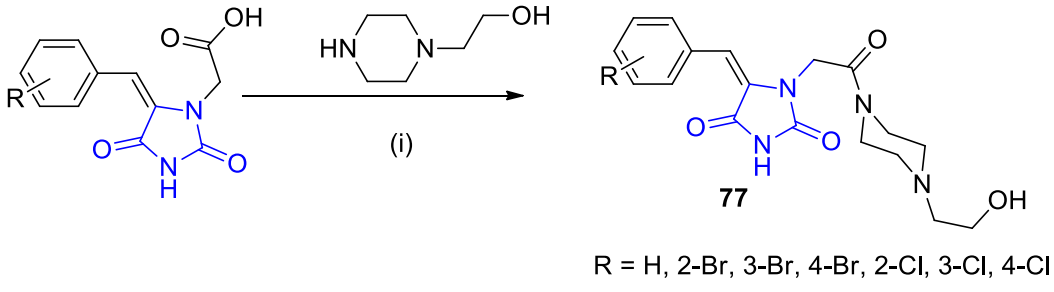
- Liu and co-workers [116] had successfully synthesized a series of hydantoin derivatives **76 (Scheme 34)** and evaluated them as potential anti-tuberculosis inhibitors. They reported that at concentration of 12.5 µg/mL, all compounds exhibited inhibition on the growth of mycobacterium tuberculosis strain H37Rv at 2 weeks.



***Scheme 34**: Synthesis of hydantoin derivatives **76**; **Reagents and conditions**; (i) RBr, K_2CO_3, DMF; (ii) $SnCl_2$, H_2O, EtOH; (iii) EDC, HOBT, DIEA, DMF; (iv) LiOH, MeOH, THF, H_2O*

1.7.6.7 Hydantoin derivatives as anti-convulsant agents

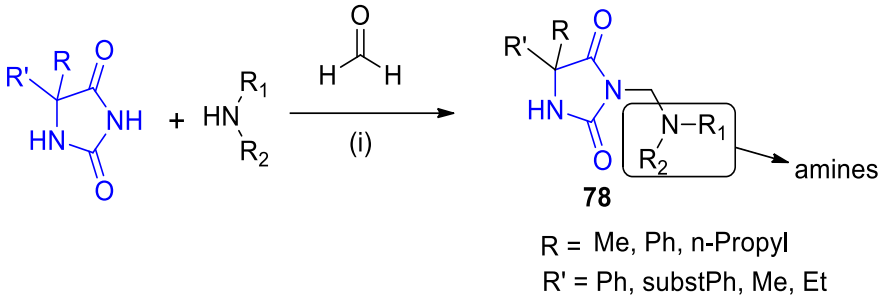
- Pekala and co-workers[117] had successfully synthesized a series of 5-arylidene imidazolidine-2,4-dione derivatives **77 (Scheme 35)** and evaluated them against anti-convulsant agent. Their results showed that only (5Z)-(3-chloro)benzylidene-3-{2-[4-(hydroxyethyl)piperazin-1-yl]-2 oxoethyl}imidazolidine-2,4-dione had the strongest cardio depressive activity which in dose of 10^{-6} M prolonged P–Q by 112%, QRS by 30% and Q–T by ca. 56%, and reduced the coronary flow by 30%.



***Scheme 35**: Synthesis of 5-arylidene hydantoin derivatives **77**; **Reagents and conditions**: (i) Et_3N, DMF, BOP, rt*

- Rishiphatak and co-workers[118] had successfully synthesized 5,5-disubstituted 2,4-imidazolidinedione derivatives **78** (**Scheme 36**) and evaluated them for their anti-convulsant activities. They reported that among the synthesized compounds both alkyl substituents are less active i.e. the latency to induce

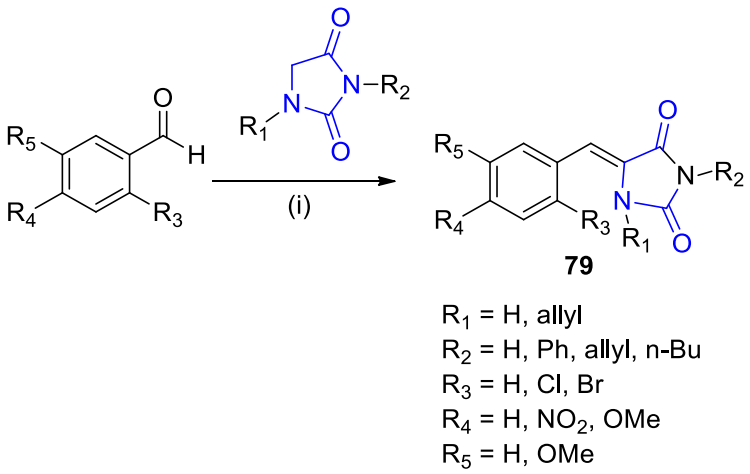
convulsions is less than that of the compounds having substituent either phenyl or substituted phenyl [S-2 $p < 0.05$ and S-4 $p < 0.001$] respectively.



***Scheme 36**: Synthesis of synthesized 5,5-disubstituted 2,4-imidazolidinedione derivatives **78**; **Reagents and conditions**: (i) MW, EtOH, 30% aq NaOH*

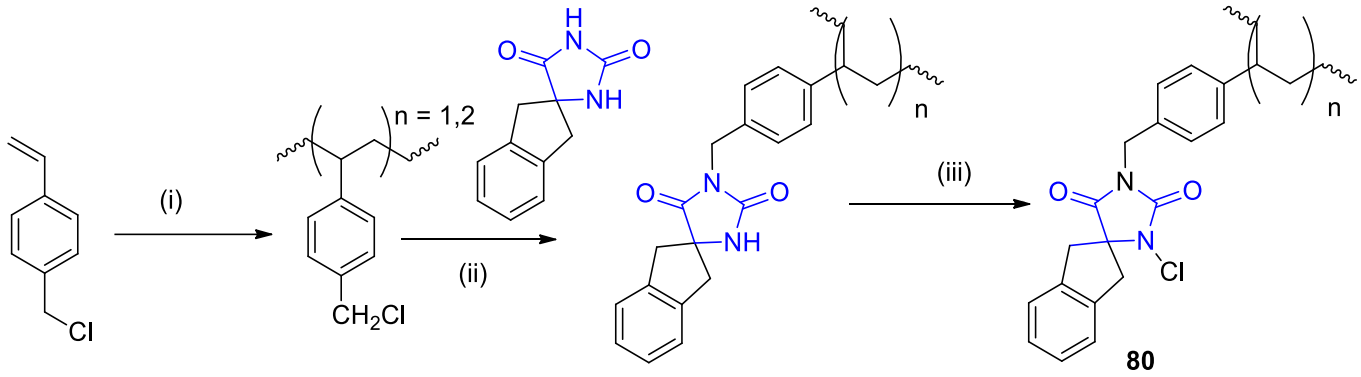
1.7.6.8. Hydantoin derivatives as anti-microbial agents

- Hidayat and co-workers[119] had successfully evaluated certain substituted hydantoins and benzalhydantoins **79** which were reported to be synthesized according to **Scheme 36** and evaluated them for their anti-microbial activities. They reported that 3-butyl-4-nitrobenzylidine hydantoin and 3-butyl-2-bromo-4,5-dimethoxybenzylidine hydantoin showed the most antimicrobial activities against *E. coli*, *M. furfur* and *S. aureus.*



***Scheme 37**: Synthesis of substituted 5-arylidene hydantoin derivatives **79**; **Reagents and conditions**: (i) MW, EtOH, 30% aq NaOH, 300w*

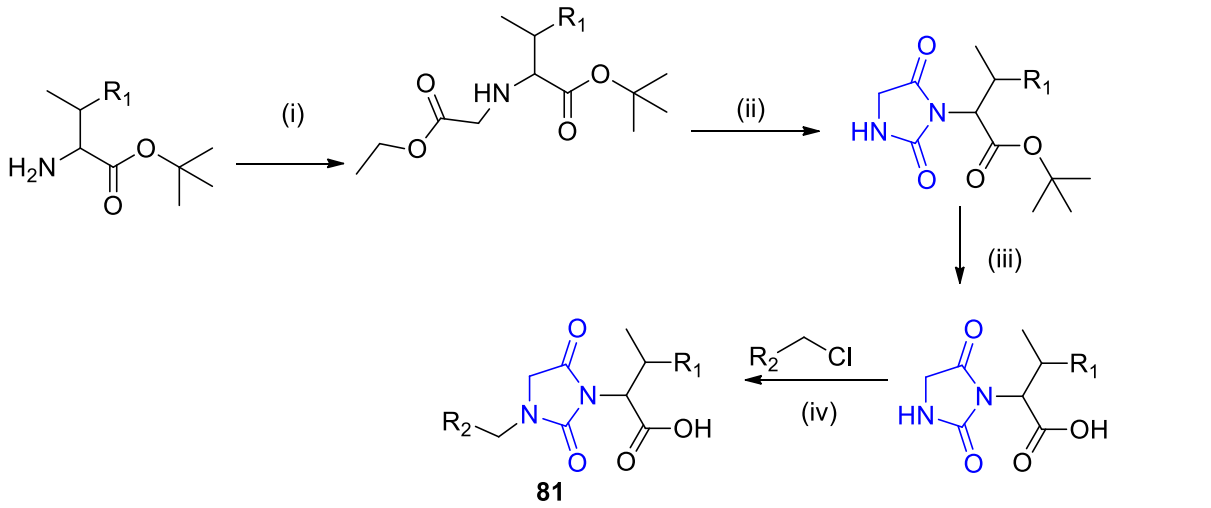
- Chylińska *et. al.*[120] had successfully synthesized homopolymers containing hydantoin **80 (Scheme 38)** and evaluated them for their anti-microbial activities. Their results showed the high biocidal activity of the obtained chlorinated polystyrene derivatives containing spirohydantoin moieties.



***Scheme 38**: Synthesis novel N-halamine hydantoin-containing polystyrenes **80**; **Reagents and conditions**; (i) AIBN, 80 ℃; (ii) K_2CO_3, rt; (iii) TCICA, acetone, rt*

1.7.6.9. Hydantoin derivatives as anti-HIV agent

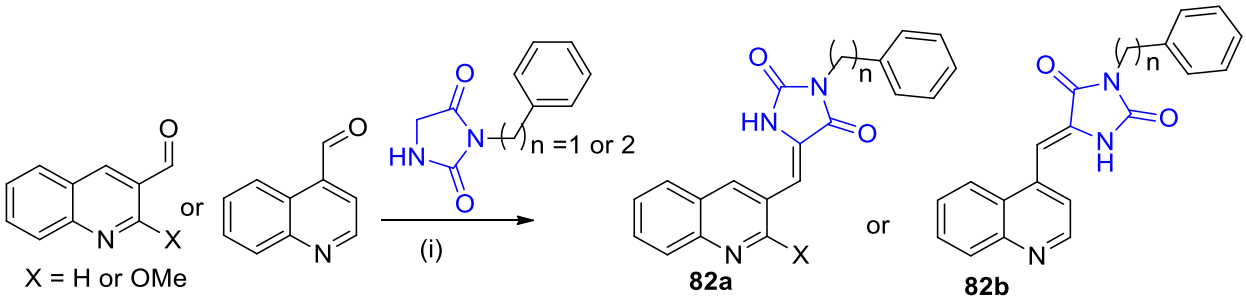
- Flosi and co-workers[121] have successfully synthesized imidazolidine-2,4-dione acid or ester isosteres **81** (**Scheme 39**) and evaluated them against lopinavir-resistant mutant HIV. They reported that all their selected imidazolidine-2,4-dione containing PIs were more effective at inhibiting highly resistant patient isolates Pt1 and Pt2 than the standard lopinavir.



***Scheme 39**: Synthesis of imidazolidine-2,4-dione acid or esters isostere **81**; **Reagents and conditions**; (i) Ethyl bromo acetate, Et_3N, NaH, stirring rt; (ii) chlorosulfonylisocyanate, CH_2Cl_2, 0 ℃; (iii) EDC, H_2O, Et_3N, MeOH, stirring rt; (iv) NaHMDs, DMF, 70 ℃*

- Ibrahim and co-workers[122]successfully synthesized 5-((substituted quinolin-3-yl/1-naphthyl) methylene)-3-substituted imidazolidin-2,4-dione **82 (Scheme 40)** and evaluated them as Human Immunodeficiency Virus Type (HIV-1)

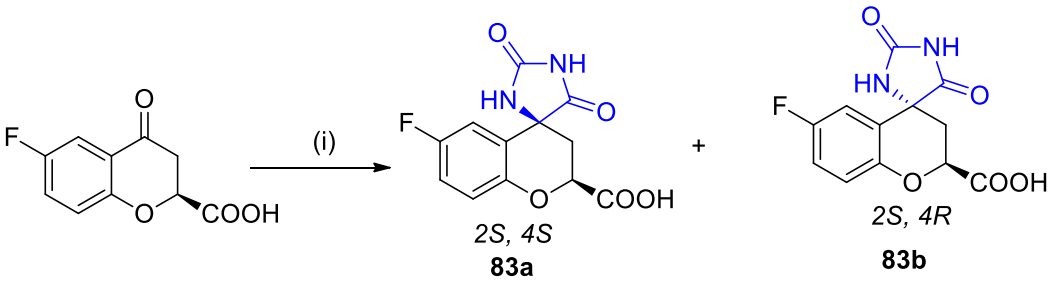
fusion inhibitors. They reported that between the two derivatives, *(Z)*-3-benzyl-5-((2-chloro-8-methylquinolin-3-yl) methylene) hydantoin was the most potent in inhibiting the HIV-1_{IIIB} infection with EC_{50} value of 0.148 µM and selectivity index of 117.36.



***Scheme 40**: Synthesis of novel 5-((substituted quinolin-3-yl-1-naphthyl) methylene)3-hydantoin **82**; **Reagents and conditions**; (i) EtOH, 2,2,6,6-Tetramethylpiperidine (TMP), Reflux*

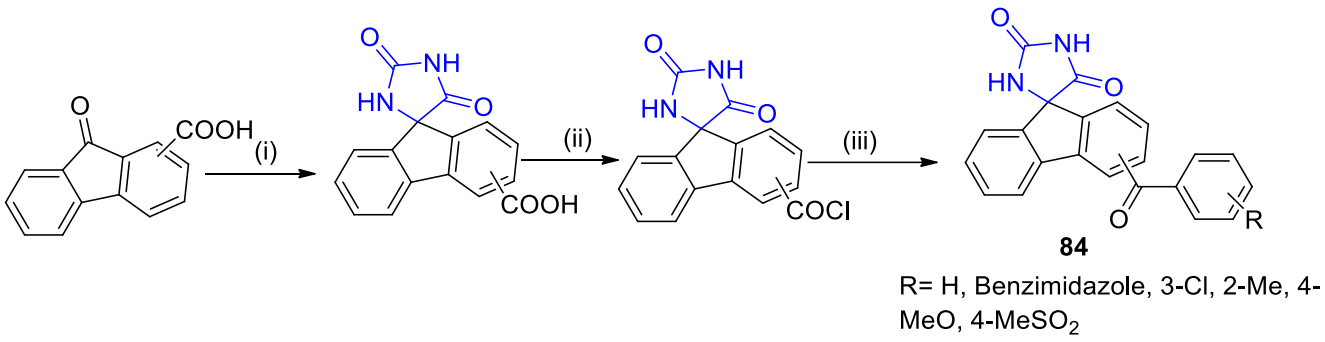
1.7.6.10. Hydantoin derivatives as aldose reductase inhibitors

- Unno and co-workers [123]successfully synthesized 2-substituted 6-fluoro-2,3-dihydrospiro[4h-1-benzopyran-4-4'-imidazolidene]-2,5-diones **83** **(Scheme 41)** and evaluated them as aldose reductase inhibitors activity. They found that the *2S, 4S* isomers was a more potent aldose reductase inhibitor than the other corresponding stereoisomers *2S, 4R.*



***Scheme 41**: Synthesis of 2-substituted 6-fluoro-2,3-dihydrospiro[4h-1-benzopyran-4-4'-imidazolidene]-2,5-diones **83**; **Reagents and conditions**; (i) KCN, $(NH_4)_2CO_3$, H_2O*

- Bovy and co-workers[124]successfully synthesized spiro [fluorene-9',5'-imidazolidine]-2',4'-diones derivatives **84** (**Scheme 42**) and evaluated them for their activities against aldose reductase. They reported that almost all of their compounds were very effective in reducing sorbitol accumulation in the lenses and sciatic nerves of rats treated with standard streptozotocin.

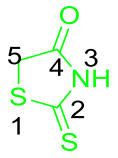


***Scheme 42**: Synthesis of spiro [fluorene-9',5' -imidazolidine]- 2',4' -diones **84**; **Reagents and conditions**; (i) $(NH_4)_2CO_3$, KCN, rt (ii) $COCl_2$, rt; (iii) Ph-R, $CHCl_3$, $AlCl_3$, reflux*

1.8. Rhodanine containing compounds

1.8.1. Chemistry of rhodanine

Rhodanine **85** (**Figure 19)**, also known as 2-thioxo-thiazolidin-4-one is a five-membered heterocyclic molecule containing sulphur and nitrogen atoms at positions 1 and 3 (thiazole nucleus) with a carbonyl group on the fourth carbon. Rhodanine **85** was first discovered in 1877 by Marceli Necki and he named it *rhodaninsaure.*[125]

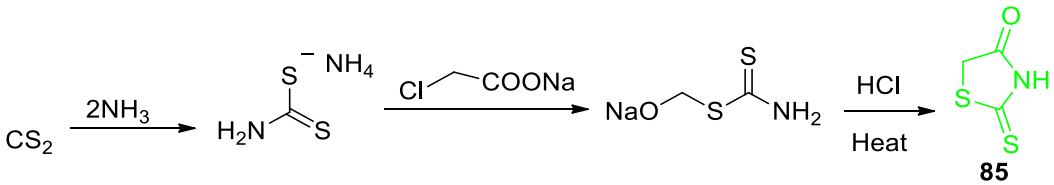


85

Figure 19**: Rhodanine structure **85

1.8.2. General method for synthesis of rhodanine

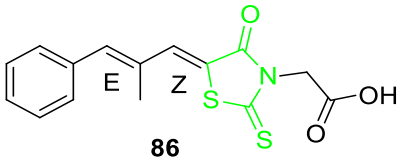
Rhodanine **85** can be synthesized by treating carbon disulfide and chloroacetic acid via dithiocarbamate intermediate.[126] **(Scheme 43)**



***Scheme 43**: Synthesis of rhodanine 85 via dithiocarbamate intermediate.*

1.8.3. Clinically approved rhodanine containing drugs

Even though rhodanine has been reported for a variety of biological activities, there is only epalrestat (carboxylic acid derivative)[127] **86 (Figure 20)** as a commercially available aldose reductase drug in the market which was discovered in 1982 by ONO pharmaceuticals in Japan. This drug is currently used for the treatment of diabetic complications, such as neuropathy, nephropathy and cataracts.



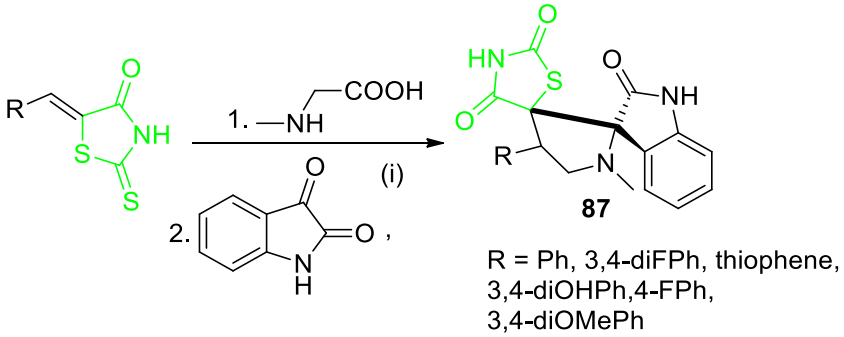
86

Figure 20: *Clinically approved epalrestat* ***86***

1.8.4. Pharmacological applications of rhodanine derivatives

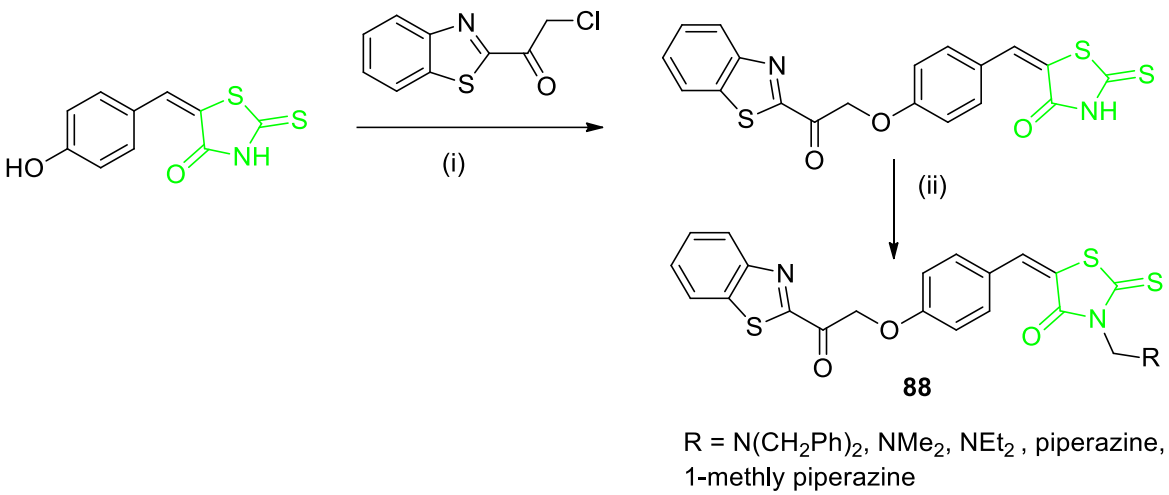
1.8.4.1. Rhodanine derivatives as anti-diabetic agents

- Murugan *et al*.[128] synthesized a series of novel dispiropyrrolidines through [3+2] cycloaddition reactions with rhodanine derivatives **87 (Scheme 44)** and evaluated them for their in vivo antidiabetic activities. They reported that all their selective compounds showed better reduction in glucose levels than rosiglitazone as a standard drug.



Scheme 44: *Synthesis of novel dispiropyrrolidines through [3+2] cycloaddition reactions with rhodanine derivatives* ***87****;* ***Reagents and conditions*** *(i) MeOH, Reflux*

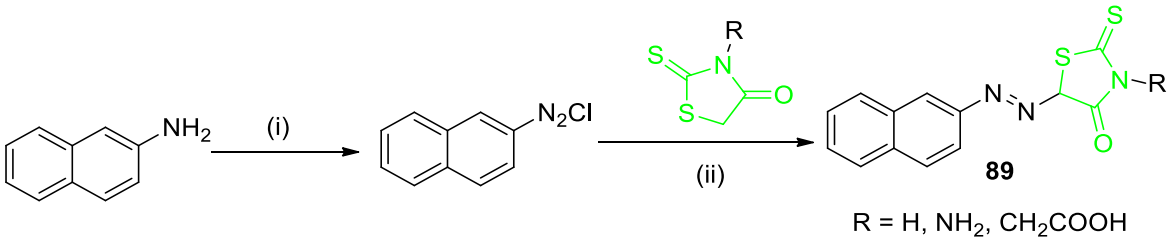
- Kapoor and Khare[129] have successfully synthesized a novel series of rhodanine derivatives **88** (**Scheme 45**) and evaluated them for their antidiabetic activities. They reported that all their synthesized derivatives showed promising anti-diabetic activities.



Scheme 45: *Synthesis of novel series of rhodanine derivatives* ***88****;* ***Reagents and conditions*** *(i) K_2CO_3, DMF, rt, (ii) Formaldehyde, amines, rt*

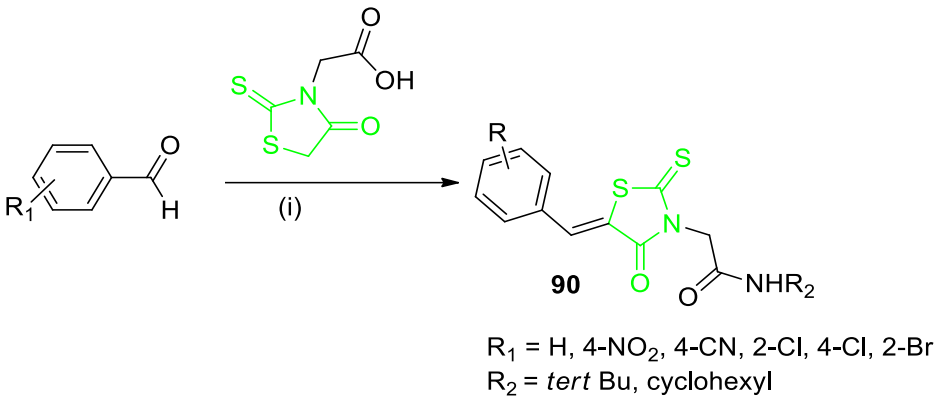
1.8.4.2. Rhodanine derivatives as antioxidant/ viral agents

- Akram and co-workers[130] have successfully synthesized a series of derivatives for rhodanine azo compounds containing a naphthalene ring **89 (Scheme 46)** and evaluated them for their antioxidant/ viral activities. Their results showed that all of their compounds have moderate-to-good anti-oxidant activities against the *Escherichia coli Pseudomonas aeruginosa and Staphylococcus aureus* bacteria.



Scheme 46*: Synthesis of azo rhodanine compounds containing a naphthalene ring* ***89****;* ***Reagents and conditions*** *(i) $NaNO_2$, HCl, H_2O, 0-5 ℃; (ii) AcONa, EtOH, 0-5 ℃*

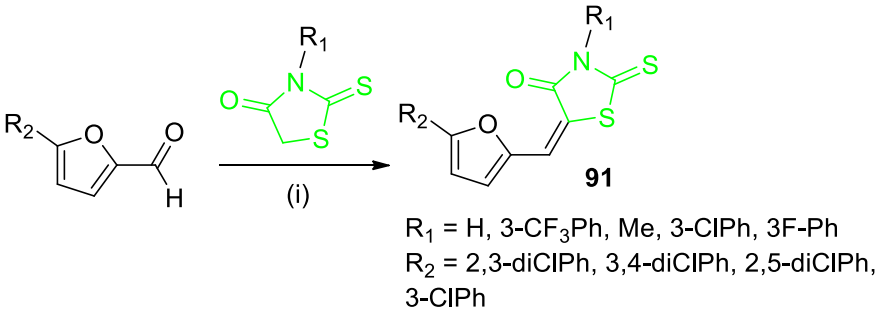
- Tarahomi *et al*.[131] have successfully synthesized a series of rhodanine based amide derivatives **90** (**Scheme 47**) and evaluated them for their anti-bacterial activities. Their results showed that the antibacterial activities of all their synthesized compounds tended to be more potent against gram-positive species than gram-negative bacteria.



***Scheme 47**: Synthesis of novel rhodanine based amide derivatives **90**; **Reagents and conditions** (i) Aniline, R_2-N ≡C, THF, rt*

1.8.4.3. Rhodanine derivatives as anti-malarial agent

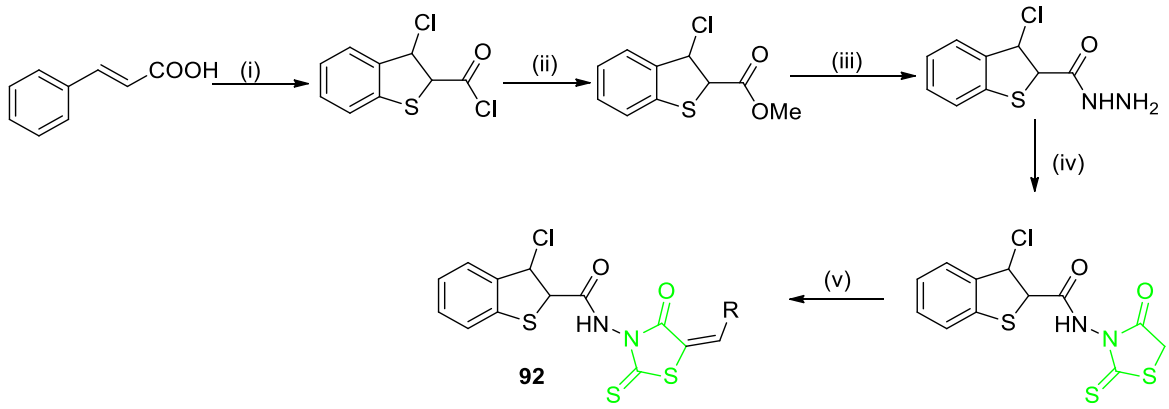
- Kumar and co-workers[132] have successfully synthesized a series of 5-((Furan-2-yl)methylene)-2-thioxothiazolidin-4-one **91** (**Scheme 48**) and evaluated them for their anti-malarial activities. They reported that most compounds of this class were found to inhibit *Plasmodium falciparum* enoyl ACP reductase (PfENR) at low nanomolar to low micromolar concentrations.



***Scheme 48**: Synthesis of 5-((Furan-2-yl)methylen)-2-thioxothiazolidin-4-one **91**; **Reagents and conditions** (i) NaOAc, Acetic acid, reflux*

1.8.4.4. Rhodanine derivatives as anti-inflammatory agents

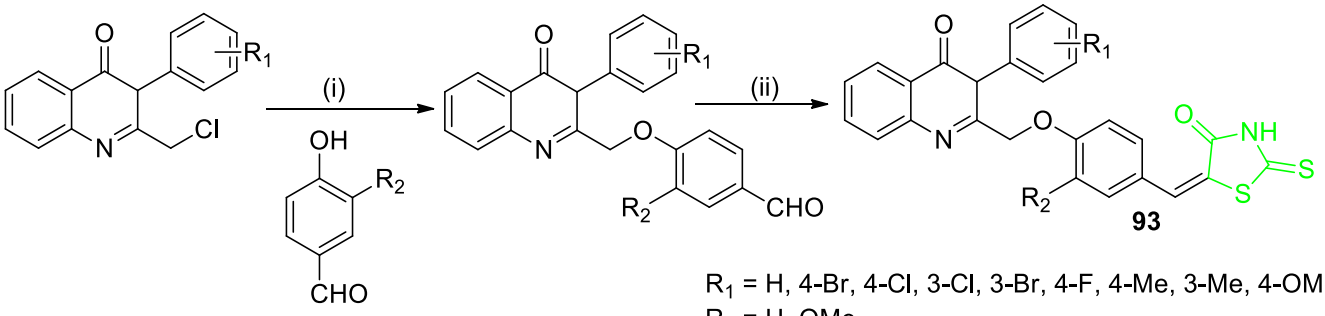
- Miligy and co-workers[133] have successfully synthesized new molecular hybrids by combining benzothiophene with rhodanine **92 (Scheme 49)** and evaluated them for their anti-inflammatory agents. Their results revealed that the only 3,4-dimethoxyphenyl derivative exhibited significant anti-inflammatory activity higher than the standard celecoxib.



R = 1,3-diphenyl-1H-pyrazole-4-yl, 3-(4-Methoxyphenyl)-1-phenyl-1H-pyrazole-4-yl, 3-(4-bromophenyl)-1-phenyl-1H-pyrazole-4-yl, Ph, 4-OMePh, 4-ClPh, 3-OH,4-OMePh, 3,4-diOMePh

***Scheme 49**: Synthesis of new molecular hybrids combining benzothiophene with rhodanine **92**; **Reagents and conditions**: (i) $SOCl_2$/pyridine, reflux; (ii) Methanol: benzene, rt; (iii) $NH_2NH_2.H_2O$, EtOH, reflux; (iv) bis(carboxymethyl)trithiocarbonate, H_2O, reflux; (v) Different aldehydes or Isatin, piperidine, benzene, reflux*

- In their synthesis and anticancer activities of novel quinazolinone-based rhodanine, El-Sayed *et. al.* [134] have successfully synthesized a novel series of twenty quinazolinone-based rhodanine derivatives **93 (Scheme 50)** and evaluated them for their anti-inflammatory activities. They found that all their target compounds were active with IC_{50} values roughly in the range of 10–60 µM.



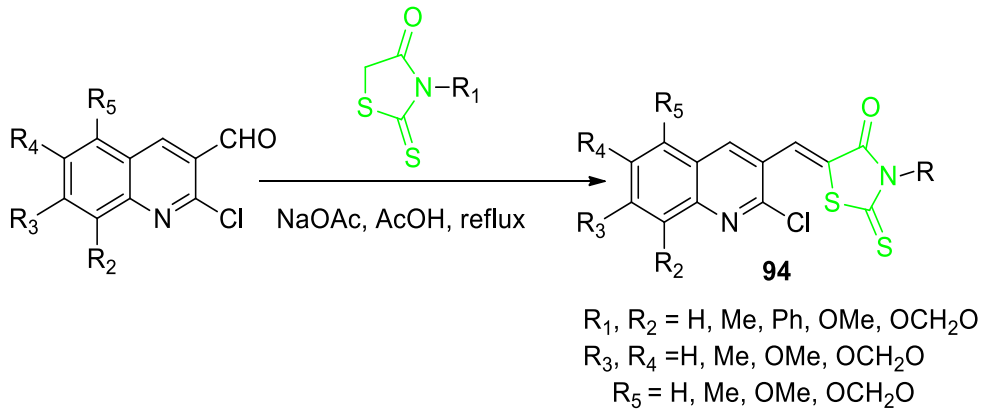
R_1 = H, 4-Br, 4-Cl, 3-Cl, 3-Br, 4-F, 4-Me, 3-Me, 4-OMe
R_2 = H, OMe

***Scheme 50**: Synthesis of novel quinazolinone-based rhodanine derivative **93**; **Reagents and conditions**: (i) K_2CO_3, KI, acetonitrile, reflux, (ii) Rhodanine, sodium acetate, glacial acetic acid, reflux*

1.8.4.5. Rhodanine derivatives as anti-cancer agents

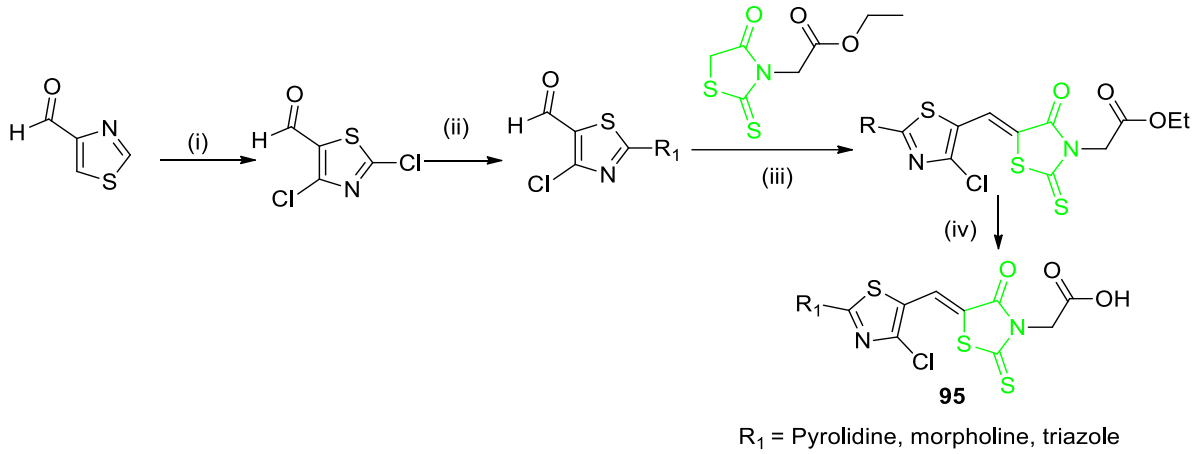
- In their synthesis and biological evaluation of new rhodanine analogues bearing 2-chloroquinoline and benzo[h]quinoline scaffolds **94** (**Scheme 51**), Ramesh *et. al.*[135] have evaluated compounds **94** for their anti-cancer activities. They reported that some of their compounds with methyl derivative were

capable of inhibiting the proliferation of cancer cell lines at a micromolar concentration.



Scheme 51: *Synthesis of new rhodanine analogues bearing 2-chloroquinoline and benzo[h]quinoline scaffolds* ***94***

- Ozen and co-workers[136] have successfully synthesized series of thiazolyl-2,4-rhodanine compounds **95** (**Scheme 52**) and evaluated them for their anti-cancer activities. They reported that the carboxylic ester derivatives having morpholine and triazole had very strong anticancer effects at 10 µM concentration in Huh7 cell line.

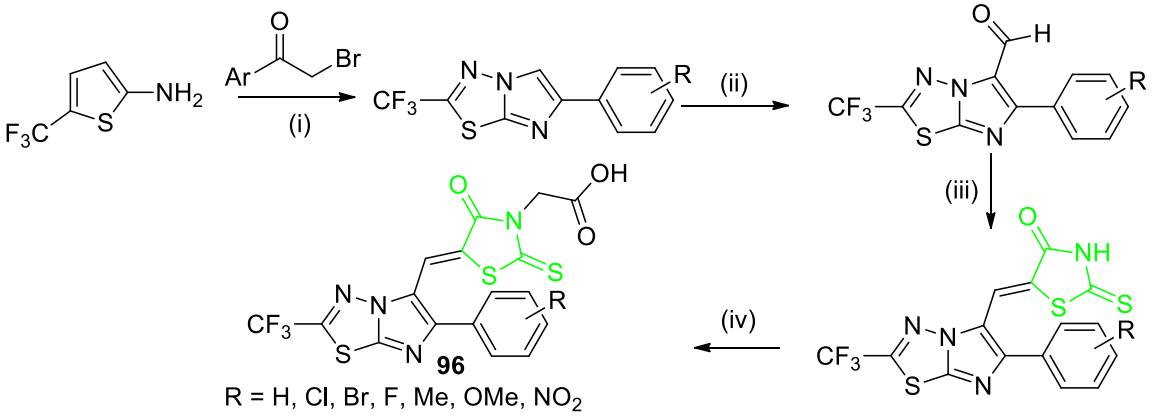


Scheme 52*: Synthesis of thiazolyl-2,4- rhodanine compounds* ***95****:* ***Reagents and conditions****: (i) $POCl_3$, DMF, rt; (ii) R_1-H, Na_2CO_3, CH_3CN, reflux; (iii) AcOH, AcONa, reflux; (iv) AcOH, HCl, reflux.*

1.8.4.6. Rhodanine derivatives as anti-tuberculosis agents

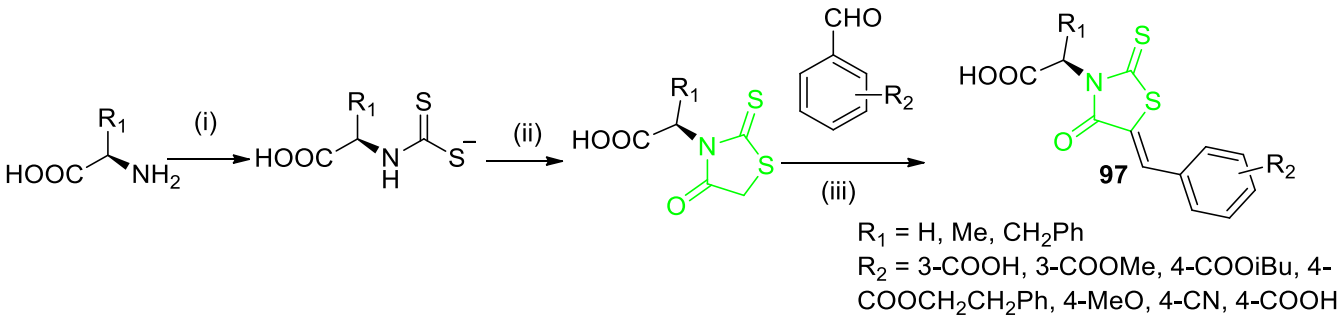
- Alegaon *et. al*.[137] have successfully synthesized a series 1,2-imidazo-1,3,4-thiadiazole-2,4-rhodanine-3-acetic acid conjugates **96 (Scheme 53)** and evaluated them for their antitubercular activities. They obtained five

compounds (chloro, bromo, fluoro, methyl and methoxy phenyl derivatives) which exhibited good activity with MIC in range 3.12-1.56µg/ml.



***Scheme 53**: Synthesis of novel 1,2-imidazo-1,3,4-thiadiazole-2,4-rhodanine-3-acetic acid conjugates **96**: **Reagents and conditions**: (i) M.W. 600 watts (ii) Vilsmeier–Haack reagent, (iii) rhodanine, M.W. 600 watts, (iv) acetic acid, M.W. 600 watts*

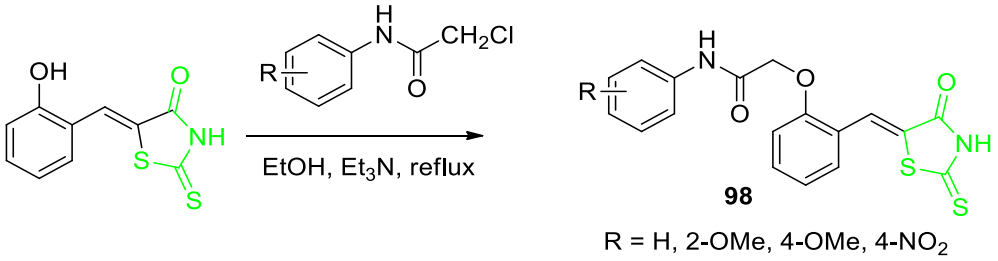
- Mori and co-workers[138] have successfully synthesized a series of 3-(carboxymethyl) rhodanine and aminothiazole **97** (**Scheme 54**) and evaluated them for their activities against mycobacterium tuberculosis (Zmp1). They reported that almost all their rhodanine target compounds exhibited Zmp1 inhibition with IC_{50} values in the range 1.3-43.9 µM.



***Scheme 54**: Synthesis of 3-(carboxymethyl) rhodanine and aminothiazole **97**: **Reagents and conditions**: (i) carbon disulfide, NaOH, H_2O, rt; (ii) sodium chloroacetate solution, 6 N HCl, cat. $POCl_3$, 75 ℃; (iii) B-alanine, AcOH, reflux*

1.8.4.7. Rhodanine derivatives as anti-convulsant agents

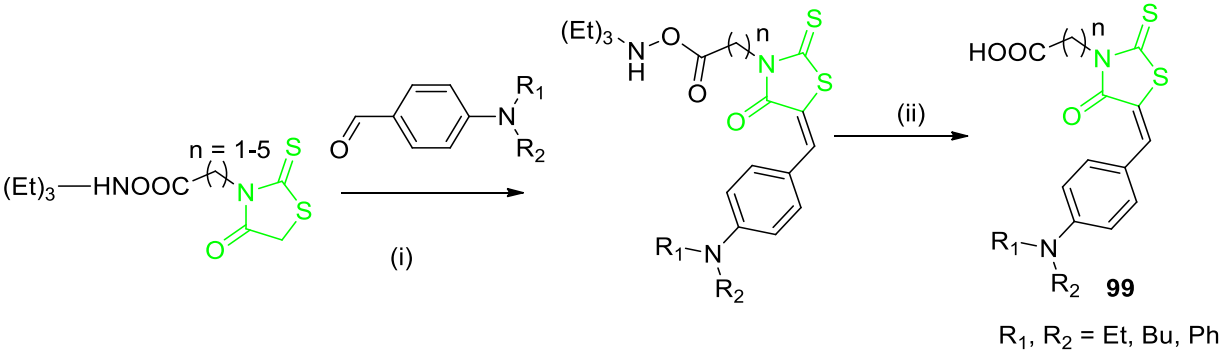
- Gagoria and co-workers[139] have successfully synthesized a series of benzylidene rhodamine derivatives **98 (Scheme 55)** and evaluated them for their anti-convulsant activities. They reported that all their compounds exhibited remarkable anti-convulsant activity at a lower dose of 50 mg/kg.



Scheme 55**: Synthesis of benzylidene rhodanine derivatives **98

1.8.4.8. Rhodanine derivatives as anti-microbial agents

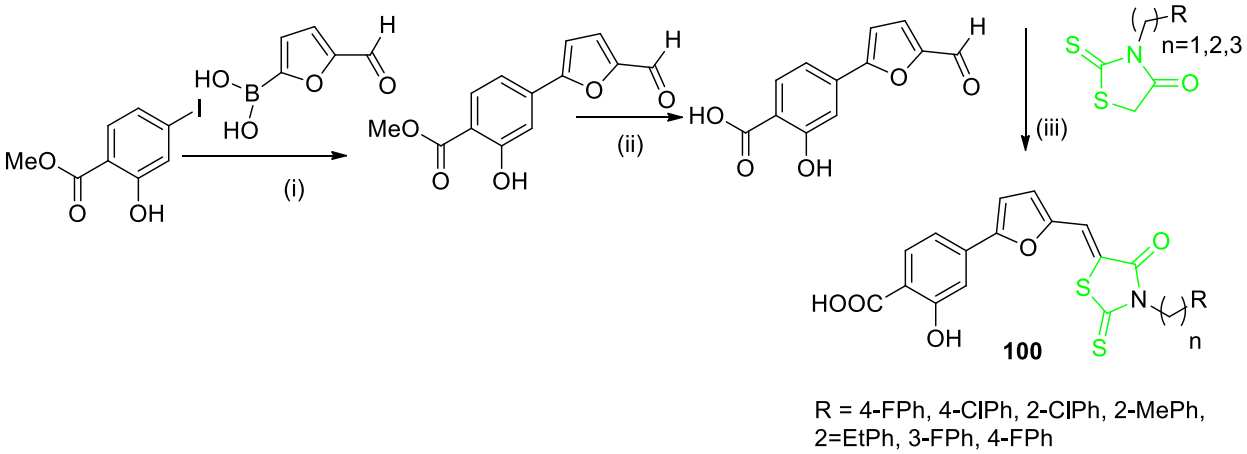
- Tejchman and co-workers [140] have successfully synthesized a series of 2,4-rhodanine 3-carboxyalkanoic analogues with 4-(*N,N*)-dialkyl-amino or diphenylamino)-benzylidene moiety (**99**, **Scheme 56**) and evaluated them for their anti-microbial activities. They reported that all their rhodanine derivatives showed good anti-bacterial activity when compared to the gram-positive bacterial strains and lacked activity to the reference gram-negative bacterial strains and yeast strains.



***Scheme 56**: Synthesis of rhodanine 3-carboxyalkanoic acid derivatives **99**; **Reagents and conditions**: (i) Et_3N, Isopropyl alcohol, reflux (ii) HCl, H_2O, rt*

1.8.4.9. Rhodanine derivatives as anti-HIV agents

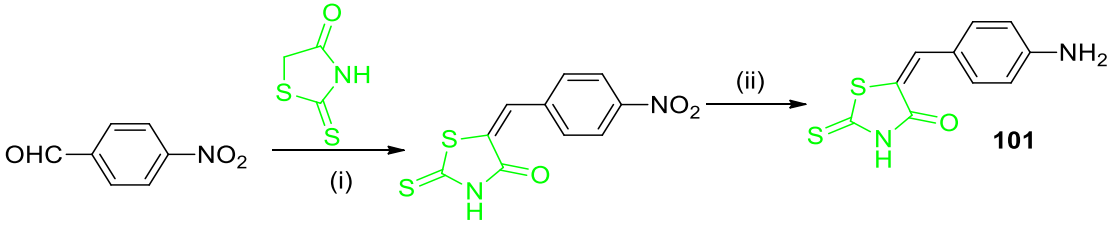
- Tintori and co-workers[141] have successfully synthesized rhodanine derivatives **100** (**Scheme 57**) and evaluated them for their anti-HIV activities. They observed that all their compounds showed a considerable reduction of activity in presence of serum due to a high binding to serum albumin.



***Scheme 57**: Synthesis of rhodanine derivatives **100**: **Reagents and conditions**: (i) Pd $(PPh_3)_2Cl_2$, Na_2CO_3, DMF/EtOH, rt, (ii) 1N NaOH (aq), MeOH/THF, reflux (iii) MW 300 W, 110˚C*

1.8.4.10. Rhodanine derivatives as aldose reductase inhibitors

- In their synthesis and biological evaluation of some new rhodanine analogues as aldose reductase inhibitors (ARIs), Khan and co-workers[142] have successfully synthesized as series of 2-thiaoxozolidin-4-one benzylidene derivatives **101** (**Scheme 58**) and evaluated them for their aldose reductase activities. They reported that their 4-amino benzylidene derivatives showed most promising ALR2 inhibitory efficacy (83.00% at 10µg/mL).

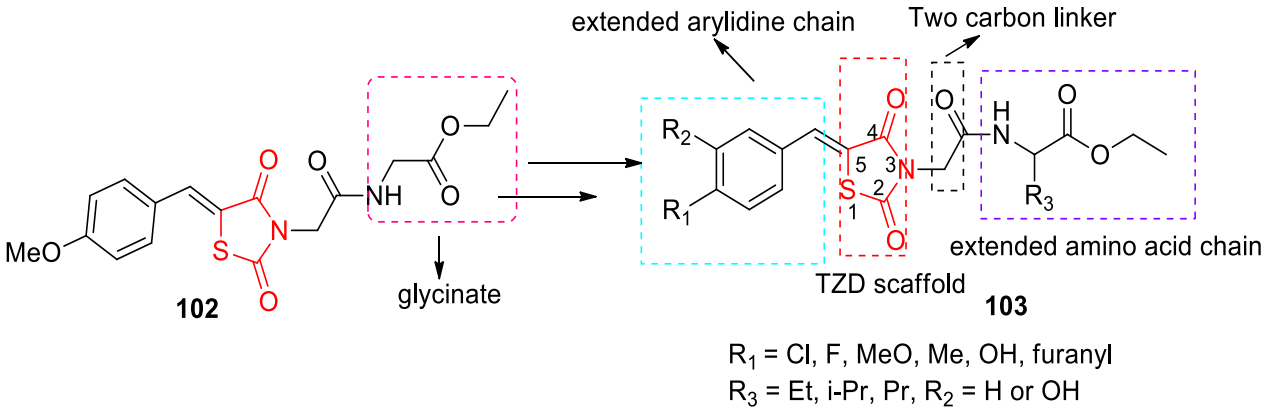


***Scheme 58:** Synthesis of 2-thiaoxozolidin-4-one benzylidene derivatives **101**; **Reagents and conditions**: (i) AcONa, AcOH, reflux; (ii) tin granules, HCl, heat, NaOH*

1.9. Aims, origin and objectives of the project

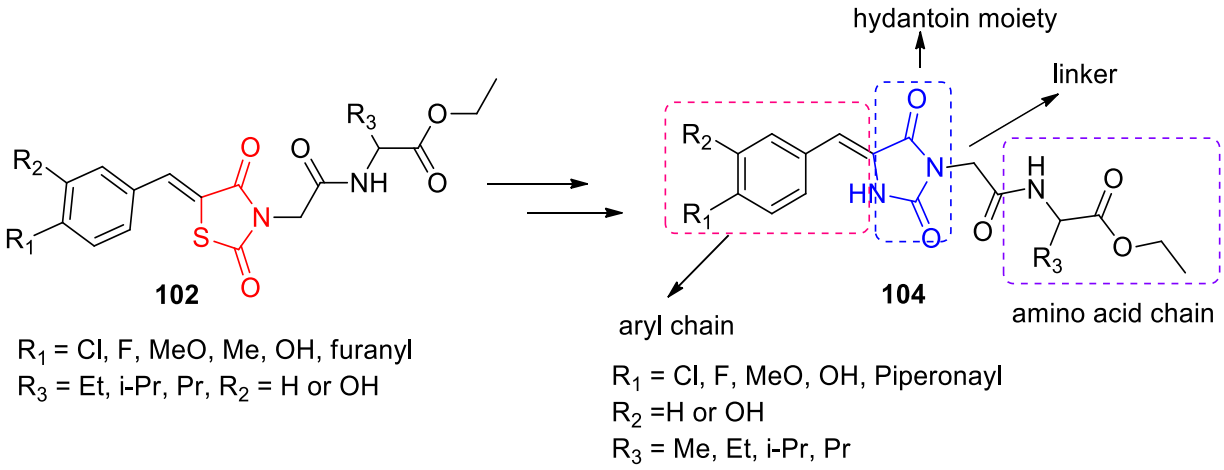
Compounds containing glitazone were widely reported as anti-diabetic agents.[143] Recent studies involving the structure activity relationship (SAR) of novel glitazones as antidiabetic agents by Srinivas *et. al.*[144] revealed that substituting glitazone at N-H (3) position together with electron-releasing groups (OCH_3) at the *para* position or halogens at the *meta* position of the aromatic ring showed a good biological response in the bioassays performed.

Having this in mind, our first aim of this project was to modify a *para* methoxy glycinate **102**, previously prepared by Kumar and co-workers[145] which showed significant anti-diabetic activity. The modification is carried by extending the arylidene chain with various aldehydes via a six-step synthetic protocols as well as introducing racemic amino acids instead of glycine due to their sustainability and green properties as well as their chiral properties. **(Scheme 59)** It was our plan to evaluate all the synthesized compounds their *in vitro* α-glucosidase activities as potential anti-diabetic agents.



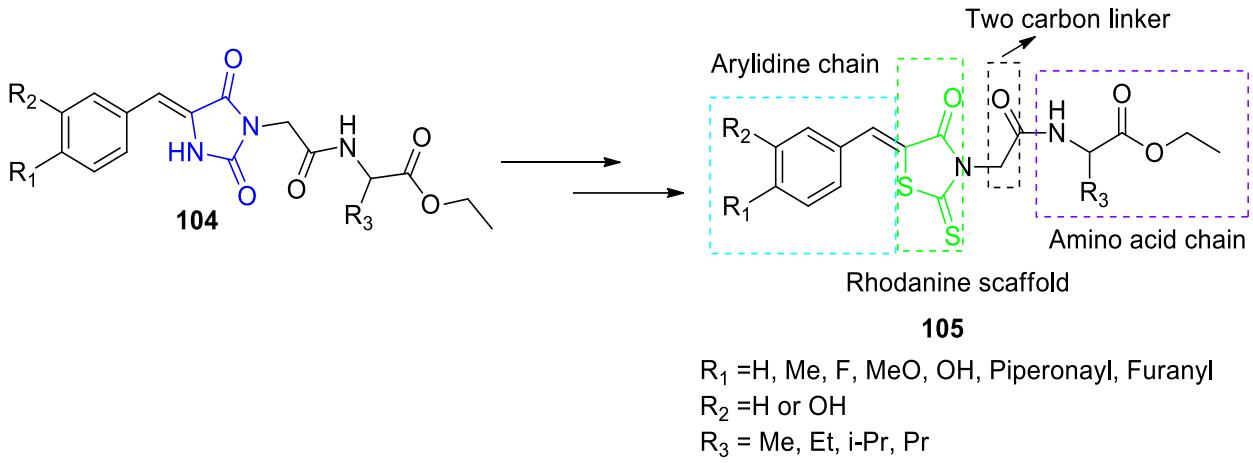
Scheme 59**: Synthesis of glitazones containing compounds **103

Having successfully synthesized the new series of 5-(arylidene)-thiazolidine-2,4-dione esters[146] **103** (**Scheme 60**). Our second aim of this project was to synthesize a new series of 5-(arylidene)-imidazolidine-2,4-dione esters **104** as envisaged potential anti-diabetic agents by replacing the glitazone moiety with a hydantoin moiety as depicted in **Scheme 60**.



Scheme 60**: Synthesis of compounds containing the hydantoin moiety **104

Lastly, our final aim was to synthesize a novel series of some new (5-arylidene) rhodanine -2,4-dione esters **105** as potential anti-diabetic agents, by replacing the hydantoin moiety with the rhodanine moiety as depicted in **scheme 61**.



Scheme 61**: Synthesis of target rhodanine containing compounds **105

Our main **objectives** were as follows:

- To compare bioactivity of different phenyl derivatives, 5-membered heterocyclic ring skeleton and extended amino acids chain in order to probe the influences.
- Purification of final synthesized compounds using recrystallization techniques.
- Characterization of final target compounds using a combination of NMR (^{1}H and ^{13}C), IR as well as high resolution mass spectroscopies.
- *In-vitro* anti-diabetic evaluation of targeted synthesized compounds.

Chapter II

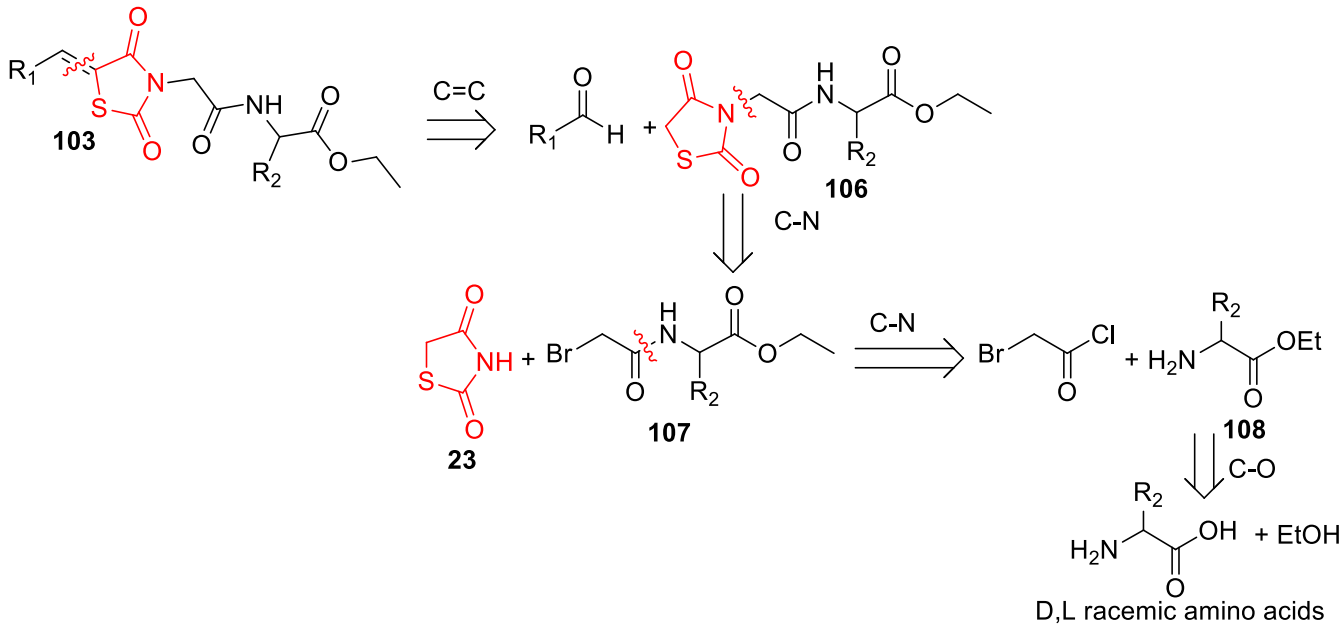
2. Results and discussion glitazones derivatives

This chapter describes results obtained in the synthesis of glitazone-containing compounds. Furthermore, it also describes results obtained from different characterization techniques, and most importantly the *in vitro* anti-diabetic results.

2.1. Chemistry of glitazone

2.1.1. Retrosynthesis of target glitazone containing moiety

In an attempt to select which commercially available starting reagents were needed for preparing our envisaged target molecule **103**, we have conducted a retrosynthesis guided by the general method used by Kumar and co-workers[145] as depicted in **scheme 62**.



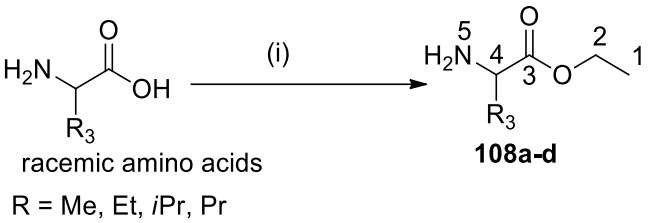
***Scheme 62**: A retrosynthetic analysis of the target glitazone containing compounds.*

Target glitazone containing molecule **103** could be derived from ethyl 2-(2,4-dioxothiazolidin-3-yl) acetyl esters **106** and different commercially available substituted aldehydes by Knoevenagel condensation. Compounds **106** would in turn be prepared from commercially available 2,4-thiazolidinediones **23** and ethyl 2-(2-bromoacetyl) esters **107** by nucleophilic substitution. Ethyl 2-(2-bromoacetamido)

esters **107** could be derived from commercially available bromo acetyl chloride and an ester protected amino acids **108** by acetylation of protected amino acids. Finally, **108** could be derived from commercially available racemic amino acids namely, α-amino butyric acid, valine and norvaline.

2.1.2. Protection of racemic amino acids (108a-d)

The initial step towards the synthesis of the targeted **103** started by protecting alanine, α-amino butyric acid, valine, and norvaline as esters. The synthesis was carried out by modifying a method reported by Li and Sha.[147] The amino acids were treated with thionyl chloride, instead of hydrochloric acid in refluxing ethanol to give desired compound **108a-d** (**scheme 63**) as oils as shown in good yields as shown in **Table 3**.



***Scheme 63**: Synthesis of ester-protected amino acids **108a-d**; **Reagents and conditions** (i) $SOCl_2$, EtOH, Reflux, 12h*

Table 3: Ester protected-amino acids **108a-d** data obtained from **scheme 63**

Compound	R_3	Yield (%)
108a[145]	Me	68
108b[148]	Et	79
108c[149]	*i*Pr	93
108d[150]	Pr	97

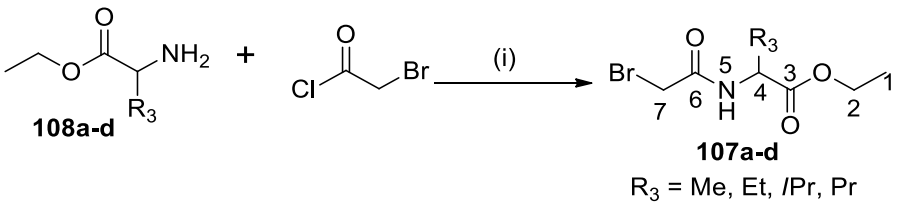
The newly formed esters **108a-d** were confirmed using the combination of ^{1}H NMR, ^{13}C NMR and IR spectroscopies. The appearance of new formed signals **H-1** and **H-2** confirmed the presence of ethoxy group. This was seen in ^{1}H NMR spectra as a quartet appearing at ~3.74-4.17 ppm integrating for 2H for **H-2**. In addition, a triplet appearing at ~1.19-1.21 ppm integrating for 3H for **H-1** was observed. The ^{13}C NMR spectra of these compounds **108a-d** showed a peak corresponding to **C-1** appearing

at a range of ~13.23-14.48 ppm and a methylene **C-2** ranging from ~ 60.99 - 63.47 ppm.

Each ester has its distinctive group indicated by R_3 with **108b** R_3 being the ethyl group, **108c** (R_3 being an isopropyl group) and **108d** norvalinate R_3 being the propyl group. The chemical shifts of these distinctive signals as observed in the NMR (^{1}H and ^{13}C) corresponded with the ones reported in literature.[151]

2.1.3. Synthesis of ethyl 2-(2-bromoacetyl) esters (107a-d)

Esters derivatives **108a-d** needed to react with electrophile in a simple nucleophilic acyl substitution to afford corresponding ethyl 2-(2-bromoacetamido) esters **107a-d (Scheme 64)** by modifying a method outlined by Kumar *et al.*[145] The addition of bromo acetyl chloride to a cooled solution of protected amino esters **108a-d** in the presence of potassium carbonate afforded the desired ethyl 2-(2-bromoacetamido) esters **107a-d** in yields ranging from 68%-99% as summarised in **Table 4**.



Scheme 64: *Synthesis of ethyl 2-(2-bromoacetamido) esters* ***107a-d; Reagents and conditions*** *(i) K_2CO_3, H_2O, DCM, 0 ℃-rt, 16h*

Table 4: Ethyl 2-(2-bromoacetamido) esters **107a-d** data obtained from **scheme 64**

Compound	R_3	Yield (%)
107a[145]	Me	73
107b[146]	Et	86
107c[146]	*i*Pr	99
107d[146]	Pr	76

Compound **107a-d** were isolated as colourless oils and confirmed by ^{1}H, ^{13}C and infra-red spectroscopies. The ^{1}H NMR spectra showed a total of 6-8 signals with the inclusion of the NH group: A doublet at ~ 7.17-8.68 ppm integrated for 1H due to N-H. A singlet at ~ 3.74-3.94 ppm was assigned to methylene protons **(H-7)** confirmed

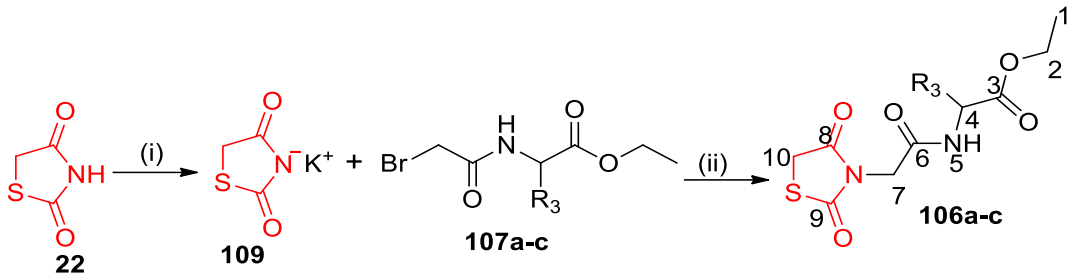
the presence of the bromo acetamido group. A quintet observed at ~ 4.17-4.56 ppm integrating for 1H was assigned to **H-4**, a quartet appearing at ~ 3.94-4.22 ppm integrating for 2H was assigned to methylene protons **(H-2)** and a triplet assigned to a methyl proton (H-1) observed at ~ 1.22-1.45 ppm integrating for 3H. Alaninate (**107a**) was confirmed by the presence of methyl signal integrating for 3H appearing at 1.45 ppm, whereas butanoate **(107b**) was confirmed by the signals representing ethyl groups which appeared as a multiplet integrated for 2H at 1.81-1.91 ppm and a triplet integrated for 3H at 0.86 ppm. Valinate (**107c**) was confirmed by the presence of the isopropyl moiety appearing as a doublet integrated for 6H at 0.88 ppm and a quintet integrated for 1H appearing at 2.07 ppm whereas norvalinate **(107d)** was confirmed by the presence of propyl signals appearing as a multiplet integrated for 4H at 1.64-1.70 ppm and a triplet integrated for 3H appeared at 0.88 ppm.

The ^{13}C NMR spectra of compounds **107a-d** showed a total of 7-9 carbon signals with an inclusion of the carbonyl carbons. Among the signals, the most prominent signals were methylene carbon signals **(C-7)** observed at ~28.67-29.32 ppm and the carbonyl carbons signal **C-3 and C-6** appearing at ~171.70-172.32 (ester) and 165.09-167.39 (amide) ppm respectively. Finally, the IR spectra of compound **107a-d** showed N-H stretches at ~3391-3393 cm^{-1}, a C=O stretches at ~1765-1663 cm^{-1} and C-O stretches at ~1597-1573 cm^{-1}. All the observed signals were in agreement with the data reported in literature[145, 146]

2.1.4. Displacement of bromine group (on compounds 107b-d) with glitazone salt 109

The next step was to access a series of new ethyl 2-(2-(2,4-dioxothiazolidin-3-yl) acetamido) esters **106a-c** by reacting ethyl 2-(2-bromoacetamido) esters **107b-d** with potassium glitazone salt **109**. However, all the compounds in this series containing alanine **107a** (R_3=Me) were not included since they were previously reported in my MSc dissertation.[152] The synthesis was carried out by modifying conditions described by Ali *et al.*[153] wherein a solution (in ethanol) of 2,4-thiazolidenedione afforded the potassium salt of glitazone **109**. The resultant **109** was subsequently added to **107** and the mixture was heated at refluxed to afford the desired ethyl 2-(2-(2,4-dioxothiazolidin-3-yl) acetamido) esters **106a-c** in good yields ranging from 81-92%.

The results and distinct characterization are captured in **Scheme 65** and the accompanying **Table 5**.



Scheme 65: *Synthesis of ethyl 2-(2-(2,4-dioxothiazolidin-3-yl)acetamido) esters* ***106a-d****;* ***Reagents and conditions*** *(i) KOH, EtOH, 70 ℃, 1h (ii) THF, reflux, 6h*

Table 5: Ethyl 2-(2-(2,4-dioxothiazolidin-3-yl) acetamido) esters **106a-c (scheme 65)**

Compound	R_3	M.p (℃)	Yields (%)
106a	Et	110-101	92
106b	*i*Pr	118-120	81
106c	Pr	119-120	81

Compounds **106a-c** were isolated as white solids. There was a total of 10 signals in the ^{1}H NMR spectra of each of compounds **106a-c**. The appearance of new singlet methylene signals **H-10** integrated for 2H appeared at ~3.84-4.17 ppm confirmed the presence of the glitazone moiety in compounds **106a-c**. Furthermore, there was a shift of the methylene signals **H-7** which were observed at 4.24-4.28 ppm as compared to the starting materials **107a-c** where they appeared at ~4.12 ppm which confirmed that the reactions had been successful. As for the rest, the doublet signals integrated for 1H assigned to **H-5** appeared at ~ 6.55-8.62 ppm; quintet signals integrating for 1H due to **H-4** were observed at ~4.16-4.35 ppm; quartet signals **H-2** integrating for 2H appeared at ~ 4.12ppm-4.23 ppm, and **H-1** signal appeared as a triplet integrating for 3H at ~ 1.18-1.30 ppm.

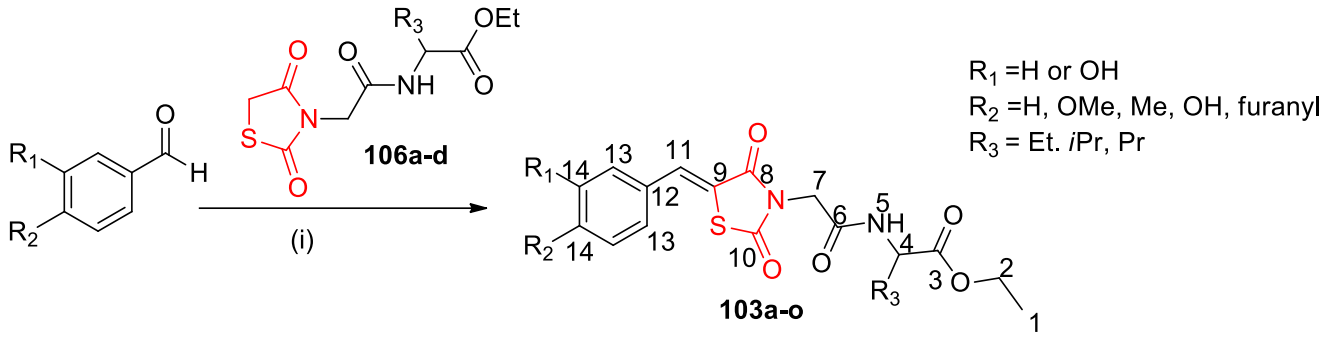
The ^{13}CNMR spectra of compounds **106a-c** exhibited a total of 8-12 signals. Methylene signals **(C-10)** assigned to the newly introduced glitazone moiety were observed at ~34.36-34.39 ppm. Methylene signals **(C-7)** were observed at ~43.25-

43.34 ppm whereas, two carbonyl signals from the glitazone ring were observed ~172.27-171.60 ppm confirmed that compounds **106a-c** were obtained successfully.

IR spectra of compounds **106a-c** were characterized by N-H stretches appearing at ~ 3262-3282 cm^{-1}, four carbonyls stretches observed at ~ 1737-1688 cm^{-1} and C-O stretches at ~1298-1214 cm^{-1}. However, the HRMS of all these compounds **106a-c** were not done on the basis that they were just intermediates.

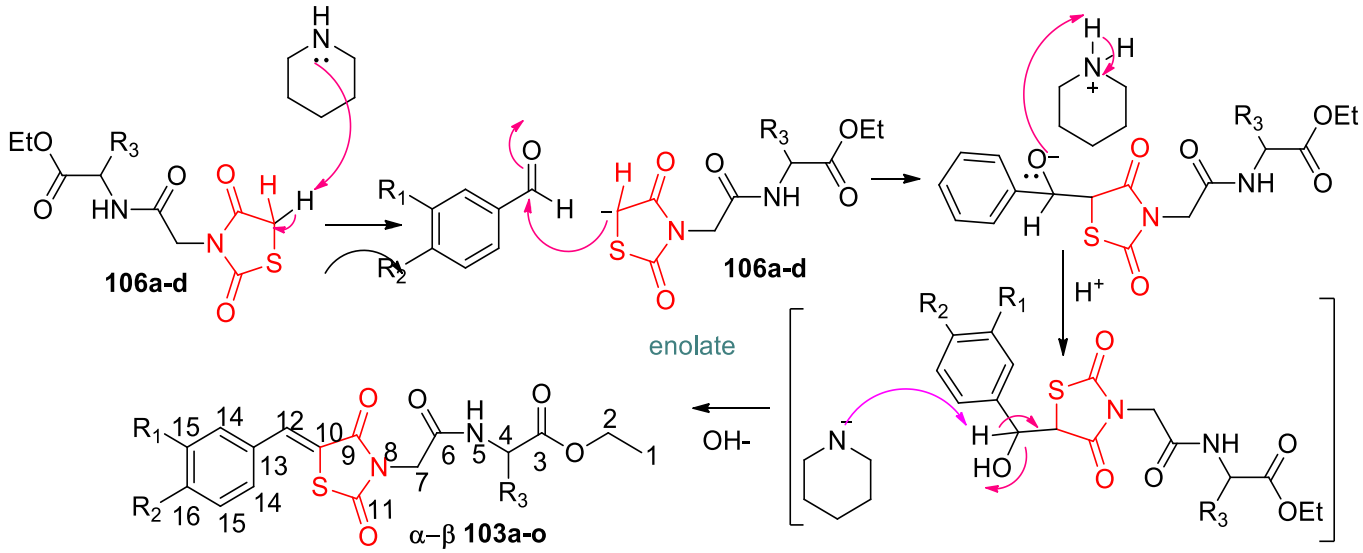
2.1.5. Synthesis of novel 5-benzylidine glitazone esters (103a-o).

To synthesize final compounds containing glitazone, a method by Van Beurden[154] was successfully utilized. The method involved Knoevenagel condensation of ethyl 2-(2-(2,4-dioxothiazolidin-3-yl) acetamido) esters **106a-c** with different aldehydes (benzaldehyde, *p*-anisaldehyde, *p*-toulaldehyde, *p*-hydroxybenzaldehyde, furfural, vanillin and *p*-fluorobenzaldehyde). The aldehydes and compound **106a-c** were reacted in the presence of a catalytic amount of piperidine and heated at reflux in ethanol for 12 hours to give the **103a-o (Scheme 66).** The successful reaction afforded our products which afforded butanoates **103a-e**, valinates **103f-j** and norvalinates **103k-o** with unoptimized yields ranging from 4-65%. **(Table 6)**.



Scheme 66: *Synthesis of target ethyl (2-(5-arylidene-2,4-dioxothiazolidin-3-yl)acetamido) esters* ***103a-o****;* ***Reagents and conditions*** *(i) Piperidine, EtOH, Reflux, 12h*

Mechanistically, the reaction occurs in the presence of piperidine as a base to abstract the acidic protons of the giltazone moiety in compound **106a-c** to form an enolate. The resultant enolate reacts with various aldehydes to give an aldol product of which after undergoing base induced elimination results in forming an intermediate. Finally, the resultant intermediate afford the desired α, β-unsaturated ethyl (2-(5-arylidene-2,4-dioxothiazolidin-3-yl) acetamido) esters **(103a-o**) via hydrolysis or dehydration. (**Scheme 67)**



Scheme 67: *Proposed mechanism for the synthesis of target glitazones* ***103a-o***

Lending credence to the successful preparation of these compounds was that ^{1}H NMR spectra of the synthesized ethyl (2-(5-arylidene-2,4-dioxothiazolidin-3-yl)acetamido) esters **103a-o** did not contain signals showing the benzaldehydic proton at ~ 9 ppm from the starting material which is a clear indication of the success of the reactions. The presence of arylidene ring was confirmed spectroscopically for all the compounds **103a-o**. The arylidene proton was observed at chemical shifts of ~7.80-7.99 ppm. Which were, *para* substituted phenyl rings (**103b**, **103c**, **103d**, **130g**, **103i**, **103j**, **103k**, **103l, 103m**, **103n)** showed two doublets in the range of ~6.93-7.92 ppm (*J* =7.6-8.8 Hz and *J* =8.0-8.8 Hz) integrating for 4H assigned to **H-14** and **H-15**.

The furanyl derivatives **103e** and **103o** exhibited a doublets (*J* = 21.2 Hz and *J* =7.6 Hz) at ~8.10-8.65 ppm and two doublets observed at ~7.17-8.05 ppm (*J* =3.6 Hz) and at 6.78-6.77 ppm (*J* =1.6 Hz).

Of interest was compounds **103j and 103n** which were isolated as a yellow solids with melting points of 216-217 ℃ and 240-241 ℃ respectively. The ^{1}H NMR spectrum of compound **103j** showed a coupling between the fluorine and the *ortho* proton **H-15** at 7.42 ppm as doublet of doublets with a higher coupling constant ($^{3}J_{F\text{-}H}$ =17.6 Hz). Coupling between fluorine and the *meta* proton was observed to be lower at 7.74 ppm ($^{3}J_{F\text{-}H}$ =17.6 Hz). This confirmed that proton *ortho* to fluorine **(H-15)** experiences stronger coupling than the *meta* **H-14** proton since the coupling constant decrease as the number of bonds increases further way from fluorine.

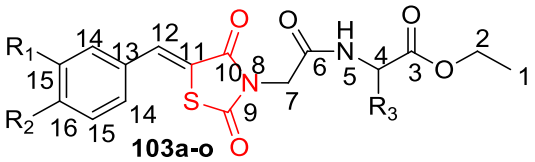


Table 6: New ethyl (2-(5-arylidene-2,4-dioxothiazolidin-3-yl)acetamido)esters **(103a-o)** obtained from **scheme 67**

Compound	R_1	R_2	R_3	M.p (℃)	Yield (%)	HRMS (m/z)
103a	H	H	Et	175-176	10	found 377.1096, calcd 377.1093
103b	H	OMe		207-208	37	found 407.1272, calcd 407.1199
103c	H	Me		206-207	8	found 391.1251, calcd 390.1249
103d	H	OH		185-186	6	found 393.1045, calcd 392.1042
103e	furanyl			184-185	11	found 367.0889, calcd 367.0886
103f	H	H	*i*Pr	167-168	24	found 391.1251, calcd 391.1249
103g	H	OMe		182-183	38	found 421.1352, calcd 421.1355
103h	OH	OMe		187-188	12	found 437.1307, calcd 436.1304
103i	H	OH		178-179	4	found 407.1202, calcd 407.1199
103j	H	F		216-217	11	found 409.1158, calcd 409.1155
103k	H	OMe	Pr	204-205	17	found 421.1358, calcd 421.1355
103l	H	Me		196-197	13	found 405.1409, calcd 405.1406
103m	H	OH		125-126	26	found 407.1202, calcd 407.1199
103n	H	F		240-241	21	found 409.1158, calcd 409.1155
103o	furanyl			181-182	65	found 381.1045, calcd 381.1042

The spectra of compounds **103a-e** on the up-field region (aliphatic chain) were characterized by a quintet appearing at ~4.18-4.22 ppm (*J* =3.2 Hz) integrating for 1H assigned to H-4, a quartet observed at 4.07-4.09 ppm (*J* =3.6 Hz) assigned to **H-2** integrating for 2H, a singlet methylene **(H-7**) integrating for 2H and lastly a triplet appearing at 1.21-1.23 ppm (*J* =7.2 Hz) integrating for 3H assigned to **H-1**

Their ^{13}C NMR spectra of products **103a-o** were characterised by a total of 13-16 signals including carbonyl carbons. As with the ^{1}H NMR, ^{13}C NMR spectra of compounds **103a-o** showed no starting benzaldehyde signal which would normally appear at ~190 ppm whereas new arylidene carbon **(C-11)** carbon signals were observed at ~ 132 ppm. The ^{13}C NMR spectrum of compound **103j** showed that the presence of fluorine splittting the signals for the *ipso*, *ortho*, *meta* and *para* positioned carbons as doublets. The *ipso* carbon (**C-16**) appeared at ~ 163.47 ppm ($^{1}J_{C-F}$ =250.0 Hz), *ortho* carbon **(C-16)** at 117.06 ppm ($^{2}J_{C-F}$ =22.0 Hz), *meta* carbon at 133.23 ppm ($^{3}J_{C-F}$ =9.0 Hz) whereas the *para* carbon was observed at 130.24 ppm ($^{4}J_{C-F}$ =3.0 Hz) which revealed that the closer the fluorine the higher the coupling constant.

The coupling between proton and fluorine was further confirmed by ^{19}F NMR. The ^{19}F spectrum of compound **103j** showed **F-C$_{16}$** signal coupling with protons **H-15** and **H-16** appearing as a multiplet at -108.02-(-108.36) ppm integrating for 1F instead of the expected singlet signal due to its coupling with the corresponding protons.

HRMS of compounds **103a-o** gave further evidence of the successful synthesis of the compounds with molecular ions corresponding to expected calculated molecular masses and data is shown in **Table 6.** The IR spectra of all compounds **103a-o** were characterized by the presence of N-H bands at ~3304-3297 cm^{-1}, four carbonyl bands at ~1600-1799 cm^{-1}, as well as C-O bands ranging from ~1100-1300cm^{-1}

2.2. Biology

In line with one of the main aims of this study, the new glitazone derivatives **103a-o** synthesized in this project were submitted for variety of biological assays. The assays used were for the *in vitro* cytotoxicity screening and the *in vitro* α-glucosidase screening. These experiments were done by Prof Maryna van de Venter of Bioassaix at the Nelson Mandela University according to the procedure described in chapter six of the experimental section.

2.2.1. *In vitro* cytotoxic evaluation

Cytotoxicity is defined as the ability of an agent to produce a toxic effect on a cell. Cytotoxicity assays are used to test the ability of cells to continue proliferating in the presence of a test compound or substance over a specific time period.[155] All the compounds **103a-o** were evaluated against human colorectal adenocarcinoma cell line (CaCo-2) at 100 µM in comparison with the untreated (UT) and melphalan as a control using MTT assay. Any compound with the number of live cells less than 2000 was considered to be toxic whereas with more than 2000 live cells is said to be nontoxic. All the synthesized compounds did not show any toxicity with exception of **103d** and **103h** which exhibited moderate toxicity of 1374.556±168.976 and 1782.722±157.3676 µM living cells respectively. **(Table 7)**

Table 7: Cytotoxicity data of glitazone containing compounds **103a-o**

Compounds	Number of live cells (µM)
103a	2725±239
103b	2479±150
103c	2513±285
103d	1374 ±168
103e	2749±289
103f	2826±189
103g	2694±113
103h	1782 ±157
103i	2485±600
103j	2544±960
103k	2753±680
103l	2842±610
103m	2153±113
103n	2939±360
103o	2544±960
Melphalan	397±730
UT	2811±237

2.2.2. *In vitro* α-glucosidase evaluation

α-Glucosidase is a membrane-bound enzyme located at the brush border of epithelial cells in the small intestine in the form of maltase-glucoamylase (MGAM) and sucrose-isomaltase.[156] The primary function of α-glucosidase inhibitors is to prevent postprandial hyperglycaemia by slowing down the digestion of carbohydrates and consequently the rate at which glucose can be absorbed and enter general circulation.[157] Due to their functioning in glucose control, α-glucosidase inhibitors are frequently used as oral antidiabetic drugs in the early stages of T2D to combat postprandial hyperglycaemia and obesity.[158]

All synthesized compounds **103a-o** were screened to evaluate their α-glucosidase inhibitory activities, in comparison with the marketed α-glucosidase inhibition standard drug Epigallocatechin gallate (EGCG) at the concentration of 10, 50, 100 µM. **(Table 8).** However, selected compounds were screened at higher concentration of 200 µM

For the butanoates **103a-e**, none of the derivatives showed any α-glucosidase inhibition at 10 µM, 50 µM and 100µM. Nevertheless, slight improvement in terms of α-glucosidase inhibition was exhibited at the higher concentration of 200 µM. This was seen in compounds containing unsubstituted phenyl **103a** and the *para* hydroxy phenyl rings **103d**, compounds which exhibited α-glucosidase inhibition of 33.38±5.65% and 37.69±0.39% respectively.

Among synthesized valinates **103f-j**, there was no inhibition against α-glucosidase at 10 µM and 50 µM. After the concentration was increased to 100 µM, there was a weak α-glucosidase inhibition observed for the *para* methoxy phenyl derivative **103g** which had a value of 8.81±13.30% and *para* hydroxy phenyl containing ring derivative **103j** exhibiting α-glucosidase inhibition of 8.8±15.50%. Only the phenyl containing ring derivative **103f** with the α-glucosidase inhibition of 32.66±4.31% and 3-hydroxy-4-methoxy phenyl derivative **103h** exhibiting 29.67±3.09% showed inhibition at 200 µM.

With regards to norvalinates **103k-o**, no α-glucosidase inhibition was observed at 10 µM while only the furanyl derivative **103o** exhibited weak α-glucosidase inhibition of 12.032±6.63% at 50 µM. At 100 µM, the α-glucosidase inhibition of furanyl derivative **103o** increased to 41.66±7.078%. At the highest concentration of 200 µM, only the *para* hydroxy phenyl derivative **103m** showed an improved inhibition exhibiting

31.83±2.85% whereas the furanyl derivative **103o** was found to be the most active derivative exhibiting 51.49±5.05% which is an improvement from 100 µM.

Overall, it was observed that the presence of electron-donating *para* hydroxy derivatives **103d** and **103m** increases the activity as compared to the unsubstituted phenyl derivatives **103a** and **103f**. Introducing both electron-donating hydroxy group at position 3 and methoxy group at position 4 decreased the α-glucosidase activities. The electron-donating furanyl derivative **103o** was the most active derivative exhibiting α-glucosidase activities in an increased trend at 50 µM, 100 µM and 200 µM respectively. As the amino acid chain was extended from butanoate (Et) to norvaline (Pr) the α-glucosidase activities decreased.

Table 8: α-glucosidase inhibition assay of compounds **103a-o** (%)

Compound	10 µM	50 µM	100 µM	200 µM
103a	-10.10±12.14	-14.40 ±16.50	-6.85±14.20	33.38±5.65
103b	-18.30±12.20	-15.30±12.90	-11.10±13.70	-
103c	-12.80 ±18.10	-8.47±19.50	-0.46±14.50	-
103d	-21.50 ±13.50	-17.50 ±12.50	-18.10±12.80	37.69±0.39
103e	-6.67±12.90	-11.16±9.54	-10.3±17.60	-
103f	-20.01±12.90	-11.01±15.50	1.36±18.50	32.66±4.31
103g	-15.10±10.40	-3.30±14.30	8.81±13.30	-
103h	-21.30±14.40	-19.60±14.47	-12.70±14.40	29.67±3.09
103i	-16.20±14.20	-11.30±11.20	-2.65±14.20	-
103j	-17.20±11.80	-9.74±15.30	8.8±15.50	-
103k	-6.75±19.70	-15.90±13.10	-24.60±21.60	-
103l	-26.17±16.75	-24.16±9.89	-22.83±10.89	-
103m	-24.01±20.55	-20.26±8.82	-14.15±8.92	31.83±2.85
103n	-19.10±11.40	-18.10±15.01	-8.46±19.70	-
103o	-22.84±12.18	12.03±6.63	41.66±7.078	51.49±5.05
EGCG	97.03±0.408	97.03±0.408	97.03±0.408	97.03±0.408

2.3. Conclusion

Three series of fifteen novel ethyl (2-(5-arylidene-2,4-dioxothiazolidin-3-yl) acetamido) esters **103a-o** were successfully synthesized by employing known conventional methods. After employing a Knoevenagel condensation five butanoates, **103a-e** were successfully obtained in yields, ranging from 6%-37%, whereas valinates **103f-j** were isolated with yields of 4-38% yields and norvalinate **103k-o** were obtained in 13-65% yields. All the synthesized target compounds **103a-o** were found to be non-toxic except for *para* hydroxy phenyl derivative **103d** and 3-hydroxy-4-methoxy phenyl derivative **103h** due to the presence of oxygen at *para* position. *In vitro* α-glucosidase screening revealed that compounds containing unsubstituted phenyl derivatives **103a** and **103f**, *para* hydroxy phenyl derivatives **103d** and **103m** as well as 3-hydroxy-4-methoxy phenyl derivative showed moderate inhibition at 200 µM. The furanyl derivative **103o** was found to be the most active compound showing α-glucosidase inhibition at 10 µM, 50 µM and 100 µM respectively in an increased trend. However the biological data was not conclusive as molecules tested tended to be weak at lowest concentrations and requires hinger concentrations to show their anti-diabetic effects.

Chapter III

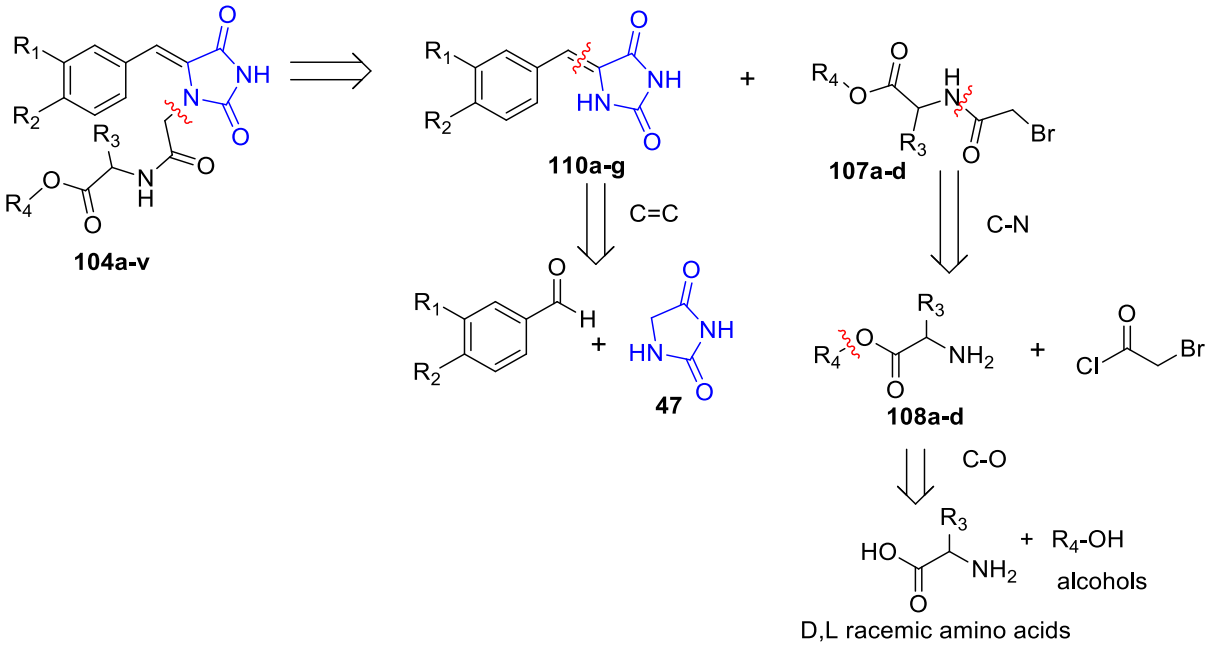
3. Results and discussion of hydantoin derivatives

This chapter looks describes the results obtained from employing various synthetic methods towards the synthesis of a set of target hydantoins. It also describes the results obtained from various characterization techniques, as well as *in vitro* anti-diabetic assays results.

3.1. Chemistry of hydantoin

3.1.2. Retrosynthesis analysis of target hydantoin-containing compounds

The general synthetic method used by Han *et al*.[159] provided the most versatility for preparing the analogues needed for the synthesis of our designed hydantoin-containing compounds **104a-v**. The analogues were thus prepared following the general retrosynthesis analysis shown in **Scheme 68.**



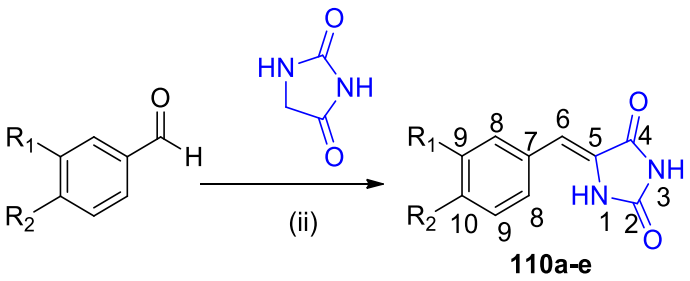
Scheme 68: *A retrosynthetic analysis of the target hydantoin containing compounds* ***104a-v***

Target hydantoin-containing compounds **104a-v** can be derived from 5-arylidene-imidazolidine-2,4-dione **110a-e** and the corresponding ethyl bromo acetamido esters **107a-d** by cleavage of the nitrogen carbon bond as depicted in **Scheme 68**.

Furthermore, 5-arylidene-imidazolidine-2,4-dione **110a-e** could be further substituted by the use of commercially available aldehydes and hydantoin by the cleavage of the carbon-to-carbon double bond between the phenyl ring and the imidazolidine-2,4-dione moiety. Moreover, ethyl bromo acetamido esters **107a-d** would then be derived from bromo acetyl chloride and ester-protected amino acids **108a-d** through the cleavage of the carbonyl carbon and nitrogen bond. Ester-protected amino acids **108a-d** could be derived from commercially available amino acids, namely, alanine, alpha amino butyric acid, valine and norvaline.

3.1.3. Synthesis of arylidene imidazolidine-2,4-diones (110a-e)

The initial step involved the fusion of commercially available hydantoin with various aldehydes using the Knoevenagel condensation reaction.[154] **(Scheme 69)** The results of unoptimized products **110a-e** were obtained in percentage yields ranging from low to good (20-65%), as summarised in **Table 9**.



***Scheme 69**: Synthesis of 5-arylidene hydantoins **110a-e**; **Reagents and conditions** (i) Piperidine, reflux, 12h*

Table 9: 5-Arylidene-imidazolidine-2,4-diones **110a-e** as described in **Scheme 69**

Compound	R_1	R_2	M.P. (℃)	Lit. M.P (℃)	Yield (%)
110a	H	Me	240-253	245-247[160]	27
110b	H	MeO	248-250	250-252[160]	52
110c	Piperonyl		254-257	258-260[161]	40
110d	H	F	270-272	273-275[162]	25
110e	OH	MeO	246-248	247-248[163]	53

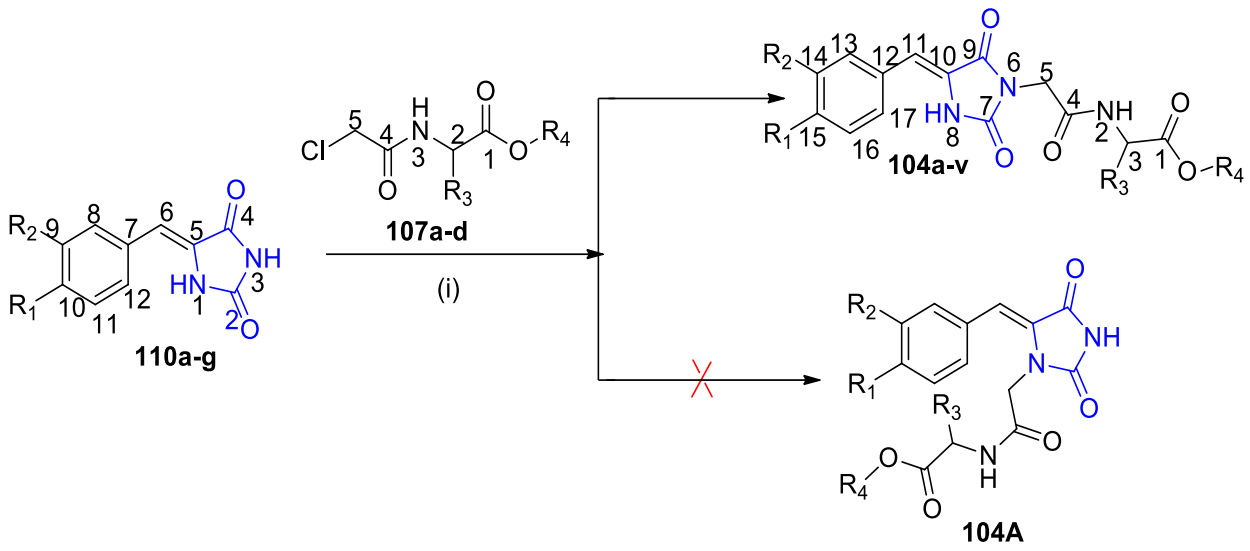
5-Arylidene hydantoins **110a-e** obtained from **Scheme 69** were characterised using a combination of ^{1}H NMR, ^{13}C NMR, as well as IR spectroscopies. ^{1}H NMR spectra of

compounds **110a-e** were characterised by a total of 5-7 signals. As a confirmation that this reaction was successful, there was an absence of the characteristic aldehydic proton peak at ~ 9 ppm indicating the consumption of aldehydes. In addition, the ^{1}H NMR spectra of compounds **110a-e** were further characterized by a newly formed singlet arylidene signal **(H-6)** integrating for 1H appearing at ~ 6.06 - 6.43 ppm, which confirmed that indeed a condensation reaction took place. The ^{13}C NMR spectra of compounded **110a-e** were characterized by the absence of the aldehydic carbon signal at ~ 190 ppm also confirming that aldehydes were fully reacted. However, there was a new arylidene carbon signal **(C-6)** peak at ~ 107.64 - 110.24 ppm indicating that condensation reactions were successful. In addition, the ^{1}H NMR spectra of compounds **110a-e** exhibited **N-1** signals at ~ 10.44-10.58 ppm integrated for 1H whereas **N-3** proton were observed at ~11.15-11.28 ppm, also with integration of 1H. The IR spectra of compounds **110a-e** indicated the N-H stretches at ~ 3152-3289 cm-1, and the C-H stretches were observed at ~ 2839-2947 cm^{-1}. Furthermore, the carbonyl stretches appeared at ~1750-1789 cm^{-1}, while the C=C stretches were observed at ~1626-1669 cm^{-1}. The melting points in the ranged between 215-284 ℃ **(Table 9)** whereas all the spectroscopic data observed were corresponding very well with the literature values.[160, 161, 162, 163, 164]

3.1.4. Synthesis of novel ethyl or methyl 5-benzylidine-hydantoin esters (104a-v)

The final step involved the synthesis of target ethyl or methyl (2-(5-(4-benzylidene)-2,5-dioxoimidazolidin-1-yl)acetamido) esters **104a-v** by modifying conditions outlined by Han *et al.*[159] Compounds **110a-e** were subjected to nucleophilic substitution in the presence of KOH in order to afford the expected ethyl or methyl (2-(5-(4-substituted benzylidene)-2,5-dioxoimidazolidin-3-yl)acetyl) esters **104A** at the N-1 position of hydantoin moiety **(Scheme 70).** Unfortunately, this reaction did not take place. After intensive studying of 2D NMR spectroscopic (^{1}H-^{15}N HMBC) results of this reaction, it was observed that the substitution took place at the N-H (1) position to give ethyl or methyl (2-(5-(4-substituted benzylidene)-2,5-dioxoimidazolidin-1-yl)acetamido) esters **104a-v** in low to good yields as summarized in **Table 10**. The regioselectivity of N-alkylation of hydantoins at the N-H (3)-position was established by analogy with the literature. Bases such as potassium hydroxide lead to selective alkylation at the more

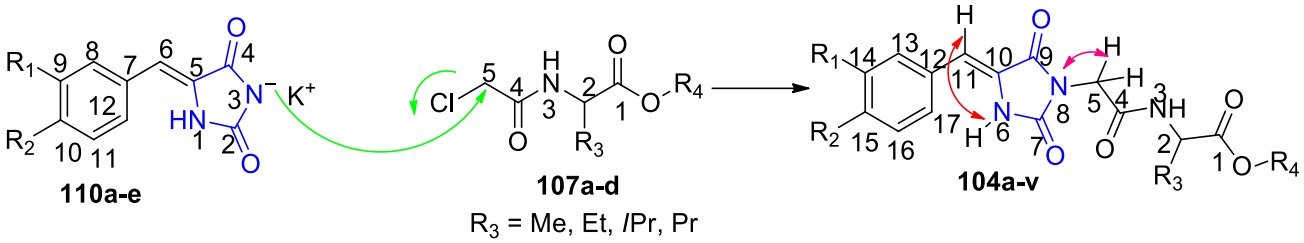
acidic N-H (3)-position; only stronger bases that effect double deprotonation give alkylation at the more hindered N-H (1)-position.[164]



Scheme 70: *Synthesis of Ethyl or Methyl (2-(5-(4-substituted benzylidene)-2,5-dioxoimidazolidin-1-yl)acetamido) esters;* ***Reagents and conditions*** *(i) MeOH, reflux, 12h*

Structurally, all the synthesized target hydantoin containing compounds **104a-v** were classified into six main categories based on the substituents of the phenyl ring and the protected amino acids: methyl (2-(5-(4-methylbenzylidene)-2,5-dioxoimidazolidin-1-yl) acetamido) esters **104a-d,** ethyl (2-(5-(4-methylbenzylidene)-2,5-dioxoimidazolidin-1-yl) acetamido) esters **104e-h**, methyl and ethyl (2-(5-(4-methoxybenzylidene)-2,5-dioxoimidazolidin-1-yl) acetamido) esters **104i-k** and **104l**, ethyl and methyl (2-(5-piperonyl)-2,5-dioxoimidazolidin-1-yl) acetamido) esters **104m-o** and **104p,** ethyl (2-(5-(4-fluorobenzylidene)-2,5-dioxoimidazolidin-1-yl) acetamido) esters **104q-t**. **(table 10)** as well as ethyl (2-(5-(3-hydroxy-4-methoxybenzylidene)-2,5-dioxoimidazolidin-1-yl) acetamido) esters **104u-v**

Mechanistically, the substitution reaction favours the more reactive N-H (3) position of hydantoin moiety rather than the N-H (1) position due to its hinger acidity. The reaction took place in refluxing methanol in the presence of potassium hydroxide as strong base where the leaving group chlorine atom in compounds **107a-d** is displaced with the nucleophiles **110a-e** to afford the desired product **104a-v**. **(Scheme 71)**

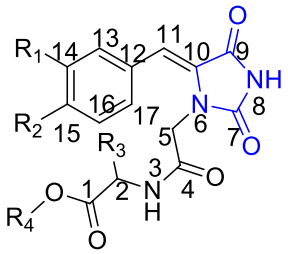


Scheme 71: *Proposed mechanism for the synthesis of ethyl or methyl (2-(5-(4- benzylidene)-2,5-dioxoimidazolidin-1-yl)acetamido) esters* ***104a-v***

Compounds **104a-v** were confirmed using a combination of ^{1}H NMR, ^{13}C NMR, IR spectroscopies as well as 2D NMR spectroscopies. ^{1}H NMR spectra of compounds **104a-v** were characterized by a total of 9-11 signals. Furthermore, the ^{1}H NMR spectra exhibited the absence of the N-H signal **(H-3)** integrating for 1H which appeared at ~11.21-11.28 ppm in the starting material **110a-e**. This observation confirmed that nucleophilic substitution with compounds **107a-d** has happened at position one **N-H (3)** of the hydantoin moiety. The N-H at position three **N-H (1)** of the hydantoin moiety was observed ~9.78-10.80 ppm integrating for 1H. Also observed at the ^{1}H NMR spectra of compounds **104a-v** was a doublet accounting for one proton ~ 8.41-8.75 ppm confirming the amidic proton **(H-3)**. In addition to this, there was a shift of chemical shift of singlet signals **H-8** integrated for 2H which were observed to be appearing at ~ 4.12-4.31 ppm, in the ^{1}H NMR spectra of compounds **104a-v** as compared to the starting material **104a-d** where it was appearing at ~3.75 -3.89 ppm, confirming that indeed the product was obtained. The ^{13}C NMR spectra of compounds **104a-v** were characterized by a total of 15-18 signals.

Additionally, heteronuclear multiple bond correlation (^{1}H-^{15}N HMBC) two-dimensional NMR spectroscopy showed that at N-H position where the proton of compounds **110a-e** was displaced, the protons **(H-5)** generated showed a correlation with **N-8**. Most importantly, there was a correlation between **N-6** and **H-11** which are three bonds away as shown in **Scheme 71**. This confirmed that nucleophilic acyl substation took place at the N-H (3) position of the hydantoin moiety as there cannot be a correlation of **N-8** with **H-11** at position N-H (1) since these protons are more than three bonds away from each other.

Table 10: Novel ethyl or methyl (2-(5-(4-benzylidene)-2,5-dioxoimidazolidin-1-yl) acetamido) esters **104a-v** data obtained from **Scheme 70**



104a-v

Compound	R_1	R_2	R_3	R_4	M.p (℃)	Yield (%)	HRMS (m/z)
104a	H	Me	Me	Me	215-234	25	found 368.0700, calcd 368.0788
104b	H	Me	Et	Me	264-266	52	found 382.1059, calcd 382.1035
104c	H	Me	*i*Pr	Me	224-226	37	found 396.1029, calcd 396.1021
104d	H	Me	Pr	Me	240-242	30	found 408.1256, calcd 408.1230
104e	H	Me	Me	Et	254-256	57	found 360.1540, calcd 360.1560
104f	H	Me	Et	Et	245-247	52	found 374,1638, calcd 374.1600
104g	H	Me	iPr	Et	254-256	36	found 388.1715, calcd 388.1794
104h	H	Me	Pr	Et	255-257	63	found 374.1601, calcd 374.1697
104i	H	OMe	Me	Me	218-220	44	found 390.1538, calcd 390.1587
104j	H	OMe	Et	Me	259-261	40	found 376.1495, calcd 376.1430
104k	H	OMe	*i*Pr	Me	260-262	40	found 404.1723, calcd 404.1743
104l	H	OMe	Pr	Et	218-220	44	found 390.1538, calcd 390.1587

104m	piperonyl		Me	Et	246-248	49	found 390.1292, calcd 390.1223
104n			Et	Et	261-263	47	found 404.1315, calcd 404.1380
104o			*i*Pr	Et	225-227	51	found 418.1515, calcd 418.1536
104p			Pr	Me	357-359	34	found 404.1339, calcd 404.1380
104q	H	F	Me	Et	238-240	25	found 388.1727, calcd 388.1798
104r	H	F	Et	Et	284-286	20	found 378.1787, calcd 378.1723
104s	H	F	*i*Pr	Et	275-277	52	found 391.1518, calcd 391.1543
104t	H	F	Pr	Et	256-258	40	found 391.1518, calcd 391.1543
104u	OH	OMe	Me	Et	296-298	44	found 391.1375, calcd 391.1380
104v	OH	OMe	Et	Et	242-244	24	found 405.1531, calcd 405.1536

. Furthermore, there was a change observed in ^{13}C NMR spectra of compounds **102a-v** where C-7 signal appeared at ~ 167.00 - 167.90 ppm, as compared to the starting material **107a-d** where it appeared at ~ 165.09 ppm. Additionally, there was a shift of **C-5** signals chemical shift which was observed at ~ 40.07 - 40.51 ppm, as compared to starting material **107a-d** where it appeared at ~ 48.78 ppm

The IR spectra of all final novel compounds **104a-v** indicated characteristic peaks for N-H stretching in the range of 3200 - 3300 cm^{-1}, C=O stretch at 1600-1790 cm^{-1}, C-H stretch at ~ 2858 - 3096 cm^{-1}, C=C at ~ 1601 -1616 cm^{-1} and the C-O stretch at ~ 1115 - 1348 cm^{-1}. HRMS of compounds **104a-v** gave further evidence of the successful synthesis of the compounds with the molecular ion corresponding to the calculated values and the data is shown in **Table 9**.

3.2. Biology

The biological screening of all the synthesized hydantoin-containing intermediates **110a-e** and new target compounds **104a-v** were done in a similar manner as described in the biological assays of the glitazone chapter 2, section 2.2.

3.2.1. *In vitro* cytotoxic evaluation

The cytotoxicity evaluation of the intermediates **110a-e** and the selected target compounds **104a-v** were evaluated against human colorectal adenocarcinoma cell line, (CaCo-2) cell line using MTT assay at 100 µM and their results are presented in **table 11**. In general, all the synthesized intermediates **110a-e** were found to be nontoxic displaying living cells of >2000 at 100 µM in comparison with the melphalan as the control. For the synthesized intermediates **110a-e**, only the electron-withdrawing *para* fluoro phenyl derivative **110d** and the electron donating-piperonyl derivative**110c** were found to be toxic, with living cells of 1997±79 and 464±78 µM respectively.

Among the ethyl or methyl (2-(5-(4-benzylidene)-2,5-dioxoimidazolidin-1-yl) acetamido) esters **104a-v**, four compounds namely: electron-donating piperonyl alaninate derivative **104m**, *para* fluoro phenyl butanoate derivative **104r**, and *para* fluoro phenyl norvalinate derivative **104t**, as well as 3-hydroxy-4-methoxy phenyl alaninate derivative **104u** with living cells of 1732±179, 249±13, 283±47 and 249±47 µM respectively were found be toxic.

Table 11: Cytotoxicity results of intermediates **110a-e** and compounds **104a-v**

Compound	Number of live cells (µM)
110a	2291±125
110b	2070±183
110c	464±780
110d	1997±790
110e	2535±242
104a	2567 ±299
104b	2230±570
104c	234±179
104d	2234±720
104e	2595±289
104f	2669±166
104g	2565±198
104h	2730±213
104i	2605±293
104j	2643±311
104k	2703±270
104l	2503±276
104m	1732±179
104n	2354±176
104o	2039±740
104p	2033±467
104q	2489±137
104r	249±130
104s	2118±153
104t	283±470
104u	249±490
105v	2119±154
melphalan	397±720
UT control	2814±237

3.2.2. *In vitro* α-glucosidase evaluation

All the intermediates (**110a-e**) and the newly synthesized compounds **104a-v** were evaluated for their *in vitro* α-glucosidase inhibition in comparison with the marketed standard drug Epigallocatechin gallate (EGCG) as summarised in **Table 12**. Results revealed that the synthesized intermediates **110a-e** showed weak or no inhibition against α-glucosidase at 10 µM, 50 µM and 100 µM. As the concentration was increased to 200 µM, there was a better improvement of α-glucosidase inhibition with 4-methyl phenyl derivative **110a** (48.37±1.03%) exhibiting highest activity followed by the 3-hydoxy-4-methoxy phenyl derivative **110e** with the inhibition of 45.81±1.76%. Electron donating *para* 4-methoxy phenyl derivative **104b** exhibited inhibition of 41.92±1.16% which was higher than piperonyl derivative **110c** and the electron withdrawing *para* fluoro derivative **110d** with inhibition of 39.74±2.37% and 36.12±2.74% respectively.

With the methyl (2-(5-(4-methylbenzylidene)-2,5-dioxoimidazolidin-1-yl) acetamido) esters **104a-d,** there was weak or no α-glucosidase inhibition at 10 and 50 µM. As the concentration was increased to 100 µM, only the valinate derivative **104c** and norvalinate derivative **104d** showed some improvement in the α-glucosidase inhibition, exhibiting 18.00±3.95% and 25.40±7.88% respectively. At 200 µM there was in increase in inhibition with the alaninate derivative **104a** exhibiting higher α-glucosidase inhibition of 51.65±2.92%, followed by valinate derivative **104c** which exhibited 45.23±3.60% and norvlinate derivative **104d** with α-glucosidase inhibition of 42.76±3.60%.

Among the synthesized ethyl (2-(5-(4-methylbenzylidene)-2,5-dioxoimidazolidin-1-yl) acetamido) esters **104e-h**, results revealed that there was only weak or no activity at 10 µM and 50 µM. Only the butanoate derivative **104f** showed some improvement of 22.60±6.83% in the α-glucosidase inhibition at 100 µM. At the highest concentration 200 µM, the α-glucosidase inhibition of the butanoate derivative **104f** increased to 52.05±2.83% while alaninate derivative **104e**, valinate derivative **104g** and norvalinate **104h** exhibited 44.58±0.99%, 48.47±2.33% and 41.94±3.31% respectively.

With the methyl and ethyl (2-(5-(4-methoxybenzylidene)-2,5-dioxoimidazolidin-1-yl) acetamido) esters **104i-k** and **104l**, there no α-glucosidase inhibition observed at 10 µM and 50 µM. As the concentration was increased to 100 µM, only the norvalinate derivative **104l** showed α-glucosidase inhibition of 24.30±29.20%. At 200 µM, the α-gluosidase inhibition of compound **104l** showed an increase in the activity to 54.21±1.55%, which is a good improvement, followed by alaninate derivative **104i** exhibiting 51.65±2.92%.

Among the synthesized methyl (2-(5-piperonyl)-2,5-dioxoimidazolidin-1-yl) acetamido) esters **104m-p**, weak α-glucosidase activity which were observed at 10µM and 50µM. At 100µM, the inhibition of valinate derivative **104o** and **104p** increase to 28.30±17.30% and 37.40±24.90% respectively. At the highest concentration of 200 µM, there was an increased in the α-glucosidase inhibition of valinate **104o** and norvalinate **104p,** exhibiting 57.77±2.79% and 57.41±6.38% respectively.

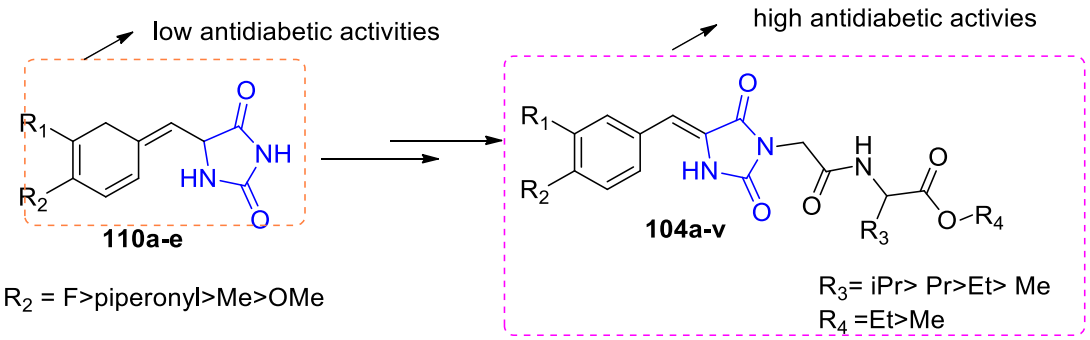
With the ethyl (2-(5-(4-fluorobenzylidene)-2,5-dioxoimidazolidin-1-yl) acetamido) esters **104q-t**. there was weak or no α-glucosidase activities which were observed at 10 µM, 50 µM and 100 µM respectively. At 200 µM, butanoate derivative **104r** exhibited highest α-glucosidase activity of 56.93±1.58%, followed by valinate derivative **104s** exhibiting 56.78±2.47%. Alaninate derivative **104q** and norvalinate derivative **104t** exhibited almost moderate inhibition of 43.78±0.89% and 47.36±4.23% respectively.

In general, all the synthesized hydantoin-containing compounds **104a-v** did not show any activity against α-glucosidase at 10 and 50µM. As the concentration was increased to 100 µM, there was a slight improvement in the α-glucosidase activities of some compounds among the synthesized compounds **104a-v**. At the highest concentration of 200 µM, piperonyl valinate derivative **104o** showed the highest α-glucosidase activity of 57.77±2.79%, followed by piperonyl norvalinate derivative **104p**, exhibiting 57.41±6.38%. Piperonyl alaninate derivative **104m**, with inhibition of 57.37±4.05%, *para* fluoro phenyl butanoate **104r** with inhibition of 56.93±1.58% and *para* fluoro phenyl valinate **104s** exhibited 56.78±2.47%. Following this was *para* methyl phenyl butanoate **104f** with α-glucosidase inhibition of 52.05±2.83%, *para* methyl phenyl alaninate **104a** exhibiting 51.65±2.92% and *para* methoxyphenyl alaninate **104i** exhibited moderate α-glucosidase activity of 51.65±2.92%.

Table 12: α-glucosidase inhibition of intermediates **110a-e** and final compounds (%) **104a-v**

Compounds	10 µM	50 µM	100 µM	200 µM
110a	-5.32±9.01	2.80±5.43	11.40±7.13	48.37±1.03
110b	-5.93±10.70	-7.28±9.94	4.06±10.60	41.92±1.16
110c	-13.60±14.50	-7.69±7.26	-2.42±6.27	39.74±2.37
110d	-6.27±6.04	-1.24±6.17	3.16±7.70	36.12±2.74
110e	-12.80±10.30	-2.03±15.70	-6.45±4.62	45.81±1.76
104a	-4.98±11.40	9.01±16.40	9.75±6.40	51.65±2.92
104b	4.92±18.00	8.87±21.30	12.2±10.90	-
104c	1.80±13.80	4.13±4.26	18.00±3.95	45.23±3.60
104d	3.45±13.60	16.30±15.20	25.40±7.88	42.76±4.30
104e	-7.03±13.20	-7.75±11.30	-6.91±4.21	44.58±0.99
104f	1.38±13.00	12.40±9.22	22.60±6.83	52.05±2.83
104g	6.49±17.80	13.80±9.60	19.20±10.20	48.47±2.33
104h	-2.22±10.20	14.50±5.42	17.00±8.54	41.94±3.31
104i	-15.80±11.10	-7.97±14.30	-10.90±28.70	51.65±2.92
104j	-9.09±11.10	-0.43±16.80	-11.40±29.60	39.32±1.01
104k	-4.18±6.32	3.37±11.50	9.51±19.70	41.04±2.62
104l	-3.33±9.06	13.10±16.00	24.30±29.20	54.21±1.55
104m	-3.85±8.78	7.70±17.80	9.60±23.00	57.37±4.05
104n	-3.19±7.41	6.94±10.90	11.20±11.00	37.65±10.04
104o	0.73±11.40	14.00±16.40	28.30±17.30	57.77±2.79
104p	11.30±15.20	16.00±7.90	37.40±24.90	57.41±6.38
104q	1.91±9.11	6.25±8.29	14.10±12.60	43.78±0.89
104r	-4.40±11.10	1.41±7.84	8.28±6.25	56.93±1.58
104s	-5.10±7.80	-0.39±8.21	6.37±3.12	56.78±2.47
104t	-12.80±5.62	-10.90±1.41	-8.03±5.32	47.36±4.23
104u	-	-	-	-
104v	-	-	-	-
EGCG	97.03±0.41	97.03±0.41	97.03±0.41	97.03±0.41

There was an improvement in α-glucosidase inhibition of synthesized compounds **104a-v** as compared to the intermediates **110a-e**. From this we deduced that the introduction of the amino acids plays a role in the α-glucosidase activities of the synthesized methyl or ethyl (2-(5-(4-substituted benzylidene)-2,5-dioxoimidazolidin-1-yl)acetamido) esters **104a-v** as shown in **Scheme 75.**



Scheme 72: *SAR of target hydantoin containing compounds* ***104a-v***

3.3. Conclusion

Six series of twenty three novel methyl or ethyl (2-(5-(4-substituted benzylidene)-2,5-dioxoimidazolidin-1-yl)acetamido) esters **104a-v (Scheme 68)** were successfully synthesized using known conventional methods. The synthesized series involved methyl (2-(5-(4-methylbenzylidene)-2,5-dioxoimidazolidin-1-yl) acetamido) esters **104a-d** with the overall yields of 25-52%, ethyl (2-(5-(4-methylbenzylidene)-2,5-dioxoimidazolidin-1-yl) acetamido) esters **104e-h** (36-63%), methyl and ethyl (2-(5-(4-methoxybenzylidene)-2,5-dioxoimidazolidin-1-yl) acetamido) esters **104i-k** (40-44%) and **104l** (44%), ethyl and methyl (2-(5-piperonyl)-2,5-dioxoimidazolidin-1-yl) acetamido) esters **104m-o** (49-51%) and **104p** (34%)**,** ethyl (2-(5-(4-fluorobenzylidene)-2,5-dioxoimidazolidin-1-yl) acetamido) esters **104q-t** (25-52%), as well as ethyl (2-(5-(3-hydroxy-4-methoxybenzylidene)-2,5-dioxoimidazolidin-1-yl) acetamido) esters **104u-v** (24-44%). *In vitro* antidiabetic screening of the synthesized compounds **104a-v** revealed that piperonyl-bearing valinate derivative **104o,** norvalinate derivative **104p,** alaninate derivative **104m** showed the highest α-glucosidase activity of 57.77±2.79%, 57.41±6.38% and 57.37±4.05% respectively due their electron rich character as compared to electron-withdrawing *para* substituted phenyl derivatives and the EGCG (97.0326±0.408) used as a standard.

Chapter IV

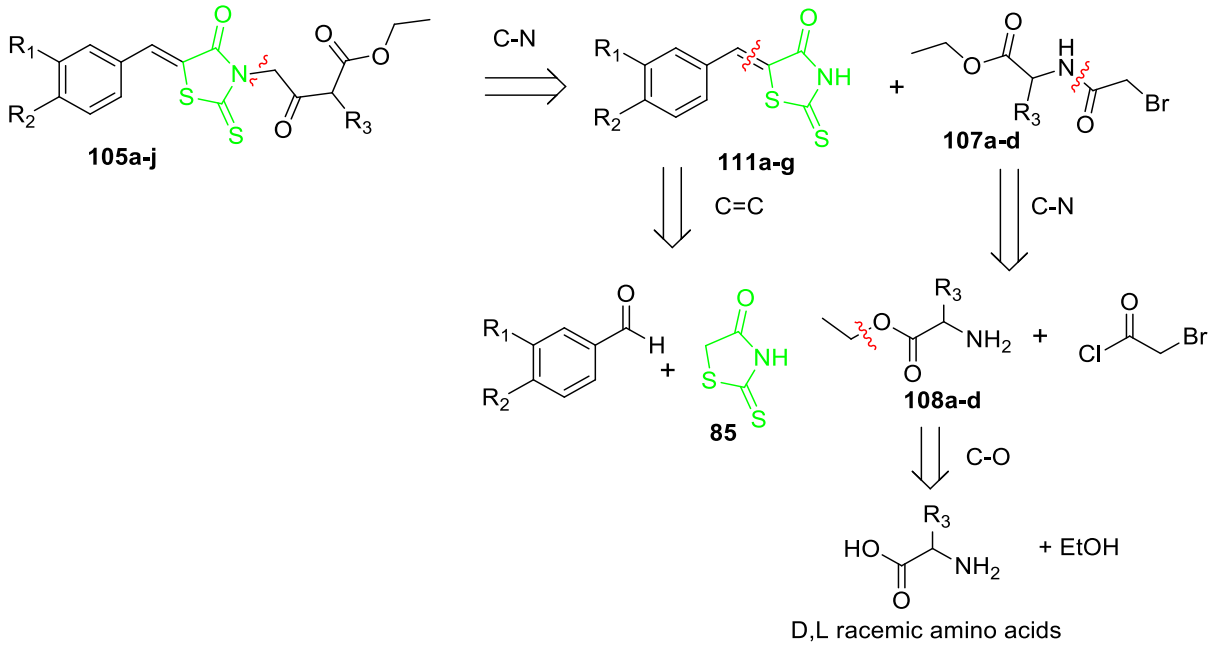
4. Results and discussion of rhodanine derivatives

This chapter deals with discussion of results obtained from the synthesis of rhodamine-containing compounds. The chapter further describes the type of reaction employed in various reaction schemes and the results obtained from different characterisation techniques thereof, and most importantly the *in vitro* anti-diabetic screening.

4.1. Chemistry of rhodanine

4.1.1. Retrosynthesis of rhodanine containing compounds

In order to determine commercially available starting material needed for the preparation of target rhodanine analogues for this study, we have conducted or contemplated a retrosynthesis **(Scheme 73)** guided by the general method used by Opletalova *et al.*[165]



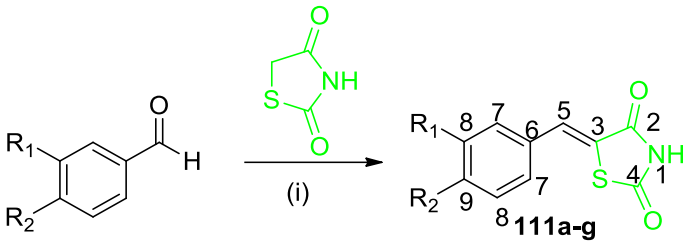
Scheme 73**: A retrosynthetic analysis of the target hydantoin containing compounds **105a-j

Target compounds **105a-j** could be substituted by nucleophilic substitution of 5-arylidene-rhodanine **111a-g** and the ethyl bromo acetamido esters **107a-d**. Furthermore, 5-arylidene-rhodanine **111a-g** could be substituted by Knoevenagel condensation of commercially-available aldehydes and 2-thioxothiazolidin-5-one

(rhodanine) **85** as starting materials. Moreover, ethyl bromo acetamido esters **107a-d** would then be substituted by acetylation of bromo acetyl chloride and ester-protected amino acids **108a-d**. Finally, ester-protected amino acids **108a-d** could be substituted by protection of commercially-available racemic amino acids alanine, α-amino butyric acid, valine and norvaline in ethanol. **(Scheme 73)**

4.1.2. Synthesis of 5-arylidine-rhodanine (111a-g)

The initial step towards the synthesis of target rhodanine analogues began by subjecting rhodane to a Knoevenagel condensation[154] reaction on acidic conditions as to synthesize 5-(4-arylidene)-2,4-rhodanine **111a-g**. **(Scheme 74)** The percentage yields of the products **111a-g** from poor to excellent with melting point ranging from 202-309 ℃ and were comparable with literature values as shown in **Table 13.**



Scheme 74: *Synthesis of 5-arylidene-2,4-rhodanines* ***111a-g****;* ***Reagents and conditions*** *(i) AcOH, AcONa, reflux, 3h*

Table 13: 5-(4- Arylidene)-2,4-rhodanines **111a-g** with respect to **Scheme 74**

Compound	R_1	R_2	M.P. (℃)	Lit M.P. (℃)	Yield (%)
111a	H	H	196-198	198-200[166]	44
111b	H	Cl	219-221	217-220[166]	69
111c	H	F	304-306	300-302[167]	77
111d	H	NO_2	190-193	192-194[168]	21
111e	Piperonyl		249-251	246-249[159]	42
111f	OH	OMe	192-195	195-198[169]	23
111g	Furanyl		232-234	235-237[166]	40

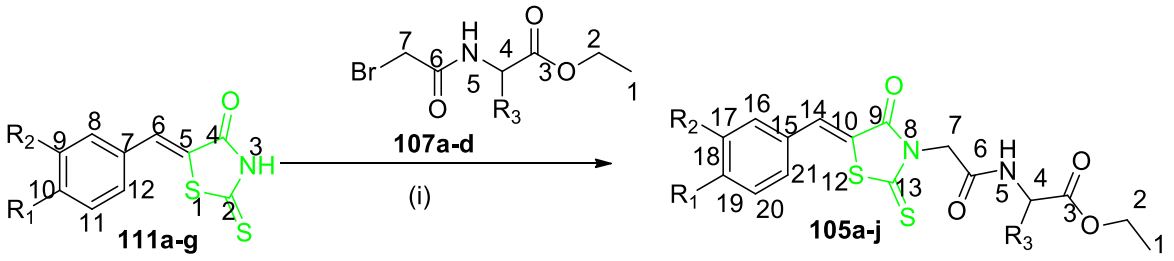
The structures of compounds **111a-g** were confirmed using a combination of ^{1}H NMR, ^{13}C NMR as well as IR spectroscopies. The lack of a characteristic aldehydic proton peak at ~ 9 ppm observed in the ^{1}H NMR spectra of products **111a-g** confirmed the

consumption of aldehydes. Furthermore, ^{1}H NMR spectra of compounds **111a-g** were also characterized by the appearance of new arylidene methylene signals **(H-5)** integrating for 1H and appearing as a singlet at ~ 7.49-7.65 ppm, confirming that the condensation reaction had been successful. Moreover, the spectra of compounds **111a-g** were also characterised by the presence of the N-H protons **(H-1)** which were observed as a singlet at ~ 13.51-13.86 ppm accounting for one proton.

^{13}C NMR spectra of compounds **111a-g** were further characterized by the absence of aldehydic carbon peak at ~ 190 ppm confirming that aldehydes were consumed during the reaction. Furthermore, all the spectra of compounds **111a-g** showed new arylidene carbon signal **(C-6)** at ~119.79-133.20 ppm which confirm the successful formation of the condensation products. The IR spectra of compounds **111a-g** were characterised by N-H stretches at ~ 3031-3155 cm^{-1}, a C-H stretch at ~ 2839-3007 cm^{-1}, a C=O stretch at ~ 1694-1741 cm^{-1} and a C=S stretch at ~ 1057-1371 cm^{-1}. All the spectroscopic data observed were in correspondence with those reported in literature.[166, 167, 168, 169]

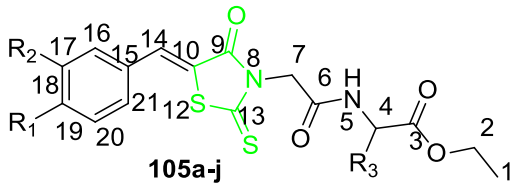
4.1.3. Synthesis of target novel 5-beylidine-rhodanine esters (105a-j)

Having successfully synthesized 5-(4-arylidene)-2,4-rhodanine **111a-g**, the last step was an alkyl substitution with compounds **107a-d** to synthesize a series of ethyl-2-(2-(5-(4-substitutedarylidene)-2-oxo-4-thioxothiazolidin-3-yl) acetamido) esters **105a-j**. **(Scheme 75)** This reaction was carried out by modifying conditions outlined by Opletalova *et al*..[165] Products **105a-j** were obtained in good to excellent yields of 32-99% as outlined in **Table 14**. Synthesized compounds **105a-j** were grouped into three categories: alaninates **105a-d**, butanoates **105e-h** and valinate **105i**. However, the derivatives on the phenyl ring were also changed to probe their influence on the α-glucosidase inhibition.



Scheme 75: *Synthesis of ethyl-2-(2-(5-(4-arylidene)-2-oxo-4-thioxothiazolidin-3-yl) acetamido) esters* ***105a-j****; Reagents and conditions (i) dioxane, KOH, reflux, 12h*

Table 14**: New ethyl-2-(2-(5-(4-arylidene)-2-oxo-4-thioxothiazolidin-3-yl) acetamido) esters **103a-j** with respect to **Scheme 75.



105a-j

Compound	R_1	R_2	R_3	M.p (℃)	Yield (%)	HRMS (m/z)
103a	H	Cl	Me	149-151	32	found 413.0364, cald 413.0318
103b	H	F		129-131	68	found 397.0647, calcd 397.0634
103c	H	NO_2		181-183	92	found 424.0531, calcd 424.0559
103d	Piperonyl			272-275	65	found 423.0642, calcd 423.0606
103e	H	H	Et	143-145	40	found 393.0673, calcd 393.0640
103f	H	NO_2		175-177	99	found 438.0759, calcd 438.0715
103g	H	F		245-247	30	found 407.1015, calcd 407.1021
103h	OH	OMe		178-181	3	found 438.0969, calcd 438.0919
103i	H	NO_2	*i*Pr	180-181	88	found 452.0272, calcd 452.0291
103j	Furanyl		Pr	272-274	63	Found 397.0674, calcd 397.0663

Lending further credence to the successful preparation of these compounds was that ^{1}H NMR spectra of compounds **105a-j** were characterised by a total of 9-12 signals. Furthermore, ^{1}H NMR spectra of these compounds showed the absence of the aromatic N-H signals **(H-3)** integrating for 1H which was appeared at ~13.78-13.71 ppm in the starting material **107a-d**. This observation confirmed that indeed the nucleophilic substitution by the N-H (3) of the rhodanine **85** on the starting material ethyl 2-(2-bromoacetamido) esters **107a-d** was successful. In addition, there was a shifts of singlet signals **H-7** integrated for 2H appearing at ~ 4.25-4.29 ppm in the ^{1}H spectra of compounds **105a-j.** These signals were compared with starting material ethyl 2-(2-bromoacetamido) esters **107a-d** where it appeared at ~ 3.75-3.89 ppm and this confirmed that indeed the products was obtained.

^{13}C NMR spectra of compounds **105a-j** were characterized by a total of ~16-20 signals. The ^{13}CNMR spectra of the compounds **105a-j** exhibited a change for the **C-7** signal which was observed at ~ 37.15 ppm as compared to the starting material ethyl 2-(2-bomoacetamido) esters **107a-d** where it previously appeared at ~ 48.78 ppm. The IR spectra of all the final novel compounds **105a-j**, showed characteristic peaks for N-H stretching in the range of ~ 3309-3302 cm^{-1}, and C=O stretching in the range of ~1738-1729 cm^{-1} and C=S stretching in the range of ~1098-1021 cm^{-1}. HRMS of compounds **105a-j** provided further evidence of the successful synthesis of the compounds and the data is shown in **Table 14**.

4.2. Biology

As part of the aim of biological evaluation of synthesized rhodamine-containing compounds, *In vitro* cytotoxicity and antidiabetic screening of the synthesized intermidiates **111a-g** and target compounds **105a-j** were done in a similar manner as described in the biology of glitazone chapter 2, section 2.2.

4.2.1. *In vitro* cytotoxic evaluation

Cytotoxicity evaluation of the intermediates **111a-g** and the target compounds **105a-j** was performed against human colorectal adenocarcinoma cell line, (CaCo-2) cell line 100 µM. Among the synthesized intermediates **111a-g**, results revealed that unsubstituted phenyl derivative **111a** with living cells of 2716±289 µM, piperonyl derivative **111e** exhibiting 2372±172 µM, *para* fluoro phenyl derivative **111f** with cell

living of 2464±132 µM and 3-hydroxy-4-methoxy phenyl derivative **111g** with living cell of 2868±132 µM were nontoxic, with the rest of intermediates being toxic. Among the synthesized final compounds **105a-j,** only three final compounds, *para* fluoro phenyl alaninate derivative **105b** exhibiting cytotoxicity of 2982±112 µM, *para* nitro phenyl butanoate derivative **105f** with live cells of 2551±158 µM and *para* fluorophenyl butanoate derivative **105g** exhibiting cytotoxicity of 2551±1864 µM were found to be nontoxic. (**Table 15**) All the other derivatives among the synthesized ethyl-2-(2-(5-(4-arylidene)-2-oxo-4-thioxothiazolidin-3-yl) acetamido) esters **105a-j** were found to be toxic.

Table 15: Cytotoxicity results of intermediates **111a-g** and target compounds **105a-j**

Compounds	Number of live cells (µM)
111a	2716±289
111b	965±229
111c	953±180
111d	1210±125
111e	2372±172
111f	2464±132
111g	2868±132
105a	295±510
105b	2982±112
105c	1936±158
105d	370±580
105e	318±570
105f	2551±158
105g	2551±1864
105h	1052±101
105i	1436±177
105j	-
melphalan	397±730
UT control	2812±237

4.2.2. *In vitro* α-glucosidase evaluation

All the synthesized intermediates **111a-g** and target compounds **105a-j** were evaluated for their *in vitro* α-glucosidase inhibition using standard methods in comparison with the marketed standard drug EGCG. The results obtained were summarised in **Table 16**. The results for synthesized intermediates **111a-g** showed that the caused no α-glucosidase inhibition at 10 µM and 50 µM. Among the synthesized 5-arylidene-rhodanine **111a-g**, only unsubstituted phenyl-containing derivative **111a** and piperonyl derivative **111e** exhibited weak inhibitions of 22.90±7.31% and 22.70±10.20% respectively. At 200 µM, unsubstituted phenyl derivative **111a** was the most active compound with an improved α-glucosidase inhibition of 50.44±1.31% followed by furanyl derivative **111g** exhibiting 47.49±1.89%. Piperonyl derivative **111e** also showed an increased α-glucosidase inhibition of 46.67±0.54, whereas 3-hydroxy-4-methoxy phenyl derivative **111g** exhibited α-glucosidase inhibition of 44.49±1.28%.

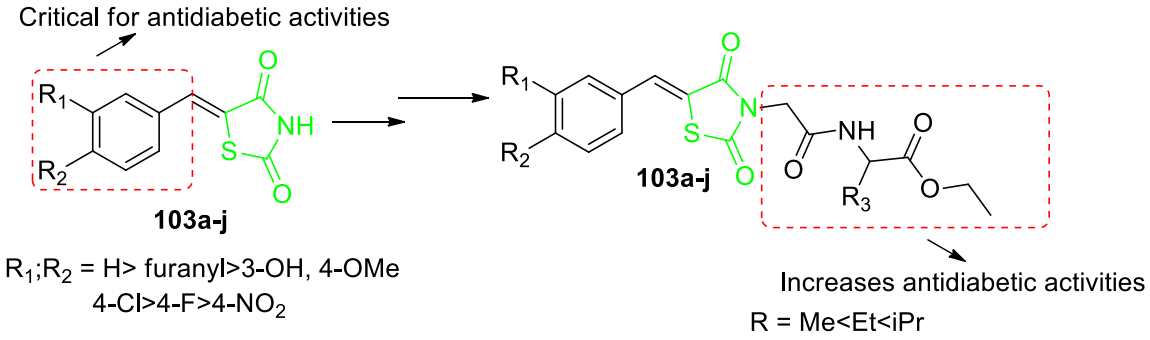
All the synthesized compounds **105a-j** did not show any α-glucosidase inhibition at 10 µM, 50 µM and 100 µM.

Among the synthesized alaninates **105a-d**, electron-poor *para* chloro phenyl derivative **105a**, nitro phenyl derivative **105c** and electron-rich piperonyl derivative **105d** showed weak α-glucosidase inhibition of 30.00±23.90%, 21.60±15.50%, 36.20±11.00 % and 30.00±23.90% respectively at 100 µM. At highest concentration of 200 µM, surprisingly electron-poor *para* fluoro phenyl derivative **105b** was the most active, exhibiting 42.88±4.33% followed, by the *para* chloro phenyl derivative **105a** which exhibited 40.20±1.65%.

Among the synthesized butanoates **105e-h**, only the electron-poor *para* nitro phenyl derivative **105f** exhibited weak activity of 34.80±14.20% at 100 µM. As the concentration was increased to 200 µM, the α-glucosidase activity of *para* nitrophenyl alaninate **105f** moderately increased to 51.32±3.62%, which was the highest among all the synthesized compounds **105a-j**. Following this compound was the *para* fluoro phenyl derivative **105g** exhibiting weak α-glucosidase inhibition of 36.12±3.37%.

In addition, *para* nitro phenyl valinate derivative **105i** exhibited weak α-glucosidase inhibition of 20.80±13.90%, with no α-glucosidase activity observed at 200 µM.

Generally, among the synthesized intermediates **111a-g**, unsubstituted phenyl derivative **111a** exhibited highest α-glucosidase activity of 50.44±1.31%, as compared to the rest of the intermediates. From these results which assisted in constructing structure activity relationship (SAR), it was deduced that introduction of different derivatives at position 3 and 4 of the phenyl rings plays no role in the α-glucosidase activities. **(Scheme 76)** Among the synthesized target rhodanine-containing compound **105a-j**, butanoate derivative **105f** exhibited highest activity of 51.32±3.62% as compared to alaninate derivative **105b** and alaninate derivative **105c** exhibiting 42.88±4.33% and 40.20±1.65% respectively. This suggested that the α-glucosidase increases as the amino acid chain increases from methyl to propyl.



Scheme 76: *SAR evaluation of intemidiates* ***111a-g*** *and target compouds* ***105a-j***

4.3. Conclusion

A series of ten novel ethyl-2-(2-(5-(4-substitutedarylidene)-2-oxo-4-thioxothiazolidin-3-yl) acetamido) esters **105a-j (Scheme 75)** was successfully synthesized using known conventional methods. Five novel alaninates **105a** with a final yield of 32%, **105b** (68%), **105c** (92%) and **105d** (65%), **105f** (99%) and **105g** (30%), two butanoates **105e** (40%) and **105h** (33%), one valinate **105i** (88%) and norvalinate **105j** (63%) were obtained in their unoptimized yields. *In vitro* antidiabetic assay results showed that *the para* nitro phenyl butanoate **105f** exhibited the highest moderate α-glucosidase activities of 51.32±3.62% followed by *para* fluoro phenyl alaninate **105b** exhibiting 42.88±4.33% and *para* nitrophenyl alaninate **105c** exhibiting 40.20±1.65% at 200 µM.

4.4. SAR summary of glitazone, hydantoin and rhodanine

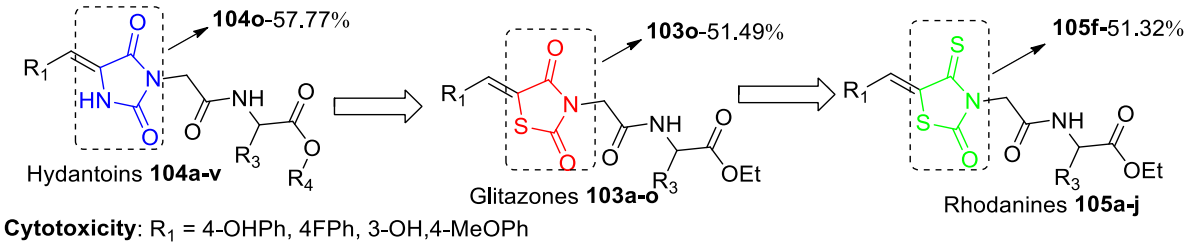
4.4.1. Cytotoxicity comparison.

Cytotoxicity screening results of all the libraries of **103a-o**, **104a-v** and **105a-j** (**Scheme 77)** has revealed that the *para* hydroxy glitazone **103d**, vallinate glitazone **103m**; piperonyl hydantoin **104m**, vallinate hydantoin **104u** and *para* fluoro hydantoin **104t** as well as *para* fluoro rhodhanine **105b** were found to be toxic**.** This implied that the introduction of benzodioxole, hydroxy and fluoro groups at para position as well as exchanging hydrogens at position 3 and 4 with 3-hydroxy-3-methoxy derivatives were associated with cytotoxicity effects.

4.4.2. α-Glucosidase inhibition comparison

The α-glucosidase inhibition showed that hydantoins **104a-v** series had the highest inhibition which was exhibited by piperonyl norvalinate **104o** (57.77%) followed by glitazone series with furanyl vallinate **103o** exhibiting 51.49% and lastly rhodanine series with *para* nitro butanoate **105f** exhibiting (51.32%) **(Scheme 77)**. From these results we have deduced that increasing amino acid chain induces α-glucosidase activity whereas the presence of oxygen on the phenyl ring plays some role in the α-glucosidase activity.

All compounds with negative α-glucosidase inhibition values were considered not active and this was due to solubility issues. However, even if the syntheses of libraries of **103a-o**,**104a-v** and **105a-j** did not produce compounds with significant α-glucosidase inhibition, however, they can be used for the development of a new series of α-glucosidase inhibitors.



Scheme 77**: SAR comparison of glitazones **103a-o**, hydantoins **104a-v** and rhodanines **105a-j

Table 16: *α-glucosidase inhibition data of intermediates* ***111a-g*** *and final compounds* ***105a-j*** *(%)*

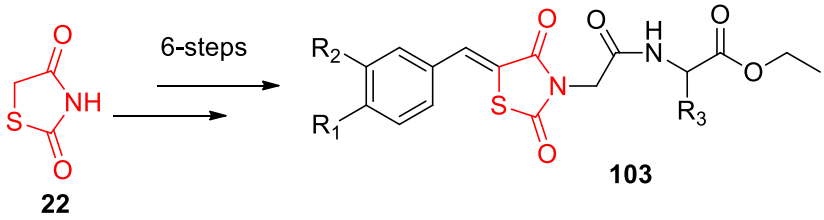
Compounds	10 µM	50 µM	100 µM	200 µM
111a	-5.33±14.90	11.90±11.70	22.90±7.31	50.44±1.31
111b	-13.50±12.70	-13.50±12.70	-8.55±14.10	27.24±6.47
111c	-4.68±10.70	-8.66±12.40	-11.20±18.80	31.83±0.98
111d	-10.90±14.80	16.50±13.90	-8.55±14.10	26.93±2.54
111e	-9.67±13.20	7.25±13.00	22.70±10.20	46.67±0.54
111f	-8.77±13.10	-8.04±15.60	-4.31±17.20	44.49±1.28
111g	-10.70±12.20	-10.10±11.80	1.56±8.10	47.49±1.89
105a	-12.10±9.46	3.87±6.07	21.60±15.50	40.20±1.65
105b	-15.20±14.90	-3.09 ±7.98	1.13±12.60	42.88±4.33
105c	-5.14±8.86	17.10±3.86	36.20±11.00	35.68±2.19
105d	-1.38±2.83	24.40±7.26	30.00±23.90	27.03±2.06
105e	-13.90±16.20	-7.10±11.40	12.80±11.20	28.39±5.65
105f	-5.14±11.90	19.50±10.70	34.80±14.20	51.32±3.62
105g	-6.62±6.54	8.00±11.40	14.60±5.82	36.12±3.37
105h	-7.15±8.91	-0.67±2.58	6.90±5.15	-7.20±2.90
105i	-11.20±18	3.62±13.20	20.80±13.90	-
105j	-	-	-	-
EGCG	97.03±0.41	97.03±0.41	97.03±0.41	97.03±0.41

Chapter V

5. Overall conclusion

This chapter looks at the main general conclusion of this study. The study has explored a series of glitazones, hydantoins as well as rhodanines analogous. The chemistry and biology of the target synthesized compounds were studied extensively and gave promising % α-glucosidase inhibition results.

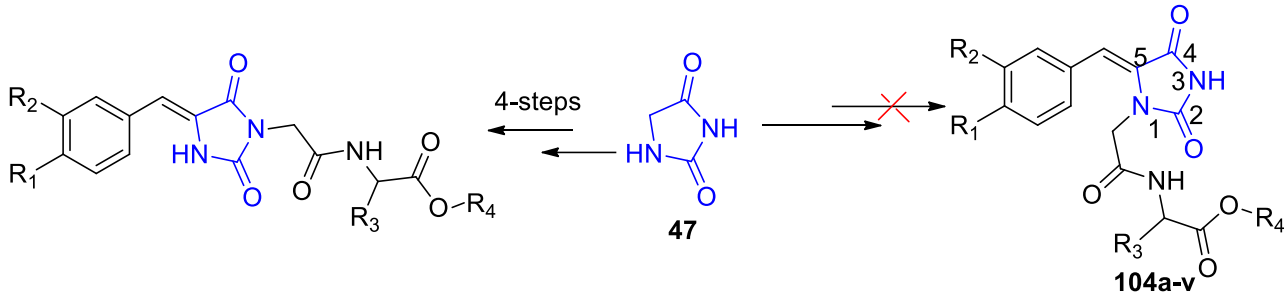
5.1. Synthesis of novel glitazone containing compounds



Scheme 78: *Synthesis of novel ethyl (2-(5-arylidene-2,4-dioxothiazolidin-3-yl)acetamido) esters*

The first part of this study involved the synthesis of novel ethyl (2-(5-arylidene-2,4-dioxothiazolidin-3-yl)acetamido) esters **103** with variations at position 3 and 4 of the phenyl ring nucleus and extended amino acid chain. **(Scheme 78)**. This was to determine if these change influence anti-diabetic and cytotoxicity activity. The compounds obtained were synthesized using known conventional methods with Knoevenagel condensation as the final step. The synthesis produced a series of new ethyl (2-(5-arylidene-2,4-dioxothiazolidin-3-yl)acetamido) esters **103a-o** (6-65%) via six synthetic step protocols from the commercially available 2,4-thiazolidinediones Most of the newly synthesized compounds did not show any significant anti-diabetic activities at 10 µM, 50 µM, 100µM concentrations. However, compounds **103a**, **103d**, **103f**, **103h**, **103m** and **103o** showed an antidiabetic activity of 33.38±5.65%, 37.69±0.39%, 32.66±4.31%, 29.31±3.09% and 31.83±2.85% respectively at the highest concentration (200 µM).

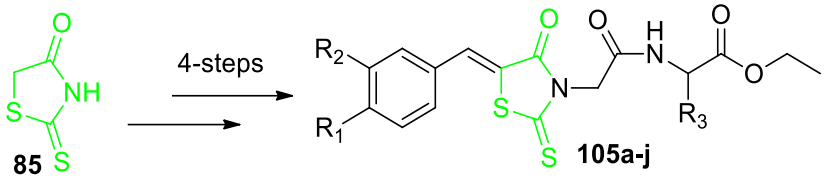
5.2. Synthesis of novel hydantoin containing compounds



Scheme 79: *Synthesis of novel ethyl or methyl (2-(5-(4-substituted benzylidene)-2,5-dioxoimidazolidin-1-yl) acetyl) esters*

The next task was of the project was to prepare a novel series of ethyl or methyl (2-(5-(4-substituted benzylidene)-2,5-dioxoimidazolidin-1-yl) acetyl) esters **104a-v**. This was achieved by starting from commercially available hydantoin **47** via four synthetic steps using known conventional methods **(Scheme 79)**. At the final synthetic step, surprisingly the nucleophilic substitution did not take place at the most acidic at N-3 position as expected but rather at the most highly reactive N-1 position of the hydantoin moiety due to the neighbouring double bonds to give a new series of novel ethyl or methyl (2-(5-(4-benzylidene)-2,5-dioxoimidazolidin-1-yl) acetyl) esters **104a-v** in yields ranging from 24-63%. All our newly synthesized ethyl or methyl (2-(5-(4-benzylidene)-2,5-dioxoimidazolidin-1-yl) acetyl) esters **104a-v** were subjected to antidiabetic screening against the α-glucosidase enzyme. All compounds did not show promising antidiabetic activity at the concentrations of 10 µM, 50 µM and 100 µM. Compounds **104a**, **104c**, **104d**, **104e**, **104f**, **104g**, **104h**, **104i**, **104l**, **104o**, **104p**, **104q**, **104r** and **104t** exhibited anti-diabetic activities of 51.65±2.92%, 45.23±3.60%, 42.76±20%, 44.58±0.99%, 52.65±2.83%, 48.47±2.33%, 41.94±3.31%, 51.65±2.92%, 54.21±1.55%, 57.41±2.79%, 57.77± 2.79%, 56.93±1.58%, 43.78±0.89% and 47.38±0.89% respectively at 200µM.

5.4. Synthesis of rhodanine containing compounds



Scheme 80*: Synthesis of novel ethyl-2-(2-(5-(4-substitutedarylidene)-2-oxo-4-thioxothiazolidin-3-yl) acetamido) esters*

This project was completed by the synthesis of a series of novel ethyl-2-(2-(5-(4-arylidene)-2-oxo-4-thioxothiazolidin-3-yl) acetamido) esters **105a-j**. The synthesis began by reacting commercially available rhodanine **85** with various aldehydes via a four-step synthetic protocol using conventional known methods **(Scheme 80)**. After applying the final nucleophilic substitution step, ethyl-2-(2-(5-(4-arylidene)-2-oxo-4-thioxothiazolidin-3-yl) acetamido) esters **105a-j** were obtained successfully with the yields ranging from 3-88%. All the newly synthesized compounds **105a-j** did not show any anti-diabetic activities at 10 µM, 50 µM and 100 µM. However, compound **105a**, **105b**, **105f** and **105g** showed some activities of 40.20±1.65%, 42.88±4.32%, 51.32±3.62% and 36.12±3.37% at a maximum concentration of 200 µM.

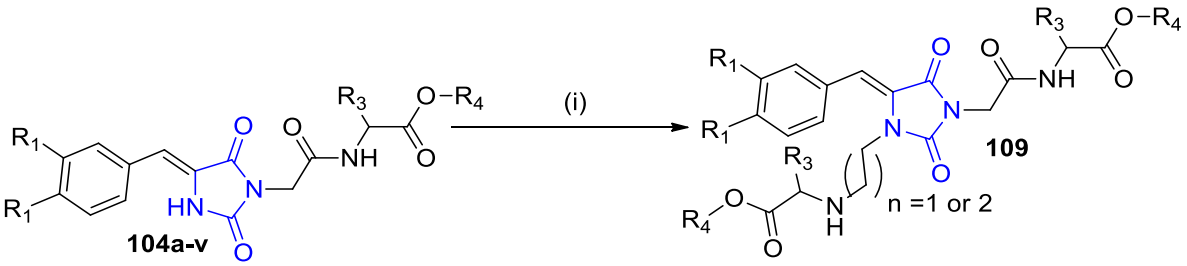
5.5. Overall conclusion and future work of the project

5.5.1. Overall conclusion

This project has explored a new series of novel glitazone **103a-o**, hydantoin **104a-v** and rhodamine **105a-j** containing compounds. The syntheses were achieved using known conventional methods using four to six step synthetic protocols. All the target compounds **103a-o**, **104a-v** and **105a-j** were successfully synthesized with low to high yields. Cytotoxicity screening results of compounds **103a-o**, **104a-v** and **105a-j** revealed that compounds containing oxygen at position 3 and 4 of the phenyl ring were toxic. With regards to the antidiabetic screening, hydantoin analogues **104a-v** was most active derivative displaying moderate α-glucosidase activities followed by glitazone **103a-o** and rhodanine **105a-j** conjugates.

5.5.2. Future work

The future aim of this project will be to synthesize a new library of hydantoin **109** as anti-diabetic agents. The synthesis will be achieved by using dibromoalkanes as a linker instead of bromoacetyl chloride at N-H (1) position. **(Scheme 81).**



Scheme 81: *Synthesis of envisaged hydantoin* ***109****;* ***Reagents and conditions*** *(i) NaH, DMF,rt*

Chapter VI

6. Experimental procedures

This chapter describes the general synthetic methods and characterisation techniques used in preparing and characterising compounds.

6.0. General procedure

All reagents used were analytical grade reagents from Sigma-Aldrich and Fluka. Thin layer chromatography (TLC) was carried out using Macherey-Nagel Alugram Sil G/UV_{254} plates, pre-coated with 0.25 mm silica gel 60. Detection was done under ultraviolet light at 254 nm.

For column chromatography, Macherey-Nagel silica gel (32–63 µm) was used, with silica gel mass 30 times that of sample, eluting with stated solvent mixtures. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker 400 MHz spectrometer using DMSO-d_6, $CDCl_3$ or D_2O as solvents and TMS at 0.00 ppm as an internal standard. Values for the chemical shifts are expressed in parts per million (ppm).

The following abbreviations are used: br.s for broad singlet, s for singlet, d for doublet, dd for doublet of doublets, q for quartet, quint for quintet and m for multiplet and coupling constant (*J*) measured in hertz (Hz). All the melting points were determined on a Buchi melting point B-540 apparatus using capillary tubes and were uncorrected.

Infrared spectra were run on a Bruker platinum 22 vector Fourier Transform spectrometer (FTIR). Mass spectra (High Resolution) were recorded on a Waters GCT using a column called the Restek Rxi Wintegra Guard (15 m, 0.25 mm ID, 0.25 µm film thickness) mass spectrometer. Samples were dissolved in a mixture of acetone and dichloromethane and injected at a volume of 1 µL at mode of 10:1 at a temperature of 280 °C. The source temperature was 100 °C and the desolvation temperature was set at 300 °C. Helium gas was used as the carrier gas. The software used to control the hyphenated system and to do all data manipulation was Masslynx 4.1 (SCN 704).

6.0.1. Thin Layer Chromatography

Thin layer chromatography (TLC) was used to monitor the reactions using aluminium backed Macherey-Nagel ALUGRAM Sil G/UV254 plates or Aldrich or Merck TLC plates, silica gel on aluminium. The most used solvent system was a mixture of hexane and ethyl acetate. Spray reagents or stains were used on thin layer chromatography plates for the detection of compounds that were not highly UV active, commonly, vanillin reagent, which was prepared by a solution of 15 g vanillin in 250 mL ethanol and 2.5 mL conc. sulfuric acid utilized for staining.

6.0.2. Purification of compounds by recrystallization techniques

Synthesized novel final compounds were purified using recrystallization methods. The solvent systems used were methanol, acetone and mixture of hexane and ethyl acetate (3:1)

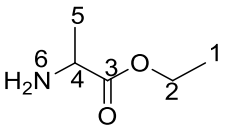
6.0.3. Nomenclature of compounds

Compounds prepared during the course of this project are named in the following experimental sections according to systematic nomenclature wherever possible. However, the numbering system used to illustrate the diagrams of these compounds is one adopted for convenience and is not meant to reflect the systematic numbering of these compounds.

6.1. General Method for esterification of amino acids (108a-d)

A solution of an amino acid (1 mmol) in ethanol was cooled to 0-5 ℃ in an ice bath. To this reaction mixture was added thionyl chloride (1 mmol) dropwise. The mixture was then allowed to warm up to room temperature before heated at reflux for 12 hours. The resulting mixture was allowed to cool down to room temperature after which a colourless liquid (**108a, 108b and 108d**) together with a light brown liquid (**108c**) were obtained at reduced pressure. These compounds were used without further purification.

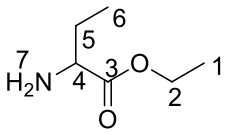
6.1.1. Ethyl propanoate (108a)



108a

A mixture of alanine (5.03 g, 56.1 mmol) and thionyl chloride (4.09 ml, 56.1 mmol) in ethanol (50 ml) gave compound **108a** as a colourless liquid[170] (4.80 g, 70%), **^{1}H NMR (400 MHz, $CDCl_3$)** δ_H (ppm) 7.58 (d, 2H, *J* = 6.8 Hz, H-6), 4.19-4.16 (m, 1H, H-4) 3.74 (q, 2H, *J* = 6.6 Hz, H-2), 1.10 (d, 3H, *J* = 7.2 Hz, H-5), 1.05 (t, 3H, *J* = 7.0 Hz, H-1); **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C (ppm) 170.27 (C-3), 62.14 (C-2), 58.3 (C-4), 16.17 (C-5), 14.36 (C-1) : **IR** (KBr cm^{-1}): 3385 (N-H), 2978 (C-H), 1720 (C=O).

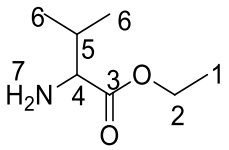
6.1.2. Ethyl butanoate (108b)



108b

A reaction of alpha amino butyric acid (5.02 g, 48.51 mmol) and thionyl chloride (3.51 ml, 48.51 mmol) in ethanol gave compound **108b** as a brown oil[171] (5.91 g, 93%), **^{1}H NMR (400 MHz, $CDCl_3$)** δ_H (ppm) 8.67 (d, 2H, *J* = 7.8 Hz, H-7), 4.22 (t, 1H, *J* = 7.2 Hz, H-4), 3.99 (q, 2H, *J* = 6.4 Hz, H-2), 1.89 (quint, 2H, *J* = 7.2 Hz, H-5), 1.19 (t, 3H, *J* = 7.2 Hz, H-1), 0.91 (t, 3H, *J* = 7.6 Hz, H-6), **^{13}C NMR (100 MHz, $CDCl_3$)** δ_C (ppm) 170.30 (C-3), 63.47 (C-2), 54.07 (C-4), 23.26 (C-5), 13.23 (C-1), 8.43 (C-6), **IR** (KBr cm^{-1}): 3397 (N-H), 1761 (C=O), 1171 (C-O).

6.1.3. Ethyl valinate (108c)

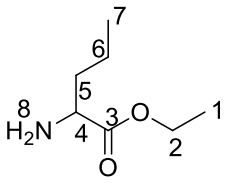


108c

A reaction of valine (10.03 g, 85.41 mmol) and thionyl chloride (5.01 ml, 85.41 mmol) in ethanol gave compound **108c** as a light brown oil[172] (9.88 g, 79 %), **^{1}H NMR (400 MHz, $CDCl_3$)** δ_H (ppm) 8.58 (d, 2H, *J* = 8.0 Hz, H-7), 4.17 (q, 1H, *J* = 5.6 Hz, H-4),

3.93 (q, 2H, *J* = 10.8 Hz, H-2), 2.06-2.04 (m, 1H, H-5), 1.21 (t, 3H, *J* = 7.2 Hz, H-1), 0.87 (d, 6H, *J* = 3.2 Hz, H-6), **^{13}C NMR (100 MHz, CDCl$_3$) δ$_C$** (ppm) 171.31 (C-3), 62.09 (C-2), 58.07 (C-4), 29.32 (C-5), 19.27 (C-6), 18.39 (C-6), 14.30 (C-1), **IR** (KBr cm^{-1}): 3352 (N-H), 1745 (C-3), 1231 (C-O).

6.1.4. Ethyl norvalinate (108d)



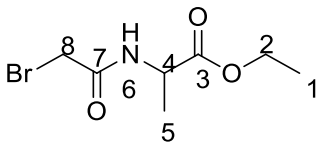
108d

A reaction of norvaline (10.01 g, 85.41 mmol) and thionyl chloride (5.01 ml, 85.41 mmol) in ethanol gave compound **108d** as a colourless oil[150] (9.76 g, 79%), **^{1}H NMR (400 MHz, CDCl$_3$) δ$_H$** (ppm) 8.66 (d, 2H, *J* = 6.2 Hz, H-8), 4.22 (t, 1H, *J* = 4.8 Hz, H-4), 4.12 (q, 2H, *J* = 7.2 Hz, H-2), 1.70– 1.61 (m, 2H, H-5), 1.36-1.35 (m, 2H, H-6), 1.21 (t, 3H, *J* = 6.8 Hz, H-1), 0.89 (t, 3H, *J* = 7.2 Hz , H-7), **^{13}C NMR (100 MHz, CDCl$_3$) δ$_C$** (ppm) 172.18 (C-3), 60.99 (C-2), 52.54 (C-4), 29.31 (C-5), 27.64 (C-6), 14.48 (C-1), 13.85 (C-7), **IR** (KBr cm^{-1}): 3397 (N-H), 1732 (C=O), 1121 (C-O).

6.2. General procedure for synthesis of ethyl (2-bromo acetamido) esters (107a-d)

A solution of bromoacetyl chloride (1 mmol) in a mixture of dichloromethane and water was added to a cooled mixture (-10 °C) of the corresponding protected amino acids (1 mmol) and potassium carbonate (3 mmol) in DCM. The resulting mixture was allowed to warm up to room temperature, before being stirred for 16 hours at the same temperature. After the completion of the reaction the organic layer was extracted and washed with water (2 x 30 ml) and brine (1 x 30 ml), dried with $MgSO_4$ and concentrated on a rotary vapour. The crude products were recrystallized from ethyl acetate to afford the corresponding bromo acetamide derivatives (**107a-d**).

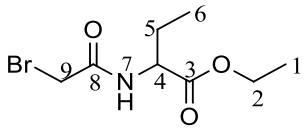
6.2.1. Ethyl 2-(2-bromoacetamido) alaninate (107a)



107a

Ethyl propanoate **108a** (5.05 g, 42.71 mmol) reacted with bromo acetylchloride (3.56 ml, 42.7 mmol) in a mixture of DCM (20 ml) and water (20 ml) to give compound **107a** as a white solid (7.60 g, 98 %), mp = 82.2-83.6 °C (lit. m.p. = 71-74 °C);[170] **^{1}H NMR (400 MHz, CDCl$_3$) δ_H** (ppm) 7.19 (d, 1H, *J* = 8.0 Hz, H-6), 4.56 (quint, 1H , *J* = 7.2 Hz, H-4), 4.22 (q, 2H, *J* = 7.1 Hz, H-2), 3.89 (s, 2H, H-8), 1.45 (d, 3H, *J* = 3.2 Hz, H-5), 1.29 (t, 3H, *J* = 4.5 Hz, H-1); **^{13}C NMR (100 MHz, CDCl$_3$) δ_C** (ppm) 172.36 (C-7), 165.09 (C-3), 61.74 (C-2), 48.78 (C-4), 28.72 (C-8), 18.27 (C-5), 14.09 (C-1): **IR** (KBr cm^{-1}): 3396 (N-H), 1723 (C=O), 1646 (C=O), 1541 (C-O).

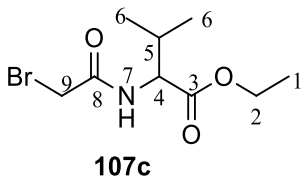
6.2.2. Ethyl 2-(2-bromoacetamido) butanoate (107b)



107b

Ethyl butanoate **108b** (2.02 g, 15.24 mmol) reacted with bromoacetyl chloride (1.27 ml, 15.24 mmol) was added in a mixture of DCM (30 ml) and water (30 ml) to give compound **107b** as a brown oil[146] (3.01 g, 95 %), **^{1}H NMR (400 MHz, CDCl$_3$) δ_H** (ppm) 7.17 (d, 1H, *J* = 6.8, Hz, H-7), 4.45 (quint, 1H, *J* = 4.0 Hz, H-4), 4.21-4.03 (m, 2H, H-2), 3.84 (s, 2H, H-9), 1.81-1.91 (2H, m, H-5), 1.22 (t, 3H, *J* = 6.0 Hz, H-1), 0.86 (t, 3H, *J* = 7.2 Hz, H-6); **^{13}C NMR (100 MHz, CDCl$_3$) δ_C** (ppm) 171.70 (C-8), 165.90 (C-3), 61.47 (C-2), 42.42 (C-4), 28.67 (C-9), 25.28 (C-5), 14.08 (C-1), 9.33 (C-6); **IR** (KBr cm^{-1}): 3389 (N-H), 1746 (C=O), 1670 (C=O), 1548 (C-O).

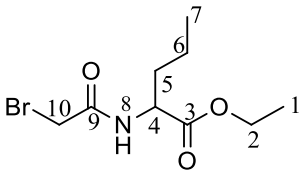
6.2.3. Ethyl 2-(2-bromoacetamido) valinate (107c)



107c

Ethyl valinate **108c** (5.03 g, 34.44 mmol) reacted with bromoacetyl chloride (2.89 ml, 34.44 mmol) in a mixture of DCM (30 ml) and water (30 ml) to give compound **107c** as a colourless oil [146] (6.74 g, 88 %), **^{1}H NMR (400 MHz, $CDCl_3$) δ_H** (ppm) 8.58 (d, 1H, *J* = 8.0, Hz, H-7), 4.17 (dd, 1H , *J* = 3.8 Hz *and J* = 4.0 Hz, H-4), 3.94 (q, 2H, *J* = 3.2 Hz, H-2), 3.74 (s, 2H, H-9), 2.07 (m, 1H, H-5), 1.22 (t, 3H, *J* = 2.4 Hz, H-1), 0.88 (d, 6H, *J* = 3.2 Hz, H-6), **^{13}C NMR (100 MHz, $CDCl_3$) δ_C** (ppm) 171.32 (C-8), 167.39 (C-3), 62.09 (C-2), 58.08 (C-5), 30.58 (C-4), 29.32 (C-9), 19.27 (C-6), 18.39 (C-6), 14.52 (C-1); 3391 (N-H), 1724 (C=O), 1663 (C=O), 1497 (C-O).

6.2.4. Ethyl 2-(2-bromoacetamido) norvalinate (107d)



107d

Ethyl norvalinate **108d** (12.20 g, 84.13 mmol) reacted with bromoacetyl chloride (6.94 ml, 84.1 mmol) in a mixture of DCM (30 ml) and water (30 ml) to give compound **107d** as a colourless oil [146] (14.13 g, 76%), **^{1}H NMR (400 MHz, $CDCl_3$) δ_H** (ppm) 8.68 (d, 1H, *J* = 3.6 Hz, H-8), 4.23-4.14 (m, 1H, H-4), 4.13-4.08 (m, 2H, H-2), 3.90 (d, 2H, *J* = 4.0 Hz, H-10), 1.70-1.64 (2H, m, H-6), 1.38-1.31 (m, 2H, H-5), 1.22 (t, 3H, *J* = 2.0 Hz, H-1); 0.88 (t, 3H, *J* = 7.2 Hz, H-7), **^{13}C NMR (100 MHz, $CDCl_3$) δ_C** (ppm) 172.18 (C-9), 166.68 (C-3), 62.10 (C-2), 52.54 (C-4), 33.40 (C-6), 29.32 (C-10), 18.92 (C-5), 14.48 (C-1), 13.85 (C-7); 3393 (N-H), 1765 (C=O), 1689 (C=O), 1573 (C-O)

6.5. Synthesis of potassium salt of glitazone (109).

109

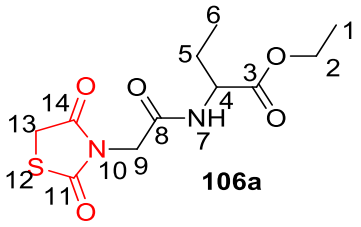
To a hot solution of 1,3-thiazolidine-2,4-dione (5.04 g, 42.70 mmol) in ethanol (50 ml) was added a solution of KOH (2.40 g, 42.70 mmol) in ethanol (50 ml) and the mixture was stirred at 70 °C for one hour. After this reaction time the mixture was allowed to cool to room temperature before being cooled in an ice bath. While cooling in ice, a white precipitate was formed which was collected by filtration and washed with cold

ethanol and dried to afford the potassium salt of 1,3-thiazolidine-2,4-dione (**109**) as a white solid (7.80 g, 78%); m.p = 260-266 °C (lit m.p = 247-250 °C);[173] **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$** (ppm) 4.14 (s, 2H, H-5); **^{13}C NMR (100 MHz, DMSO-d$_6$), δ$_C$** (ppm) 174.26 (C-2), 173.43 (C-4), 36.12 (C-5).

6.6. General procedure for synthesis of ethyl 2-(2,4-dioxothiazolidin-3-yl) acetamido esters (106a-c)

A mixture of potassium salt of 1,3-thiazolidine-2,4-dione **109** (1 mmol) and ethyl (2-bromoacetamido) derivatives **107a-c** (1 mmol) in tetrahydrofuran (THF) were heated at reflux for 12 hours. After cooling to room temperature, the mixtures were filtered through a cotton wool and the filtrates were concentrated on a rotary evaporator to provide the desired corresponding ethyl 2-(2,4-dioxothiazolidin-3-yl) acetyl derivatives **106a-c**, which were used without further purification.

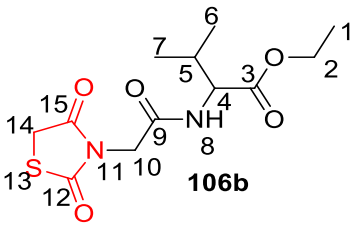
6.6.2. Ethyl 2-(2-(2,4-dioxothiazolidin-3-yl)acetamido)butanoate (106a).



106a

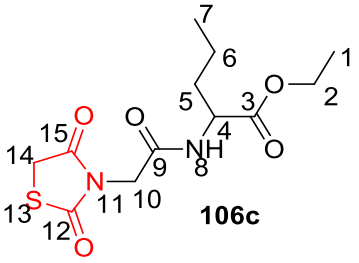
A reaction of potassium salt of 1,3-thiazolidine-2,4-dione **109** (4.50 g, 21.71 mmol) and **107b** (2.54 g, 21.7 mmol) gave compound **106a** as a white solid (5.43 g, 79%); m.p = 109-110 °C; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$ (ppm)** 6.55 (d, 1H, *J* = 6.4 Hz, H-7), 4.35 (quint, 1H, *J* =7.2 Hz, H-4), 4.24 (s, 2H, H-9), 4.23 (q, 2H, *J* =4.0 Hz, H-2), 4.06 (s, 2H, H-13), 1.94-1.86 (m, 1H, H-5a), 1.79-1.68 (m, 1H, H-5b), 1.30 (t, 3H, *J* =8.0 Hz, H-1), 0.91 (t, 3H, *J* =7.6 Hz, H-6); **^{13}C NMR (100 MHz, DMSO-d$_6$), δ$_C$ (ppm)** 172.13 (C-14), 171.51 (C-11), 171.04 (C-8), 165.89 (C-3), 60.90 (C-2), 58.04 (C-4), 43.34 (C-9), 34.37 (C-13), 30.61 (C-5), 19.29 (C-6), 18.45 (C-5), 14.51 (C-1); **IR** (KBr cm^{-1}): 3279 (NH), 3077 (C-H), 1738 (C=O), 1682 (C=O), 1298 (C-O)

6.6.3. Ethyl (2-(2,4-dioxothiazolidin-3-yl)acetamido)valinate (106b).



A reaction of potassium salt of 1,3-thiazolidine-2,4-dione **109** (3.79 g, 32.36 mmol) and **107c** (7.16 g, 32.3 mmol) gave compound **106b** as a white solid (7.91 g, 81%); m.p = 118-120 °C; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$ (ppm)** 8.57 (d, 1H, *J* =7.6 Hz, H-8), 4.28 (s, 2H, H-10), 4.16 (quint, 1H, *J* =8.4 Hz, H-4), 4.12 (q, 2H, *J* =4.0 Hz, H-2), 4.09 (s, 2H, H-14), 2.04-2.03 (m, 2H, H-5), 1.19 (t, 3H, *J* =6.4 Hz, H-1), 0.87 (s, 6H, H-6, H-7); **^{13}C NMR (100 MHz, DMSO-d$_6$), δ$_C$ (ppm)** 172.33 (C-15), 171.98 (C-12), 171.60 (C-9), 165.92 (C-3), 61.94 (C-2), 58.98 (C-4), 43.25 (C-10*)*, 34.36 (C-14), 30.60 (C-5), 19.62 (C-7), 18.46 (C-6), 14.55 (C-2); **IR** (KBr cm^{-1}): 3262 (NH), 3080 (C-H), 1736 (C=O), 1685 (C=O). 1214 (C-O)

6.6.3. Ethyl (2-(2,4-dioxothiazolidin-3-yl)acetamido)norvalinate (106c).

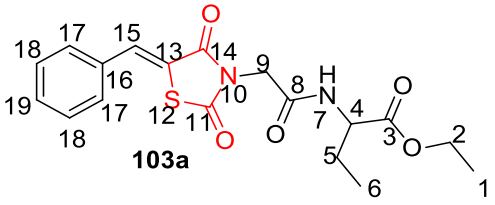


A reaction of potassium salt of 1,3-thiazolidine-2,4-dione **109** (3.97 g, 33.89 mmol) and **107d** (7.50 g, 33.8 mmol) gave compound **106c** as a white solid (8.08 g, 81%); m.p = 119-120 °C; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$ (ppm)** 8.62 (d, 1H, *J* =6.4 Hz, H-8), 4.27 (s, 2H, H-10), 4.22 (quint, 1H, *J* =8.0 Hz, H-4), 4.17 (s, 2H, H-14), 4.08 (q, 2H, *J* =5.6 Hz, H-2), 1.65-1.59 (m, 2H, H-5), 1.31-1.19 (m, 2H, H-6), 1.18 (t, 3H, *J* =6.4 Hz, H-1), 0.87 (t, 3H, *J* =6.4 Hz, H-7); **^{13}C NMR (100 MHz, DMSO-d$_6$), δ$_C$ (ppm)** 172.33 (C-15), 172.97 (C-12), 172.22 (C-9), 165.76 (C-3), 61.99 (C-2), 52.35 (C-4), 43.25 (C-10), 34.39 (C-14), 33.53 (C-5), 18.90 (H-6), 14.46 (C-2), 13.87 (C-7); **IR** (KBr cm^{-1}): 3282, (NH), 3076, (C-H) 1737, (C=O) 1685, (C=O), 1245 (C-O)

4.7. General procedure for the synthesis of ethyl (2-(5-arylidene-2,4-dioxothiazolidin-3-yl)acetamido)ester (103a-o).

To a suspension of compounds **106a-c** (1 mmol) in ethanol was added appropriate aldehydes (1 mmol) followed by catalytic amount of piperidine (10%). The resultant mixture was refluxed for 12 hours. After being allowed to cool down to room temperature, the mixture was poured into ice water and the resultant precipitate collected by filtration. The solids obtained were purified by washing with methanol to afford the desired compounds (**103a-o)**.

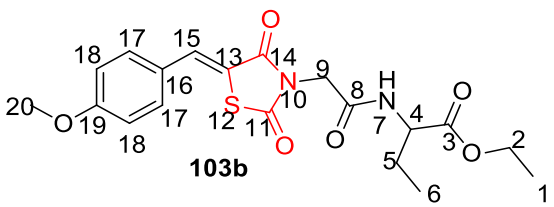
6.7.1. Ethyl 2-(2-(5-benzylidene-2,4-dioxothiazolidin-3-yl)acetamido)butanoate (103a).



103a

A reaction of benzaldehyde (0.32 ml, 3.12 mmol) and **106a** (0.90 g, 3.12 mmol) gave compound **103a** as a white solid (0.16 g, 10%); m.p = 175-176 °C; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$ (ppm)** 8.71 (d, 1H, *J* =7.6 Hz, H-7), 7.97 (s, 1H, H-15), 7.65 (d, 2H, *J* =7.2 Hz, H-17), 7.61-7.50 (m, 3H, H-18+H-19), 4.35 (s, 2H, H-9), 4.20 (quint, 1H, *J* =3.6 Hz, H-4), 4.09 (q, 2H, *J* =3.6 Hz, H-2), 1.76-1.71 (m, 1H, H-5a) 1.68-1.62 (m, 1H, H-5b), 1.23 (t, 3H, *J* =7.2 Hz, H-2), 0.90 (t, 3H, *J* =7.2 Hz, H-6); **^{13}C NMR (100 MHz, DMSO-d$_6$), δ$_C$ (ppm**) 172.02 (C-14), 167.45 (C-11), 165.72 (C-8), 165.13 (C-3), 133.84 (C-15), 133.34 (C-13), 131.22 (C-19), 130.63 (C-18), 129.88 (C-17), 121.56 (C-13), 60.98 (C-2), 54.04 (C-4), 43.60 (C-9), 24.87 (C-5), 14.50 (C-2), 10.56 (C-6); **IR** (KBr cm^{-1}): 3304, 2923, 1735, 1687, 1661, 1607, 1149; **HRMS** (ESI-TOF) m/z; calculated for $C_{18}H_{20}N_2O_5S$+H: 377.1093, found M$^+$+H: 377.1096.

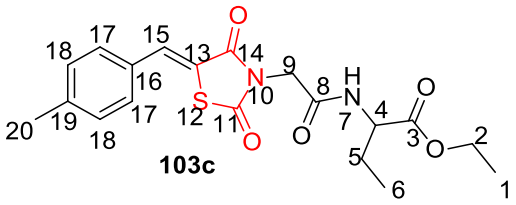
6.7.2. Ethyl 2-(2-(5-(4-methoxybenzylidene)2,4-dioxothiazolidin-3-yl)acetamido)butanoate (103b).



103b

A reaction of *p*-anisaldehyde (0.26 ml, 2.08 mmol) and **106a** (0.60 g, 2.08 mmol) gave compound **103b** as a white solid (0.31 g, 37%); m.p = 207-208 °C; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$ (ppm)** 8.70 (d, 1H, *J* = 7.6 Hz, H-7), 7.93 (s, 1H, H-15), 7.62 (d, 2H, *J* =8.8 Hz, H-18), 7.13 (d, 2H, *J* =8.8 Hz, H-17), 4.15 (s, 2H, H-9), 4.12 (quint, 1H, *J* =2.8 Hz, H-4), 4.07 (q, 2H, *J* =3.6 Hz, H-2), 3.84 (s, 3H, H-20), 1.78-1.73 (m, 1H, H-5a) 1.70-1.61 (m, 1H, H-5b), 1.19 (t, 3H, *J* = 7.2 Hz, H-1), 0.90 (t, 3H, *J* = 7.2 Hz, H-6); **^{13}C NMR (100 MHz, DMSO-d$_6$), δ$_C$ (ppm)** 172.03 (C-3), 167.54 (C-8), 165.80 (C-11+C-14), 161.73 (C-18), 133.83 (C-15), 133.79 (C-18), 125.81 (C-13), 118.24 (C-17), 115.49 (C-16), 60.98 (C-2), 56.00 (C-20), 54.03 (C-4), 43.56 (C-9), 24.88 (C-5), 14.57 (C-1), 10.6 (C-6); **IR** (KBr cm^{-1}): 3297, 2978, 1734, 1685, 1665, 1602, 1183; **HRMS** (ESI-TOF) m/z; calculated for $C_{19}H_{22}N_2O_6S$+H: 407.1199, found M$^+$+H: 407.1272.

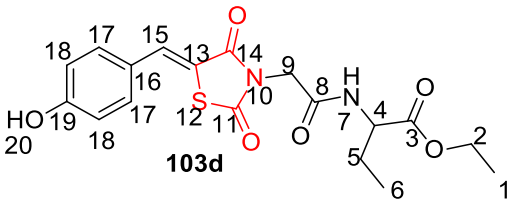
6.7.3. Ethyl 2-(2-(5-(4-methylbenzylidene)2,4-dioxothiazolidin-3-yl)acetamido)butanoate (103c).



103c

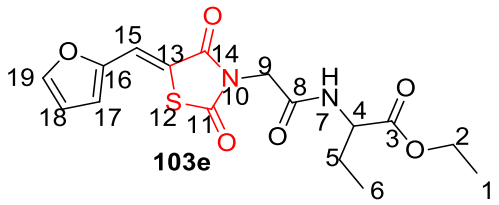
A reaction of *p*-tolualdehyde (0.40 ml, 2.77 mmol) and **106a** (0.80 g, 2.77 mmol) gave compound **103c** as a white solid (0.11 g, 8%); m.p = 206-207 °C; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$ (ppm)** 8.71 (d, 1H, *J* =7.6 Hz, H-7), 7.93 (s, 1H, H-15), 7.55 (d, 2H, *J* =8.0 Hz, H-18), 7.13 (d, 2H, *J* =8.0 Hz, H-17), 4.16 (s, 2H, H-9), 4.14 (quint, 1H, *J* =3.6 Hz, H-4), 4.08 (q, 2H, *J* = 3.6 Hz, H-2), 2.37 (s, 3H, H-20), 1.77-1.71 (m, 1H, H-5a) 1.68-1.62 (m, 1H, H-5b), 1.19 (t, 3H, *J* =7.2 Hz, H-1), 0.90 (t, 3H, *J* =7.2 Hz, H-6); **^{13}C NMR (100 MHz, DMSO-d$_6$), δ$_C$ (ppm)** 172.02 (C-14), 167.49 (C-11), 165.79 (C-8+C-3), 141.75 (C-19), 133.90 (C-15), 133.70 (C-18), 130.59 (C-16), 130.50 (C-17), 120.30 (C-13), 61.98 (C-2), 54.04 (C-4), 43.60 (C-9), 24.87 (C-5), 21.50 (C-20), 14.50 (C-1), 10.56 (C-6); **IR** (KBr cm^{-1}): 3284 (N-H), 2987 (Ar), 1749 (C=O), 1685 (C=O), 1667 (C=O), 1606 (C=O), 1149 (C-O); **HRMS** (ESI-TOF) m/z; calculated for $C_{19}H_{22}N_2O_5S$+H: 390.1249, found M$^+$+H: 391.1251.

6.7.4. Ethyl 2-(2-(5-(4-hydroxybenzylidene)2,4-dioxothiazolidin-3-yl)acetamido)butanoate (103d).



A reaction of 4-hydoxybenzaldehyde (0.42 g, 2.77 mmol) and **106a** (0.80 g, 2.77 mmol) gave compound **103d** as a white solid (0.06 g, 6%); m.p = 185-186 °C; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$ (ppm)** 10.43 (s, 1H, H-20), 8.70 (d, 1H, *J* =7.6 Hz, H-7), 7.86 (s, 1H, H-15), 7.51 (d, 2H, *J* =8.8 Hz, H-18), 6.93 (d, 2H, *J* = 8.8 Hz, H-17), 4.33 (s, 2H, H-9), 4.17 (quint, 1H, *J* =2.8 Hz, H-4), 4.09 (q, 2H, *J* =7.2 Hz, H-2), 1.77-1.71 (m, 1H, H-5a), 1.67-1.60 (m, 1H, H-5b), 1.19 (t, 3H, *J* =7.2 Hz, H-1), 0.90 (t, 3H, *J* =7.2 Hz, H-6); **^{13}C NMR (100 MHz, DMSO-d$_6$), δ$_C$ (ppm)** 172.04 (C-14), 167.66 (C-11), 165.88 (C-8), 165.85 (C-3), 160.67 (C-19, 134.27 (C-15), 133.12 (C-18), 124.26 (C-13), 116.93 (C-17), 116.84 (C-16), 60.99 (C-2), 54.04 (C-4), 43.50 (C-9), 24.86 (C-5), 14.49 (C-1), 10.56 (C-6); **IR** (KBr cm^{-1}): 3287 (NH), 3087 (Ar), 1734 (C=O), 1687 (C=O), 1660 (C=O), 1608 (C=O), 1149 (C-O); **HRMS** (ESI-TOF) m/z; calculated for $C_{18}H_{20}N_2O_6S$+H: 392.1042, found M$^+$+H: 393.1045.

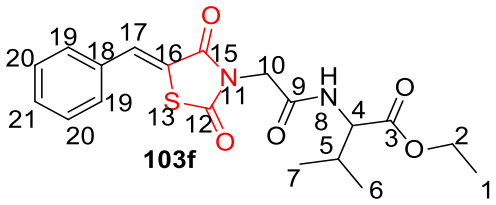
6.7.5. Ethyl 2-(2-(5-(furan-2-ylmethylene)-2,4-dioxothiazolidin-3-yl)acetamido)butanoate (103e)



A reaction of furfural (0.20 ml, 2.43 mmol) and **106a** (0.70 g, 2.43 mmol) gave compound **103e** as a white solid (0.11g g, 11%); m.p = 184-185 °C; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$ (ppm)** 8.69 (d, 1H, *J* = 8.4 Hz, H-7), 8.10 (d, 1H, *J* = 1.2 Hz, H-19), 7.80 (s, 1H, H-15), 7.17 (d, 1H, *J* = 3.6 Hz, H-17), 6.77 (dd, 1H, *J* =3.6 Hz, and *J* =1.6 Hz, H-18), 4.14 (s, 2H, H-9), 4.11 (quint, 1H, *J* = 3.6 Hz, H-4), 4.07 (q, 2H, *J* =3.6 Hz, H-2), 1.77-1.72 (m, 1H, H-5a) 1.71-1.60 (m, 1H, H-5b), 1.18 (t, 3H, *J* = 7.0 Hz, H-1), 0.90 (t, 3H, *J* =7.6 Hz, H-6); **^{13}C NMR (100 MHz, DMSO-d$_6$), δ$_C$ (ppm)** 172.03 (C-14), 168.17 (C-11), 165.79 (C-8), 165.47 (C-3), 149.63 (C-16), 148.41 (C-19), 120.24 (C-15), 119.94 (C-18), 118.93 (C-13), 114.13 (C-17), 60.97 (C-2), 43.48 (C-9), 24.87

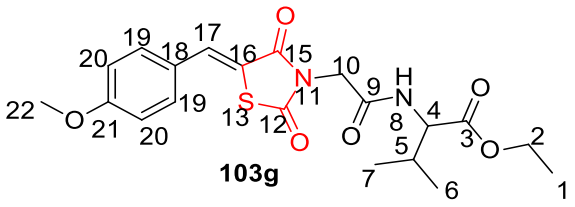
(C-5), 14.49 (C-1), 10.55 (C-6); **IR** (KBr cm^{-1}): 3292 (NH), 2971 (Ar), 1735 (C=O), 1686 (C=O), 1660 (C=O), 1615 (C=O), 1144 (C-O); **HRMS** (ESI-TOF) m/z; calculated for $C_{16}H_{18}N_2O_6S$+H: 367.0886, found M^++H: 367.0889.

6.7.8. Ethyl (2-(5-benzylidene-2,4-dioxothiazolidin-3-yl)acetyl)valinate (103f).



A reaction of benzaldehyde (0.22 ml, 2.32 mmol) and **106b** (0.70 g, 2.32 mmol) gave compound **103f** as a yellow solid (0.26 g, 24%); m.p = 167-168 °C; **^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm)** 8.67 (d, 1H, *J*= 8.4 Hz, H-8), 7.97 (s, 1H, H-17), 7.65 (d, 2H, *J* =7.2 Hz, H-19), 7.58-7.52 (m, 3H, H-20+H-21), 4.39 (s, 2H, H-10), 4.18 (quint, 1H, *J* =6.0 Hz, H-4), 4.12 (q, 2H, *J*=2.4 Hz, H-2), 2.06 (septet, 1H, *J* =4.0Hz, H-5), 1.20 (t, 3H, *J* =4.8 Hz, H-1), 0.90 (t, 6H, *J* =6.4 Hz, H-6+H-7); **^{13}C NMR (100 MHz, DMSO-d_6), δ_C (ppm)** 171.56 (C-15), 167.47 (C-12), 165.92 (C-9), 165.69 (C-3), 133.91 (C-17), 130.63 (C-18), 129.89 (C-20), 128.64 (C-21), 128.49 (C-19), 121.53 (C-16), 61.37 (C-2), 54.14 (C-4), 43.61 (C-10), 30.57 (C-5), 19.31 (C-7), 18.45 (C-6), 14.25 (C-1); **IR** (KBr cm^{-1}): 3294 (NH), 2963 (Ar), 1734 (C=O), 1688 (C=O), 1663 (C=O), 1608 (C=O), 1147 (C-O); **HRMS** (ESI-TOF) m/z; calculated for $C_{19}H_{22}N_2O_5S$+H: 391.1249, found M^++H: 391.1251.

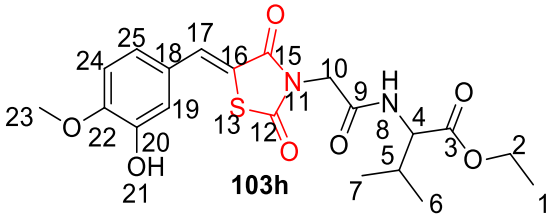
6.7.9. Ethyl (2-(5-(4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)acetyl)valinate (103g).



A reaction of *p*-anisaldehyde (0.40 ml, 2.32 mmol) and **106b** (0.70 g, 2.32 mmol) gave compound **103g** as a yellow solid (0.51 g, 38%); m.p = 182-183 °C; **^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm)** 8.66 (d, 1H, *J* =8.0 Hz, H-8), 7.92 (s, 1H, H-17), 7.61 (d, 2H, *J* =8.0 Hz, H-20), 7.12 (d, 2H, *J* 8.0 Hz, H-19), 4.18 (s, 2H, H-10), 4.15 (quint, 1H, *J* 3.6 Hz, H-4), 4.10 (q, 2H, *J* =3.6 Hz, H-2), 3.89 (s, 3H, H-22) 2.05 (septet, 1H, *J* =6.8Hz, H-5), 1.20 (t, 3H, *J* =7.2 Hz, H-1), 0.90 (t, 6H, *J* =6.4 Hz, H-7+H-6); **^{13}C NMR**

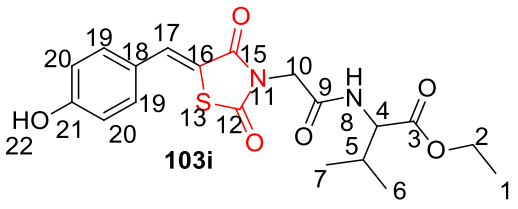
(100 MHz, DMSO-d$_6$), δ$_C$ (ppm) 171.57 (C-15), 167.52 (C-12), 165.92 (C-9), 165.89 (C-3), 161.73 (C-21), 133.80 (C-17), 132.89 (C-20), 125.77 (C-16), 118.2 (C-18), 115.47 (C-19), 60.95 (C-2), 58.04 (C-4), 55.98 (C-22), 43.54 (C-10), 30.62 (C-5), 19.33 (C-7), 18.48 (C-6), 14.45 (C-1); **IR** (KBr cm^{-1}): 3240 (NH), 2939 (Ar), 1740 (C=O), 1735 (C=O), 1663 (C=O), 1601, (C=O) 1172 (C-O); **HRMS** (ESI-TOF) m/z; calculated for $C_{20}H_{24}N_2O_6S$+H: 421.1355, found M$^+$+H: 421.1352.

6.7.10. Ethyl (2-(5-(4-hydroxy-3-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)acetyl)valinate (103h).



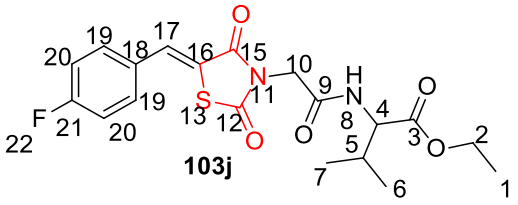
A reaction of vanillin (0.37 g, 2.32 mmol) and **103b** (0.70 g, 32 mmol) gave compound **103h** as a yellow solid (0.12 g, 12%); m.p =187-188 °C; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$ (ppm)** 10.10 (s, 1H, OH), 8.70 (d, 1H, *J* =8.4 Hz, H-8), 7.93 (s, 1H, H-17), 7.26 (d, 1H, *J* =1.6 Hz, H-19), 7.18 (dd, 2H, *J* =8.4 Hz, *J* =1.6 Hz, H-24), 7.01 (d. 1H, *J* =8.4 Hz, H-25), 4.22 (s, 2H, H-10), 4.20 (quint, 1H, *J*=3.6 Hz, H-4), 4.15 (q, 2H, *J* =3.6 Hz, H-2), 3.89 (s, 1H, H-23), 2.12 (septet, 1H, *J* =6.8 Hz, H-5), 1.20 (t, 3H, *J* =6.0 Hz, H-1), 0.95 (t, 6H, *J* =6.4 Hz, H-6+H-7); **^{13}C NMR (100 MHz, DMSO-d$_6$), δ$_C$ (ppm)** 171.60 (C-15), 167.62 (C-12), 165.96 (C-9), 165.83 (C-3), 150.25 (C-22), 148.49 (C-20), 134.58 (C-17), 124.78 (C-22), 124.70 (C-19), 117.12 (C-18), 116.71 (C-24), 114.87 (C-25), 60.95 (C-2), 58.04 (C-4), 56.12 (C-23), 43.50 (C-10), 30.61 (C-5), 19.33 (C-7), 18.49 (C-6), 14.53 (C-1); **IR** (KBr cm^{-1}): 3243 (NH), 2966 (Ar), 1733 (C=O), 1672 (C=O), 1653 (C=O), 1601 (C=O), 1170 (C-O); **HRMS** (ESI-TOF) m/z; calculated for $C_{20}H_{24}N_2O_7S$+H: 436.1304, found M$^+$+H: 437.1307.

6.7.11. Ethyl (2-(5-(4-hydroxybenzylidene)-2,4-dioxothiazolidin-3-yl)acetyl)valinate (103i).



A reaction of 4-hydroxybenzaldehyde (0.40 ml, 2.32 mmol) and **106b** (0.70 g, 2.32 mmol) gave compound **103i** as a yellow solid (0.034 g, 4%); m.p = 178-179 °C; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$ (ppm)** 10.43 (s, 1H, H-22), 8.65 (d, 1H, *J* =8.4 Hz, H-8), 7.86 (s, 1H, H-17), 7.53 (d, 2H, *J* =8.8 Hz, H-20), 6.93 (d, 2H, *J* =8.4 Hz, H-19), 4.19 (s, 2H, H-10), 4.16 (quint, 1H, *J* =3.6 Hz, H-4), 4.08 (q, 2H, *J* =3.6 Hz, H-2), 2.06 (septet, 1H, *J* =6.4 Hz, H-5), 1.21 (t, 3H, *J* =7.2 Hz, H-1), 0.90 (t, 6H, *J* =6.4 Hz, H-6+H-7); **^{13}C NMR (100 MHz, DMSO-d$_6$), δ$_C$ (ppm)** 171.59 (C-15), 167.63 (C-12), 165.96 (C-9), 165.88 (C-3), 160.70 (C-21), 134.29 (C-17), 133.12 (C-20), 124.25 (C-16), 118.23 (C-18), 116.88 (C-19), 60.99 (C-2), 58.03 (C-4), 43.50 (C-10), 30.61 (C-5), 19.34 (C-7), 18.49 (C-6), 14.48 (C-1); **IR** (KBr cm^{-1}): 3299 (N-H), 2968 (Ar), 1739 (C=O), 1730 (C=O), 1682 (C=O), 1660 (C=O), 1172 (C-O); **HRMS** (ESI-TOF) m/z; calculated for $C_{19}H_{22}N_2O_6S$+H: 407.1199, found M$^+$+H: 407.1202.

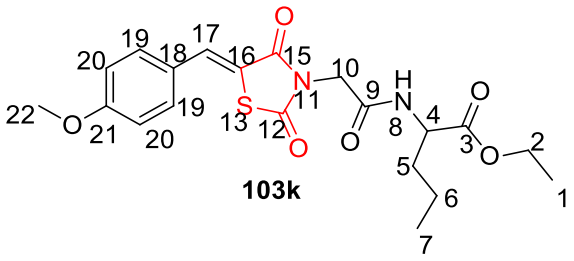
6.7.12. Ethyl (2-(5-(4-fluorobenzylidene)-2,4-dioxothiazolidin-3-yl)acetyl)valinate (103j).



103j

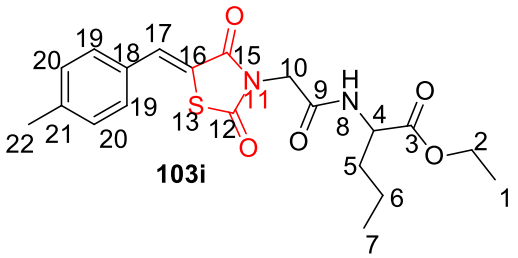
A reaction of 4-fluorobenzaldehyde (0.25 ml, 2.32 mmol) and **106b** (0.70 g, 2.32 mmol) in ethanol (20 ml) gave compound **103j** as a yellow solid (0.11 g, 4%); m.p = 216-217 °C; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$ (ppm)** 8.65 (d, 1H, *J* =8.4 Hz, H-8), 7.99 (s, 1H, H-17), 7.74 (dd, 2H, $^{3}J_{FH}$ =17.6 Hz, J_{HH} =8.8 Hz, H-20), 7.42 (dd, 2H, $^{4}J_{FH}$ =8.4 Hz and J_{HH} =5.6 Hz , H-19), 4.17 (s, 2H, H-10), 4.15 (quint, 1H, *J* =3.6 Hz, H-4), 4.10 (q, 2H, *J* =3.6 Hz, H-2), 2.05 (septet, 1H, *J* =6.8 Hz, H-5), 1.20 (t, 3H, *J* =7.2 Hz, H-1), 0.90 (t, 6H, *J* =6.4 Hz, H-6+H-7); **^{19}F NMR (376.5 MHz, DMSO-d$_6$) δ$_F$ (ppm)** -108.30-(-108.36) (m, 1F, F-22); **^{13}C NMR (100 MHz, DMSO-d$_6$), δ$_C$ (ppm)** 171.57 (C-15), 167.33 (C-12), 165.84 (C-9), 165.64 (C-3), 163.47 (d, ^{1}J =250.0 Hz, C-21), 133.23 (d, ^{2}J = 9.0 Hz, C-20), 132.04 (C-17), 130.24 (d, ^{4}J =3.0 Hz, C-18), 121.18 (C-16), 117.06 (d, ^{3}J =22.0 Hz, C-19), 60.96 (C-2), 58.05 (C-4), 43.64 (C-10), 30.61 (C-5), 19.34 (C-7), 18.54 (C-6), 14.5 (C-1); **IR** (KBr cm^{-1}): 3283 (NH), 2961 (Ar), 1736 (C=O), 1686 (C=O), 1662 (C=O), 1614 (C=O), 1149 (C=O); **HRMS** (ESI-TOF) m/z; calculated for $C_{19}H_{21}FN_2O_5S$+H: 409.1155, found M$^+$+H: 409.1158.

6.7.13. Ethyl (2-(5-(4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)acetyl)norvalinate (103k).



A reaction of *p*-anisaldehyde (0.40 ml, 2.32 mmol) and **106c** (0.70 g, 2.32 mmol) gave compound **103k** as a white solid (0.22 g, 17%); m.p = 204-205 °C; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$ (ppm)** 8.72 (d, 1H, *J* = 7.6 Hz, H-8), 7.91 (s, 1H, H-17), 7.61 (d, 2H, *J* =8.8 Hz, H-20), 7.12 (d, 2H, *J* = 8.8 Hz, H-19), 4.32 (s, 2H, H-20), 4.21 (quint, 1H, *J* =5.2 Hz, H-4), 4.09 (q, 2H, *J* = 3.6 Hz, H-2), 3.83 (s, 3H, H-22), 1.66-1.60 (m, 2H, H-6), 1.34-1.28 (m, 2H, H-5), 1.18 (t, 3H, *J* =6.8 Hz, H-1), 0.89 (t, 3H, *J* =7.8 Hz, H-7); **^{13}C NMR (100 MHz, DMSO-d$_6$), δ$_C$ (ppm)** 172.26 (C-15), 167.59 (C-12), 165.80 (C9+C-3), 161.74 (C-21), 133.86 (C-17), 132.80 (C-20), 125.77 (C-16), 118.23 (C-18), 115.49 (C-19), 60.99 (C-2), 55.04 (C-22), 52.43 (C-4), 43.46 (C-10), 33.52 (C-5), 18.92 (C-6), 14.46 (C-1), 13.87 (C-7); **IR** (KBr cm^{-1}): 3303 (NH), 2960 (Ar), 1734 (C=O), 1687 (C=O), 1663 (C=O), 1593 (C=O), 1183 (C-O); **HRMS** (ESI-TOF) m/z; calculated for $C_{20}H_{24}N_2O_6S$+H: 421.1355, found M$^+$+H: 421.1358

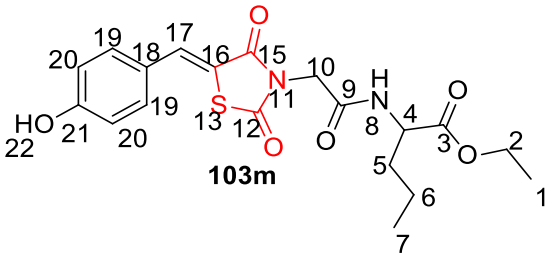
6.7.14. Ethyl (2-(5-(4-methylbenzylidene)-2,4-dioxothiazolidin-3-yl)acetyl)norvalinate (103i).



A reaction of *p*-tolualdehyde (0.29 ml, 2.32 mmol) and **106c** (0.70 g, 2.32 mmol) gave compound **103i** as a white solid (0.12 g, 13%); m.p = 196-197 °C; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$ (ppm)** 8.72 (d, 1H, *J* =7.6 Hz, H-8), 7.91 (s, 1H, H-17), 7.54 (d, 2H, *J* =8.0 Hz, H-20), 7.37 (d, 2H, *J* =8.0 Hz, H-19), 4.32 (s, 2H, H-10), 4.21 (quint, 1H, *J* =5.2 Hz, C-4), 4.09 (q, 2H, *J* =3.6 Hz, H-2), 2.37 (s, 3H, H-22), 1.69-1.57 (m, 2H, H-6), 1.37-1.34 (m, 2H, H-5), 1.18 (t, 3H, *J* =6.8 Hz, H-1), 0.89 (t, 3H, *J* =7.8 Hz, H-7); **^{13}C NMR (100 MHz, DMSO-d$_6$), δ$_C$ (ppm)** 172.26 (C-15), 167.54 (C-12), 165.79 (C-

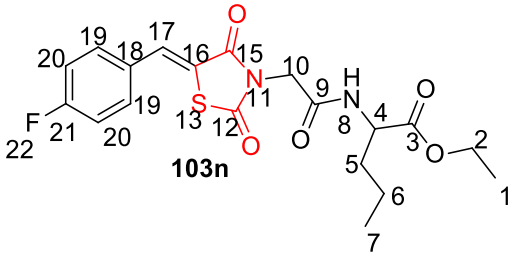
9+C-3), 141.67 (C-21), 133.93 (C-17), 130.70 (C-20), 130.52 (C-19), 120.28 (C-16), 61.04 (C-2), 52.44 (C-4), 43.53 (C-10), 33.48 (C-5), 21.56 (C-22), 18.92 (C-6), 14.46 (C-1), 13.86 (C-7); **IR** (KBr cm^{-1}): 3241, 2960, 1734, 1689, 1661, 1599, 1149; **HRMS** (ESI-TOF) m/z; calculated for $C_{20}H_{24}N_2O_5S$+H: 405.1406, found M^++H: 405.1409.

6.7.15. Ethyl (2-(5-(4-hydroxybenzylidene)-2,4-dioxothiazolidin-3-yl)acetyl)norvalinate (103m).



A reaction of 4-hydroxybenzaldehyde (0.30 g, 2.32 mmol) and **106c** (0.70 g, 2.32 mmol) gave compound **103m** as a yellow solid (0.24 g, 26%); m.p = 125-126 °C; **^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm) 10**.36 (s, 1H, H-22), 8.66 (d, 1H, *J* =7.6 Hz, H-8), 7.86 (s, 1H, H-17), 7.51 (d, 2H, *J* =8.4 Hz, H-20), 6.93 (d, 2H, *J* =8.8 Hz, H-19), 4.25 (s, 2H, H-10), 4.22 (quint, 1H, *J* =5.2 Hz, H-4), 4.10 (q, 2H, *J* =3.6 Hz, H-2), 1.69-1.59 (m, 2H, H-6), 1.36-1.30 (m, 2H, H-5), 1.18 (t, 3H, *J* =6.8 Hz, H-1), 0.88 (t, 3H, *J* =7.2 Hz, H-7); **^{13}C NMR (100 MHz, DMSO-d_6), δ_C (ppm)** 172.22 (C-15), 167.61 (C-12), 165.85 (C-9), 165.77 (C-3), 160.68 (C-21), 134.26 (C-21), 133.08 (C-20), 124.29 (C-16), 116.97 (C-18), 116.89 (C-19), 60.97 (C-2), 52.42 (C-4), 43.56 (C-10), 33.64 (C-5), 18.92 (C-6), 14.47 (C-1), 13.87 (C-7); **IR** (KBr cm^{-1}): 3308 (NH), 2989 (Ar), 1730 (C=O), 1668 (C=O), 1631 (C=O), 1601 (C=O), 1153 (C-O); **HRMS** (ESI-TOF) m/z; calculated for $C_{19}H_{22}N_2O_6S$+H: 407.1199, found M^++H: 407.1202.

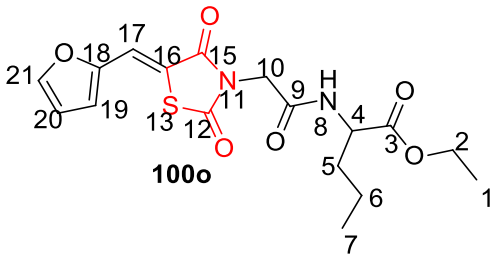
6.7.16. Ethyl (2-(5-(4-fluorobenzylidene)-2,4-dioxothiazolidin-3-yl)acetyl)norvalinate (103n).



A reaction of 4-fluorobenzaldehyde (0.16 ml, 1.32 mmol) and **106c** (0.40 g, 1.32 mmol) gave compound **103n** as a yellow solid (0.11 g, 21%); m.p = 240-241 °C; **^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm)** 8.72 (d, 1H, *J* =7.6 Hz, H-8), 7.99 (s, 1H, H-17),

7.74 (dd, 2H, $^{3}J_{FH}$ = 17.2 Hz, J_{HH} = 8.4 Hz, H-20), 7.42 (dd, 2H, $^{4}J_{FH}$ = 8.8 Hz, J_{HH} = 5.6 Hz, H-19), 4.23 (s, 2H, H-10), 4.13 (quint, 1H, *J* =3.6 Hz, H-4), 4.09 (q, 2H, *J* =5.2 Hz, H-2), 1.69-1.59 (m, 2H, H-6), 1.36-1.30 (m 2H, H-6), 1.88 (t, 3H, *J* =7.2 Hz, H-1), 0.88 (t, 3H, *J* =7.2 Hz, H-7); **^{19}F NMR (376.5 MHz, DMSO-d$_6$) δ$_F$ (ppm)** -108.28-(-108.36) (m, 1F, F-22); **^{13}C NMR (100 MHz, DMSO-d$_6$), δ$_C$ (ppm)** 172.23 (C-15), 167.34 (C-12), 165.67 (C-9), 165.63 (C-3), 163.19 (d, ^{1}J =250.0 Hz, C-21), 133.10 (d, ^{2}J =9.0 Hz, C-20), 132.79 (C-17), 130.04 (d, ^{4}J =3.0 Hz, C-18), 121.16 (C-16), 117.1 (d, ^{3}J =22.0 Hz, C-19), 61.00 (C-2), 52.42 (C-4), 43.64 (C-10), 33.93 (C-6), 18.92 (C-5), 14.48 (C-1), 13.88 (C-7); **IR** (KBr cm^{-1}): 3285 (N-H), 2964 (Ar), 1735 (C=O), 1686 (C=O), 1657 (C=O), 1609 (C=O), 1147 (C-O); **HRMS** (ESI-TOF) m/z; calculated for $C_{19}H_{21}FN_2O_5S$+H: 409.1155, found M$^+$+H: 409.1158.

6.7.17. Ethyl (2-(5-(furan-2-ylmethylene)-2,4-dioxothiazolidin-3-yl)acetyl)norvalinate (103o).



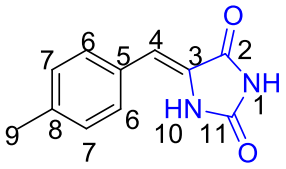
100o

A reaction of furfural (0.20 ml, 2.32 mmol) and **106c** (0.70 g, 2.32 mmol) gave compound **103o** as a brown solid (0.61 g, 65%); m.p. = 181-182 °C; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$ (ppm)** 8.65 (dd, 1H, *J* =21.2 Hz and *J* =7.6 Hz, H-20), 8.10 (s, 1H, H-8), 7.79 (s, 1H, H-17), 7.17 (d, 1H, *J* =1.2 Hz, H-21), 6.78 (dd, 1H, *J* =1.2 Hz and *J* =1.6 Hz, H-19), 4.36 (s, 2H, H-10), 4.23 (quint, 1H, *J* =5.6 Hz, H-4), 4.11 (q, 2H, *J* =3.6 Hz, H-2), 1.56-1.48 (m, 2H, H-6), 1.36-1.32 (m, 2H, H-5), 1.20 (t, 3H, *J* =6.8 Hz, H-1), 0.88 (t, 3H, *J* =7.2 Hz, H-7); **^{13}C NMR (100 MHz, DMSO-d$_6$), δ$_C$ (ppm)** 172.22 (C-15), 168.06 (C-12), 165.91 (C-9), 165.74 (C-3), 149.57 (C-18), 148.38 (C-20), 120.24 (C-17), 119.88 (C-21), 118.18 (C-16), 114.15 (C-19), 60.10 (C-2), 58.06 (C-4), 43.42 (C-10), 33.60 (C-5), 18.32 (C-6), 14.50 (C-1), 13.87 (C-7); **IR** (KBr cm^{-1}): 3285, 2959, 1735, 1684, 1662, 1615, 1147; **HRMS** (ESI-TOF) m/z; calculated for $C_{17}H_{20}N_2O_6S$+H: 381.1042, found M$^+$+H: 381.1045.

6.8. General method for synthesis of 5-arylidene hydantoins (110a-e)

Appropriate aldehydes (1 mmol) and hydantoin **47** (1 mmol) were dissolved in piperidine (10 ml). The mixture was heated to 130 °C for 6.5 hours and TLC analysis indicated that the reaction was complete. Water (200 ml) was added to the mixture after it was cooled to room temperature. This was followed by stirring until the mixture was dissolved and the undissolved substances filtered. Subsequently 10 M hydrochloric acid (20 ml) was added dropwise to the filtrate and the resultant precipitate was obtained by filtration, washed with water and dried to give the desired 5-arylidene hydantoins **110a-e**.

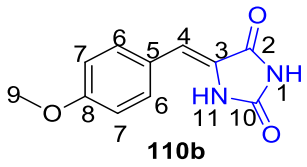
6.8.1. 5-(4-Methylbenzylidene)-2,4-hydantoin (110a)



110a

A reaction of *p*-toluylaldehyde (4.71 ml, 3.99 mmol) and hydantoin **47** (4.04 g, 3.99 mmol) gave compound **110b** as a yellow solid (2.18 g, 27 %); m.p = 240.5-253.1 ℃ (lit m.p =245-247 ℃);[160] **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$** (ppm) 11.25 (s, 1H, H-1), 10.50 (s, 1H, H-10), 7.52 (d, 2H, *J* = 8.0 Hz, H-6), 7.21 (d, 2H, *J* = 8.0 Hz, H-7), 6.39 (s, 1H, H-4); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ$_C$** (ppm) 166.04 (C-2), 156.10 (C-11), 138.61 (C-8), 130.12 (C-7), 129.71 (C-5), 127.64 (C-6), 108.98 (C-4); **IR** (KBr cm^{-1}): 3289 (N-H), 2919 (C-H), 1758 (C=O), 1715 (C=O), 1669 (C=C).

6.8.2. 5-(4-methoxybenzylidene)-2,4-hydantoin (110b)

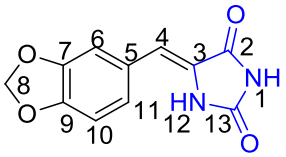


110b

A reaction of *p*-anisaldehyde (3.45 ml, 3.99 mmol) and hydantoin **47** (4.01 g, 3.99 mmol) gave compound **110b** as a yellow solid (4.51 g, 52 %); m.p = 248-250 ℃ (lit m.p = 250–252 ℃);[160] **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$** (ppm) 11.20 (s, 1H, H-1), 10.45

(s, 1H, H-11), 7.59 (d, 2H, *J* = 8.8 Hz, H-6), 6.95 (d, 2H, *J* = 8.8 Hz, H-7), 6.39(s, 1H, H-4), 2.31 (s, 3H, H-9); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ$_C$** (ppm) 166.10 (C-2), 159.90 (C-10), 156.11 (C-8), 131.01 (C-6), 126.50 (C-5), 114.70 (C-7), 109.14 (C-4); **IR** (KBr cm-1): 3157 (N-H), 2935 (C-H), 1755 (C=O), 1709 (C=O), 1650 (C=C).

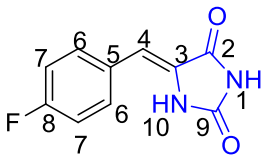
6.8.3. 5-piperonyl-2,4-hydantoin (110c)



110c

A reaction of piperonal (9.28 g, 3.99 mmol) and hydantoin **47** (4.01 g, 3.99 mmol) gave compound **110c** as a yellow solid (3.75 g, 40 %); m.p = 254-257 ℃ (lit m.p = 258-260 ℃);[161] **^{1}H NMR (400 MHz, DMSO-d6) δ$_H$** (ppm) 11.22 (s, 1H, H-1), 10.47 (s, 1H, H-12), 7.26 (d, 1H, *J* = 1.2 Hz, H-6), 7.12 (d, 1H, *J* = 3.2 Hz, H-11), 6.95 (d, 1H, *J* = 8.0 Hz, H-10), 6.36 (s, 1H, H-4), 6.06 (s, 2H, H-8); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ$_C$** (ppm) 166.10 (C-2), 156.08 (C-13), 148.25 (C-9), 148.01 (C-7), 127.45 (C-5), 126.74 (C-11), 125.26 (C-3), 109.30 (C-6+C-10), 109.29 (C-4), 101.87 (C-8); **IR** (KBr cm^{-1}): 3152 (N-H), 2933 (C-H), 1750 (C=O), 1707 (C=O), 1657 (C=C).

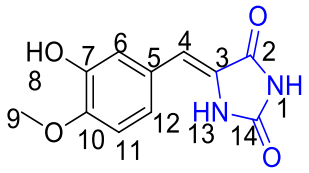
6.8.4. 5-(4-fluorobenzylidene)-2,4-hydantoin (110d)



110d

A reaction of 4-fluorobenzaldehyde (4.31 ml, 3.99 mmol) and hydantoin **47** (4.03 g, 3.99 mmol) gave compound **110d** as a yellow solid (2.14 g, 25 %); m.p = 270-272 ℃ (lit m.p = 273-275 ℃);[174] **^{1}H NMR (400 MHz, DMSO-d6) δ$_H$** (ppm) 11.28 (s, 1H, H-1), 10.58 (s, 1H, H-10), 7.67 (dd, 2H, $^3J_{FH}$ = 17.6, J_{HH}= 8.8 Hz, H-6), 7.24 (dd, 2H, $^4J_{FH}$ = 8.4 Hz, J_{HH} = 5.6 Hz, H-7), 6.43 (s, 1H, H-4), **^{13}C NMR (100 MHz, DMSO-d6) δ$_C$** (ppm) 165.95 (C-2), 162.01 (d, ^1J = 246.0 Hz, C-8) 156.17 (C-9), 132.00 (d, ^4J = 3.0 Hz, C-5), 30.01 (d, ^3J = 9.0 Hz, C-6), 128.20 (C-3), 116.10 (d, ^2J = 22.0, C-7), 107.64 (C-4), **IR** (KBr cm^{-1}): 3268 (N-H), 2947 (C-H), 1789 (C=O), 1732 (C=O), 1664 (C=C).

6.8.5. 5-(3-hydroxy-4-methoxybenzylidene)-2,4-hydantoin (110e)



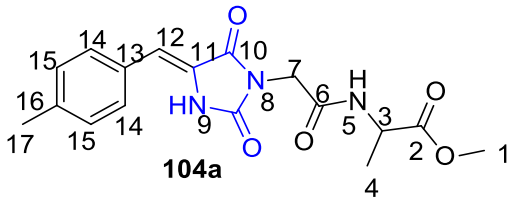
110e

A reaction of vanillin (7.60 g, 3.99 mmol) and hydantoin **47** (4.01 g, 3.99 mmol) gave compound **110e** as a yellow solid (5.08 g, 53%); m.p = 246-248 ℃ (lit m.p 24-248 ℃);[163] **^{1}H NMR (400 MHz, DMSO-d6) δ_H** (ppm) 11.15 (s, 1H, H-1), 10.44 (s, 1H, H-13), 9.48 (s, 1H, H-8), 7.11 (dd, 1H, *J* = 3.8 Hz and *J* = 4.4 Hz, H-12), 7.07 (d, 1H, *J* = 8.0 Hz, H-6); 6.70 (d, 1H, *J* = 8.0 Hz, H-11), 6.37 (s, 1H, H-4), 2.06 (s, 3H, H-9); **^{13}C NMR (100 MHz, DMSO-d6) δ_C** (ppm) 166.10 (C-2), 156.18 (C-14), 148.19 (C-10), 148.01 (C-7), 125.94 (C-5), 124.81 (C-3), 123.94 (C-12), 116.18 (C-6), 113.62 (C-11), 110.24 (C-4); **IR** (KBr cm-1): 3279 (N-H), 2839 (C-H), 1755 (C=O), 1709 (C=O), 1626 (C=C).

6.9. General method for the synthesis of ethyl 2-(2-(5-(4-benzylidene)-2,4-dioxoimidazolidin-1-yl)acetamido) esters (104a-v)

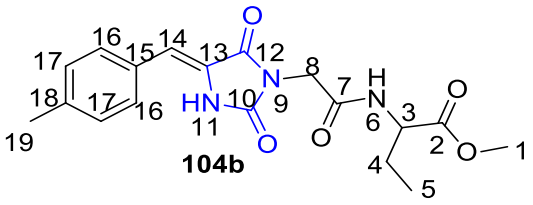
A mixture of 5–arylidene-4-thioxo-thiazolidin-2-ones **110a-e** (1 mmol) dissolved in methanol (5 ml) and potassium hydroxide (1 mmol) in methanol (3 ml) was stirred at 120 ℃ for 15 minutes. Ethyl (2-bromoacetamido) ester derivatives **107a-d** (1 mmol) were then added, and the resultant mixture were heated at reflux for 2-4 hours. After cooling, the precipitate was filtered and recrystallized from acetone (5 ml) or a mixture of *n*-hexane and ethyl acetate (6 ml) to obtain the desired target products **104a-v.**

6.9.1. Methyl-(2-(5-(4-methylbenzylidene)-2,4-dioxoimidazolidin-1-yl)acetamido)alaninate (104a)



A reaction of 5-(4-methylbenzalidene) imidazolidine-2,4-dione **110b** (0.28 g, 1.26 mmol) and methyl-2-(2-bromoacetamido) alaninate **107a** (0.24 g, 1.26 mmol) gave compound **104a** as a yellow solid (0.12 g, 25%); m.p = 232-234 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$** (ppm) 10.79 (s, 1H, H-9), 8.75 (d, 1H, *J* = 6.8 Hz, H-5), 7.58 (d, 2H, *J* = 8.0 Hz, H-14), 7.23 (d, 2H, *J* = 8.0 Hz, H-15), 6.45 (s, 1H, H-12), 4.31 (quint, 1H, *J* = 8.0 Hz, H-3), 4.20 (s, 2H, H-7), 3.64 (s, 3H, H-1), 2.43 (s, 3H, H-17), 1.30 (d, 3H, *J* = 8.0 Hz, H-4), 1.18 (t, 3H, *J* = 8.0 Hz, H-1); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ$_C$** (ppm) 173.70 (C-2), 167.00 (C-6), 166.41 (C-9), 164.70 (C-10), 155.34 (C-16), 139.30 (C-13), 130.60 (C-14), 129.51 (C-15), 126.93 (C-13), 110.50 (C-12), 52.39 (C-1), 48.17 (C-3), 40.41 (C-7), 21.4 (C-17), 19.90 (C-4), 17.50 (C-1); **IR** (KBr cm^{-1}): 3291 (N-H), 2859 (ArCH), 1770 (C=O), 1741 (C=O), 1714 (C=O), 1660 (C=O), 1604 (C=C), 1158 (C-O). **HRMS**: calcd for $C_{16}H_{16}Cl^{37}N_3O_5$ (ESI-TOF, [M]+H), 368.0788; found, 368.0700.

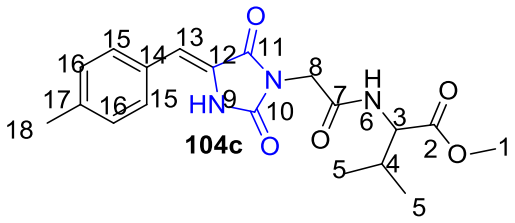
6.9.2 Methyl-(2-(5-(4-methylbenzylidene)-2,4-dioxoimidazolidin-1-yl)acetamido) butanoate (104b)



A reaction of 5-(4-methylbenzalidene) imidazolidine-2,4-dione **110b** (0.28 g, 1.26 mmol) and methyl-2-(2-bromoacetamido) butanoate **107b** (0.26 g, 1.26 mmol) gave compound **104b** as a white solid (0.14 g, 28 %); m.p = 264-266 ℃; (lit m.p) **^{1}H NMR (400 MHz, DMSO-d$_6$)**, **δ$_H$** (ppm) 10,80 (s, 1H, H-11), 8.66 (d, 1H, *J* = 8.0 Hz, H-6), 7.56 (d, 2H, *J* = 8.0 Hz, H-16), 7.24 (d, 2H, *J* = 8.0 Hz, H-17), 6.53 (s, 1H, H-14), 4.21 (quint, 1H, *J* = 8.0 Hz, H-3), 4.16 (s, 2H, H-8), 3.63 (s, 3H, H-1), 1.79 - 1.69 (m, 1H, H-4a), 1.64 - 1.60 (m, 1H, H-4b), 2.42 (s, 3H, H-19), 0.90 (t, 3H, *J* = 4.0 Hz, H-5); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ$_C$** (ppm) 173.31 (C-2), 166.40 (C-7), 164.31 (C-10), 164.02 (C-12), 155.30 (C-18), 138.62 (C-15), 130.21 (C-17), 129.53 (C-16), 126.20

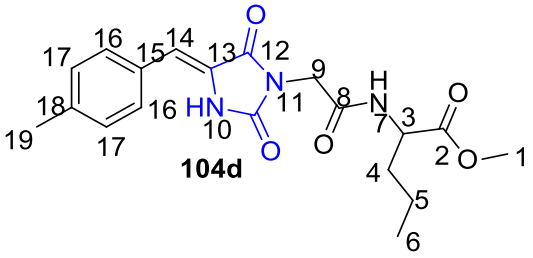
(C-15), 110.94 (C-14), 53.41 (C-3), 52.3 (C-1), 40.59 (C-8), 25.68 (C-4), 21.4 (C-19), 10.61 (C-5); **IR** (KBr cm^{-1}): 3292 (N-H), 2878 (ArCH), 1770 (C=O), 1740 (C=O), 1712 (C=O), 1660 (C=O), 1605 (C=C), 1148 (C-O). HRMS: calcd for $C_{17}H_{18}Cl^{37}N_3O_5$ (ESI-TOF, [M]+H), 382.1035; found, 382.1059.

6.9.3. Methyl-(2-(5-(4-methylbenzylidene)-2,4-dioxoimidazolidin-1-yl)acetamido)valinate (104c)



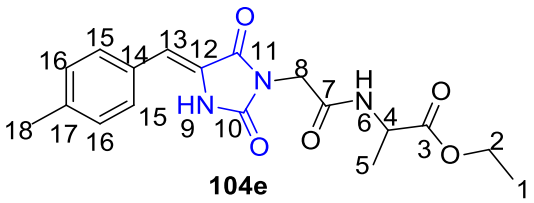
A reaction of 5-(4-methylbenzalidene) imidazolidine-2,4-dione **110b** (0.21 g, 0.90 mmol) and methyl-2-(2-bromoacetamido) valinate **107c** (0.21 g, 0.90 mmol) gave compound **104c** as a yellow solid (0.14 g, 37 %); m.p = 224-226 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$** (ppm) 10,80 (s, 1H, H-10), 8.62 (d, 1H, *J* = 4.0 Hz, H-7), 7.56 (d, 2H, *J* = 8.0 Hz, H-16), 7.24 (d, 2H, *J* = 8.0 Hz, H-15), 6.53 (s, 1H, H-13), 4.24 (quint, 1H, *J* = 4.0 Hz, H-3), 4.20 (s, 2H, H-8), 3.63 (s, 3H, H-1), 2.03 (quint, 1H, *J* = 4.0 Hz, H-4), 0.89 (t, 6H, *J* = 8.0 Hz, H-5); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ$_C$** (ppm) 172.41 (C-2), 167.02 (C-7), 166.34 (C-10), 164.30 (C-11), 155.71 (C-17), 138.63 (C-14), 130.20 (C-16), 129.61 (C-15), 126.92 (C-12), 109.90 (C-13), 52.71 (C-3), 52.3 (C-1), 21.40 (C-1) 41.30 (C-8), 30.61 (C-4), 18.90 (C-5); **IR** (KBr cm^{-1}): 3297 (N-H), 2962 (ArCH), 1771 (C=O), 1740 (C=O), 1712 (C=O), 1659 (C=O), 1609 (C=C), 1148 (C-O); **HRMS:** calcd for $C_{18}H_{20}Cl^{37}N_3O_5$ (ESI-TOF, [M]+H), 396.1591; found, 396.1521.

6.9.4. Methyl-(2-(5-(4-methylbenzylidene)-2,4-dioxoimidazolidin-1-yl)acetamido)norvalinate (104d)



A reaction of 5-(4-methylbenzalidene) imidazolidine-2,4-dione **110b** (0.22 g,1.01 mmol) and methyl-2-(2-bromoacetamido) norvalinate **107d** (0.22 g, 1.01 mmol) gave compound **104d** as a yellow solid (0.12 g, 30 %); m.p = 240-242 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$** (ppm) 10.80 (s, 1H, H-11), 8.67 (d, 1H, *J* = 8.0 Hz, H-7), 7.56 (d, 2H, *J* = 8.0 Hz, H-16), 7.24 (d, 2H, *J* = 8.0 Hz, H-17), 6.54 (s, 1H, H-14), 4.28 (quint, 1H, *J* = 8.0 Hz, H-3), 3.64 (s, 3H, H-1), 2.42 (s, 3H, H-19), 1.70-1.56 (m, 2H, H-4), 1.32 (q, 2H, *J* = 8.0 Hz, H-5), 1.09 (t, 3H, *J* = 8.0 Hz, H-6), 0.87 (t, 3H, *J* = 4.0 Hz, H-7); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ$_C$** (ppm) 172.71 (C-2), 172.40 (C-8), 167.31 (C-12), 164.63 (C-10), 155.31 (C-18), 139.01 (C-15), 130.01 (C-17), 129.89 (C-116), 126.9 (C-15), 110.51 (C-4), 52.3 (C-3), 52.2 (C-1), 40.64 (C-9), 33.50 (C-4), 21.41 (C-5), 21.40 (C-19), 18.92 (C-6); **IR** (KBr cm^{-1}): 3290 (N-H), 2957 (Ar C H), 1784 (C=O), 1740 (C=O), 1712 (C=O), 1670 (C=O), 1602 (C=C), 1097 (C-O); **HRMS**: calcd for $C_{18}H_{20}Cl^{37}N_3O_5$+H (ESI-TOF, [M]+H), 408.1230; found,408.1256.

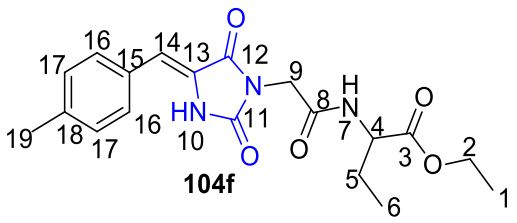
6.9.5. Ethyl 2-(2-(5-(4-methylbenzylidene)-2,4-dioxoimidazolidin-1-yl)acetamido)alaninate (104e)



104e

A reaction of 5-(4-methylbenzalidene) imidazolidine-2,4-dione **110b** (0.12 g, 0.59 mmol) and ethyl-2-(2-bromoacetamido) alaninate **107a** (0.12 g, 0.59 mmol) gave compound **104e** as a white solid (0.12 g, 57 %); m.p = 254-256 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$** (ppm) 10.81 (s, 1H, H-9), 8.67 (d, 1H, *J* = 6.8 Hz, H-6), 7.56 (d, 2H, *J* = 8.0 Hz, H-15), 7.24 (d, 2H, *J* = 8.0 Hz, H-16), 6.53 (s, 1H, H-13), 4,25 (quint, 1H, *J* = 7.7 Hz, H-4), 4.13 (s, 2H, H-8), 4.10 (q, 2H, *J* = 7.2 Hz, H-2), 2.34 (s, 3H, H-18), 1.96 (t, 3H, *J* = 7.2 Hz, H-1), 1.29 (d, 3H, *J* = 7.2 Hz, H-5); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ$_C$** (ppm) 173.01 (C-3), 166.60 (C-7), 165.04 (C-11), 164.71 (C-10), 155.32 (C-17), 138.91 (C-14), 130.52 (C-12), 130.01 (C-15), 129.93 (C-16), 110.33 (C-13), 61.04 (C-2), 48.31 (C-4), 40.56 (C-8), 17.54 (C-5), 14.53 (C-1); **IR** (KBr cm^{-1}): 3289 (N-H), 2916 (ArC-H), 1763 (C=O), 1718 (C=O), 1659 (C=O), 1630 (C=O), 1604 (C=C), 1185 (C-O); **HRMS**: calcd for $C_{18}H_{21}N_3O_5$ (ESI-TOF, [M]+H), 360.1560; found, 360.1540.

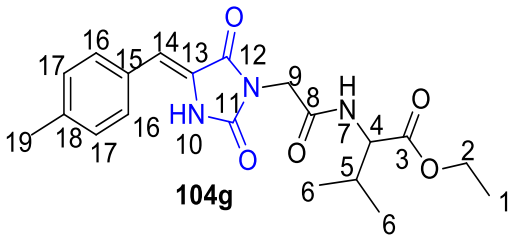
6.9.6. Ethyl 2-(2-(5-(4-methylbenzylidene)-2,4-dioxoimidazolidin-1-yl)acetamido)butanoate (104f)



104f

A reaction of 5-(4-methylbenzalidene) imidazolidine-2,4-dione **110b** (1 g, 4.95 mmol) and ethyl-2-(2-bromoacetamido) butanoate **107b** (1.02 g, 4.95 mmol) gave compound **104f** as a white solid (0.96 g, 52 %); m.p = 245–247 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$** (ppm) 10.80 (s, 1H, H-10), 8.66 (d, 1H, *J* = 8.0 Hz, H-7), 7.56 (d, 2H, *J* = 8.0 Hz, H-16), 7.24 (d, 2H, *J* = 8.0 Hz, H-17), 6.54 (s, 1H, H-14), 4.23 (quint, 1H, *J* = 8.0 Hz, H-4), 4.17 (s, 2H, H-9), 4.10 (q, 2H, *J* = 8.0 Hz, H-2), 2.33 (s, 3H, H-19), 1.77 -1.71 (m, 1H, H-5a), 1.68 -1.58 (m, 1H, H-5b), 1.19 (t, 3H, *J* = 4.0 Hz, H-1), 0.89 (t, 3H, *J* = 8.0 Hz, H-6), **^{13}C NMR (100 MHz, DMSO-d$_6$) δ$_C$** (ppm) 172.71 (C-3), 172.03 (C-8), 164.05 (C-12), 163.41 (C-11), 155.71 (C-18), 139.02 (C-15), 130.23 (C-16), 129.91 (C-17), 126.23 (C-13), 110.24 (C-14), 61.01 (C-2), 53.74 (C-4), 41.03 (C-9), 24.62 (C-19), 21.04 (C-5), 14.61 (C-1), 10.91 (C-6); **IR** (KBr cm-1): 3293 (N-H), 2917 (aro C-H), 2877 (ArC-H), 1770 (C=O), 1739 (C=O), 1710 (C=O), 1658 (C=O), 1605 (C=C), 1170 (C-O); **HRMS**: calcd for $C_{19}H_{23}N_30_5$ (ESI-TOF, [M]+H), 374.1638; found, 374.1600.

6.9.7. Ethyl 2-(2-(5-(4-methylbenzylidene)-2,4-dioxoimidazolidin-1-yl)acetamido) valinate (104g)

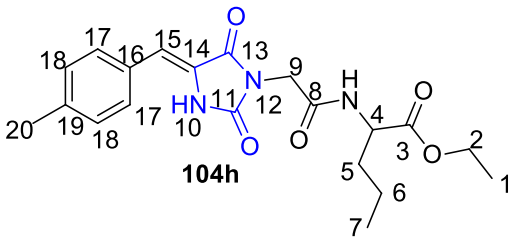


104g

A reaction of 5-(4-methylbenzalidene) imidazolidine-2,4-dione **110b** (0.35 g, 1.73 mmol) and ethyl-2-(2-bromoacetomido) valinate **107c** (0.38 g, 1.73 mmol) gave compound **104g** as a white solid (0.24 g, 36 %); m.p = 254-256 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$** (ppm) 10.78 (s, 1H, H-10), 8.56 (d, 1H, *J* = 8.0 Hz, H-7), 7.55 (d, 2H, *J*

= 8.0 Hz, H-17), 7.24 (d, 2H, *J* = 8.0 Hz, H-16), 6.50 (s, 1H, H-14), 4.20 (s, 2H, H-9), 4.14 (quint, 1H, *J* = 8.0 Hz, H-4), 4.08 (q, 2H, *J* = 4.0 Hz, H-2), 2.34 (s, 3H, H-19), 2.06 (q, 2H, *J* = 4.0 Hz, H-5), 1.18 (t, 3H, *J* = 8.0 Hz, H-1), 0.90 (d, 6H, *J* = 4.0 Hz, H-6); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ$_C$** (ppm) 172.01 (C-3), 171.07 (C-8), 166.76 (C-12), 166.34 (C-11), 155.32 (C-18), 139.34 (C-15), 138.91 (C-13), 130.07 (C-16), 129.94 (C-17), 110.52 (C-14), 60.08 (C-2), 58.09 (C-4), 40.64 (C-9), 31.07 (C-2), 21.06 (C-19), 19.25 (C-6), 18.92 (C-6), 14.64 (C-1); **IR** (KBr cm-1); 3292 (N-H), 3001 (C-H), 2978 (ArCH), 1768 (C=O),1752 (C=O), 1748 (C=O), 1702 (C=O), 1604 (C=C), 1348 (C-O); **HRMS**: calcd for $C_{20}H_{25}N_3O_5$ (ESI-TOF, [M]+H), 388.1794; found, 388.1715.

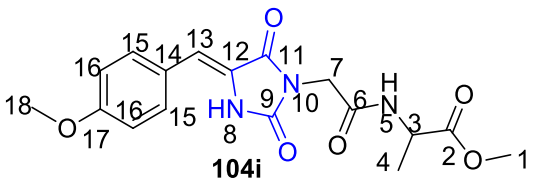
6.9.8. Ethyl 2-(2-(5-(4-methylbenzylidene)-2,4-dioxoimidazolidin-1-yl)acetamido) norvalinate (104h)



104h

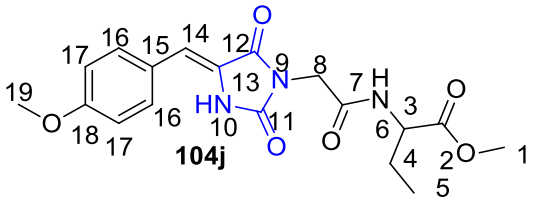
A reaction of 5-(4-methylbenzalidene) imidazolidine-2,4-dione **110b** (1 g, 4.95 mmol) and ethyl-2-(2-bromoacetamido) norvalinate **107d** (1.10 g, 4.95 mmol) gave compound **104h** as a white solid (1.23 g, 63%); m.p = 255-257 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$** (ppm) 10.80 (s, 1H, H-12), 8.65 (d, 1H, *J* = 16.0 Hz, H-8), 7.55 (d, 2H, *J* = 8.0 Hz, H-17), 7.24 (d, 2H, *J* = 8.0 Hz, H-18), 6.53 (s, 1H, H-15), 4.27 (quint, 1H, *J* = 4.0 Hz, H-4), 4.21 (s ,2H, H-10), 4.15 (q, 2H, *J* = 4.0 Hz, H-2), 2.33 (s, 3H, H-20), 1.71 - 1.56 (m, 2H, H-6), 1.30 (q, 2H, *J* = 8.0 Hz, H-5), 1.18 (t, 3H, *J* = 8.0 Hz, H-1), 0.87 (t, 3H, *J* = 8.0 Hz, H-7); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ$_C$** (ppm) 172.71 (C-3), 172.30 (C-9), 166.35 (C-13), 164.72 (C-11), 155.74 (C-19), 138.90 (C-16), 130.01 (C-17), 129.51 (C-14), 126.15 (C-18), 111.21 (C-15), 61.39 (C-2), 52.42 (C-4), 40.65 (C-10), 33.61 (C-5), 21.64 (C-20), 19.27 (C-6), 14.65 (C-1), 13.90 (C-7); **IR** (KBr cm-1): 3291 (N-H), 2957 (ArC-H), 1786 (C=O), 1740 (C=O), 1718 (C=O), 1680 (C=O), 1608 (C=C), 1119 (C-O); **HRMS**: calcd for $C_{19}H_{23}N_3O_5$ (ESI-TOF, [M]+H), 374.1638; found, 374.1601

6.9.9. Methyl-(2-(5-(4-methoxybenzylidene)-2,4-dioxoimidazolidin-1-yl)acetyl)alaninate (104i)



A reaction of 5-(4-methoxybenzalidene) imidazolidine-2,4-dione **110d** (0.84 g, 3.85 mmol) and methyl-2-(2-bromoacetamido) alaninate **107a** (0.75 g, 3.85 mmol) gave compound **104i** as a yellow solid (0.68 g, 47 %) m.p = 218-220 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$** (ppm 10.78 (s, 1H, H-8), 8.73 (d, 1H, *J* = 4.0 Hz, H-5), 7.68 (d, 2H, *J* = 8.0 Hz, H-15), 6.95 (d, 2H, *J* = 8.0 Hz, H-16), 6.40 (s, 1H, H-13), 4.30 (quint, 1H, *J* = 8.0 Hz, H-3), 4.25 (s, 2H, H-7), 3.81 (s, 3H, H-18), 3.63 (s, 3H, H-1), 1.27 (d, 3H, *J* = 4.0 Hz, H-4); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ$_C$** (ppm) 173.41 (C-2), 167.06 (C-6), 165.73 (C-9), 159.65 (C-11), 156.32 (C-17), 131.50 (C-15), 127.24 (C-14), 126.91 (C-12), 115.22 (C-16), 109.24 (C-13), 55.8 (C-18), 52.4 (C-3), 48.2 (C-1), 41.07 (C-8), 19.93 (C-4); **IR** (KBr cm^{-1}): 3286 (N-H), 2955 (ArC-H), 1762 (C=O), 1741 (C=O), 1713 (C=O), 1654 (C=O), 1601 (C=C), 1115 (C-O); **HRMS**: calcd for $C_{17}H_{19}N_3O_5$ (ESI-TOF, [M]+H),362.1274; found, 362.1234

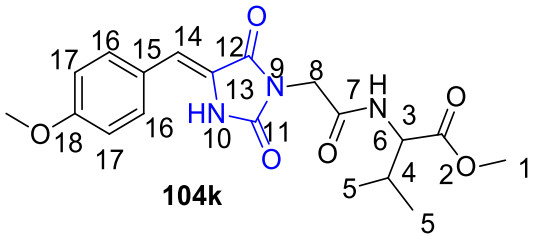
6.9.10. Methyl-2-(2-(5-(4-methoxybenzylidene)2,4-dioxoimidazolidin-1-yl)acetamido)butanoate (104j)



A reaction of 5-(4-methoxybenzalidene) imidazolidine-2,4-dione **110d** (1.02 g, 4.58 mmol) and methyl-2-(2-bromoacetamido) butanoate **107b** (0.95 g, 4.58 mmol) gave compound **104j** as a yellow solid (0.71 g, 40 %) m.p = 259-261 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$** (ppm) 10.75 (s, 1H, H-1o), 8.64 (d, 1H, *J* = 4.0 Hz, H-7), 7.56 (d, 2H, *J* = 8.0 Hz, H-16), 6.94 (d, 2H, *J* = 8.0 Hz, H-17), 6.54 (s, 1H, H-14), 4.22 (quint, 1H, *J* = 8.0 Hz, H-3), 4.15 (s, 2H, H-8), 3.81 (s, 3H, H-19) 3.64 (s, 3H, H-1), 1.79 - 1.712 (m, 1H, H-4a), 1.65 - 1.60 (m, 1H, H-4b), 0.87 (t, 3H, *J* = 8.0 Hz, H-5); **^{13}C NMR (100**

MHz, DMSO-d$_6$) δ$_C$ (ppm) 173.15 (C-2), 166.44 (C-7), 164.32 (C-12), 160.01 (C-11), 154.63 (C-18), 131.60 (C-16), 125.41 (C-15), 124.61 (C-13), 114.66 (C-17), 110.50 (C-14), 61.07 (C-2), 58.79 (C-3), 55.45 (C-19), 52.3 (H-1), 25.31 (C-4), 10.60 (C-5); **IR** (KBr cm-1): 3287 (N-H), 2972 (ArC-H), 1785 (C=O), 1739 (C=O), 1711 (C=O), 1659 (C=O), 1601 (C=C), 1169 (C-O); **HRMS**: calcd for $C_{18}H_{21}N_3O_6$ (ESI-TOF, [M]+H), 376.1430; found, 376.1495.

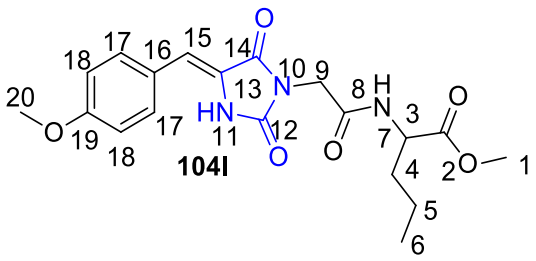
6.9.11. Ethyl 2-(2-(5-(4-methoxybenzylidene)-2,4-dioxoimidazolidin-1-yl)acetamido) valinate (104k)



104k

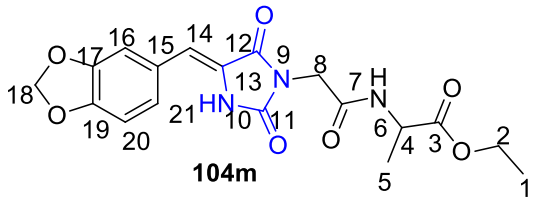
A reaction of 5-(4-methoxybenzalidene) imidazolidine-2,4-dione **110d** (0.5 g, 2.29 mmol) and methyl-2-(2-bromacetamido) valinate **107c** (0.5 g, 2.29 mmol) gave compound **104k** as a brown solid (0.43 g, 44 %); m.p = 260-262 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$** (ppm) 10.78 (s, 1H, H-10), 8.58 (d, 1H, *J* = 8.0 Hz, H-7), 7.63 (d, 2H, *J* = 8.0 Hz, H-16), 6.99 (d, 2H, *J* = 8.0 Hz, H-17), 6.54 (s, 1H, H-14), 4.22 (quint, 1H, *J* = 4.0 Hz, H-3), 4.12 (m, 3H, H-7), 3.81 (s, 3H, H-19), 3.66 (s, 3H, H-1), 2.20 (m, 1H H-4), 0.89 (t, 6H, *J* = 8.0 Hz, H-5); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ$_C$** (ppm) 171.72 (C-2), 166.74 (C-7), 164.02 (C-12), 160.31 (C-11), 155.32 (C-18), 131.61 (C-16), 125.55 (C-15), 125.23 (C-13), 114.81 (C-17), 110.50 (C-14), 58.0 (C-3), 55.8 (H-19), 48.2 (H-1), 40.7 (H-8), 30.32 (C-4), 19.3 (C-5), 18.6 (C-5); **IR** (KBr cm^{-1}): 3296 (N-H), 2977 (ArC-H), 1769 (C=O), 1740 (C=O), 1710 (C=O), 1659 (C=O), 1604 (C=C), 1213 (C-O); **HRMS**: calcd for $C_{20}H_{25}N_3O_6$ (ESI-TOF, [M]+H), 404.1743; found, 404.1723.

6.9.12. Ethyl 2-(2-(5-(4-methoxybenzylidene)-2,4-dioxoimidazolidin-1-yl)acetamido) norvalinate (104l)



A reaction of 5-(4-methylbenzalidene) imidazolidine-2,4-dione **110b** (1.03 g, 4.58 mmol) and ethyl-2-(2-bromoacetamido) norvalinate **107d** (1.02 g, 4.58 mmol) gave compound **104l** as a brown solid (0.82 g, 44 %); m.p = 218-220 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$** (ppm) 9.87 (s, 1H, H-11), 8.71 (d, 1H, *J* = 8.0 Hz, H-7), 7.66 (d, 2H, *J* = 8.0 Hz, H-18), 6.97 (d, 2H, *J* = 12.0 Hz, H-17), 6.50 (s, 1H, H-15), 4.29 (quint, 1H, *J* = 4.0 Hz, H-4), 4.14 (s, 2H, H-9), 4.03 (q, 2H, J = 4.0 Hz, H-2), 3.80 (s, 3H, H-20), 1.71 - 1.60 (m, 2H, H-4), 1.29 (q, 2H, *J* = 8.0 Hz, H-5), 1.22 (t, 3H, *J* = 4.0 Hz, H-1), 0.87 (t, 3H, *J* = 4.0 Hz, H-6); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ$_C$** (ppm) 173.45 (C-3), 170.01 (C-9), 167.31 (C-12), 160.04 (C-14), 155.78 (C-19), 132.60 (C-13), 131.31 (C-17), 125.90 (C-16), 115.23 (C-18), 110.21 (C-15), 59.10 (C-2), 55.71 (C-20), 52.05 (C-4), 40.31 (C-7), 32.62 (C-5), 18.30 (C-4), 14.61 (C-1), 13.93 (C-6); **IR** (KBr cm-1): 3280 (N-H), 2957 (ArC-H), 1789 (C=O), 1743 (C=O), 1706 (C=O), 1658 (C=O), 1600 (C=C), 1210 (C-O); **HRMS**: calcd for $C_{19}H_{23}N_3O_6$ (ESI-TOF, [M]+H), 390.1587; found, 390.1538.

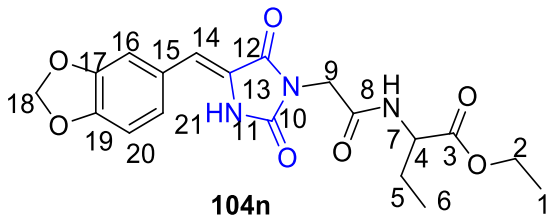
6.9.13. Ethyl 2-(2-(5-(benzo[d][1,3]dioxol-5-ylmethylene)-2,4-dioxoimidazolidin-1-yl)acetamido)alaninate (104m)



A reaction of 5-piperonyl imidazolido-2,4-dione **110f** (1.03 g, 4.31 mmol) and ethyl-2-(2-bromoacetamido) alaninate **107a** (0.95 g, 4.31 mmol) gave compound **104m** as a brown solid (0.51 g, 49%); m.p = 246-248 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$** (ppm 10.80 (s, 1H, H-10), 8.67 (d, 1H, J = 7.2 Hz, H-6) 7.32 (d, 1H, *J* = 1.4 Hz, H-16), 7.16

(dd, 1H, *J* = 1.4 Hz and *J* = 1.4 Hz, H-20), 6.97 (d, 1H, *J* = 8.0 Hz, H-21), 6.51 (s, 1H, H-14), 6.08 (s, 2H, H-17), 4.28 (quint, 1H, *J* = 4.4 Hz, H-4), 4.12 (s, 2H, H-8), 4.08 (q, 2H, *J* = 1.6 Hz, H-2), 1.29 (d, 3H, *J* = 7.6 Hz, H-5), 1.67 (t, 3H, *J* = 6.8 Hz, H-1); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ$_C$** (ppm) 172.71 (C-3), 167.90 (C-7), 166.31 (C-11), 164.42 (C-3), 159.25 (C-15), 148.36 (C-19), 148.28 (C-17), 127.26 (C-20), 125.59 (C-21), 125.21 (C-13), 110.61 (C-16), 109.32 (C-14), 101.95 (C-18), 61.05 (C-2), 48.39 (C-4), 40.51 (C-8), 17.55 (C-5), 14.44 (C-1); **IR** (KBr cm^{-1}): 3294 (N-H), 2971 (ArC-H), 1769 (C=O), 1738 (C=O), 1714 (C=O), 1658 (C=O), 1604 (C=C), 1293 (C-O); **HRMS**: calcd for $C_{18}H_{19}N_3O_7$ (ESI-TOF, [M]+H), 390.1223; found, 390.1292

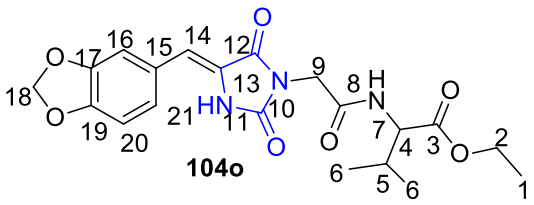
6.9.14. Ethyl 2-(2-(5-(benzo[d][1,3]dioxol-5-ylmethylene)-2,4-dioxoimidazolidin-1-yl)acetamido)butanoate (104n)



104n

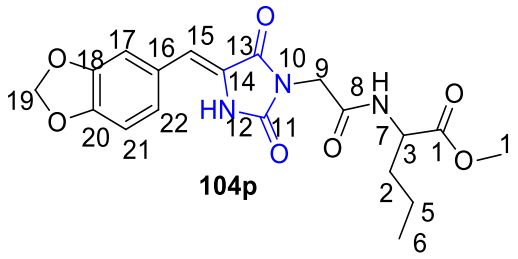
A reaction of 5-(4-benzo[d][1,3] methylene) imidazolido-2,4-dione **110f** (1 g, 4.31 mmol) and ethyl-2-(2-bromoacetamido)butanoate **107b** (0.59 g, 4.31 mmol) gave compound **104n** as a brown solid (0.82 g, 47 %); m.p = 261-263 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$** (ppm 10.75 (s, 1H, H-11), 8.62 (d, 1H, *J* = 8.0 Hz, H-7), 7.31 (d, 1H, H-16), 7.18 (d, 1H, *J* = 8.0 Hz, H-21), 6.97 (d, 1H, *J* = 8.0 Hz, H-20), 6.51 (s, 1H, H-14), 6.08 (s, 2H, H-18), 4.16 (quint, 1H, *J* = 8.0 Hz, H-4), 4.15 (s, 2H, H-9), 4.10 (q, 2H, J = 8.0 Hz, H-2), 1.74-1.71 (m, 1H, H-5a), 1.63-1.60 (m, 1H, H-5b), 1.19 (t, 3H, *J* = 8.0 Hz, H-1), 0.90 (t, 3H, *J* = 8.0 Hz, H-6); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ$_C$** (ppm) 173.01 (C-3), 167.10 (C-8), 166.74 (C-12), 164.32 (C-10), 154.04 (C-15), 148.71 (C-17), 148.43 (C-19), 127.61 (C-21), 125.55 (C-16), 110.52 (C-20), 109.52 (C-14), 102.14 (C-18), 60.72 (C-2), 54.71 (C-4), 41.03 (C-9), 25.05 (C-5) 14.66 (C-1), 10.60 (C-6); **IR** (KBr cm^{-1}): 3278 (N-H), 2958 (ArC-H), 1783 (C=O), 1732 (C=O), 1714 (C=O), 1682 (C=O), 1616 (C=C), 1204 (C-O); **HRMS**: calcd for $C_{19}H_{21}N_3O_7$ (ESI-TOF, [M]+H), 404.1380; found, 404.1315

6.9.15. Ethyl-2-(2-(5-(benzo[d][1,3]dioxol-5-ylmethylene)-2,4-dioxoimidazolidin-1-yl)acetamido)valinate (104o)



A reaction of 5-(4-benzo[d][1,3] methylene) imidazolido-2,4-dione **110f** (1.02 g, 4.31 mmol) and ethyl-2-(2-bromoacetamido)valinate **107c** (0.95 g, 4.31 mmol) gave compound **104o** as a brown solid (0.92 g, 51%); m.p = 225-227 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$)**, **δ_H** (ppm 10.62 (s, 1H, H-11), 8.63 (d, 1H, *J* = 4.0 Hz, H-7), 7.29 (d, 1H, *J* = 8.0 Hz, H-16), 7.18 (d, 1H, *J* = 8.0 Hz, H-21), 6.97 (d, 1H, *J* = 12.0 Hz, H-20), 6.41 (s, 1H, H-14), 6.07 (s, 2H, H-18), 4.22 (quint, 1H, *J* = 4.0 Hz, H-4), 4.19 (s, 2H, H-9), 4.12 (q, 2H, *J* = 12.0 Hz, H-2), 2.03 (quint, 2H, *J* = 8.0 Hz, H-5), 1.18 (t, 3H, *J* = 12.0 Hz, H-1), 0.88 (t, 6H, *J* = 8.0 Hz, H-6); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ_C** (ppm) 172.41 (C-3), 169.42 (C-8), 167.05 (C-12), 164.73 (C-10), 155.21 (C-15), 148.78 (C-17), 135.99 (C-13), 126.65 (C-19), 124.94 (C-16), 109.51 (C-14), 101.82 (C-18), 60.72 (C-2), 58.46 (C-4), 43.05 (C-9), 41.07 (C-5), 18.36 (C-1), 17.31 (C-6); **IR** (KBr cm^{-1}): 3287 (N-H), 2972 (ArC-H), 1785 (C=O), 1739 (C=O), 1712 (C=O), 1659 (C=O), 1601 (C=C), 1257 (C-O); **HRMS**: calcd for $C_{20}H_{23}N_3O_7$ (ESI-TOF, [M]+H), 418.1536; found, 418.1595

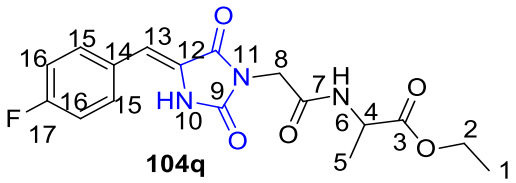
6.9.16. Methyl 2-(2-(5-(benzo[d][1,3]dioxol-5-ylmethylene)-2,4-dioxoimidazolidin-1-yl)acetamido)norvalinate (104p)



A reaction of 5-(4-benzo[d][1,3] methylene) imidazolido-2,4-dione **110f** (1.03 g, 4.31 mmol) and methyl-2-(2-bromoacetamido)norvalinate **107d** (0.95 g, 4.31 mmol) gave compound **104p** as a brown solid (0.62 g, 34%); m.p = 357-359 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$)** **δ_H** (ppm 10.75 (s, 1H, H-12), 8.64 (d, 1H, *J* = 8.0 Hz, H-8), 7.31 (d, 1H, *J*

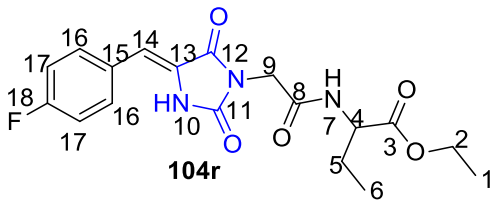
= 4.0 Hz, H-17), 7.18 (d, 1H, *J* = 8.0 Hz, H-22), 6.97 (d, 1H, *J* = 8.0 Hz, H-21), 6.52 (s, 1H, H-15), 6.08 (s, 2H, H-19), 4.27 (quint, 1H, *J* = 4.0 Hz, H-3), 4.14 (s, 2H, H-9), 3.64 (s, 3H, H-1), 1.70–1.41 (m, 2H, H-4), 1.38–1.25 (m, 2H, H-5), 0.88 (t, 3H, J = 7.6 Hz,H-6) **^{13}C NMR (100 MHz, DMSO-d$_6$) δ$_C$** (ppm) 173.30 (C-2), 167.01 (C-8), 164.74 (C-13), 164.35 (C-11), 155.32 (C-16), 148.71 (C-18, C-20), 129.50 (C-14), 126.61 (C-22), 124.91 (C-17), 111.25 (C-21),108.81 (C-15), 102.24 (C-19), 52.3 (C-3), 52.2 (C-1), 40.5 (C-9), 33.6 (C-4), 18.9 (C-5), 13.9 (C-6); **IR** (KBr cm^{-1}): 3284 (N-H), 2958 (ArC-H), 1766 (C=O), 1740 (C=O), 1710 (C=O), 1659 (C=O), 1601 (C=C), 1208 (C-O); **HRMS**: calcd for $C_{19}H_{21}N_3O_7$ (ESI-TOF, [M]+H), 404.1380; found, 404.1339.

6.9.17. Ethyl 2-(2-(5-(4-fluorobenzylidene)-2,4-dioxoimidazolidin-1-yl)acetamido) alaninate (104q)



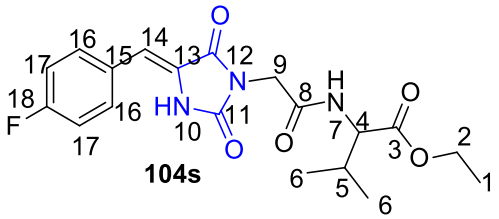
A reaction of 5-(4-Fluorobenzalidene) imidazolidine-2,4-dione **110c** (0.28 g, 1.26 mmol) and ethyl-2-(2-bromoacetamido) alaninate **107a** (0.24 g, 1.26 mmol) gave compound **104q** as a yellow solid (0.12 g, 25 %); m.p = 238-240 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$** (ppm) 10.79 (s, 1H, H-10), 8.75 (d, 1H, *J* = 4.0 Hz, H-6), 7.58 (dd, 2H, $^3J_{FH}$ = 17.6 Hz, J_{HH} =8.0, H-15), 7.23 (dd, 2H, $^4J_{FH}$ = 8.0 Hz, J_{HH} = 5.6 Hz, H-16), 6.45 (s, 1H, H-13), 4.31 (quint, 1H, *J* = 8.0 Hz, H-4), 4.20 (s, 2H, H-8), 4.10 (q, 2H, *J* = 8.0 Hz, H-2), 1.30 (d, 3H, *J* = 8.0 Hz, H-5), 1.18 (t, 3H, *J* = 8.0 Hz, H-1); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ$_C$** (ppm) 173.71 (C-3), 167.02 (C-7), 166.48 (C-11), 164.75 (C-9), 155.30 (d, ^1J = 250.0 Hz, C-17), 139.33 (d, ^4J =3.0 Hz, C-14), 130.65 (d, ^3J = 9.0 Hz, C-15), 129.55 (d, ^2J = 22.0 Hz, C-16), 126.96 (C-12), 110.52 (C-13), 58.70 (C-2), 52.45 (C-4), 40.46 (C-8), 19.91 (C-5), 18.00 (C-1); **IR** (KBr cm^{-1}): 3445 (N-H), 3062 (ArCH), 1797 (C=O), 1737 (C=O), 1713 (C=O), 1682 (C=O), 1660 (C=C), 1192 (C-O); **HRMS**: calcd for $C_{20}H_{23}N_3O_7$ (ESI-TOF, [M]+H), 388.1794; found, 388.1727.

6.9.18. Ethyl 2-(2-(5-(4-fluorobenzylidene)-2,4-dioxoimidazolidin-1-yl)acetamido)butanoate (104r)



A reaction of 5-(4-Fluorobenzalidene) imidazolidine-2,4-dione **110c** (0.32 g, 1.54 mmol) and ethyl-2-(2-bromoacetamido) butanoate **107b** (0.33 g, 1.54 mmol) gave compound **104r** as a yellow solid(0.12 g, 20%); m.p = 284-286 ℃, **^{1}H NMR (400 MHz, DMSO-d$_6$)**, **δ_H** (ppm) 10.58 (s, 1H, H-11), 8.66 (d, 1H, *J* = 8.0 Hz, H-7), 7.70 (dd, 2H, $^4J_{FH}$ = 8.0 Hz, J_{HH} = 5.6 Hz, H-16), 7.23 (dd, 2H, $^3J_{FH}$ = 17.6 Hz, J_{HH} = 8.0 Hz, H-17), 6.58 (s, 1H, H-14), 4.22 (quint, 1H, *J* = 8.0 Hz, H-4), 4.16 (s, 2H, H-9), 4.11 (q, 2H, *J* = 8.0 Hz, H-2), 1.88 - 1.67 (m, 1H, H-5a), 1.73-1.69 (m, 1H, H-5b), 1.27 (t, 3H, *J* = 8.0 Hz, H-1), 0.89 (t, 3H, *J* = 4.0 Hz, H-6); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ_C** (ppm) 172.71 (C-3), 167.03 (C-8), 164.07 (C-12), 161.31 (C-10), 155.36 (d, ^1J = 246.0 Hz, C-18), 132.33 (d, ^3J = 9.0 Hz, C-15), 128.91 (d, ^4J = 3.0 Hz C-16), 127.62 (C-13), 116.51 (d, ^2J = 22.0 Hz, C-17), 109.20 (C-14), 60.19 (C-2), 52.71 (C-4), 39.90 (C-9), 25.34 (C-5), 14.63 (C-1), 10.65 (C-6); **IR** (KBr cm^{-1}); 3183 (N-H), 2992 (ArC-H), 1736 (C=O), 1710 (C=O), 1701 (C=O), 1629 (C=O), 1611 (C=C), 1211 (C-O); **HRMS**: calcd for $C_{20}H_{23}N_3O_7$ (ESI-TOF, [M]+H), 378.1387; found, 378.1323.

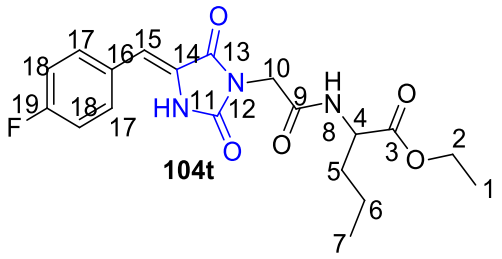
6.9.20. Ethyl 2-(2-(5-(4-Fluorobenzylidene)-2,4-dioxoimidazolidin-1-yl)acetamido) valinate (104s)



A reaction of 5-(4-fluorobenzalidene) imidazolidine-2,4-dione **101s** (0.17 g, 0.82 mmol) and ethyl-2-(2-bromoacetamido) valinate **107c** (0.19 g, 0.82 mmol) gave compound **104s** as a yellow solid (0.17 g, 52%); m.p = 275- 277 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$) δ_H** (ppm) 10.78 (s, 1H, H-11), 8.41 (d, 1H, *J* = 8.0 Hz, H-7), 7.85 (dd,

2H, $^{3}J_{FH}$ = 17.6 Hz, J_{HH} = 8.0 Hz, H-16), 7.34 (dd, 2H, $^{4}J_{FH}$= 8.0 Hz, J_{HH} = 5.6 Hz, H-17), 6.09 (s, 1H, H-14), 4.17 (quint, 1H, *J* = 4.0 Hz, H-4), 4.11 (s, 2H, H-9), 4.04 (q, 2H, *J* = 8.0 Hz, H-2), 2.06 - 1.99 (septet, 1H, H-5), 1.19 (t, 3H, *J* = 8.0 Hz, H-1), 0.88 (t, 6H, *J* = 8.0 Hz, H-6); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ**$_C$ (ppm) 172.01 (C-3), 170.15 (C-8), 168.42 (C-12), 167.30 (C-10), 151.04 (d, ^{1}J = 246.0 Hz, C-18), 135.69 (d, ^{4}J = 3.0 Hz, C-15), 134.31 (C-13), 131.35 (d, ^{3}J = 9.0 Hz, C-16), 128.91 (d, ^{2}J = 22.0 Hz, C-17), 104.22 (C-14), 61.01 (C-2), 58.01 (C-4), 40.61 (C-9), 30.60 (C-5), 18.97 (C-6), 14.66 (C-1); **IR** (KBr cm^{-1}); 3243 (N-H), 2898 (ArC-H), 1732 (C=O), 1714 (C=O), 1709 (C=O), 1627 (C=O), 1615 (C=C), 1238 (C-O); **HRMS**: calcd for $C_{20}H_{23}N_3O_7$ (ESI-TOF, [M]+H), 391.1543; found, 391.1528

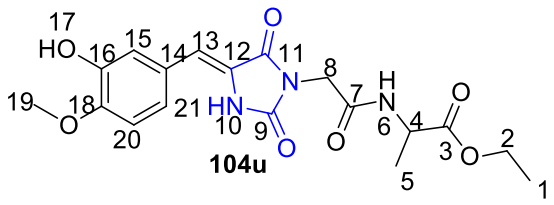
6.9.21. Ethyl 2-(2-(5-(4-Fluorobenzylidene)-2,4-dioxoimidazolidin-1-yl)acetamido) norvalinate (104t)



104t

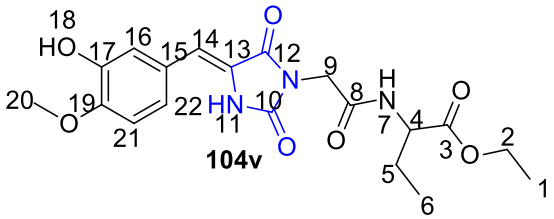
A reaction of 5-(4-Fluorobenzalidene) imidazolidine-2,4-dione **110c** (0.21 g, 1.03 mmol) and ethyl-2-(2-bromoacetamido) norvalinate **107d** (0.21 g,1.04 mmol) gave compound **104t** as a yellow solid (0.15 g, 40 %); m.p = 256-258 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$), δ**$_H$ (ppm) 10.61 (s, 1H, H-11), 8.48 (d, 1H, *J* = 8.0 Hz, H-8), 7.64 (dd, 2H, $^{3}J_{FH}$ = 17.6 Hz, J_{HH} = 8.0 Hz, H-17), 7.45 (d, 2H, $^{4}J_{FH}$ = 8.0 Hz, J_{HH} = 5.6 Hz, H-18), 6.40 (s, 1H, H-15), 4.44 (quint, 1H, *J* = 8.0 Hz, H-4), 4.36 (s, 2H, H-10), 4.28 (q, 2H, *J* = 8.0 Hz, H-2),1.31 - 1.27 (m, 2H, H-6), 1.22 (q, 2H, *J* = 4.0 Hz, H-5), 1.10 (t, 3H, *J* = 8.0 Hz, H-1), 0.88 (t, 3H, *J* = 4.0 Hz, H-7); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ**$_C$ (ppm) 170.72 (C-3), 167.31 (C-9), 165.90 (C-13), 164.35 (C-12), 155.79 (d, ^{1}J = 246.0 Hz, C-19), 139.02 (d, ^{4}J = 3.0 Hz, C-16), 131.61 (d, ^{3}J = 9.0 Hz, C-17), 129.33 (d, ^{2}J = 22.0 Hz, C-18), 127.62 (C-14), 108.80 (C-15), 61.72 (C-2), 52.75 (C-4), 48.77 (C-10), 40.69 (C-5), 21.38 (C-6), 18.39 (C-1), 17.66 (C-7); **IR** (KBr cm^{-1}); 3284 (N-H), 2958 (ArC-H), 1766 (C=O), 1740 (C=O), 1710 (C=O), 1659 (C=O), 1601 (C=C), 1208 (C-O); **HRMS**: calcd for $C_{20}H_{23}N_3O_7$ (ESI-TOF, [M]+H), 391.1543; found, 391.1507

6.9.22. Ethyl 2-(2-(5-(3-hydroxy-4-methoxybenzylidene)-2,4-dioxoimidazolidin-1-yl)acetamido)alaninate (104u)



A reaction of 5-(3-hydroxy-4-methoxybenzalidene) imidazolidine-2,4-dione **110e** (0.20 g, 0.85 mmol) and ethyl-2-(2-bromoacetamido) alaninate **107a** (0.17 g, 0.85 mmol) gave compound **104u** as a brown solid (0.15 g, 44 %); m.p = 296-298 ℃, **^{1}H NMR (400 MHz, DMSO-d$_6$) δ$_H$** (ppm 10.75 (s, 1H, H-10), 9.78 (s, 1H, H-17), 8.67 (d, 1H, *J* = 4.0 Hz, H-6), 7.16 (d, 1H, *J* = 8.0 Hz, H-15), 7.14 (d, 1H, *J* = 4.0 Hz, H-21), 6.80 (d, 1H, *J* = 8.0 Hz, H-20), 6.50 (s, 1H, H-17), 6.36 (s, 1H, H-13), 4.25 (quint, 1H, *J* = 8.0 Hz, H-4), 4.18 (s, 2H, H-8), 4.07 (q, 2H, *J* = 4.0 Hz, H-2), 3.87 (s,3H, H-19), 1.29 (d, 3H, *J* = 4.0 Hz, H-5), 1.19 (t, 3H, *J* = 8.0 Hz, H-1); **^{13}C NMR (100 MHz, DMSO-d$_6$) δ$_C$** (ppm) 172.72 (C-3), 170.34 (C-7), 166.44 (C-11), 164.36 (C-9), 157.37 (C-18), 155.76 (C-16), 142.65 (C-14), 124.61 (C-13), 123.92 (C-12), 116.24 (C-21), 113.98 (C-15), 110.26 (C-20), 61.09 (C-2), 56.87 (C-19), 55.09 (C-4), 40.33 (C-8), 17.67 (C-5), 14.61 (C-1); **IR** (KBr cm^{-1}); 3354 (N-H), 2942 (ArC-H), 1773 (C=O), 1725 (C=O), 1714 (C=O), 1667 (C=O), 1631 (C=C), 1159 (C-O); **HRMS**: calcd for $C_{20}H_{23}N_3O_7$ (ESI-TOF, [M]+H), 391.1380; found, 391.1375

6.9.23. Ethyl 2-(2-(5-(3-hydroxy-4-methoxybenzylidene)-2,4-dioxoimidazolidin-1-yl)acetamido)butanoate (104v)



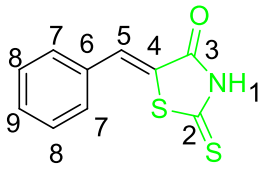
A reaction of 5-(3-hydroxy-4-methoxybenzalidene) imidazolidine-2,4-dione **110e** (1.03g, 4.27 mmol) and ethyl-2-(2-bromoacetamido) butanoate **107b** (0.88 g, 4.27 mmol) gave compound **104v** as a brown solid (0.42 g, 24 %); m.p = 242-244 ℃; **IR** (KBr cm-1); **^{1}H NMR (400 MHz, DMSO-d6), δ$_H$** (ppm) 10.72 (s, 1H, H-11), 9.89 (s,

1H, H-18), 8.65 (d, 1H, *J* = 4.0 Hz, H-7), 7.20 (d, 1H, *J* = 8.0 Hz, H-16), 7.12 (d, H, *J* = 8.0 Hz, H-22), 6.94 (d, 1H, *J* = 8.0 Hz, H-21), 6.82 (s, 1H, H-18), 6.51 (s, 1H, H-14), 4.31 (quint, 1H, *J* = 8.0 Hz, H-4), 4.25 (q, 2H, *J* = 8.0 Hz, H-2), 4.16 (s, 2H, H-9), 2.09 (s, 3H, H-20), 1.81 - 1.77 (m, 2H, H-5a), 1.71-1.60 (m, 1H, H-5b), 1.09 (t, 3H, *J* = 8.0 Hz, H-1), 0.89 (t, 3H, *J* = 8.0 Hz, H-6); **^{13}C NMR (100 Hz, DMSO-d$_6$) δ$_C$** (ppm) 173.06 (C-3), 169.78 (C-8), 166.77 (C-12), 164.75 (C-10), 149.97 (C-19), 148.07 (C-17), 124.68 (C-15), 124.69 (C-14), 123.90 (C-13), 116.51 (C-22), 114.23 (C-16), 111.24 (C-21), 61.79 (C-2), 59.45 (C-20), 56.45 (C-4), 40.62 (C-8), 24.95 (C-5), 15.91 (C-1), 10.88(C-6); **IR** (KBr cm^{-1}); 3321 (N-H), 2912 (ArC-H), 1763 (C=O), 1715 (C=O), 1703 (C=O), 1637 (C=O), 1614 (C=C), 1192 (C-O); **HRMS**: calcd for $C_{20}H_{23}N_3O_7$ (ESI-TOF, [M]+H), 405.1536; found, 405.1531

6.10. General method for synthesis of 5-arylidine rhodanines (108a-g) using Knoevenagel reaction

A mixture of rhodanine **85** (1 mmol), appropriate aldehyde (1 mmol) and sodium acetate (1 mmol) in 10 ml of acetic acid were heated at reflux for 3 hours. After cooling the resultant precipitate was washed with water (5 ml) and acetic acid (5 ml) to give the desired 5-arylidine rhodanines **111a-g**.

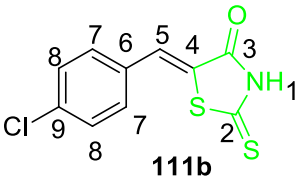
6.10.1. 5-Benzylidene-2,4-rhodanine (111a)



111a

A mixture of benzaldehyde (2.28 g, 22.52 mmol) and 2-thioxothiazolidin-5-one **85** (3.05 g, 22.52 mmol) in acetic acid gave compound **111a** as a brown solid (2.19 g, 44%); m.p = 196 -198 °C. (lit. m.p. = 198-200 ℃);[166] **^{1}H NMR (400 MHz, DMSO-d$_6$)**, **δ$_H$** (ppm) 13.86 (br.s, 1H, H-1), 7.65 (s, 1H, H-5), 7.61 (d, 2H, *J* = 7.2 Hz, H-7), 7.53-7.45 (m, 3H, H-8+H-9); **^{13}C NMR (100 MHz, DMSO-d$_6$)**, **δ$_C$** (ppm) 196.16 (C-3), 169.83 (C-2), 133.45 (C-6), 132.09 (C-5), 131.20 (C-7), 129.90 (C-8), 129.74 (C-9), 125.97 (C-4); **IR** (KBr cm^{-1}): 3156 (N-H), 3072 (C-H), 1705 (C=O), 1066 (C=C), 1086 (C=S).

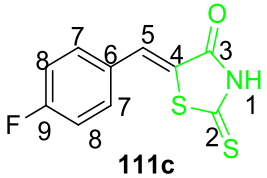
6.10.2. 5-(4-Chlorobenzylidene)-2,4-rhodanine (111b)



111b

A mixture of 4-chlorobenzaldehyde (2.79 g, 22.52 mmol) and 2-thioxothiazolidin-5-one **85** (3.04 g, 22.52 mmol) in acetic acid gave compound **111b** as a brown solid (4 g, 69%) m.p = 219-221 °C (lit. m.p. 217-220 ℃);[166] **^{1}H NMR (400 MHz, DMSO-d$_6$)**, **δ**$_H$ (ppm) 13.78 (br.s, 1H, H-1), 7.61-7.59 (m 4H, H-7 + H-8), 7.56 (s, 1H, H-5); **^{13}C NMR (100 MHz, DMSO-d$_6$)**, **δ**$_C$ (ppm) 195.97 (C-3), 170.06(C-2), 135.76 (C-9), 132.48 (C-5), 132.35 (C-8), 130.45 (C-4), 129.94 (C-7), 126.94 (C-6); **IR** (KBr cm^{-1}): 3410 (N-H), 2988 (ArC-H), 1694 (C=O), 1595 (C=C), 1084 (C=S).

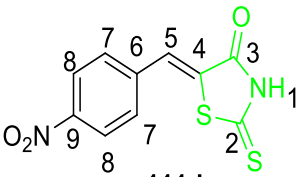
6.10.3. 5-(4- Fluorobenzylidene) 2,4-rhodanine (111c)



111c

A mixture of 4-fluorobenzaldehyde (2.43 ml, 22.5 mmol) and 2-thioxothiazolidin-5-one **85** (3.01 g, 22.5 mmol) in acetic acid gave compound **111c** as a yellow solid (3.67 g, 77%); m.p = 304-306 °C (lit. m.p. = 300-302 ℃);[167] **^{1}H NMR (400 MHz, DMSO-d$_6$)**, **δ**$_H$ (ppm) 13.72 (br.s, 1H, H-1), 7.49 (s, 1H, H-5), 7.43 (dd, 2H, $^3J_{FH}$ = 17.6 Hz, J_{HH} = 8.8 Hz, H-7), 7.31 (dd, 2H, $^4J_{FH}$ = 8.8 Hz, J_{HH} = 5.6 Hz, H-8); **^{13}C NMR (100 MHz, DMSO-d$_6$)**, **δ**$_C$ (ppm) 196.05 (C-3), 169.95 (C-2), 163.01 (d, ^1J = 250.0 Hz, C-9), 133.38 (d, ^4J = 8.0 Hz, C-6), 130.88 (C-5), 130.14 (d, ^3J =3.0 Hz, C-6), 125.81 (C-4) 116.13 (d, ^2J = 22.0 Hz, C-8); **IR** (KBr cm^{-1}): 3231 (N-H), 3095 (ArCH), 1741 (C=O), 1687 (C=C), 1371 (C=S).

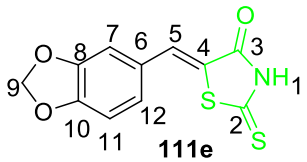
6.10.4. 5-(4-Nitrobenzylidene)-2,4-rhodanine (111d)



111d

A mixture of nitrobenzaldehyde (2.79 g, 22.5 mmol) and 2-thioxothiazolidin-5-one **85** (3.03 g, 22.5 mmol) in acetic acid gave compound **111d** as a brown solid (1.24 g, 21%); m.p = 190-193 °C; (lit. m.p. = 192-194 °C);[168] **^{1}H NMR (400 MHz, DMSO-d$_6$)**, **δ_H** (ppm) 13.71 (br.s, 1H, H-1), 7.57 (s, 1H, H-5), 7.47 (d, 2H, *J* = 8.4 Hz, H-8), 6.94 (d, 2H, *J* = 8.4 Hz, H-7) , **^{13}C NMR (100 MHz, DMSO-d$_6$)**, **δ_C** (ppm) 195.96 (C-3), 169.92 (C-2), 160.83 (C-9), 148.57 (C-6), 135.20 (C-5), 133.08 (C-8), 124.41 (C-4), 117.00 (C-7); **IR** (KBr cm^{-1}): 3395 (N-H), 2988 (ArC-H), 1692 (C=O), 1582 (C=C), 1061 (C=S).

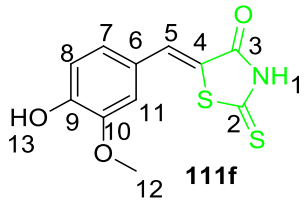
6.10.5. 5-Piperonyl-2,4-rhodanine (111e)



111e

A mixture of piperonal (2.79 g, 22.5 mmol) and 2-thioxothiazolidin-5-one **85** (3.02 g, 22.5 mmol) in acetic acid gave compound **111e** as a yellow solid (2.52 g, 42%); m.p = 249-251 °C. (lit. m.p. 246–249°C);[175] **^{1}H NMR (400 MHz, DMSO-d$_6$)**, **δ_H** (ppm) 13.74 (br.s, 1H, H-1), 7.56 (s, 1H, H-5), 7.18 (dd, 1H, *J* = 1.2 Hz and *J* = 1.2 Hz , H-12), 7.16 (d, 1H, J = 3.6, H-11), 7.07 (d, 1H, *J* = 1.2, H-7), 6.14 (s, 2H, H-9); **^{13}C NMR (100 MHz, DMSO-d$_6$)**, **δ_C** (ppm) 195.93 (C-3), 169.63 (C-2), 150.43 (C-10), 148.57 (C-8), 133.20 (C-5), 125.53 (C-6), 124.85 (C-4), 121.59 (C-7), 116.81 (C-12), 114.79 (C-11). 102.61 (C-9); **IR** (KBr cm^{-1}): 3155 (N-H), 3059 (C-H), 2909 (Ar-CH) 1688 (C=O), 1681 (C=C), 1072 (C=S).

6.10.6. 5-(3-Hydroxy-4-methoxybenzylidene)-2,4-rhodanine (111f)

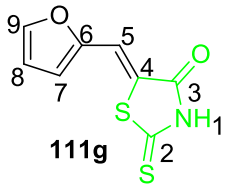


111f

A reaction of vanillin (2.79 g, 22.5 mmol) and 2-thioxothiazolidin-5-one **85** (3.01 g, 22.5 mmol) in acetic acid gave compound **111f** as a brown solid (1.37 g, 23%); m.p = 192-195 °C. (lit. m.p. = 195-198 °C);[169] **^{1}H NMR (400 MHz, DMSO-d$_6$)**, **δ_H** (ppm) 13.71 (br.s, 1H, H-1), 10.11 (s, 1H, H-13), 7.56 (s, 1H, H-5), 7.15 (d, 1H, *J* = 1.4, H-11), 7.09 (d, 1H, *J* = 8.4 Hz, H-8), 6.95 (dd, 1H, *J* = 8.4 and *J* = 1.4, H-7), 3.83 (s, 3H, H-

12); **^{13}C NMR (100 MHz, DMSO-d$_6$)**, **δ_C** (ppm) 195.93 (C-3), 169.92 (C-2), 150.44 (C-10), 148.57 (C-11), 133.20 (C-5), 125.5 (C-6), 124.85 (C-4), 121.59 (C-9), 116.81 (C-8), 114.79 (C-7), 56.78 (H-12); **IR** (KBr cm^{-1}): 3336 (N-H), 2907 (ArCH), 1775 (C=O), 1613 (C=C), 1057 (C=S).

6.10.7. 5-(Furan-2-ylmethylene)-2,4-rhodanine (111g)



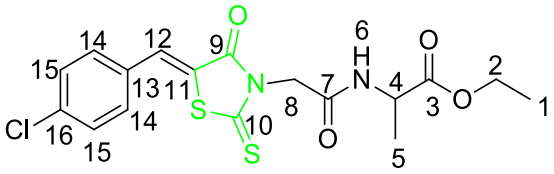
111g

A reaction of furfural (2.16 g, 22.5 mmol) and 2-thioxothiazolidin-5-one **85** (3.02 g, 22.5 mmol) gave compound **111g** as a brown solid (1.90 g, 40%); m.p = 232-234 °C (lit m.p =230-237 ◦C);[166] **^{1}H NMR (400 MHz, DMSO-d$_6$)**, **δ_H** (ppm) 13.51 (br.s, 1H, H-1), 8.07 (d, 1H, *J* = 3.6 Hz , H-9), 7.43 (s, 1H, H-5), 7.11 (d, 1H, *J* = 3.6 Hz, H-7), 6.77 (dd, 1H, *J* = 1.6 Hz and *J* = 1.4 Hz, H-8); **^{13}C NMR (100 MHz, DMSO-d$_6$)**, **δ_C** (ppm) 197.60 (C-3), 172.47 (C-2), 150.06 (C-6), 148.49 (C-9) 123.98 (C-4), 119.79 (C-5), 117.48 (C-7), 114.29 (C-8); **IR** (KBr cm^{-1}): 3324 (N-H), 2987 (ArCH), 1735 (C=O), 1603 (C=C), 1017 (C=S).

6.11. General method for Synthesis of ethyl-2-(5-(4-benzylidine)-2-oxo-5-thioxothiazolidin-3-yl) conjugates. (105a-j)

An appropriate 5-(4-benzylidene)-2-thioxothiazolidin-5-one **111a-g** (1 mmol) and potassium hydroxide (1 mmol) in dioxane (20 ml) were stirred for 20 minutes at 120 ℃. After this reaction time, ethyl 2-(2-bromoacetamido) esters **107a-d** (1 mmol) were added, and the mixture was refluxed for 12 hours. The resultant mixture was then concentrated at a reduced pressure to obtain the crude products which were washed with a mixture of hexane and ethyl acetate (10 ml) and the resultant precipitate was collected by filtration and dried at room temperature for 24 hours to give the desired products **105a-j**.

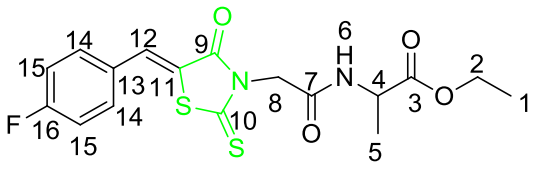
6.11.1. Ethyl-2-(2-(5-(4-chlorobenzylidene)-2-oxo-4-thioxothiazolidin-3-yl) acetamido)alaninate (105a).



105a

A reaction of 5-(4-chlorobenzylidene)-2-thioxothiazolidin-5-one **111b** (0.20 g, 3.60 mmol) and ethyl-2-(2-bromoacetamido) alaninate **107a** (0.70 g, 3.60 mmol) in dioxane gave compound **105a** as an orange solid (0.47g, 32%); m.p = 149.9-151.4 °C; **^{1}H NMR (400 MHz, DMSO-d$_6$)**, **δ_H** (ppm) 8.84 (d, 1H, *J* = 7.2 Hz, H-6), 7.87 (s, 1H, H-12), 7.69 (d, 2H, *J* = 8.4 Hz, H-14), 7.62 (d, 2H, *J* = 8.8 Hz, H-15), 4.11 (s, 2H, H-8), 4.27 (quint, 1H, *J* = 7.2 Hz, H-4), 4.09 (q, 2H, *J* = 7.2 Hz, H-2), 1.31 (d, 3H, *J* = 7.2 Hz, H-5), 1.17 (t, 3H, *J* = 7.2 Hz, H-1); **^{13}C NMR (100 MHz, DMSO-d$_6$)**, **δ_C** (ppm) 192.16 (C-10), 179.17 (C-9), 172.56 (C-7), 170.78 (C-3), 165.69 (C-16), 136.08 (C-13), 134.44 (C-12), 133.49(C-14), 129.95 (C-14), 127.27 (C-11), 60.10 (C-2), 48.70 (C-4), 36.92 (C-8), 17.41 (C-5), 14.44 (C-1) ; **IR** (KBr cm^{-1}): 3349 (N-H), 2989 (ArCH), 1732 (C=O), 1688 (C=O), 1680 (C=O), 1585 (C=C), 1086 (C=S); **HRMS**: calcd for $C_{17}H_{17}ClN_2O_4S_2$ (ESI-TOF, [M]+H), 413.0318; found, 413.0364.

6.11.2. Ethyl-2-(2-(5-(4-fluorobenzylidene)-2-oxo-4-thioxothiazolidin-3-yl) acetamido)alaninate (105b).



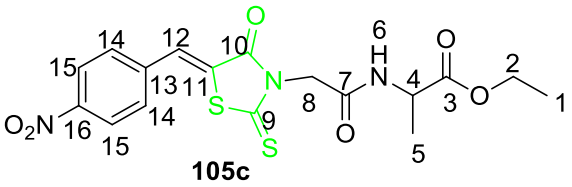
105b

A reaction of 5-(4- methylbenzylidene)-2-thioxothiazolidin-5-one **111d** (1.02 g, 4.25 mmol) and ethyl 2-(2-bromoacetamido) alaninate **107a** (0.82 g, 4.25 mmol) in dioxane gave compound **105b** as a light brown (1.12g, 68%); m.p = 129.4-131.8 °C; **^{1}H NMR (400 MHz, DMSO-d$_6$)**, **δ_H** (ppm) 8.89 (d, 1H, *J* = 7.2 Hz, H-6), 7.87 (s, 1H, H-12), 7.73 (dd, 2H, $^{3}J_{FH}$ = 17.2 Hz, and J_{HH} = 8.4 Hz, H-14), 7.40 (dd, 2H, $^{4}J_{FH}$ = 8.4 Hz and J_{HH} = 5.6 Hz, H-15), 4.31 (s, 2H, H-8), 4.27 (quint, 1H, *J* = 7.2 Hz, H-4), 4.08 (q, 2H, *J* = 7.2, H-2), 1.30 (d, 3H, *J* = 7.6 Hz, H-5) 1.16 (t, 3H, *J* = 6.8 Hz, H-1); **^{13}C NMR (100**

MHz, DMSO-d$_6$), **δ**$_C$ (ppm) 192.20 (C-10), 179.25 (C-9), 172.58 (C-7), 172.36 (C-3), 134.76 (d, ^1J = 250.0 Hz, C-15), 133.47 (d, ^2J = 22.0 Hz, C-14), 133.38 (C-12), 130.70 (d, ^4J = 3.0 Hz, C-12), 126.30 (C-11), 116.99 (d, ^3J = 9.0 Hz, C-13), 61.07 (C-2), 48.71 (C-4), 36.89 (C-8), 17.39 (C-16), 14.45 (C-1); **IR** (KBr cm^{-1}): 3352 (N-H), 2979 (ArCH), 1733 (C=O), 1688 (C=O), 1660 (C=O), 1595 (C=C), 1029 (C=S); **HRMS**: calcd for $C_{17}H_{17}FN_2O_4S_2$ (ESI-TOF, [M]+H), 397.0634; found, 397.0647

6.11.3. Ethyl-2-(2-(5-(4-nitrobenzylidene)-2-oxo-4-thioxothiazolidin-3-yl) acetamido)alaninate (105c)

Chemical Formula: $C_{17}H_{17}N_3O_6S_2$

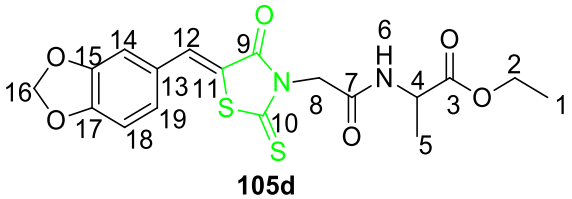


105c

A reaction of 5-(4-nitrobenzylidene)-2-thioxothiazolidin-5-one **111c** (0.31 g, 1.13 mmol) and ethyl-2-(2-bromoacetamido) alaninate **107a** (0.25 g, 1.13 mmol) in dioxane gave compound **105c** as a brown solid (0.87 g, 92%); m.p = 181.2-183.4 °C; **^{1}H NMR (400 MHz, DMSO-d$_6$)**, **δ**$_H$ (ppm) 8.88 (d, 1H, *J* = 6.8 Hz, H-6), 8.33 (d, 2H, *J* = 8.8 Hz, H-14), 7.95 (s, 1H, H-12), 7.90 (d, 1H, *J* = 8.8 Hz, H-14), 4.34 (s, 2H, H-8), 4.28 (quint, 1H, *J* = 4.0 Hz, H-4), 4.09 (q, 2H, *J* = 2.0 Hz, H-2), 1.31 (d, 3H, *J* = 7.2 Hz, H-5), 1.17 (t, 3H, *J* = 7.2 Hz, H-1); **^{13}C NMR (100 MHz, DMSO-d$_6$)**, **δ**$_C$ (ppm) 192.71 (C-9), 178.90 (C-10), 172.58 (C-7), 165.62 (C-3), 148.20 (C-16), 139.88 (C-13), 132.93 (C-12), 131.75 (C-14), 130.64 (C-11) 124.76 (C-15), 61.07 (C-2), 48.71 (C-4), 37.19 (C-8), 17.42 (C-5), 14.46 (C-1); **IR** (KBr cm^{-1}): 3342 (N-H), 2973 (ArCH), 1729 (C=O), 1698 (C=O), 1658 (C=O), 1604 (C=C), 1021 (C=S); **HRMS**: calcd for $C_{17}H_{17}N_3O_6S_2$ (ESI-TOF, [M]+H), 424.0559; found, 424.0531

6.11.4. Ethyl-2-(2-(5-benzol[d][1.3]dioxol-5-ylmethylene)-2-oxo-4-thioxothiazolidin-3-yl)acetamido)alaninate (105d)

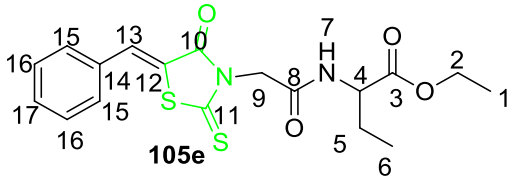
Chemical Formula: $C_{18}H_{18}N_2O_6S_2$



105d

A reaction of 5-benzol[d][1.3]dioxol-5-ylmethylene-2-thioxothiazolidin-5-one **111e** (0.7 g, 2.64 mmol) and ethyl-2-(2-bromoacetamido) alaninate **107a** (0.51 g, 2.64 mmol) in dioxane gave compound **105d** as a yellow solid (0.73 g, 65%); m.p = 272.3-275.1 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$)**, **δ_H** (ppm) 8.87 (d, 1H, *J* = 6.8 Hz, H-6), 7.55 (s, 1H, H-12), 7.23 (d, 1H *J* = 8.4 Hz, H-18), 7.14 (dd, 1H, *J* = 1.4 Hz and *J* = 8.0 Hz, H-19), 6.99 (d, *J* = 7.6 Hz, H-14) 6.14 (s, 2H, H-16), 4.33 (s, 2H, H-8), 4.10 (quint, 1H, *J* = 3.6 Hz, H-4), 4.09 (q, 1H, *J* = 6.8 Hz, H-2), 1.30 (d, 3H, *J* = 7.2 Hz, H-16), 1.16 (t, 3H, *J* = 7.2 Hz, H-1); **^{13}C NMR (100 MHz, DMSO-d$_6$)**, **δ_C** (ppm) 191.47 (C-10), 179.42 (C-9), 169.75 (C-7), 165.38 (C-3), 150.14 (C-20), 148.76 (C-15), 136.08 (C-14), 132.41 (C-12), 127.20 (C-11), 123.59 (C-18), 109.50 (C-16), 102.62 (C-19), 61.08 (C-2), 48.71 (C-4), 36.74 (C-8), 17.39 (C-5), 14.45 (C-1); **IR** (KBr cm^{-1}): 3302 (N-H), 2990 (ArCH), 1738 (C=O), 1692 (C=O), 1654 (C=O), 1628 (C=C), 1098 (C=S); **HRMS**: calcd for $C_{18}H_{18}N_2O_6S_2$ (ESI-TOF, [M]+H), 423.0606; found, 423.0642

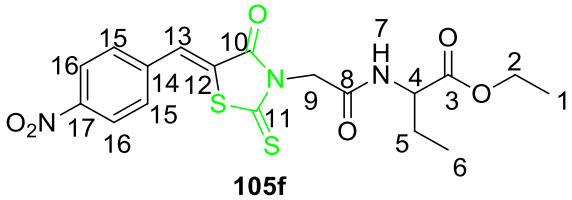
6.11.5. Ethyl-2-(2-(5-benzylidene-2-oxo-4-thioxothiazolidin-3-yl) acetamido)butanoate (105e)



105e

A reaction of 5-benzylidene-2-thioxothiazolidin-5-one **111a** (0.7 g,3.16 mmol), and ethyl-2-(2-bomoacetamido) butanoate **107b** (0.66 g, 3.16 mmol) in dioxane gave compound **105e** as a white solid (0.5 g, 40%); m.p = 143.8-152.8 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$)**, **δ_H** (ppm) 8.86 (d, 1H, *J* = 8.0 Hz, H-7), 7.85 (s, 1H, H-13), 7.67 (d, 2H, *J* = 8.0 Hz, H-15), 7.57-7.52 (m, 3H, H-16+H-17), 4.36 (s, 2H, H-7), 4.19 (quint, 1H, *J* = 4.0 Hz, H-4), 4.08 (q, 2H, *J* = 8.0 Hz, H-2), 1.75-1.72 (m, 1H, H-5a), 1.68-1.58 (m,b1H, H-5b), 1.16 (t, 3H, *J* = 8.0 Hz, H-1), 0.91 (t, 3H, *J* = 4.0 Hz, H-6), **^{13}C NMR (100 MHz, DMSO-d$_6$)**, **δ_C** (ppm) 192.78 (C-11), 179.07 (C-10), 172.37 (C-8), 165.68 (C-3), 135.88 (C-13), 131.26 (C-15), 129.52 (C-16+C-117), 126.90 (C-14) 61.02 (C-2), 54.72 (C-4), 37.01 (C-9), 24.59 (C-5), 14.58 (C-1), 10.60 (C-6)); **IR** (KBr cm^{-1}): 3342 (N-H), 2979 (ArCH), 1736 (C=O), 1693 (C=O), 1661 (C=O), 1604 (C=C),1071 (C=S); **HRMS**: calcd for Chemical Formula: $C_{18}H_{20}N_2O_4S_2$ (ESI-TOF, [M]+H), 393.064; found, 393.0673

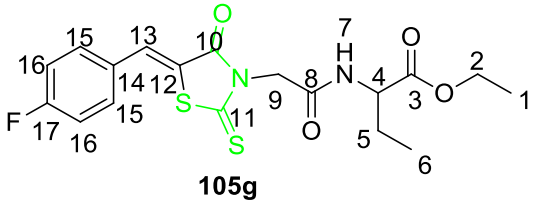
6.11.6. Ethyl-2-(2-(5-(4-nitrobenzylidene)-2-oxo-4-thioxothiazolidin-3-yl) acetamido)butanoate (105f)



105f

A reaction of 5-(4-nitrobenzylidene)-2-thioxothiazolidin-5-one **111c** (0.32 g, 1.13 mmol) and ethyl-2-(2-bromoacetamido)butanoate **107b** (0.23 g, 1.13 mmol) in dioxane gave compound **105f** as a brown solid (0.51 g, 99%); m.p = 175.3-177.4 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$)**, **δ_H** (ppm) 8.86 (d, 1H, *J* = 7.6 Hz, H-7), 8.33 (d, 2H, *J* = 8.4 Hz, H-16), 7.94 (s, 1H, H-13), 7.90 (d, 1H, *J* = 8.8 Hz, H-15), 4.23 (d, 2H, *J* = 4.8 Hz, H-9), 4.10 (quint, 1H, *J* = 7.2 Hz, H-4), 4.07 (q, 2H, *J* = 6.8 Hz, H-2), 1.80-1.63 (m, 1H, H-5a), 1.62-1.56 (H-5b), 1.17 (t, 3H, *J* = 7.2 Hz, H-1), 0.91 (t, 3H, *J* = 7.2 Hz, H-6); **^{13}C NMR (100 MHz, DMSO-d$_6$)**, **δ_C** (ppm) 192.86 (C-11), 178.492 (C-10), 171.96 (C-8), 165.99 (C-3), 148.19 (C-17), 139.88 (C-15), 132.92 (C-13), 131.77 (C-15), 130.60 (C-14), 124.75 (C-12), 61.01 (C-2), 54.40 (C-4), 37.16 (C-9), 24.77 (C-5), 14.57 (C-1), 10.59 (C-6); **IR** (KBr cm^{-1}): 3288 (N-H), 2975 (ArCH), 1727 (C=O), 1703 (C=O), 1654 (C=O), 1554 (C=C), 1023 (C=S); **HRMS**: calcd for $C_{18}H_{19}N_3O_6S_2$ (ESI-TOF, [M]+H), 438.0715; found, 438.0759

6.11.7. Ethyl-2-(2-(5-(4-fluorobenzylidene)-2-oxo-4-thioxothiazolidin-3-yl) acetamido)butanoate (105g)

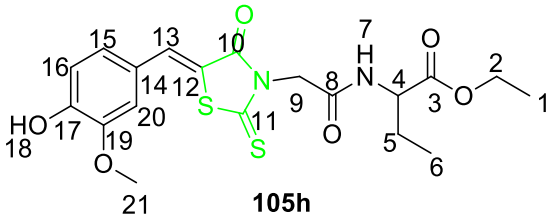


105g

A reaction of 5-methylbenzylidene-2-thioxothiazolidin-5-one **111d** (1.03 g, 4.25 mmol) and ethyl-2-(2-bromoacetamido) butanoate **107b** (0.88 g, 4.25 mmol) in dioxane gave compound **105g** as a brown solid (0.52 g, 30%); m.p = 245.8-256.7 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$)**, **δ_H** (ppm) 8.80 (d, 1H, *J* = 7.2 Hz, H-7), 7.87 (s, 1H, H-13), 7.74 (dd, 2H, $^{3}J_{FH}$ = 17.2 Hz, and J_{HH} = 8.4 Hz, H-15), 7.40 (dd, 2H, J_{FH} = 8.4 Hz and J_{HH} = 5.6 Hz, H-16, 4.21 (s, 2H, H-9), 4.10 (quint, 1H, *J* = 5.6 Hz, H-4), 4.08 (q, 2H, *J* = 3.6 Hz, H-2), 1.77-1.70 (m, 1H, H-5a), 1.67-1.62 (m, 1H, H-5b), 1.17 (t, 3H, *J* = 7.2 Hz, H-1),

0.90 (t, 3H, *J* = 7.2 Hz, H-6), **^{13}C NMR (100 MHz, DMSO-d$_6$)**, **δ$_C$** (ppm) 192.24 (C-11), 179.22 (C-10), 171.95 (C-8), 166.01 (C-3), 134.75 (C-117), 133.45 (d, *J* = 9.0 Hz, C-15), 130.99 (C-13), 130.32 (C-14), 126.32 (C-12), 116.99 (d, *J* = 250.0 Hz, C-116), 61.00 (C-16), 54.38 (C-4), 36.90 (C-9), 24.79 (C-5), 14.50 (C-1) 10.57 (C-6); **IR** (KBr cm^{-1}): 3495 (N-H), 2999 (ArCH), 1732 (C=O), 1682 (C=O), 1599 (C=O), 1566(C=C), 1022 (C=S); **HRMS**: calcd for $C_{18}H_{19}FN_2O_4S_2$ (ESI-TOF, [M]+H), 407.1021; found, 407.1015

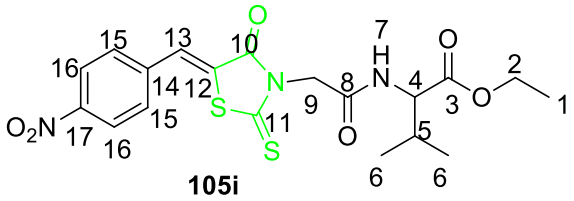
6.11.8. Ethyl-2-(2-(5-(3-hydroxy-4-methoxybenzylidene)-2-oxo-4-thioxothiazolidin-3-yl) acetamido)-3-methylbutanoate (105h)



105h

A reaction of 5-(3-hydroxy-4-methoxybenzylidene)-2-thioxothiazolidin-5-one **111f** (0.5g, 1.87 mmol) and ethyl-2-(2-bromoacetamido) butanoate **107b** (0.39 g, 1.87 mmol) in dioxane gave compound **105h** as a brown solid (0.20 g, 3%); m.p = 178.9-181.2 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$)**, **δ$_H$** (ppm) 10.31 (s, 1H, H-18), 8.65 (d, 1H, *J* = 8.0 Hz, H-7), 7.90 (s, 1H, H-13), 7.56 (d, 1H, *J* = 4.0 Hz, H-15), 7.09 (dd, 1H, *J* = 4.0 Hz and *J* = 1.3 Hz, H-16), 6.95 (d, 1H, *J* = 8.0 Hz, H-20), 4.26 (s, 2H, H-9), 4.17 (quint, 1H, *J* = 4.0 Hz, H-4), 4.10 (q, 2H, *J* = 8.0 Hz, H-2), 3.87 (s, 3H, H-20), 1.75-1.72 (m, 1H, H-5a), 1.68-1.58 (m, 1H, H-5b), 1.18 (t, 3H, *J* = 8,0 Hz, H-1), 0.88 (t, 3H, *J* = 8.0 Hz, H-6); **^{13}C NMR (100 MHz, DMSO-d$_6$)**, **δ$_C$** (pp) 196.10 (C-11), 169.35 (C-10), 168.38 (C-8), 166.65 (C-3), 156.60 (C-19), 148.28 (d, ^1J = 250.0 Hz, C-17), 132.90 (C-13), 125.94 (d, ^4J = 3.0 Hz, C-14), 124.61 (C-20), 119.22 (C-12), 116.50 (d, ^2J = 22.0 Hz, C-16), 114.52 (d, ^3J = 9.0 Hz, C-15), 61.63 (C-2), 56.07 (C-21), 53.70 (C-4), 36.72 (C-7), 24.97 (C-6), 14.53 (C-1), 10.38 (C-6); **IR** (KBr cm^{-1}): 3193 (N-H), 2934 (ArCH) , 1723 (C=O), 1682 (C=O), 1659 (C=O), 1588 (C=C), 1029 (C=S); **HRMS**: calcd for $C_{19}H_{22}N_2O_6S_2$, [M]+H), 438.0919; found, 438.0969

6.11.9. Ethyl-3-methyl-2-(2-(5-(4-nitrobenzylidene)-2-oxo-4-thioxothiazolidin-3-yl) acetamido) valinate (105i)

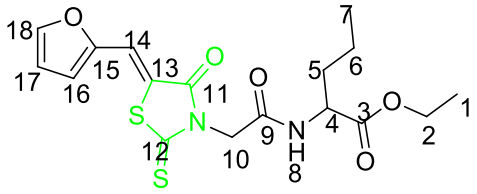


105i

A reaction of 5-(4-nitrobenzylidene)-2-thioxothiazolidin-5-one **111c** (0.30 g, 1.13 mmol) and ethyl-2-(2-bromoacetamido) valinate **107c** (0.25 g, 1.13 mmol) in dioxane gave compound **105i** as a yellow solid (0.42 g, 88%); m.p = 180.8-181.9 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$)**, **δ_H** (ppm) 8.75 (d, 1H, *J* = 8.4 Hz, H-7), 8.31 (d, 2H, *J* = 8.4 Hz, H-16), 7.91 (s, 1H, H-13), 7.88 (d, 1H, *J* = 8.8 Hz, H-15), 4.17 (d, 2H, *J* = 6.8 Hz, H-9), 4.11 (quint, 1H, *J* = 3.6 Hz, H-4), 4.07 (q, 2H, *J* = 3.2 Hz, H-2), 2.05 (septet, 1H, H-5), 1.16 (t, 3H, *J* = 7.2 Hz, H-1), 0.90 (t, 6H, *J* = 6.8 Hz, H-6); **^{13}C NMR (100 MHz, DMSO-d$_6$)**, **δ_C** (ppm) 192.94 (C-11), 178.40 (C-10), 171.51 (C-8), 166.22 (C-3), 148.16 (C-17), 139.84 (C-14), 132.90 (C-13), 132.01 (C-15) 130.66 (C-16), 124.72 (C-12), 61.00 (C-2), 58.42 (C-4), 37.17 (C-9), 30.49 (C-5), 19.33 (C-6), 18.46 (C-6), 14.01 (C-1); **IR** (KBr cm^{-1}): 3293 (N-H), 2938 (ArCH), 1721 (C=O), 1704 (C=O), 1658 (C=O), 1547(C=C), 1026 (C=S); **HRMS**: calcd for $C_{19}H_{21}N_3O_6S_2$ (ESI-TOF, [M]+H), 452.0872; found, 452.0891

6.11.10. Ethyl 2-(2-(5-(furan-2-ylmethylene)-2-oxo-4-thioxothiazolidin-3-yl)acetamido)norvalinate (105j)

Chemical Formula: $C_{17}H_{20}N_2O_5S_2$



102j

A reaction of 5-(furan-2-ylmethylene)-2-thioxothiazolidin-5-one **111g** (0.71 g, 3.16 mmol) and ethyl 2-(2-bromoacetamido) norvalinate **105d** (0.61 g, 3.16 mmol) in dioxane gave compound **105j** as a yellow solid (0.73 g, 63 %); m.p = 272.3-274.5 ℃; **^{1}H NMR (400 MHz, DMSO-d$_6$)**, **δ_H** (ppm) 8.70 (d, 1H, *J* = 7.2 Hz, H-8), 7.44 (d, 1H, *J* = 8.8 Hz, H-18), 7.30 (d, 1H, *J* = 2.4 Hz, H-17), 7.23 (s, 1H, H-14), 6.90 (d, 1H, J = 8.8 Hz, H-15), 4.17 (s, 2H, H-10), 4.08 (quint, 1H, *J* = 3.6 Hz, H-4), 4.05 (q, 2H, *J* =

1.6 Hz, H-2), 1.76-1.55 (m, 2H, H-5), 1.35-1.30 (m, 2H, H-6), 1.17 (t, 3H, *J* = 2.8 Hz, H-1), 0.85 (t, 3H, *J* = 7.2 Hz, H-7); **^{13}C NMR (100 MHz, DMSO-d$_6$)**, **δ_C** (ppm) 188.53 (C-11), 178.35 (C-12), 172.75 (C-9), 171.02 (C-3), 132.69 (C-14), 131.82 (C-18), 130.82 (C-15), 126.22 (C-17), 122.90 (C-13), 116.75 (C-15), 61.01 (C-2), 52.68 (C-4), 36.78 (C-9), 33.39 (C-5), 18.94 (C-6), 14.49 (C-1), 13.90 (C-7); **IR** (KBr cm^{-1}): 3302 (N-H), 2990 (ArCH), 1738 (C=O), 1692 (C=O), 1659 (C=O),1588 (C=C), 1098 (C=S); **HRMS**: calcd for $C_{17}H_{20}N_2O_5S_2$ (ESI-TOF, [M]+H), 397.0174; found, 397.0163.

6.2. Biological assays

6.2.1. General in vitro cytotoxic assays method

Dulbecco's Modified Eagle Media – Low glucose (DMEM-LG) and Foetal Bovine Serum (FBS) were purchased from GE Healthcare Life Sciences (Logan, UT, USA). PBS with and without Ca2+ and Mg2+ and trypsin was purchased from Lonza (Wakersville, MD, USA). Bis-benzamide H 33342 trihydrochloride (Hoechst) and propidium iodide (PI) was purchased from Sigma (St. Louis, MO, USA). All the synthesized compounds were dissolved to give a final concentration of 100 µM. Samples were sonicated if solubility was a problem and stored at 4°C until required. The human colorectal adenocarcinoma cell line ($Caco_2$) was used for cytotoxicity screening. Cells were maintained in 10 cm culture dishes in 10 mL complete medium (DMEM-LG + 10% FBS + 1x penicillin/streptomycin) and incubated at 37°C, 5% CO2, and 100% relative humidity until needed and were seeded in 96 well plates at 4000 cells/well (100 µL aliquots) and left overnight to attach. 200 µM dilutions of each compound were prepared in complete medium and 100 µL aliquots of 200 µM dilution were added to the 100 µL of medium in which 96 cells were seeded in 96 well plates at 4000 cells/well (100 µL aliquots) and left overnight to attach, thus yielding a final concentration of 100 µM before being treated for 48 hours at 37°C, 5% CO2. 100 µL staining solution Hoechst (5 µg/mL) and 10 µL PI solution was used as stains dyes and plates were incubated for 30 minutes after which were acquired immediately using the DAPI and Texas Red filters. Quantification of live and dead cells were performed using the ImageXpress Micro XLS Widefield Microscope (Molecular Devices) using a 10x Plan Fluor objective and DAPI and Texas Red filter cubes. Acquired images were analysed using the MetaXpress software and Multi-Wavelength Cell Scoring Application Module. Acquired data was transferred to an excel spreadsheet and data

analysed and processed. The green line in appendix 29,31 and 33 indicated the average number of lives cell in the untreated (UT) control population. The red line indicated 70% of the average number of lives cell in the untreated (UT) control population and the black line showed the average number of live cells treated with melphalan whereas error bars indicated standard deviation of the mean of 4 replicate wells from a single experiment.

6.2.2. General in vitro α-glucosidase assay method

Saccharomyces cerevisiae α-glucosidase (50 µg/mL) and substrate (p-nitrophenyl glucopyranoside) were purchased from Sigma-Aldrich. Enzyme was prepared in potassium phosphate buffer (pH 6.3, 3 mM), and the synthesized compounds and were dissolved in DMSO at a final concentration of 100 µM. Samples were sonicated if solubility was a problem and stored at 4°C until required. Sample concentrations tested were 10, 50 and 100 µM. The various concentrations of compounds (5 µL), enzyme solution (20 µL), and potassium phosphate buffer (60 µL), were added in the 96-well plate and incubated at 37 °C for 5 min. Thereafter, the substrate (10 µL, 4 mM) was added to the mixture and allowed to incubate at 37 °C for 20 min followed by the addition of sodium carbonate (25 µl). Eventually, the change in absorbance was measured by BioTek® PowerWave XS spectrophotometer (Winooski, VT, USA). Epigallacatechin gallate (EGCG) (200 µM) was used as control whereas error bars indicated the standard deviation of the mean. No enzyme and no substrate controls were included, and the percentage α-glucosidase inhibition was calculated using as follows:

$$\%\ \alpha\text{-glucosidase inhibition} = \frac{(\text{A405nm of control} - \text{A405nm of test sample})}{\text{A405nm of control}}\ x\ 100$$

References

1. Lakhtakia, R. The history of diabetes mellitus. *Sultan Qaboos Univ. Med. J* **13**, 368–370 (2013).
2. Ahmed, A. M. History of diabetes mellitus. *Saudi Med. J* **23**, 373–378 (2002).
3. Pathak, A. K., Sinha, P. K. & Sharma, J. Diabetes – A Historical review. *J. Drug Deliv. Ther.* **3**, 83–84 (2013).
4. Robertson, R. Perspectives in Diabetes History of Diabetes. *Diabetes* **41**, 1–5 (1992).
5. Tamilnadu, C. Evaluation of retinal ganglion cell activity by pattern visual evoked potentials in type 2 diabetic patients. (2017).
6. Gutteridge, I. . Diabetes mellitus : a brief history , epidemiology ,definition and classification. *Clin. Exp. Optom.* **82**, 102–106 (1999).
7. Brown, T. Design thinking. *Harv. Bus. Rev.* **86**, 84–92 (2008).
8. Shouhip, H. A. Diabetes mellitus. *Rev. Bras. Med.* **62**, 60–71 (2005).
9. Zhang, X. F. & Tan, B. K. Effects of an ethanollic extract of Gynura procumbens on serum glucose, cholesterol and triglyceride levels in normal and streptozotocin-induced diabetic rats. *Singapore Med. J.* **41**, 9–13 (2000).
10. Windecker, R., Kindermann, S. & Spraul, M. Tip Therm: A simple screening method for Temperature sensation. *Diabetologia* **40**, 483 (1997).
11. Chiang, J. L., Kirkman, M. S., Laffel, L. M. B. & Peters, A. L. Type 1 diabetes through the life span: A position statement of the American Diabetes Association. *Diabetes Care* **37**, 2034–2054 (2014).
12. Gregory, J. M., Moore, D. J. & Simmons, J. H. Type 1 diabetes mellitus. *Pediatr. Rev.* **34**, 203–215 (2013).
13. Selvin E, Parrinello CM, Sacks DB, C. J. Trends in Prevalence and Control of Diabetes in the U.S., 1988-1994 and 1999-2010. *Ann. Intern Med.* **160**, 517–525 (2015).

14. Catarino, D. *et al.* Non-immune-mediated versus immune-mediated type 1 diabetes: Diagnosis and long-term differences - Retrospective analysis. *Diabetol. Metab. Syndr.* **12**, 4–9 (2020).

15. Dreyer, M. Type 1 diabetes. *Diabetologe* **15**, 400–407 (2019).

16. Kaneto, H. Pathophysiology of type 2 diabetes mellitus. *Nihon Rinsho.* **73**, 2003–2007 (2015).

17. Cantley, J. & Ashcroft, F. M. Q&A: Insulin secretion and type 2 diabetes: Why do β-cells fail? *BMC Biol.* **27**, 269 (2015).

18. Olokoba, A. B., Obateru, O. A. & Olokoba, L. B. Type 2 Diabetes: a Review of current trends -. *Oman Med. J.* **7**, 61–66 (2015).

19. Chawla, A., Chawla, R. & Jaggi, S. Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum? *Indian J. Endocrinol. Metab.* **20**, 546–553 (2016).

20. Hauner, H. & Scherbaum, W. A. Type 2 diabetes. *medlineplus* **127**, 1003–1005 (2002).

21. Buchanan, T. A. & Xiang, A. H. Gestational diabetes mellitus. *J. Clin. Invest.* **115**, 485–491 (2005).

22. Catalano, P. M., Kirwan, J. P., Haugel-De Mouzon, S. & King, J. Gestational diabetes and insulin resistance: Role in short- and long-term implications for mother and fetus. *J. Nutr.* **133**, 1674–1683 (2003).

23. Dias, S., Pheiffer, C., Rheeder, P. & Adam, S. Screening and diagnosis of gestational diabetes mellitus in South Africa: What we know so far. *S.A. Med. J.* **109**, 457–462 (2019).

24. Chukwuemeka, S. C. Adverse foetal outcomes in gestational diabetes : a systematic review and meta-analysis . (2020).

25. Li, S., Wang, J., Zhang, B., Li, X. & Liu, Y. Diabetes Mellitus and Cause-Specific Mortality : A Population-Based Study. *J Diabetes metab* **43**, 319–341 (2019).

26. Saeedi, P. *et al.* Global and regional diabetes prevalence estimates for 2019

and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res. Clin. Pract.* **157**, 107843 (2019).

27. Unwin, N., Gan D., Mbanya J.C., Ramachandran, A. & Roglic, G., Shaw, J., Soltèsz, G., Whiting, D., Zgibor, j., Zhang, P., Zimmet, P. IDF Diabetes Atlas Fourth Edition. in *IDF* 1–527 (2009).

28. Zimmet, P. Z. Diabetes and its drivers: The largest epidemic in human history? *Clini. Diabetes Endocrinol.* **3**, 1–8 (2017).

29. IDF Atlas, I. D. F. IDF Diabetes atlas. in *8th edition* 1–150 (2017).

30. Sheleme, T., Mamo, G., Melaku, T. & Sahilu, T. Glycemic Control and its predictors among adult diabetic patients attending mettu karl referral hospital, southwest ethiopia: a prospective bservational study. *Diabetes Ther.* **11**, 1775–1794 (2020).

31. Asmelash, D. & Asmelash, Y. The burden of undiagnosed diabetes mellitus in adult African population: A systematic review and meta-analysis. *J. Diabetes Res.* **1**, 1–8 (2019).

32. Feachem, R. G., Jamison, D. T. & Bos, E. R. *Disease and mortality in sub-Saharan Africa*. *The world bank* (The World Bank, 1991).

33. Assah, F. K. & Mbanya, J.-C. Diabetes in Sub-Saharan Africa – Overview of a looming health challenge. *Eur. Endocrinol.* **5**, 13 (2009).

34. Peer, N., Kengne, A. P., Motala, A. A. & Mbanya, J. C. Diabetes in the Africa region: An update. *Diabetes Res. Clin. Pract.* **103**, 197–205 (2014).

35. El-Kebbi MI, Bidikian NH, Hneiny L, N. M. Epidemiology of type 2 diabetes in the Middle East and North Africa: Challenges and call for action. *J. Diabetes* **12**, 1401–25 (2021).

36. Werfalli, M. *et al.* Diabetes in South African older adults: prevalence and impact on quality of life and functional disability–as assessed using SAGE Wave 1 data. *Glob. Health Action* **11**, 1449–924 (2018).

37. Hill, J. *et al.* Findings from community-based screenings for type 2 diabetes

mellitus in at risk communities in Cape Town, South Africa: A pilot study. *Int. J. Environ. Res. Public Health* **17**, 1–15 (2020).

38. Mutyambizi, C., Booysen, F., Stokes, A., Pavlova, M. & Groot, W. Lifestyle and socio-economic inequalities in diabetes prevalence in South Africa: A decomposition analysis. *PLoS One* **14**, 1–21 (2019).

39. Pheiffer, C. *et al.* The prevalence of type 2 diabetes in South Africa: A systematic review protocol. *BMJ* **8**, 2–5 (2018).

40. Statistics South Africa. Selected health indicators among the elderly: Findings from the SADHS 2016, GHS 2016 and MACoD 2016. *Stats South Africa* 56 www.statssa.gov.za (2019).

41. Vecchio, I., Tornali, C., Bragazzi, N. L. & Martini, M. The discovery of insulin: An important milestone in the history of medicine. *Front. Endocrinol. (Lausanne).* **9**, 1–8 (2018).

42. Best, C. H. The history of insulin. *Diabetes* **11**, 495–503 (1962).

43. Dave, J. A. & Delport, S. V. Prescribing insulin in type 1 diabetes mellitus: An update for general practitioners. *S.A. Fam. Pr.* **48**, 30–36 (2006).

44. Marín-Peñalver, J. J., Martín-Timón, I., Sevillano-Collantes, C. & Cañizo-Gómez, F. J. Update on the treatment of type 2 diabetes mellitus. *World J. Diabetes* **7**, 354–359 (2016).

45. Scheen, A. J. Sulphonylureas in the management of type 2 diabetes: To be or not to be? *Diabetes Epidemiol. Manag.* **1**, 1000–02 (2021).

46. Krentz, A. J. & Bailey, C. J. Oral antidiabetic agents: Current role in type 2 diabetes mellitus. *Drugs* **65**, 385–411 (2005).

47. R.A DeFronzo. Pharmacologic therapy for type 2 diabetes mellitus. *Ann. Intern. Med.* **131**, 281–303 (1999).

48. McLeod, J. F. Clinical Pharmacokinetics ofof Nateglinide. *Clin. pharmcokinet.* **43**, 97–120 (2004).

49. Rabasa-Lhoret, R. & Chiasson, J. L. Potential of α-glucosidase inhibitors in

elderly patients with diabetes mellitus and impaired glucose tolerance. *Drugs and Aging* **13**, 131–143 (1998).

50. Brown, T. Deisign thinking. *Harv. Bus. Rev.* **86**, 84–92 (2008).

51. Lee, Y. H., Wang, M. Y., Yu, X. X. & Unger, R. H. Glucagon is the key factor in the development of diabetes. *Diabetologia* **59**, 1372–1375 (2016).

52. Ludvik, B., Kautzky-willer, A., Prager, R., Thomaseth, K. & Pacini, G. Amylin : History and Overview. *Diabet. Med.* 9–13 (1997).

53. Renukuntla, V. S., Ramchandani, N., Trast, J., Cantwell, M. & Heptulla, R. A. Role of Glucagon-like peptide-1 analogue versus Amylin as an adjuvant therapy in type 1 diabetes in a closed loop setting with ePID algorithm. *J. Diabetes Sci. Technol.* **8**, 1011–1017 (2014).

54. Boer, G. A. & Holst, J. J. Incretin Hormones and Type 2 Diabetes—Mechanistic Insights and Therapeutic Approaches. *Biology (Basel).* **9**, 473–485 (2020).

55. Klonoff, D. C. Incretin therapy for type 2 diabetes mellitus. *Adv. Ther.* **27**, 881–894 (2010).

56. Abbott, C. A., McCaughan, G. W., Baker, E. & Sutherland, G. R. Genomic organization, exact localization, and tissue expression of the human CD26 (dipeptidyl peptidase IV) gene. *Immunogenetics* **40**, 331–338 (1994).

57. Lorenzi, M. The polyol pathway as a mechanism for diabetic retinopathy: Attractive, elusive, and resilient. *Exp. Diabesity Res.* **2007**, (2007).

58. Aslan, H. E. & Beydemir S. Phenolic compounds: The inhibition effect on polyol pathway enzymes. *Chem. Biol. Interact.* **266**, 47–55 (2017).

59. Carper, D. *et al.* Aldose reductase and ϱ-crystallin belong to the same protein superfamily as aldehyde reductase. *FEBS Lett.* **220**, 209–213 (1987).

60. Bailey, C. J. Metformin: historical overview. *Diabetologia* **60**, 1566–1576 (2017).

61. Lehrer, S. Inhaled biguanides and mTOR inhibition for influenza and coronavirus (Review). *J. World Acad. Sci.* **2**, 1–11 (2020).

62. Rashid, M., Shrivastava, N. & Husain, A. Synthesis and SAR strategy of

thiazolidinedione: a novel approach for cancer treatment. *J. Chil. Chem. Soc.* **65**, 4817–4832 (2020).

63. Gale, E. A. M. Lessons from the glitazones: A story of drug development. *Lancet* **357**, 1870–1875 (2001).

64. Devchand, P. R., Liu, T., Altman, R. B., FitzGerald, G. A. & Schadt, E. E. The pioglitazone trek via human PPAR gamma: From discovery to a medicine at the FDA and beyond. *Front. Pharmacol.* **9**, 1–9 (2018).

65. Chaudhury, A. *et al.* Clinical review of antidiabetic drugs: implications for type 2 diabetes mellitus management. *Front. Endocrinol. (Lausanne).* **8**, 1–12 (2017).

66. Desouza, C. V. & Shivaswamy, V. Pioglitazone in the treatment of type 2 diabetes: safety and efficacy review. *Clini. Med. Insights Endocrinol, Diabetes* **3**, 43–51 (2010).

67. Wang, C. *et al.* The skeletal effects of thiazolidinedione and metformin on insulin-resistant mice. *J Bone Miner. Metab* **30**, 630–637 (2012).

68. Soccio R.E, C. E. . and L. M. . Thazolidinediones and the Promise of Insulin Sensitization in Type 2 Diabetes. *Cell. Metab.* **20**, 573–591 (2014).

69. Bae, J., Park, T., Kim, H., Lee, M. & Cha, B. S. Lobeglitazone: A novel thiazolidinedione for the management of type 2 diabetes mellitus. *J. Diabetes Metab.* **45**, 326–336 (2021).

70. Olefsky, J. M. Treatment of insulin resistance with peroxisome proliferator-activated receptor γ agonists. *J. Clin. Invest.* **106**, 467–472 (2000).

71. Nanjan, M. J., Mohammed, M., Prashantha Kumar, B. R. & Chandrasekar, M. J. N. Thiazolidinediones as antidiabetic agents: A critical review. *Bioorg. Chem.* **77**, 548–567 (2018).

72. Bhatti, R. S., Shah, S., Suresh, Krishan, P. & Sandhu, J. S. Recent pharmacological developments on rhodanines and 2,4-thiazolidinediones. *Int. J. Med. Chem.* **3**, 1–16 (2013).

73. Thangavel, N., Al Bratty, M., Akhtar Javed, S., Ahsan, W. & Alhazmi, H. A. Targeting peroxisome proliferator-activated receptors using thiazolidinediones:

strategy for design of novel antidiabetic drugs. *Int. J. Med. Chem.* **2**, 1–20 (2017).

74. Yasmin, S. *et al.* Novel benzylidene thiazolidinedione derivatives as partial PPARγ agonists and their antidiabetic effects on Type 2 Diabetes. *Sci. Rep.* **7**, 1–17 (2017).

75. Ahmadi, A., Khalili, M., Samavat, S., Shahbazi, E. & Nahri-Niknafs, B. Synthesis and evaluation of the hypoglycemic and hypolipidemic activity of novel arylidene thiazolidinedione analogson a type 2 diabetes model. *J. Pharm. Chem.* **50**, 165–171 (2016).

76. Marc, G. *et al.* New phenolic derivatives of thiazolidine-2,4-dione with antioxidant and antiradical properties: Synthesis, characterization, in vitro evaluation, and quantum studies. *Molecules* **24**, 1–19 (2019).

77. Aneja, D. K. *et al.* Synthesis of new pyrazolyl-2, 4-thiazolidinediones as antibacterial and antifungal agents. *Org. Med. Chem. Lett.* **1**, 15 (2011).

78. Sharma, R. K. *et al.* Synthesis and structure-activity-relationship studies of thiazolidinediones as antiplasmodial inhibitors of the Plasmodium falciparum cysteine protease falcipain-2. *Eur. J. Med. Chem.* **90**, 507–518 (2015).

79. Rekha, S. & Shantharam, U. & V. C. Synthesis and evaluation of novel thiazolidinediones for anti inflammatory activity. *Int. Res. J. Pharm.* **2**, 81–84 (2011).

80. Da Rocha Junior, L. F. *et al.* Synthesis of a novel thiazolidinedione and evaluation of its modulatory effect on IFN- γ, IL-6, IL-17A, and IL-22 production in PBMCs from rheumatoid arthritis patients. *Biomed Res. Int.* **13**, 1–8 (2013).

81. Kumar, K. S., Reddy, B. M. & Babu, V. H. Synthesis of some novel 2,4-thiazolidinedione incoparated pyrazole derivatives as cancer agents. *Int. J. Pharm. Pharm. Sci.* **6**, 6–9 (2014).

82. Corigliano, D. M. *et al.* indole and 2,4-thiazolidinedione conjugates as potential anticancer modulators. *Peer. J.* **1**, 1–19 (2018).

83. Chilamakuru, N. B., Shankarananth, V., Rajasekhar, K. K. & Singirisetty, T.

Synthesis, characterisation and anti-tubercular activity of some new 3,5-disubstituted-2,4-thiazolidinediones. *Asian J. Pharm. Clin. Res.* **6**, 29–33 (2013).

84. Mishchenko, M., Shtrygol, S., Kaminskyy, D. & Lesyk, R. Thiazole-bearing 4-thiazolidinones as new anticonvulsant agents. *Sci. Pharm.* **88**, 1–14 (2020).

85. Alhameed, R. A. *et al.* Synthesis and antimicrobial activity of a new series of thiazolidine-2,4-diones carboxamide and amino acid derivatives. *Molecules* **25**, 1–17 (2020).

86. Alagawadi, K. R. & Alegaon, S. G. Synthesis, characterization and antimicrobial activity evaluation of new 2,4-Thiazolidinediones bearing imidazo[2,1-b][1,3,4]thiadiazole moiety. *Arab. J. Chem.* **4**, 465–472 (2011).

87. Bahare, R. S., Ganguly, S., Choowongkomon, K. & Seetaha, S. Synthesis, HIV-1 RT inhibitory, antibacterial, antifungal and binding mode studies of some novel N-substituted 5-benzylidine-2,4-thiazolidinediones. *DARU, J. Pharm. Sci.* **23**, 6 (2015).

88. Rawal, R. K., Prabhakar, Y. S., Katti, S. B. & De Clercq, E. 2-(Aryl)-3-furan-2-ylmethyl-thiazolidin-4-ones as selective HIV-RT Inhibitors. *Bioorg. Med. Chem.* **13**, 6771–6776 (2005).

89. Daş-Evcimen, N., Bozdağ-Dündar, O., Sarikaya, M. & Ertan, R. In vitro aldose reductase inhibitory activity of some flavonyl-2,4-thiazolidinediones. *J. Enzyme Inhib. Med. Chem.* **23**, 297–301 (2008).

90. Gore, S., Chinthapally, K., Baskaran, S. & König, B. Syntheis of substituted hydantoins in low melting mixtures. *Chem. Commun.* **49**, 5052–5054 (2013).

91. Shimizu, T. Studies on the use of hydantoin-related compounds as slow release fertilizers. *Soil Sci. Plant Nutr.* **32**, 373–382 (1986).

92. Meusel, M. & Gütschow, M. Recent developments in hydantoin chemistry: A review. *Org. Prep. Proced. Int.* **36**, 391–443 (2004).

93. Monteiro, J. L., Pieber, B., Corrêa, A. G. & Kappe, C. O. Continuous synthesis of hydantoins: Intensifying the Bucherer-Bergs Reaction. *Synlett* **27**, 83–87 (2016).

94. Patching, S. Synthesis, NMR analysis and applications of isotope-labelled hydantoins. *J. Diagnostic Imaging Ther.* **4**, 3–26 (2017).

95. Colacino, E., Lamaty, F., Martinez, J. & Parrot, I. Microwave-assisted solid-phase synthesis of hydantoin derivatives. *Tetrahedron Lett.* **48**, 5317–5320 (2007).

96. Paul, S., Gupta, M., Gupta, R. & Loupy, A. Microwave assisted synthesis of 1,5-disubstituted hydantoins and thiohydantoins in solvent-free conditions. *Synthesis (Stuttg).* **28**, 75–78 (2002).

97. Muccioli, G. G. *et al.* A rapid and efficient microwave-assisted synthesis of hydantoins and thiohydantoins. *Tetrahedron* **59**, 1301–1307 (2003).

98. Bialonska, D. & Zjawiony, J. K. Aplysinopsins - Marine indole alkaloids: Chemistry, bioactivity and ecological significance. *Mar. Drugs* **7**, 166–183 (2009).

99. Walters, E. W. *et al.* Adenylosuccinate synthetase from maize. *Plant Physiol.* **114**, 549–555 (1996).

100. D. Skropeta, N. P. & Ana, Z. Kinase inhibitors from marine sponges. *Mar. Drugs* **9**, 2131–2154 (2011).

101. Looper, Ryan EJoseph B. Gibbons, Justin M. Salvant, Rachel M. Vaden, Ki-Hyeok Kwon, B. E. W. Synthesis of Naamidine A and selective access to N2 -Acyl-2- aminoimidazole analogues. *J. Org. Chem.* **80**, 10076–10085 (2016).

102. Grosso, C., Valentão, P., Ferreres, F. & Andrade, P. B. Review: Bioactive marine drugs and marine biomaterials for brain diseases. *Mar. Drugs* **12**, 2539–2589 (2014).

103. Jaromin, A. *et al.* Synthesis and antiplasmodial activity of novel bioinspired imidazolidinedione derivatives. *Biomolecules* **11**, 1–13 (2021).

104. Snead, O. C. & Donner, E. J. A new generation of anticonvulsants for the treatment of epilepsy in children. *Paediatr. Child Health (Oxford).* **12**, 741–744 (2007).

105. Cho, S. H., Kim, S. H. & Shin, D. Recent applications of hydantoin and

thiohydantoin in medicinal chemistry. *Eur. J. Med. Chem.* **164**, 517–545 (2019).

106. Kotha, S., Gupta, N. K. & Aswar, V. R. Multicomponent approach to hydantoins and thiohydantoins involving a deep eutectic solvent. *Asian J. Chem.* **14**, 3188–3197 (2019).

107. Cheng, X. C. *et al.* Synthesis of novel imidazolidine-2,4-dione derivatives as potential antidiabetic agents. *Asian J. Chem.* **23**, 4114–4116 (2011).

108. Ahmad, R. *et al.* Chiral aryl sulfonyl hydantoins as hypoglycemic agents. *J. Chem. Sci.* **55**, 203–207 (2000).

109. Rajic, Z. *et al.* Hydantoin derivatives of L- and D-amino acids: Synthesis and evaluation of their antiviral and antitumoral activity. *Molecules* **11**, 837–848 (2006).

110. Fujisaki, F. *et al.* Synthesis of new 5-substituted hydantoins and symmetrical twin-drug type hydantoin derivatives. *Chem. Pharm. Bull.* **62**, 429–438 (2014).

111. Araújo, M. J. *et al.* Imidazolidin-4-one derivatives of primaquine as novel transmission-blocking antimalarials. *J. Med. Chem.* **48**, 888–892 (2005).

112. Xu, Y. *et al.* Antitussive and anti-inflammatory dual-active agents developed from natural product lead compound 1-methylhydantoin. *Molecules* **24**, 1–12 (2019).

113. Abdel-Aziz, A. A. M. *et al.* Synthesis, anti-inflammatory, analgesic and COX-1/2 inhibition activities of anilides based on 5,5-diphenylimidazolidine-2,4-dione scaffold: Molecular docking studies. *Eur. J. Med. Chem.* **115**, 121–131 (2016).

114. Zhang, M., Liang, Y. R., Li, H., Liu, M. M. & Wang, Y. Design, synthesis, and biological evaluation of hydantoin bridged analogues of combretastatin A-4 as potential anticancer agents. *Bioorg Med. Chem.* **25**, 6623–6634 (2017).

115. Angelova, V. . T. *et al.* Synthesis, in vitro antiproliferative and antimycobacterial activity of thiazolidine -2,4-dione and hydantoin derivatives. *Chem. Commun.* **49**, 643–651 (2017).

116. Liu, Y., Zhong, W. & Li, S. Syntheses and studies of hydantoin derivatives as potential anti-tuberculosis inhibitors. *Chem. Lett.* **23**, 133–136 (2012).

117. Pękala, E. *et al.* Synthesis, structure-activity relationship of some new anti-arrhythmic 5-arylidene imidazolidine-2,4-dione derivatives. *Eur. J. Med. Chem.* **40**, 259–269 (2005).

118. Rishipathak D.D, P. S. . and B. D. . Microwave assisted synthesis and biological evaluation of hydantoin derivatives. *Asian J. Chem.* **19**, 5450–5458 (2007).

119. Hidayat, I. W., Thu, Y. Y., Black, S. C. D. & Read, R. W. Biological evaluation of certain substituted hydantoins and benzalhydantoins against microbes. *Mater. Sci. Eng.* **107**, 6–13 (2016).

120. Chylińska, M. *et al.* Synthesis and biocidal activity of novel N-halamine hydantoin-containing polystyrenes. *E-Polymers* **14**, 15–25 (2014).

121. Flosi, W. J. *et al.* Discovery of imidazolidine-2,4-dione-linked HIV protease inhibitors with activity against lopinavir-resistant mutant HIV. *Bioorg Med. Chem.* **14**, 6695–6712 (2006).

122. Ibrahim, T. S. *et al.* Design, synthesis and biological evaluation of novel 5-((substituted quinolin-3-yl/1-naphthyl) methylene)-3-substituted imidazolidin-2,4-dione as HIV-1 fusion inhibitors. *Bioorg. Chem.* **99**, 103–782 (2020).

123. Yamaguchi T, Miura K, Usui T, Unno R, Matsumoto Y, Fukushima M, Mizuno K, Kondo Y, Baba Y, K. M. Synthesis and aldose reductase inhibitory activity of 2-substituted-6-fluoro-2,3-dihydrospiro [4H-1-benzopyran-4, 4'-imidazolidine]-2',5'-diones. *Chem. Pharm. Bull.* **40**, 1569–1572 (1992).

124. Iqbal, Z. *et al.* Synthesis, characterization, hypoglycemic and aldose reductase inhibition activity of arylsulfonylspiro[fluorene-9,5′-imidazolidine]-2′,4′-diones. *Eur. J. Med. Chem.* **98**, 127–138 (2015).

125. Kerru, N., Gummidi, L., Maddila, S., Gangu, K. K. & Jonnalagadda, S. B. A review on recent advances in nitrogen-containing molecules and their biological applications. *Molecules* **25**, 1909–1950 (2020).

126. Panuwat Padungros, A. W. Practical synthesis of aromatic dithiocarbamates. *Synth. Commun.* **44**, 2336–2343 (2015).

127. Hotta, N. *et al.* Long-term clinical effects of epalrestat, an aldose reductase

inhibitor, on diabetic peripheral neuropathy: The 3-year, multicenter, comparative aldose reductase inhibitor-diabetes complications trial. *Diabetes Care* **29**, 1538–1544 (2006).

128. Murugan, R., Anbazhagan, S. L. S. & Narayanan, S. S. Synthesis and in vivo antidiabetic activity of novel dispiropyrrolidines through[3+2] cycloaddition reactions with thiazolidinedione and rhodanine derivatives. *Eur. J. Med. Chem.* **45**, 35–48 (2009).

129. Kapoor, A. & Khare, N. Anti-hyperglycemic evaluation of 2,4-thiazolidinedione and rhodanine derivatives. *Pharm. Chem.* **8**, 224–235 (2016).

130. Akram, D., Elhaty, I. A. & AlNeyadi, S. S. Synthesis and antibacterial activity of rhodanine-based azo dyes and their use as spectrophotometric chemosensor for Fe3+ Ions. *Chemosensors* **8**, 1–13 (2020).

131. Tarahomi, M., Baharfar, R. & Mohseni, M. Synthesis and antibacterial activity evaluation of novel rhodanine based amide derivatives. *Clin. Microbiol. Infect. Dis.* **4**, 1–5 (2019).

132. Kumar, G. *et al.* Discovery of a rhodanine class of compounds as inhibitors of Plasmodium falciparum enoyl-acyl carrier protein reductase. *J. Med. Chem.* **50**, 2665–2675 (2007).

133. Rahman, L. & Qasim, M. The biological activity of 5, 5'-Imidazolidine-2,4-Dione derivatives. *Int. J. Pharm. Sci.* **5**, 494–504 (2013).

134. El-Sayed, S. *et al.* Synthesis and anticancer activity of novel quinazolinone-based rhodanines. *Chem. Cent. J.* **11**, 1–10 (2017).

135. Ramesh, V. *et al.* Synthesis and biological evaluation of new rhodanine analogues bearing 2-chloroquinoline and benzo[h]quinoline scaffolds as anticancer agents. *Eur. J. Med. Chem.* **83**, 569–580 (2014).

136. Ozen, C., Ceylan Unlusoy, M., Aliary, N., Ozturk, M. & Bozdag Dundar, O. Thiazolidinedione or rhodanine: a study on synthesis and anticancer activity comparison of novel thiazole derivatives. *J. Pharm. Pharm. Sci.* **20**, 415–427 (2017).

137. Alegaon, S. G. *et al.* Novel imidazo[2,1-b][1,3,4]thiadiazole carrying rhodanine-3-acetic acid as potential antitubercular agents. *Bioorg Med. Chem. Lett.* **22**, 1917–1921 (2012).

138. Mori, M. *et al.* Design, synthesis, SAR and biological investigation of 3-(carboxymethyl)rhodanine and aminothiazole inhibitors of Mycobacterium tuberculosis Zmp1. *Bioorg Med. Chem. Lett.* **28**, 637–641 (2018).

139. Gagoria, J., Singh, K., Jain, S. K., Khatkar, G. & Khatkar, A. Synthesis and anti-convulsant study of benzylidine Rhodanine derivatives. *Orient. J. Chem.* **24**, 713–716 (2008).

140. Tejchman, W., Korona-Glowniak, I., Malm, A., Zylewski, M. & Suder, P. Antibacterial properties of 5-substituted derivatives of rhodanine-3-carboxyalkyl acids. *Med. Chem. Res.* **26**, 1316–1324 (2017).

141. Tintori, C. *et al.* Rhodanine derivatives as potent anti-HIV and anti-HSV microbicides. *PLoS One* **13**, 1–19 (2018).

142. Khan, N., Gautam, G. & Gupta, A. K. Synthesis and biological evaluation of some new rhodanine analogues as aldose reductase inhibitors (ARIs). *J. Drug Deliv. Ther.* **9**, 161–167 (2019).

143. Chan, C. W. *et al.* Glitazones and alpha-glucosidase inhibitors as the second-line oral anti-diabetic agents added to metformin reduce cardiovascular risk in Type 2 diabetes patients: A nationwide cohort observational study. *Cardiovasc. Diabetol.* **17**, 1–13 (2018).

144. Srinivas, M. G. *et al.* Development of Novel Glitazones as Antidiabetic Agents: Molecular Design, Synthesis, Evaluation of Glucose Uptake Activity and SAR Studies. *Lett. Drug Des. Discov.* **17**, 840–849 (2019).

145. Kumar, B. R. P. *et al.* Synthesis, glucose uptake activity and structure-activity relationships of some novel glitazones incorporated with glycine, aromatic and alicyclic amine moieties via two carbon acyl linker. *Eur. J. Med. Chem.* **46**, 835–844 (2011).

146. Tshiluka, N. R., Bvumbi, M. V, Ramaite, I. I. & Mnyakeni-Moleele, S. S. Synthesis of some new 5-arylidene-2 ,4-thiazolidinedione esters. *Arkivoc* **1**,

161–175 (2020).

147. Li, J. & Sha, Y. A convenient synthesis of amino acid methyl esters. *Molecules* **13**, 1111–1119 (2008).

148. Mangawa, S. K., Singh, A. K. & Awasthi, S. K. Design and synthesis of a s-triazene based asymmetric organocatalyst and its application in enantioselective alkylation. *RSC Adv.* **5**, 61144–47 (2015).

149. Rachedi, K. O. *et al.* Synthesis, DFT and POM analyses of cytotoxicity activity of α-amidophosphonates derivatives: Identification of potential antiviral O,O-pharmacophore site. *J. Mol. Struct.* **1197**, 196–203 (2019).

150. Shmatova, O. I., Shevchenko, N. E., Balenkova, E. S., Röschenthaler, G. V. & Nenajdenko, V. G. Friedel-Crafts alkylation of natural amino acid-derived pyrroles with CF3-substituted cyclic imines. *Mendeleev Commun.* **23**, 92–93 (2013).

151. Ansari, A. M. & Ugwu, S. O. Efficient synthesis of α-substituted amino acid ester: Alkylation and hydrogenation removal of Schiff's base protecting group. *Synth. Commun.* **38**, 2330–2340 (2008).

152. Tshiluka, N. Synthesis of glitazone analogues as anti-diabetic drugs. (2018).

153. Ali, A. M. *et al.* Synthesis and Three-dimensional Qualitative Structure Selectivity Derivatives As COX2 Inhibitors. *Arch. Pharmacal Res.* **30**, 1186–1204 (2007).

154. Van Beurden, K., de Koning, S., Molendijk, D. & van Schijndel, J. The Knoevenagel reaction: a review of the unfinished treasure map to forming carbon–carbon bonds. *Green Chem. Lett. Rev.* **13**, 85–100 (2020).

155. Kamiloglu, S., Sari, G., Ozdal, T. & Capanoglu, E. Guidelines for cell viability assays. *Food Front.* **1**, 332–349 (2020).

156. Rose, D. R., Chaudet, M. M. & Jones, K. Structural studies of the intestinal alpha-glucosidases, maltase-glucoamylase and sucrase-isomaltase. **66**, 11–13 (2018).

157. Roskar, I. *et al.* Peptide modulators of alpha-glucosidase. **6**, 625–631 (2015).

158. Gong, L., Feng, D., Wang, T., Ren, Y. & Liu, Y. Inhibitors of α -amylase and α -glucosidase : Potential linkage for whole cereal foods on prevention of hyperglycemia. *Food Sci. Nutr.* **8**, 6320–6337 (2020).

159. Han, J., Dong, H., Xu, Z., Lei, J. & Wang, M. Facile synthesis of 5-arylidene thiohydantoin by sequential sulfonylation/desulfination reaction. *Int. J. Mol. Sci.* **14**, 12484–12495 (2013).

160. Lamiri, M. *et al.* Microwave-assisted solvent-free regiospecific synthesis of 5-alkylidene and 5-arylidenehydantoins. *Synth. Commun.* **36**, 1575–1584 (2006).

161. J. Posner, B. S. P. & J. . R. Synthesis and Pharmacological Evaluation of 4-Iminothiazolidinones for Inhibition of PI3 Kinase. *Bone* **23**, 1–7 (2008).

162. Lozynskyi, A. *et al.* Synthesis, antioxidant and antimicrobial activities of novel thiopyrano[2,3-d]thiazoles based on aroylacrylic acids. *Mol. Divers.* **21**, 427–436 (2017).

163. Ha, Y. M. *et al.* Analogs of 5-(substituted benzylidene)hydantoin as inhibitors of tyrosinase and melanin formation. *Biochim. Biophys. Acta - Gen. Subj.* **1810**, 612–619 (2011).

164. Shintani, Y., Kato, K., Kawami, M., Takano, M. & Kumamoto, T. Direct N1-selective alkylation of hydantoins using potassium bases. *Chem. Pharm. Bull.* **69**, 407–410 (2021).

165. Opletalova, V. *et al.* Synthesis and characterization of (Z)-5-arylmethylidenerhodanines with photosynthesis-inhibiting properties. *Molecules* **16**, 5207–5227 (2011).

166. Metwally, N. H. A simple green synthesis of (Z)-5-arylmethylene-4-thioxothiazolidines and thiopyrano[2,3-d]thiazolidine-2-thiones in PEG-400 under catalyst-free conditions. *J. Sulfur Chem.* **35**, 528–537 (2014).

167. Sirivolu, V. R., Vernekar, S. K. V, Marchand, C., Pommier, Y. & Wang, Z. 5-Arylidenethioxothiazolidinones as Inhibitors of Tyrosyl–DNA Phosphodiesterase I. **55**, 8671–8684 (2020).

168. Guarda, V. L. M. *et al.* Synthesis and structural study of arylidene thiazolidine

and benzothiazine compounds. *Sulfur Lett.* **26**, 17–27 (2003).

169. Wen, J. *et al.* Synthesis, biological evaluation and molecular modeling studies of psammaplin A and its analogs as potent histone deacetylases inhibitors and cytotoxic agents. *Bioorganic Med. Chem. Lett.* **26**, 4372–4376 (2016).

170. Rashamuse, T. J. *et al.* Design, synthesis and biological evaluation of novel 2-(5-aryl-1H-imidazol-1-yl) derivatives as potential inhibitors of the HIV-1 Vpu and host BST-2 protein interaction. *Eur. J. Med. Chem.* **190**, 111–112 (2020).

171. Mangawa, S. K., Singh, A. K. & Awasthi, S. K. Design and synthesis of a s-triazene based asymmetric organocatalyst and its application in enantioselective alkylation. *RSC Adv.* **5**, 61144–61147 (2015).

172. Rachedi, K. O. *et al.* Synthesis, DFT and POM analyses of cytotoxicity activity of α-amidophosphonates derivatives: Identification of potential antiviral O,O-pharmacophore site. *J. Mol. Struct.* **1197**, 196–203 (2019).

173. Ma, L. *et al.* Structural exploration, synthesis and pharmacological evaluation of novel 5-benzylidenethiazolidine-2,4-dione derivatives as iNOS inhibitors against inflammatory diseases. *Eur. J. Med. Chem.* **92**, 178–190 (2015).

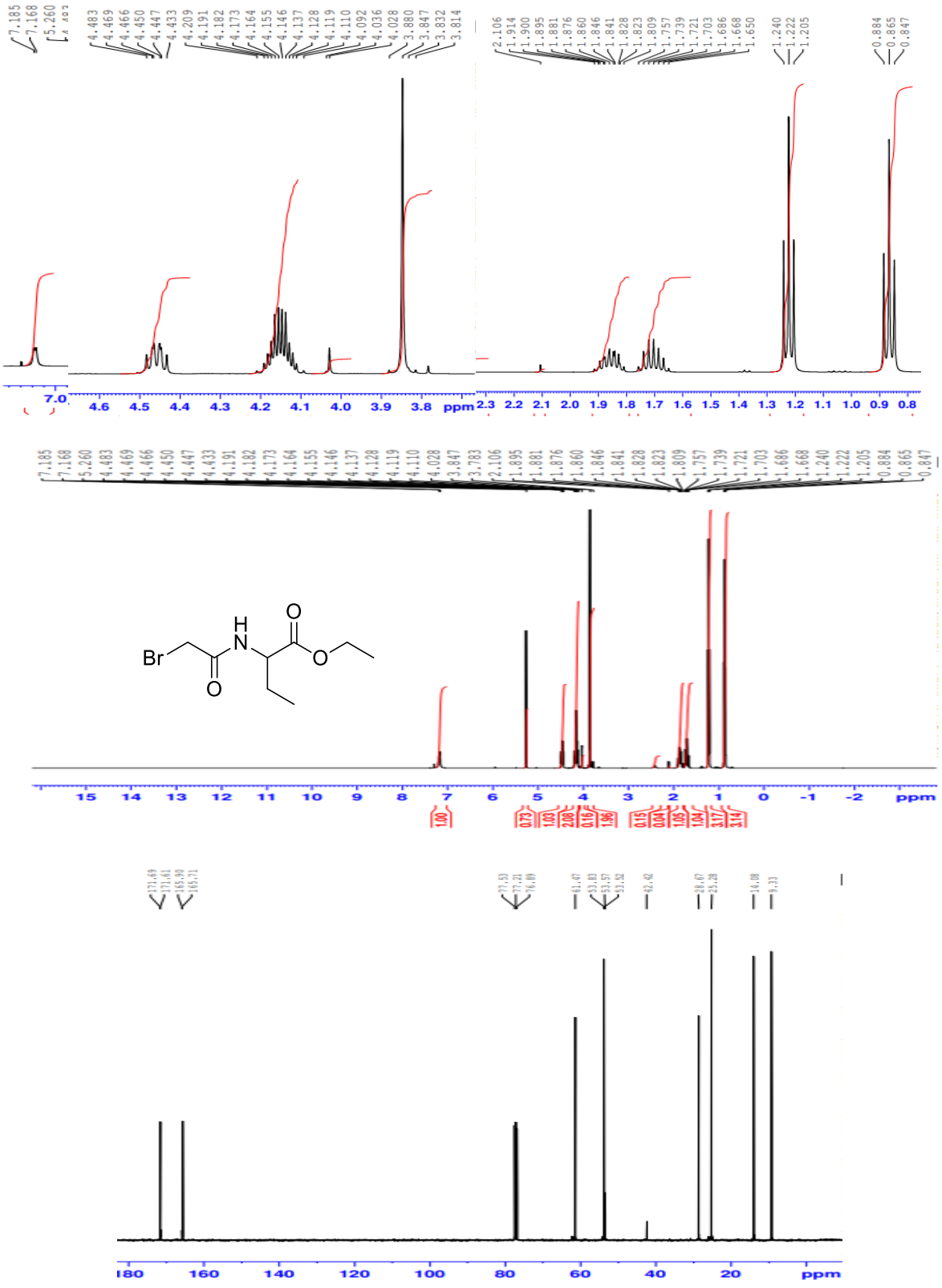
174. Wang, H. *et al.* Design, Synthesis and biological evaluation of hydantoin derivatives. *J. Chem. Pharm. Res.* **6**, 424–427 (2014).

175. Dong, S. *et al.* Design and synthesis of 5-substituted benzo[d][1,3]dioxole derivatives as potent anticonvulsant agents. *Arch. Pharm.* **350**, 1–7 (2017).

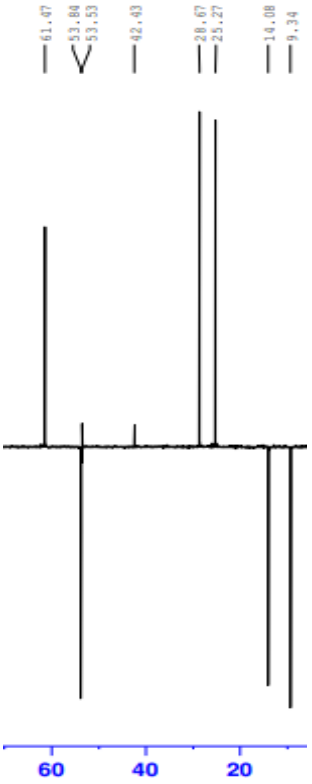
Appendix

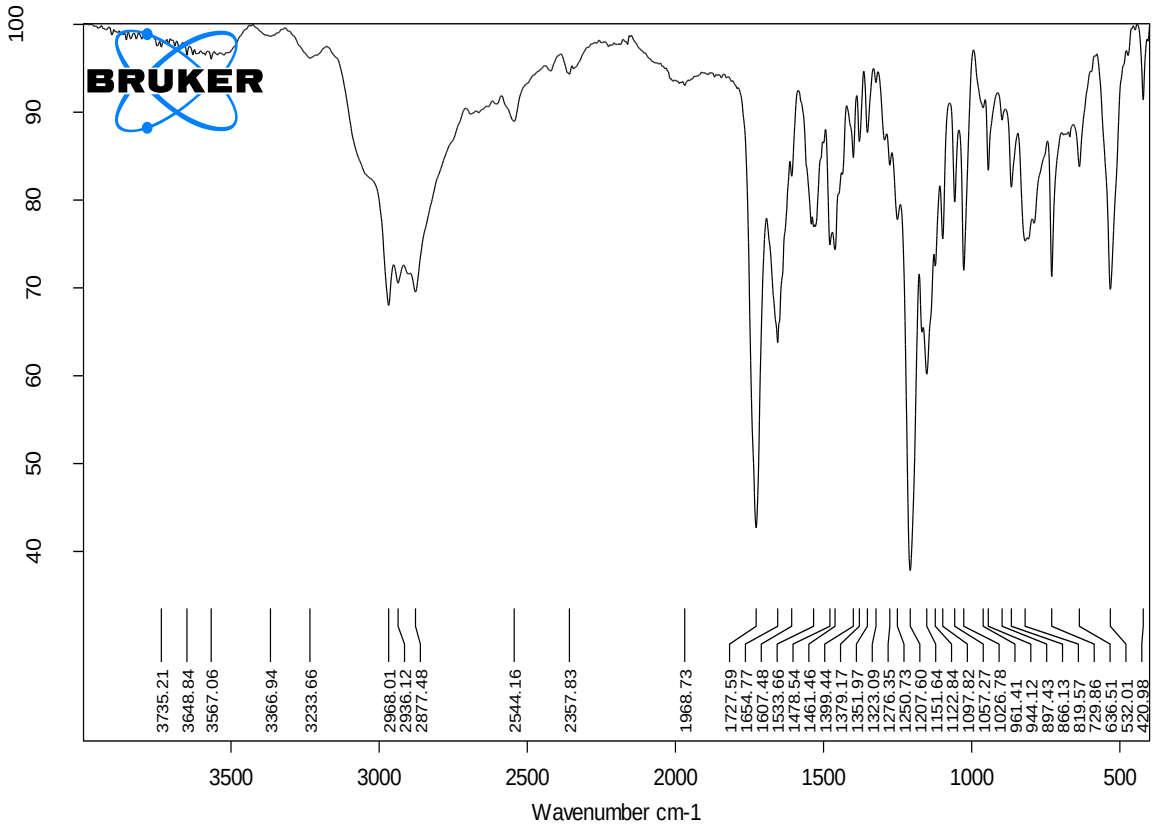
Selected ^{1}H, ^{13}C, dept NMR, IR spectra and biological assays charts

A1. ^{1}H and ^{13}C NMR spectra of compound **107b**

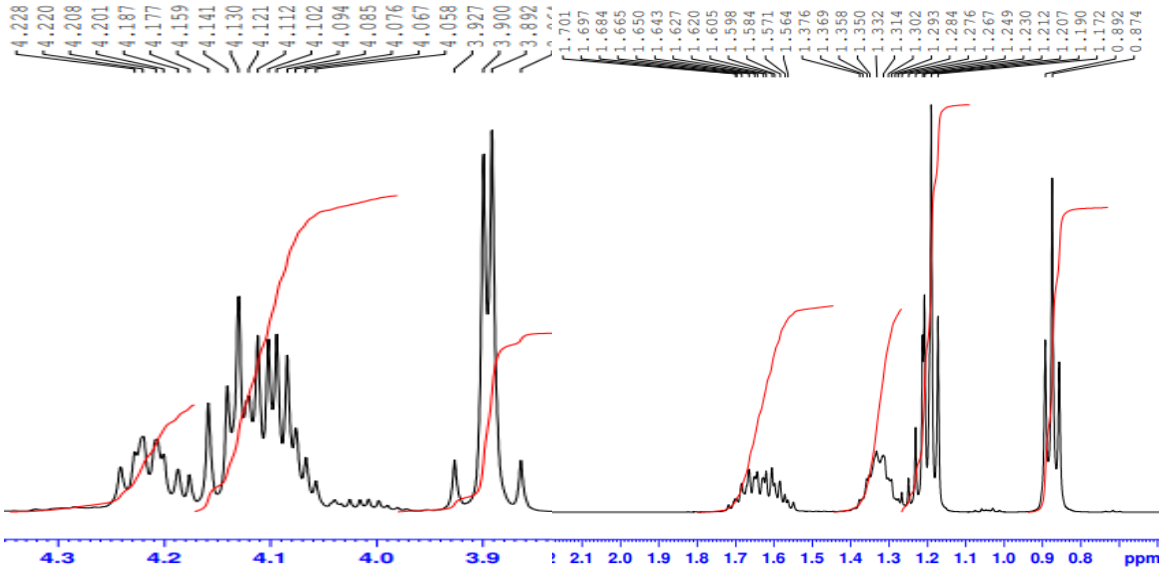


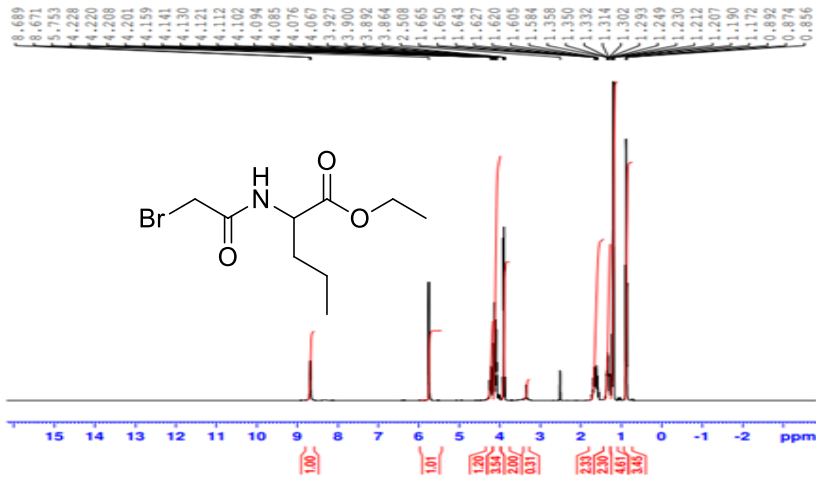
A2. DEPT-135 and IR spectra of compound **107b**

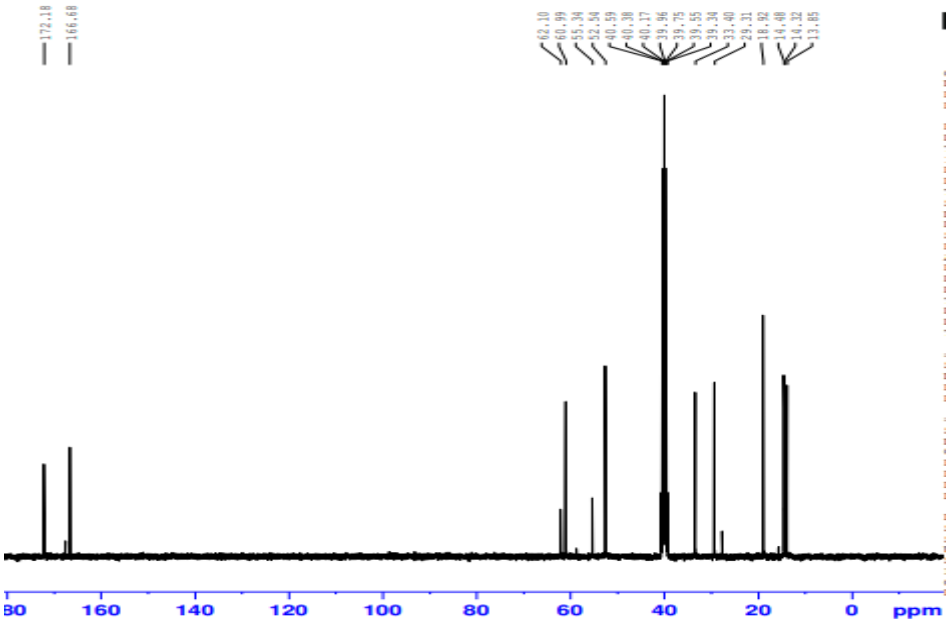




A3. ^{1}H and ^{13}C NMR spectra of compound **107d.**

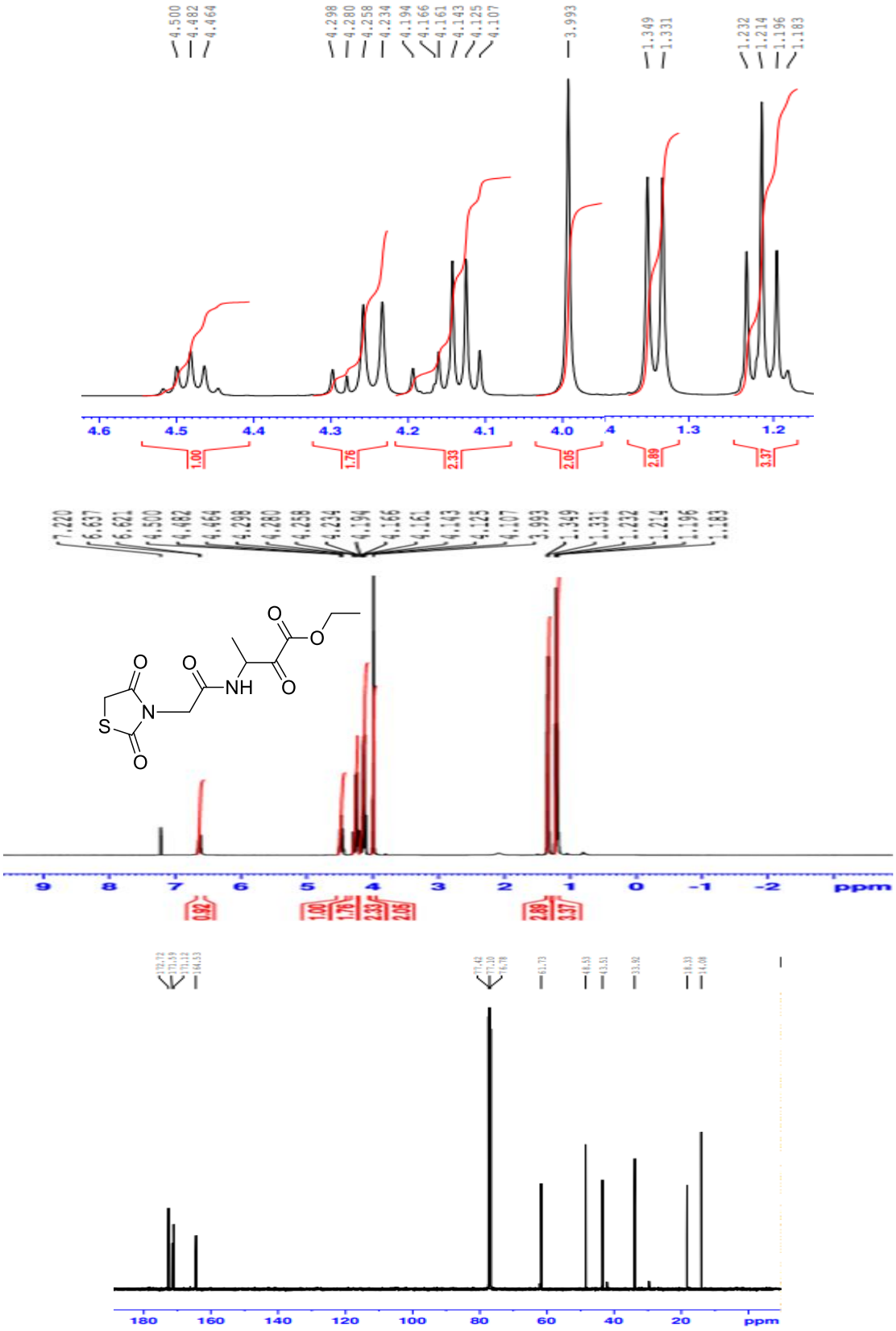




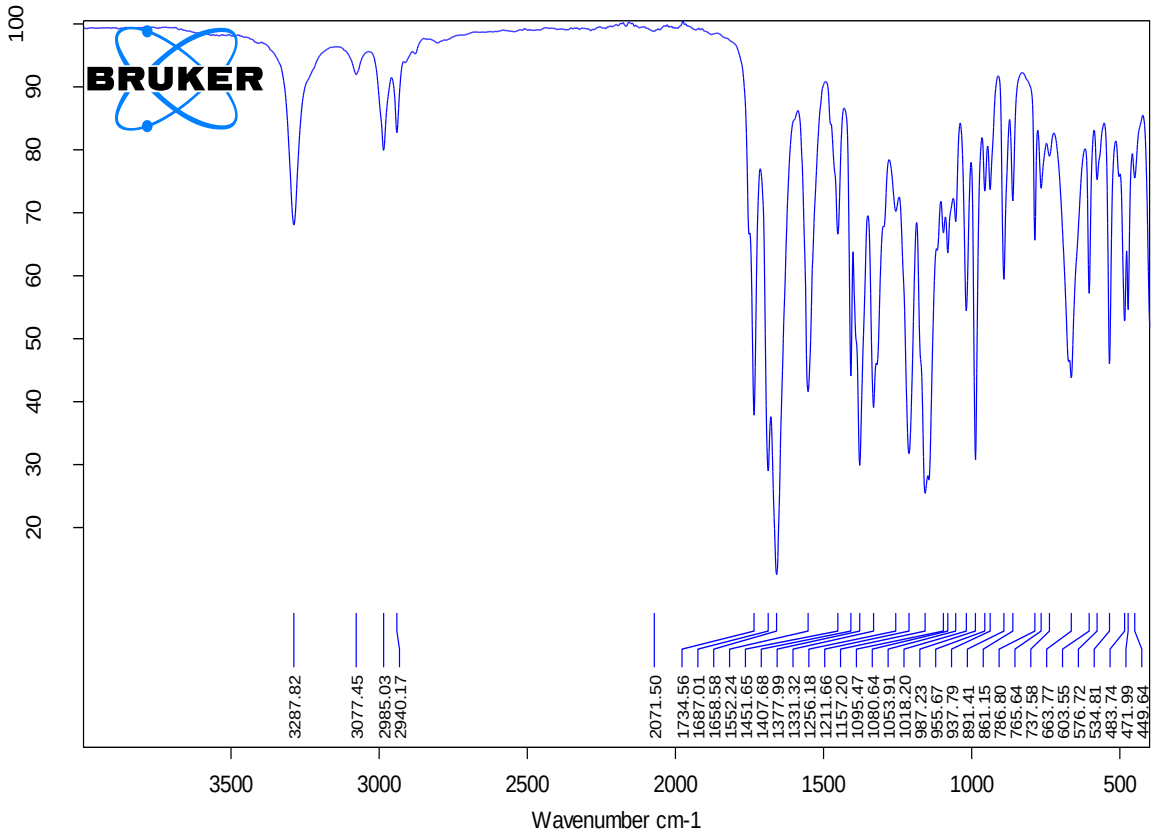


A4. DEPT-135 and IR spectra of compound **107d.**

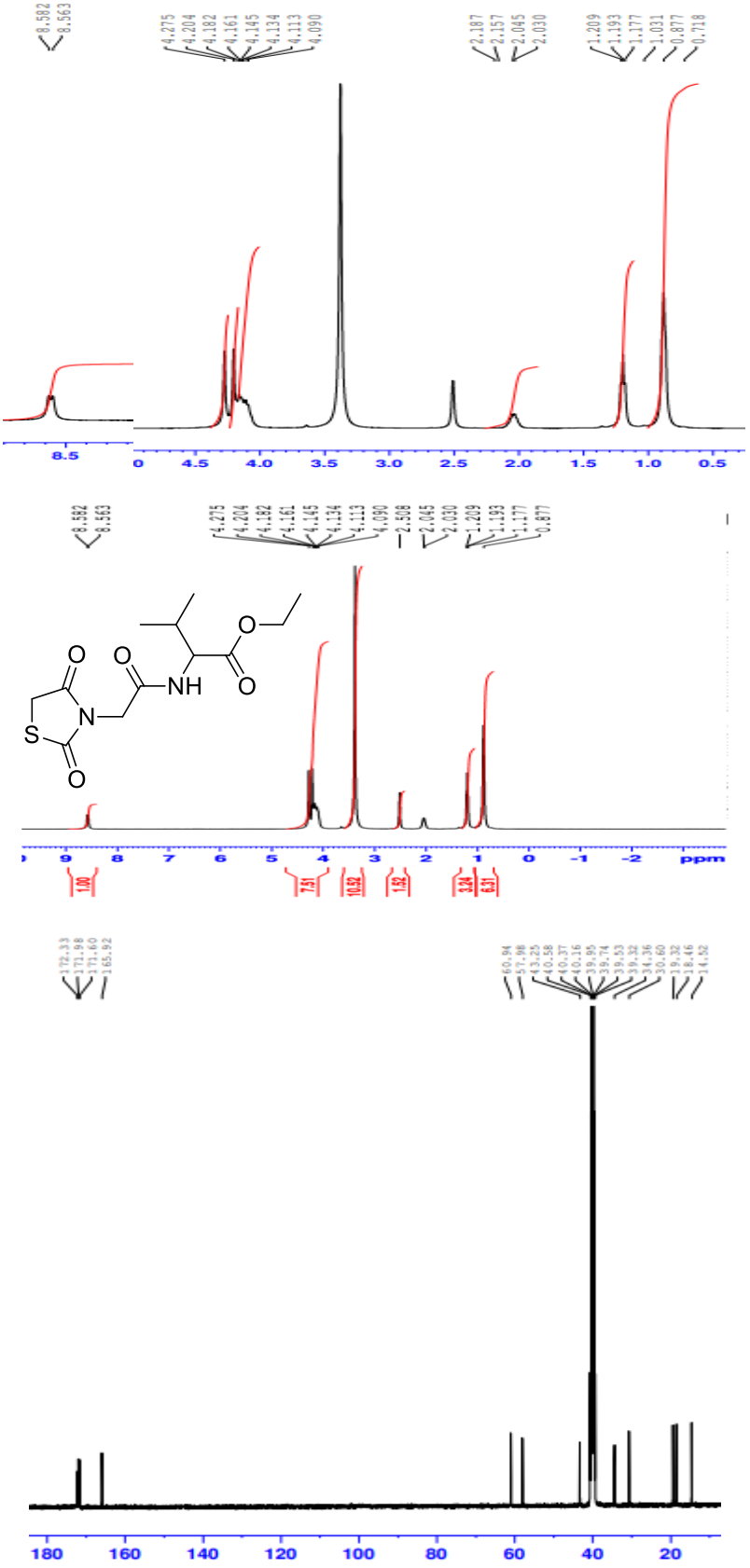
S5. ^{1}H and ^{13}C NMR spectra of compound **106b**



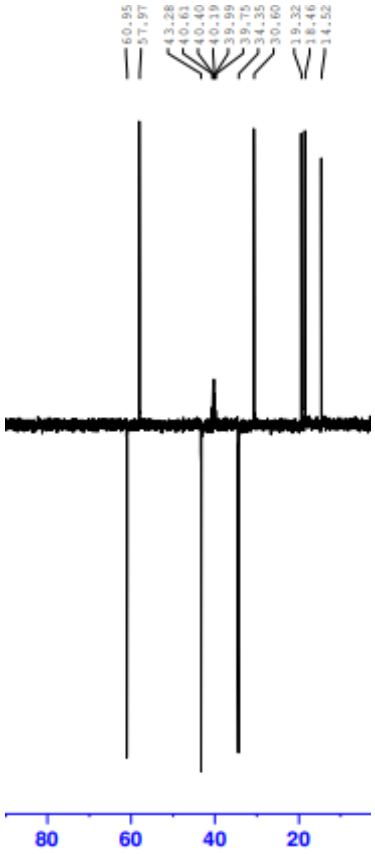
A6. DEPT-135 and IR spectra of compound **106c**

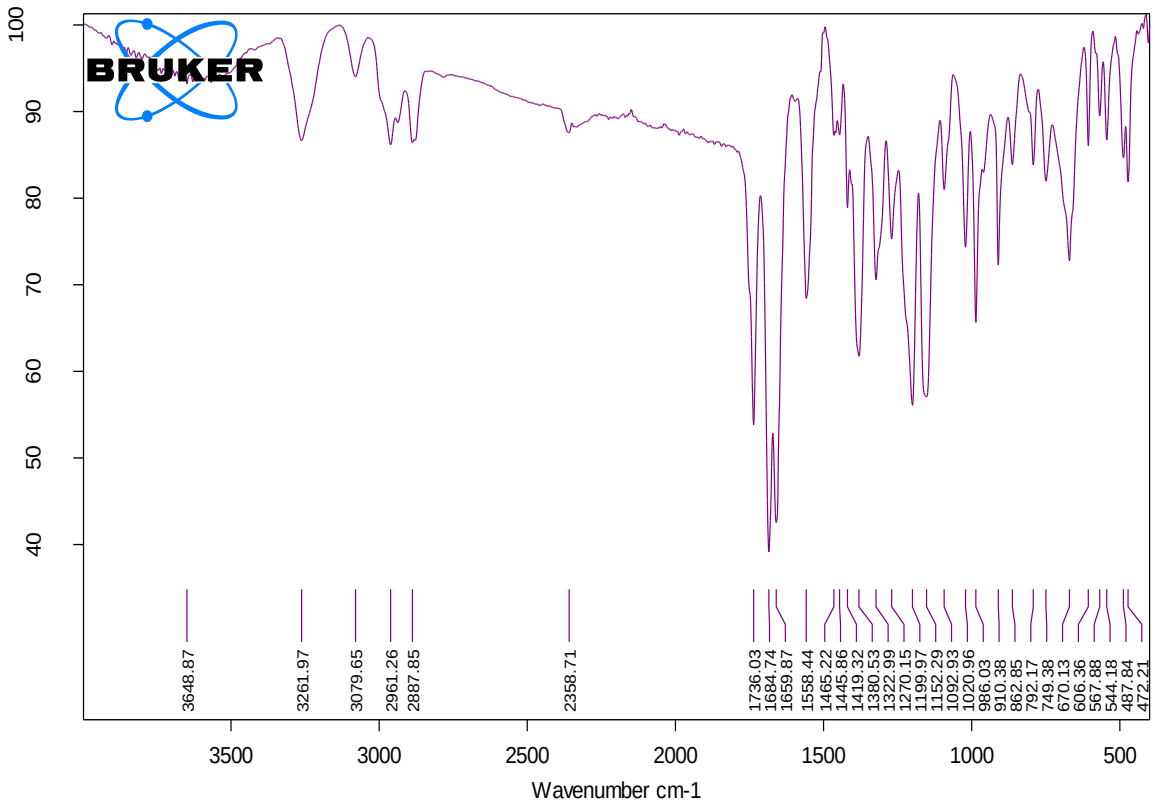


A7. ^{1}H and ^{13}C NMR spectra of compound **106c**

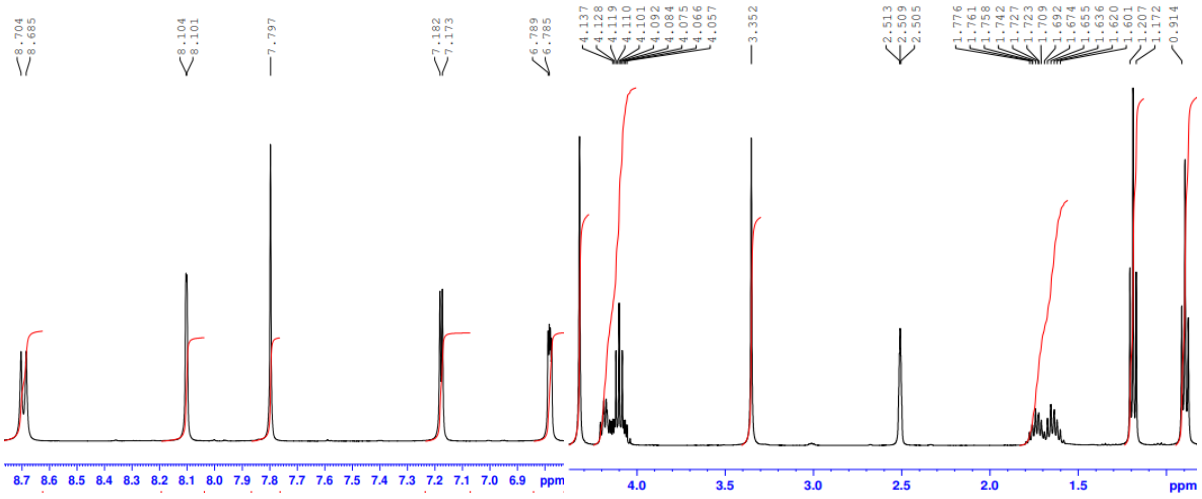


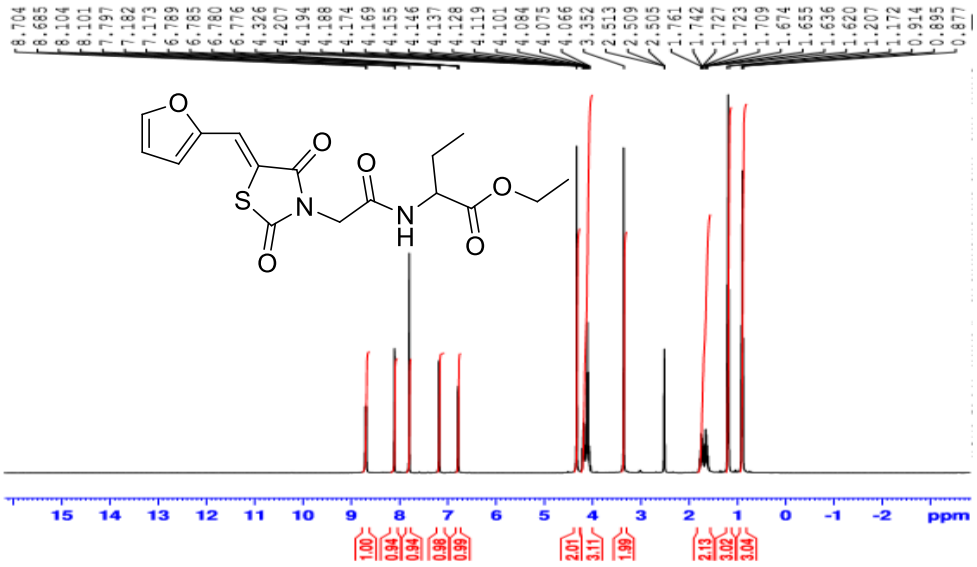
A8. DEPT-135 and IR spectra of compound **105c**

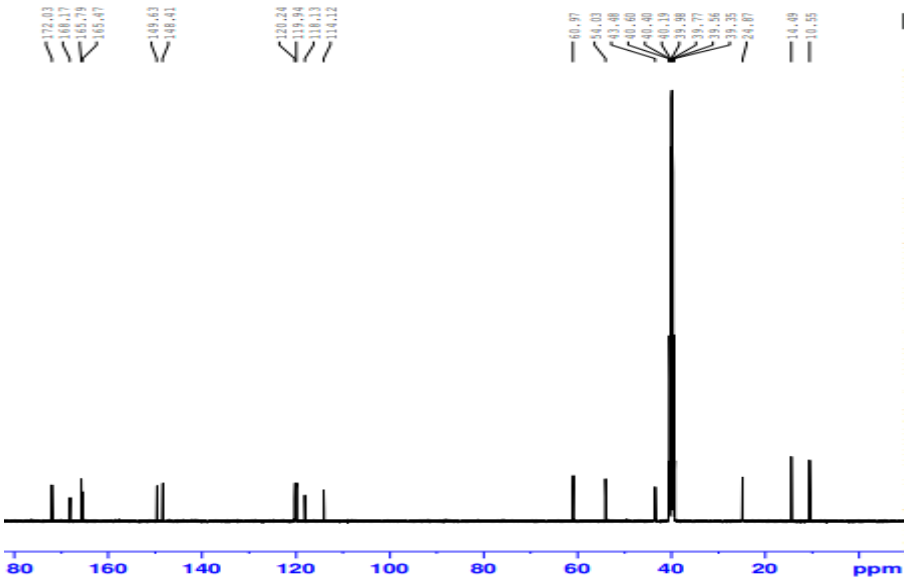




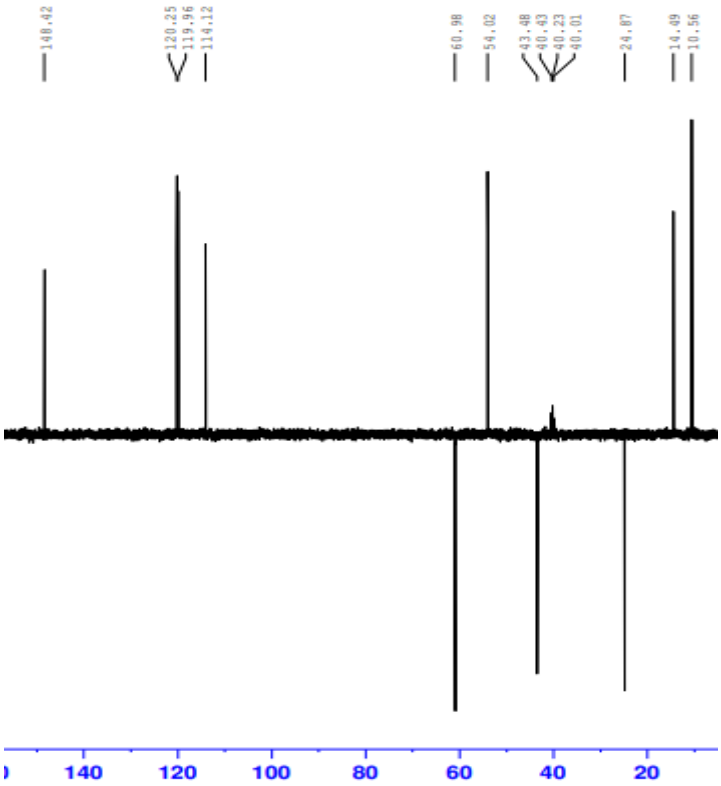
A9. ^{1}H and ^{13}C NMR spectra of compound **103e**

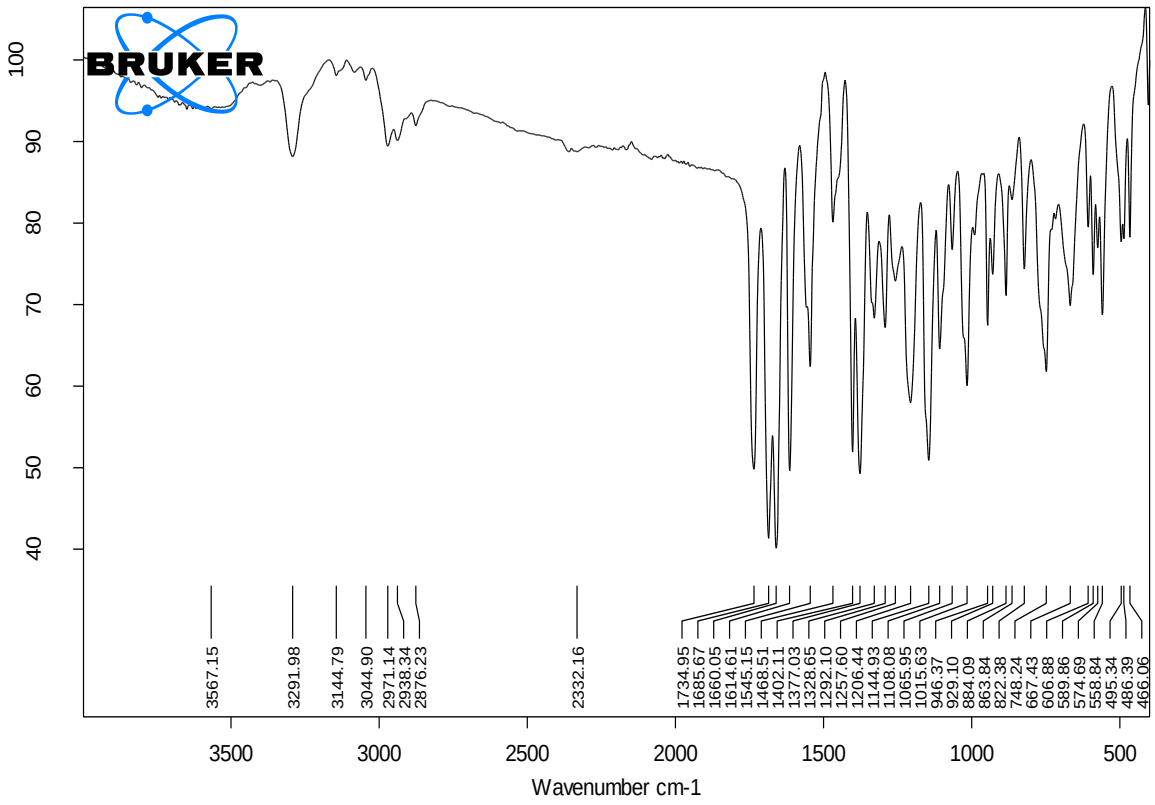




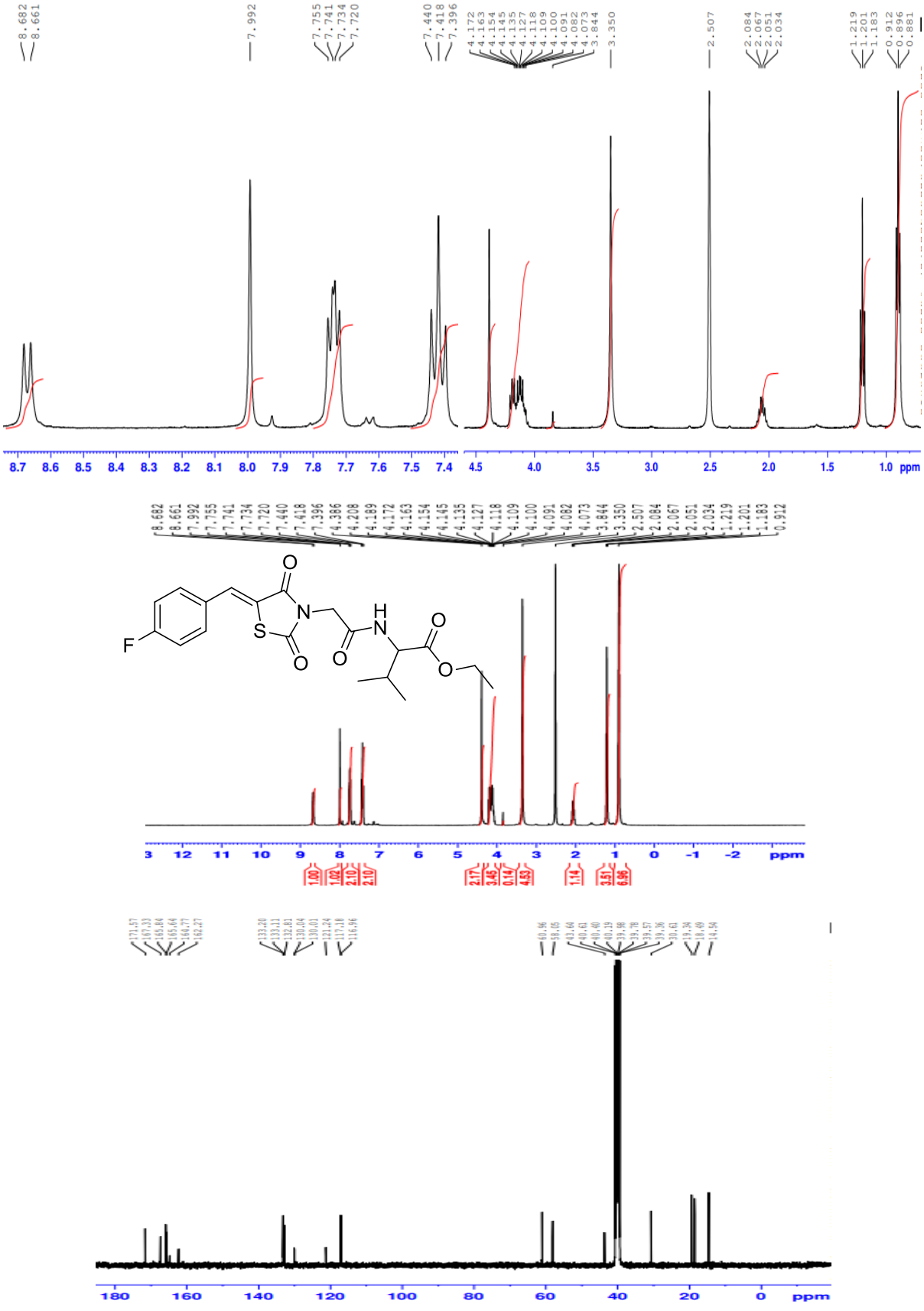


S10. DEPT-135 and IR spectra of compound **103j**

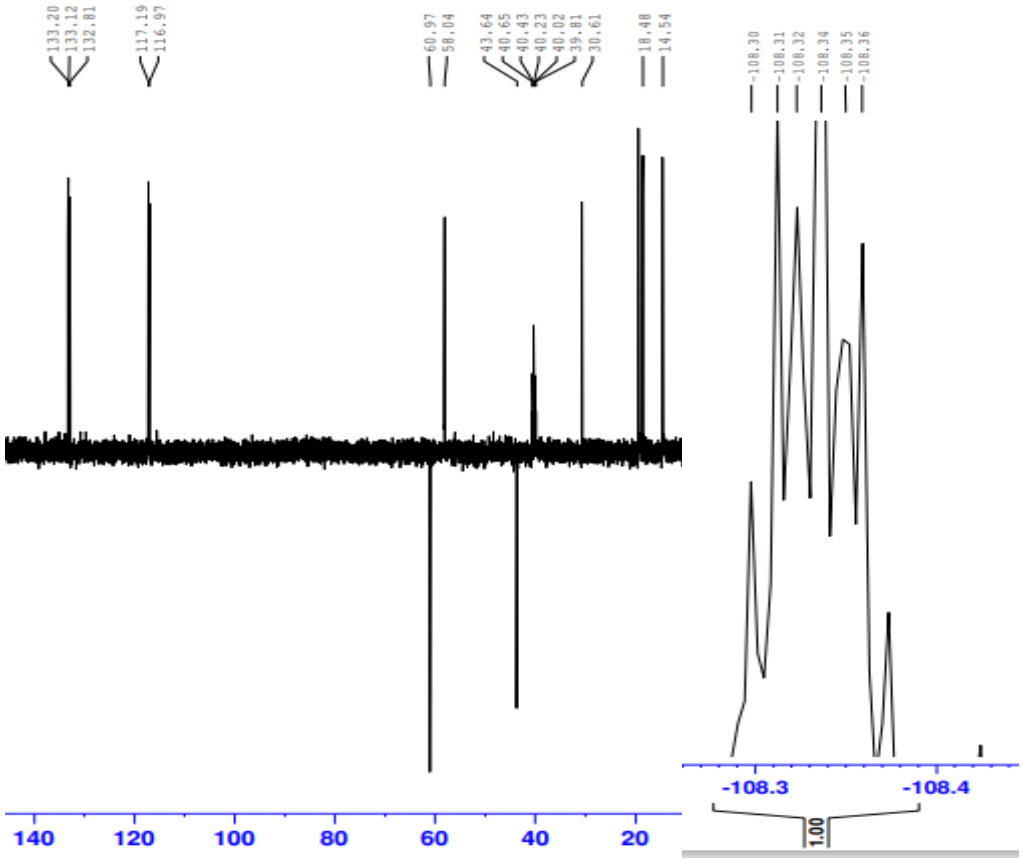


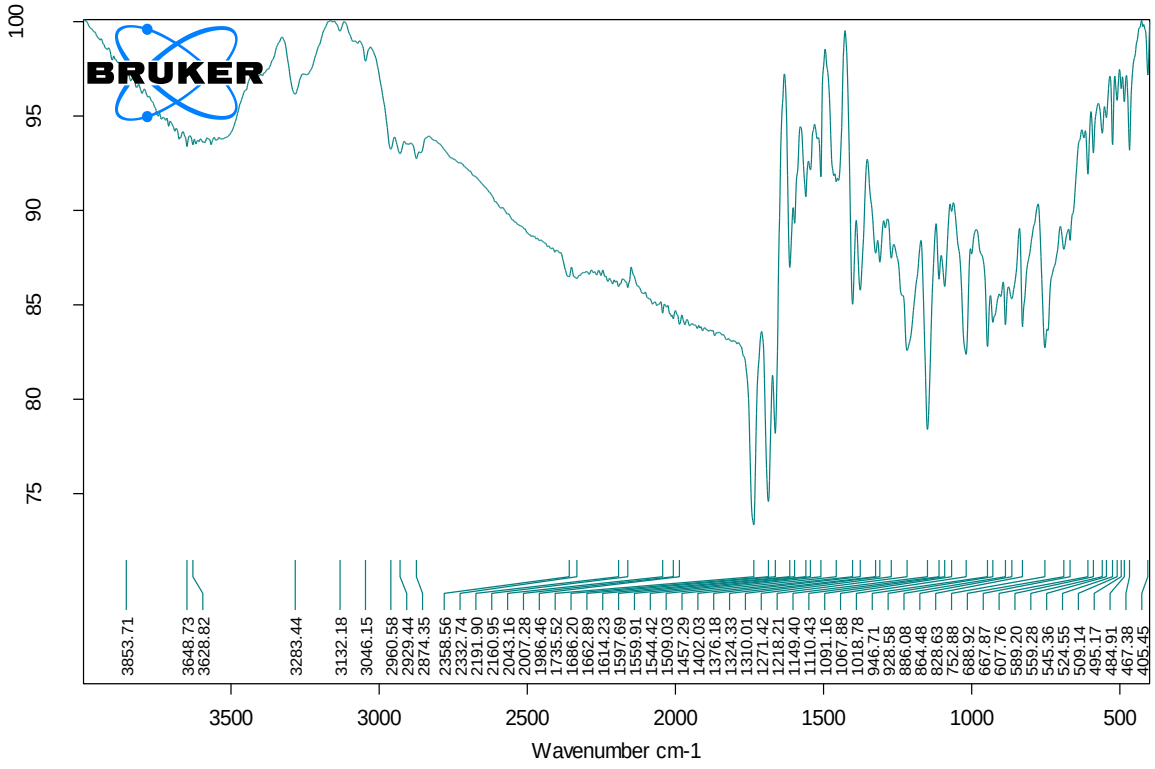


A11. ^{1}H and ^{13}C NMR spectra of compound **103j**

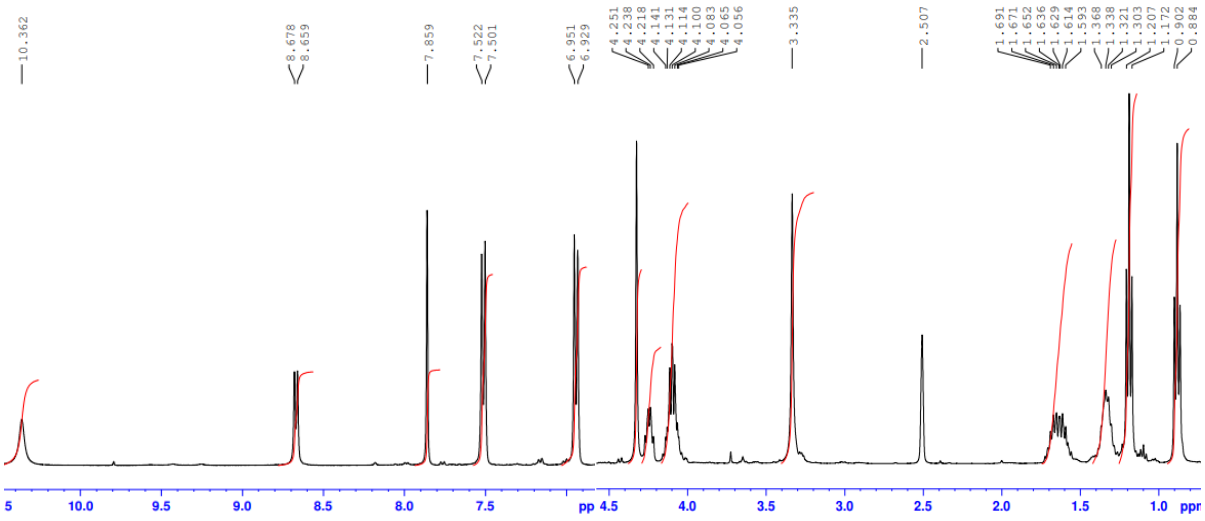


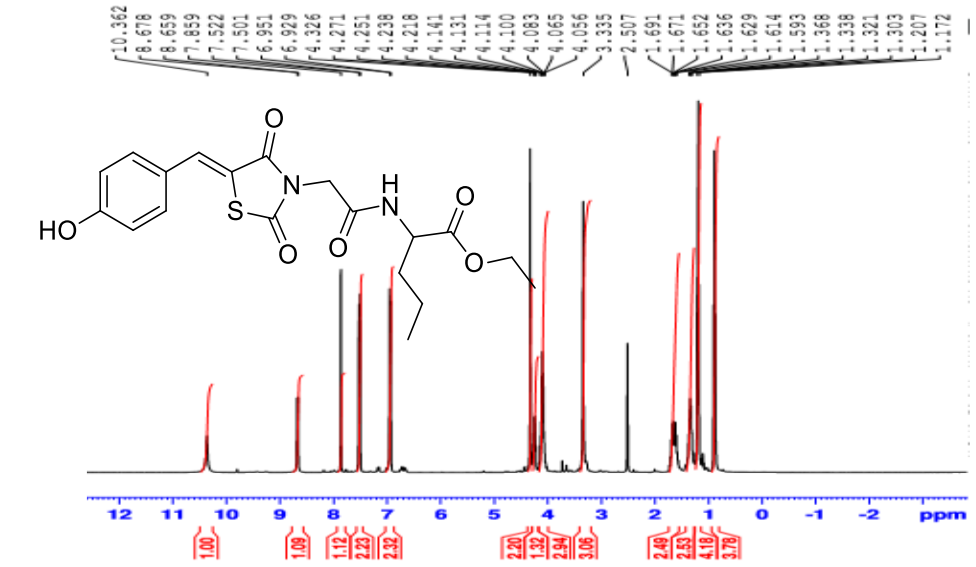
A12. DEPT-135, ^{19}F and IR spectra of compound **103j**

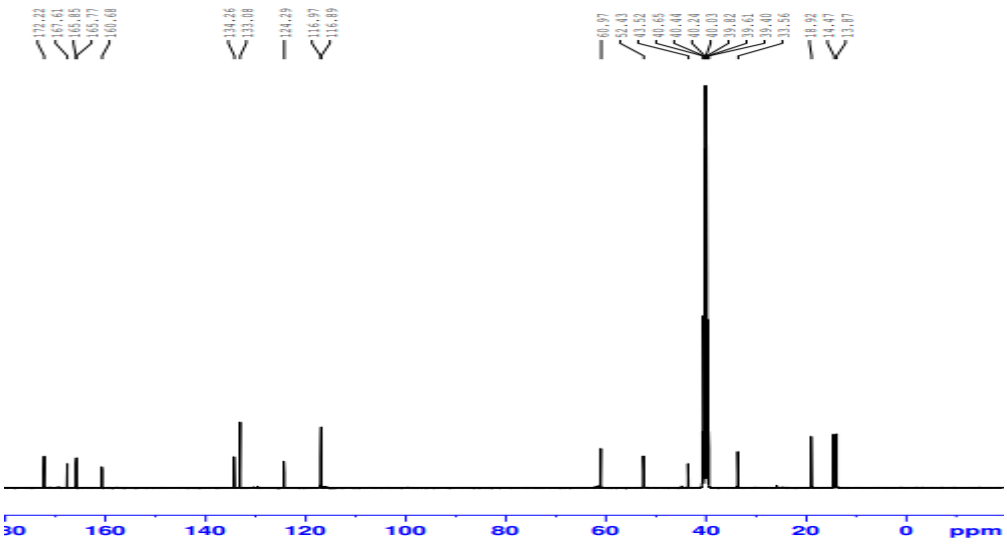




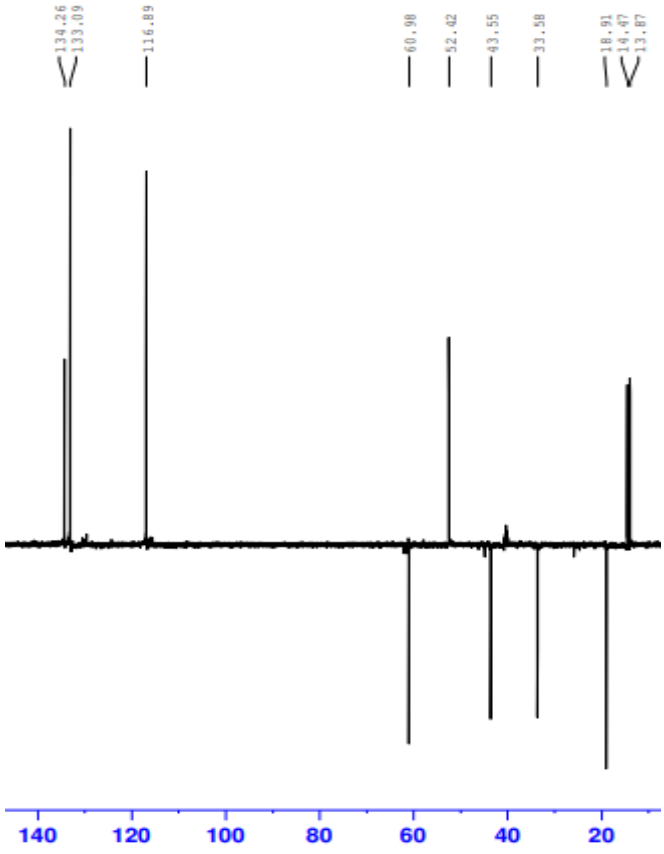
A13. ^{1}H and ^{13}C NMR spectra of compound **103m**

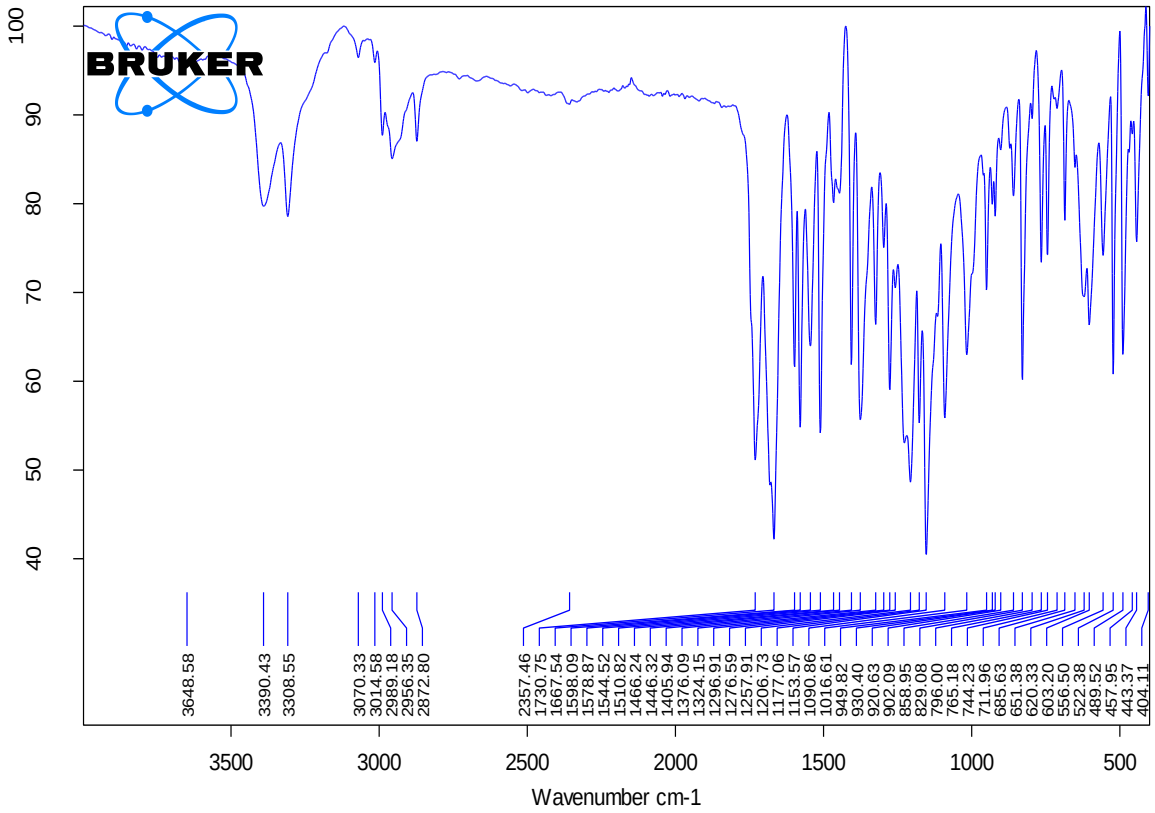




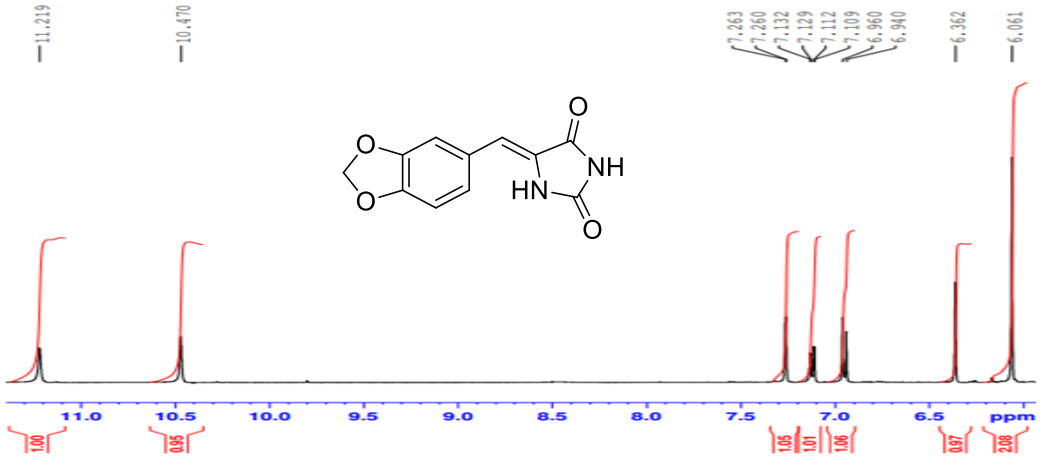


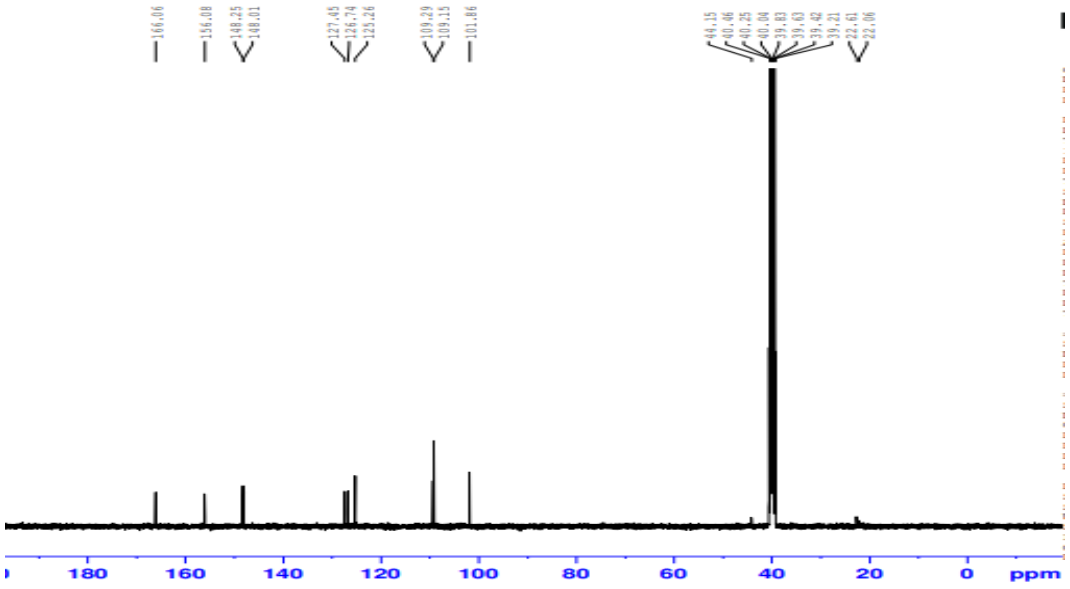
A14. DEPT-135 and IR spectra of compound **103m**

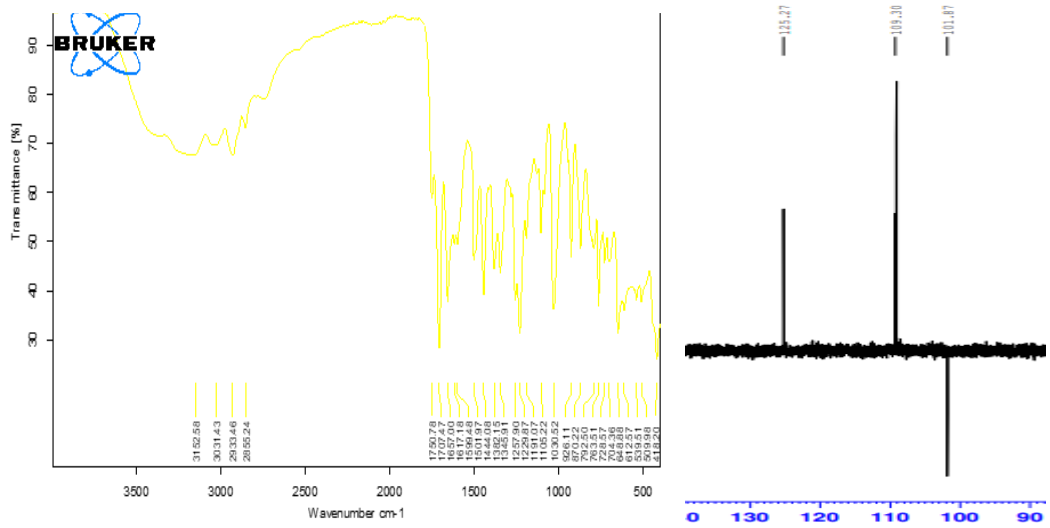




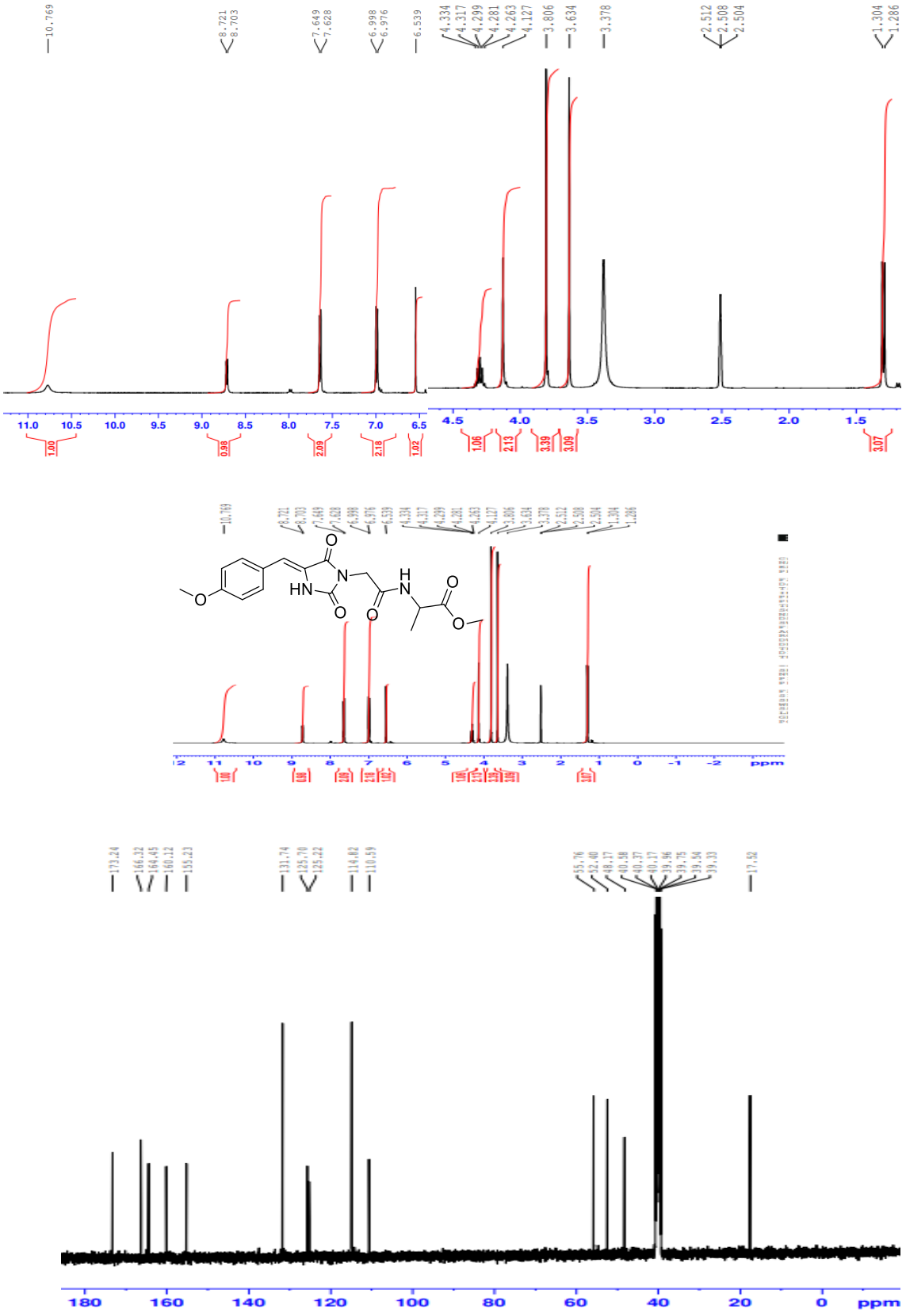
A15. ^{1}H, ^{13}C NMR, IR and DEPT-135 spectra of compound **110f**



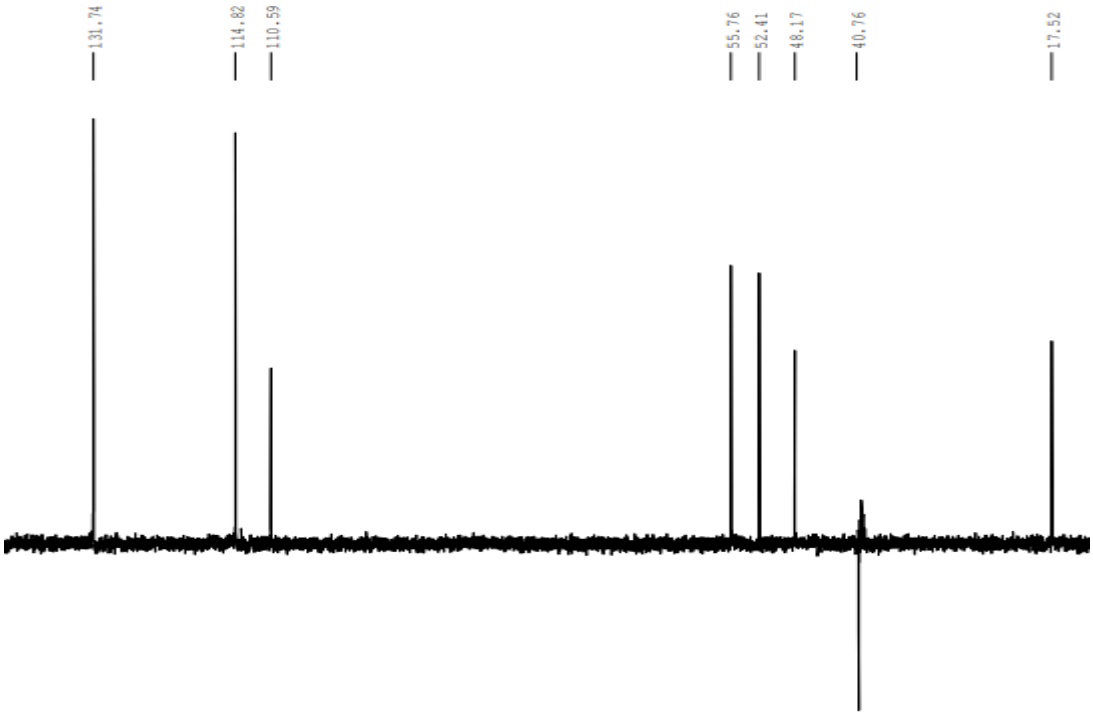


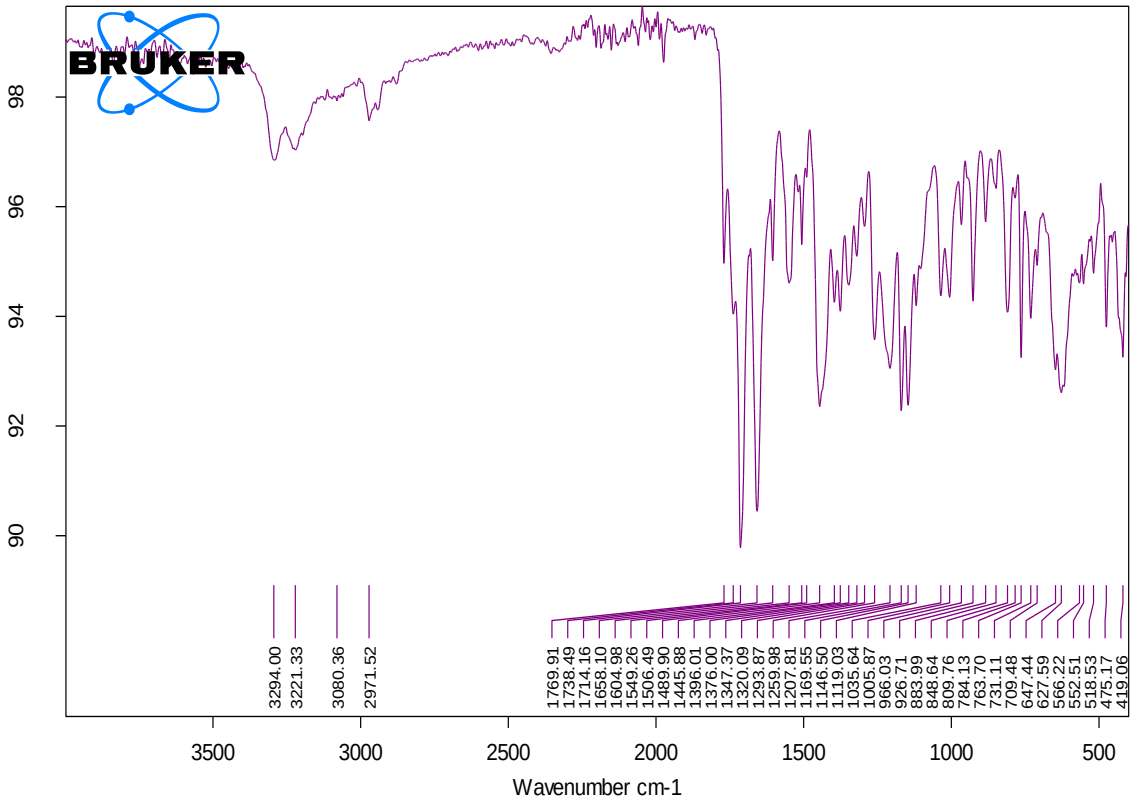


A16. ^{1}H and ^{13}C NMR spectra of compound **104a**

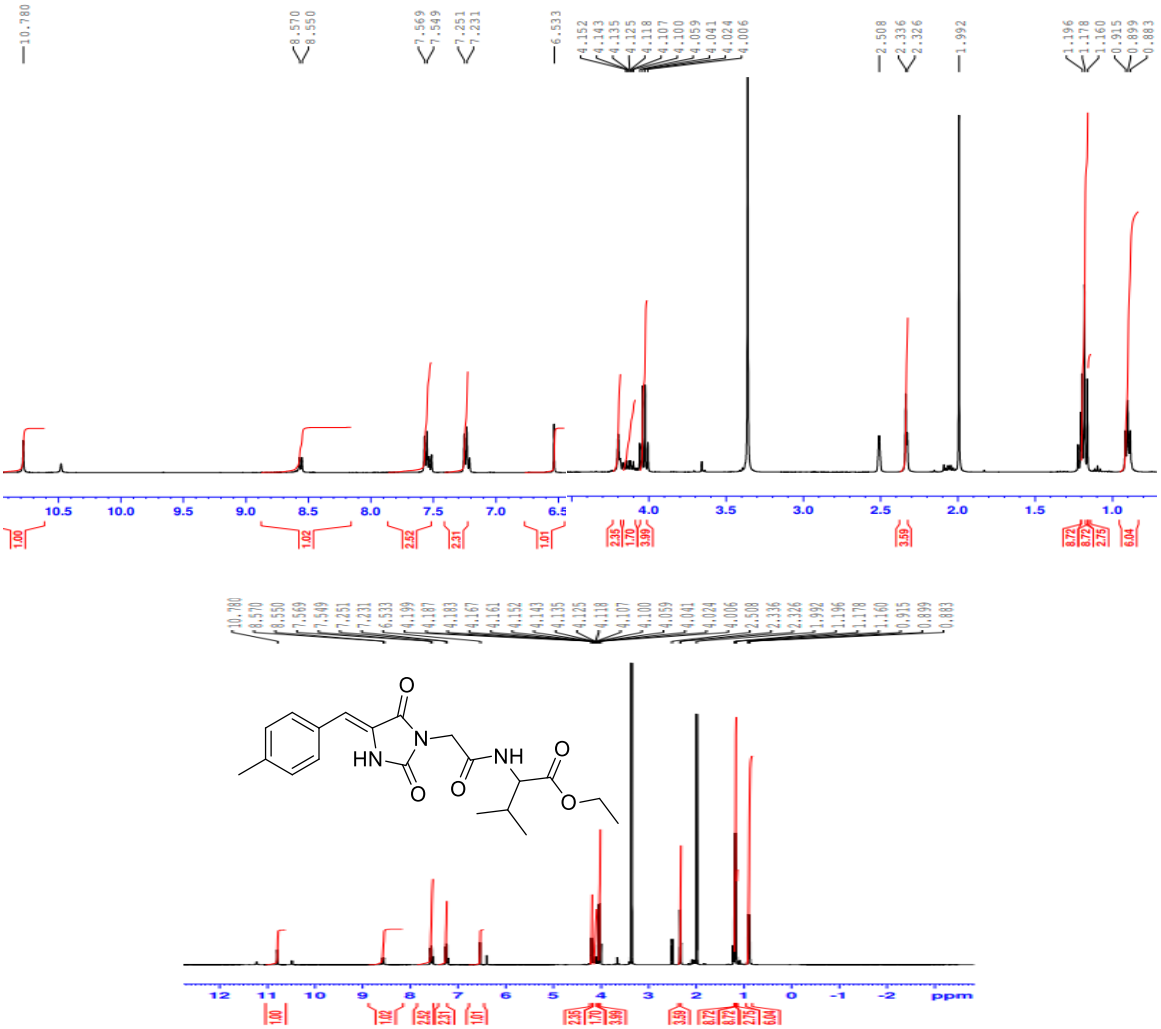


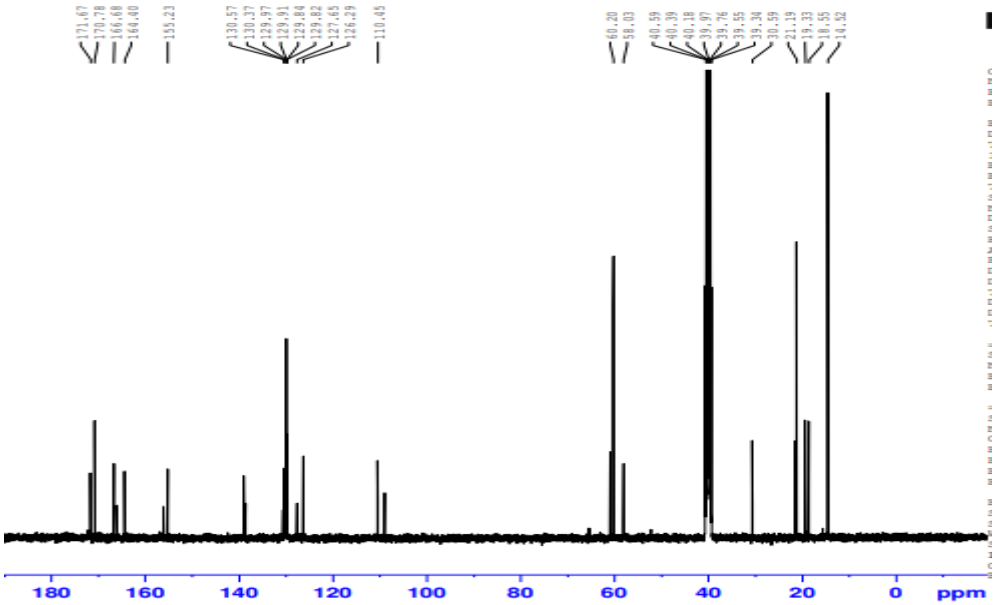
A17. DEPT-135 and IR spectra of compound **104a**



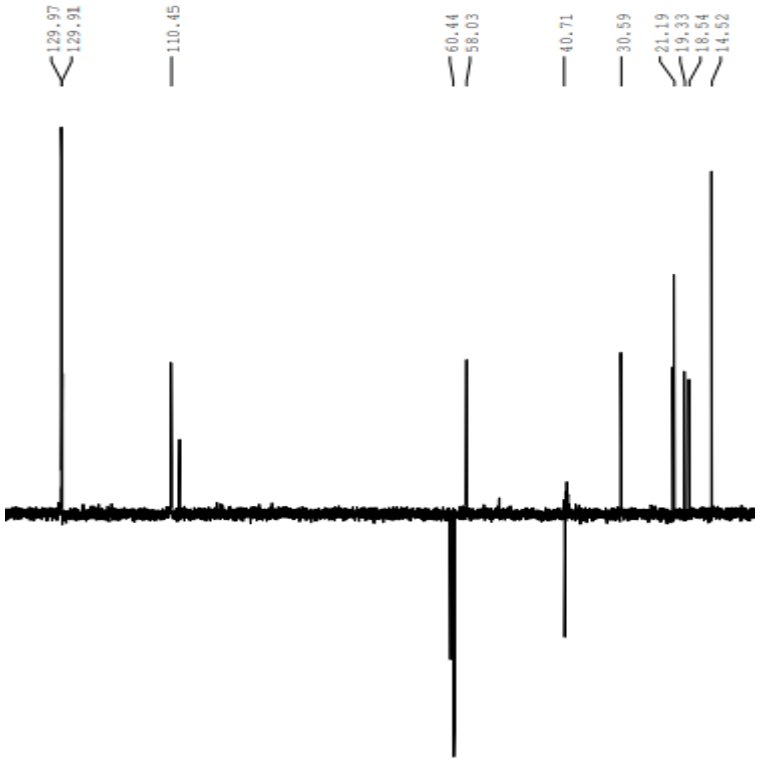


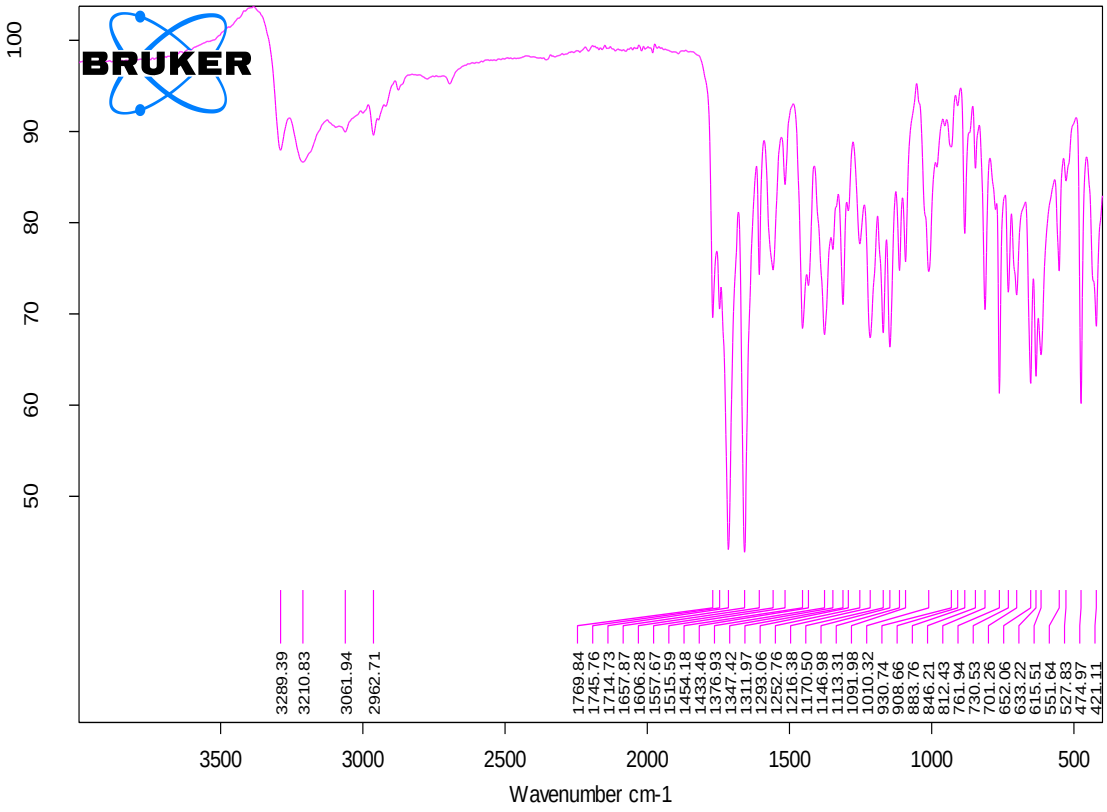
A18. ^{1}H and ^{13}C NMR spectra of compound **104g**



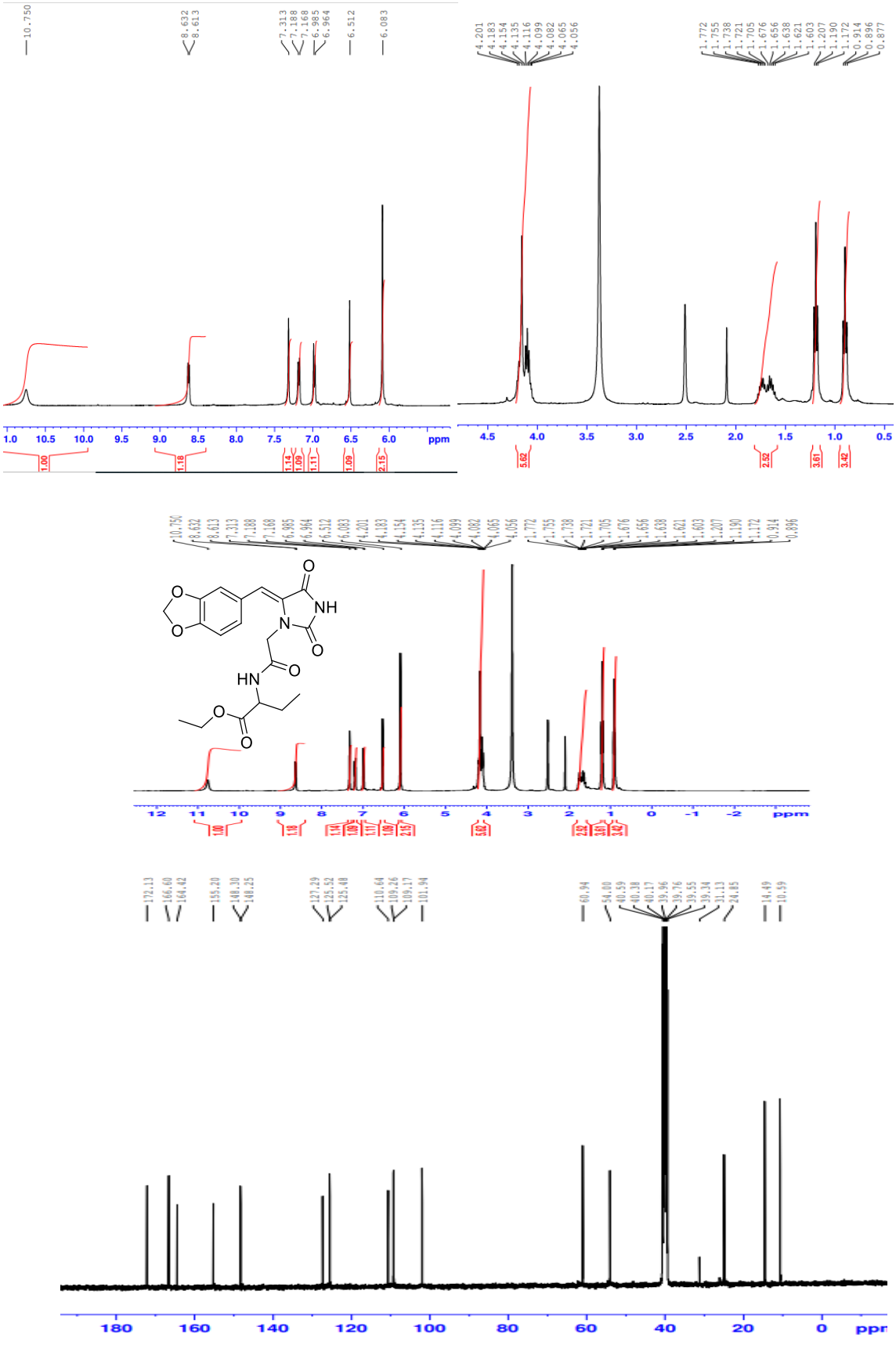


A19: Dept 135 and IR spectra of compound **104g**

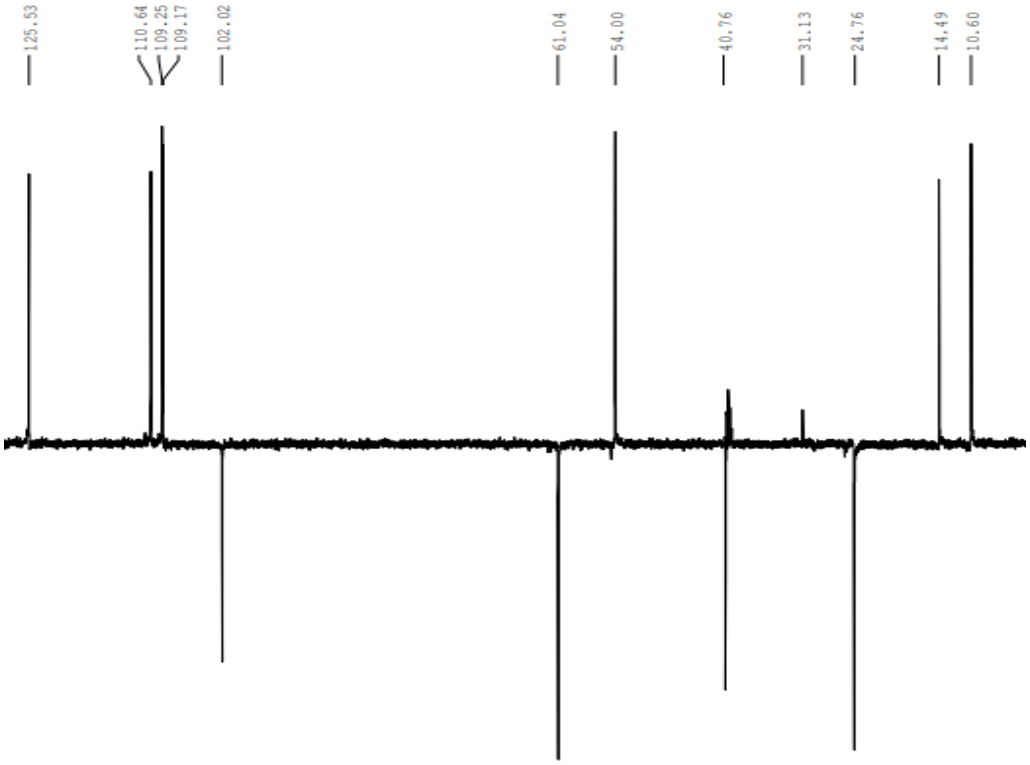


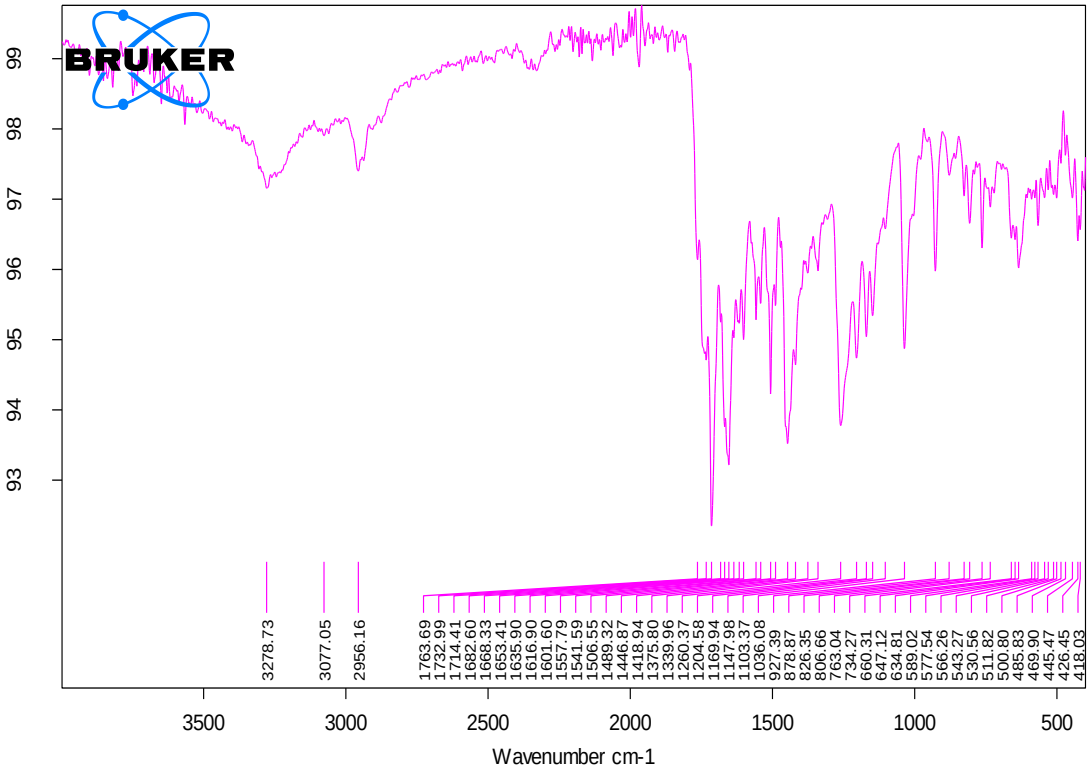


A20. ^{1}H and ^{13}C NMR spectra of compound **104n**

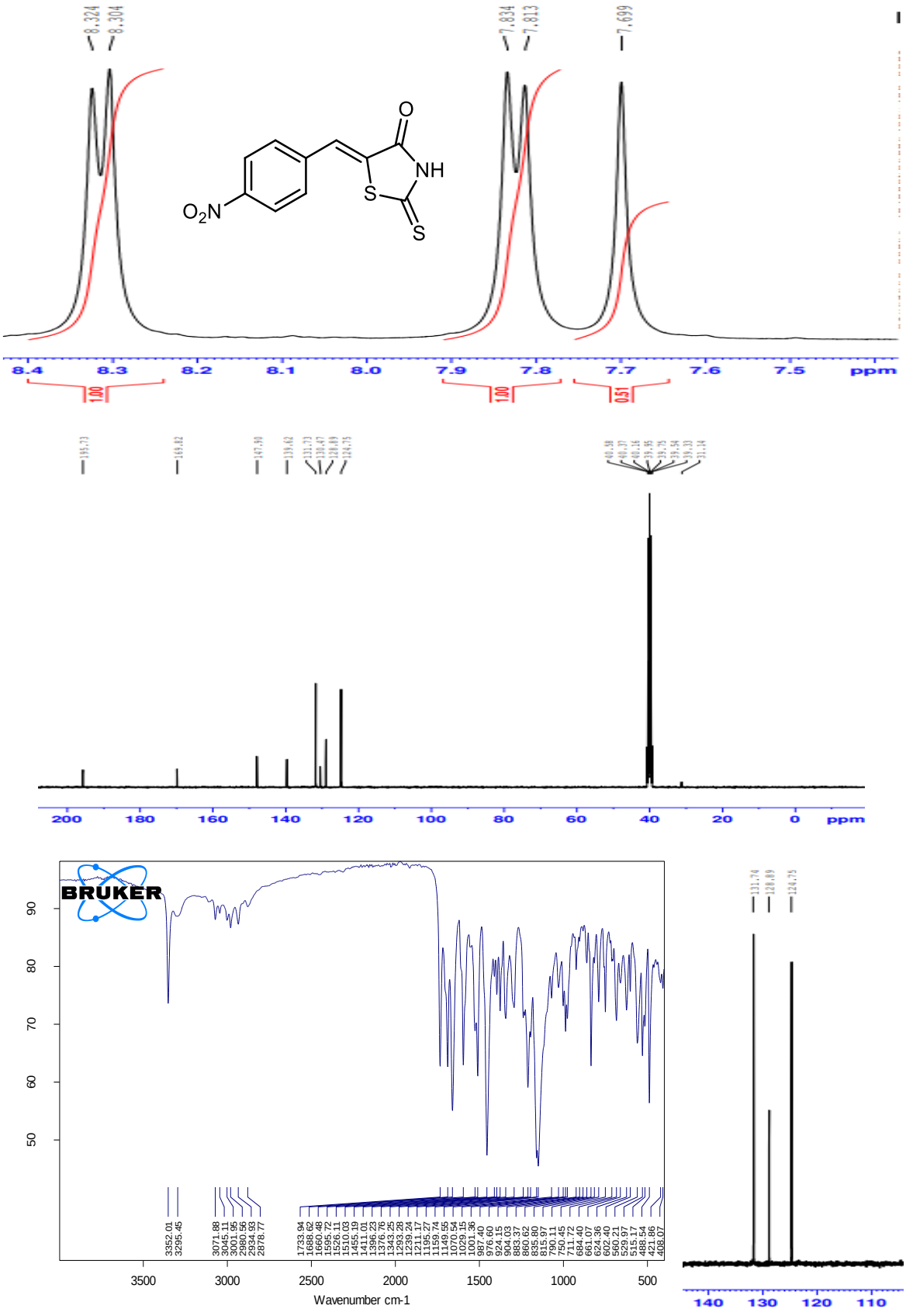


A21. DEPT-135 and IR spectra of compound **104n**

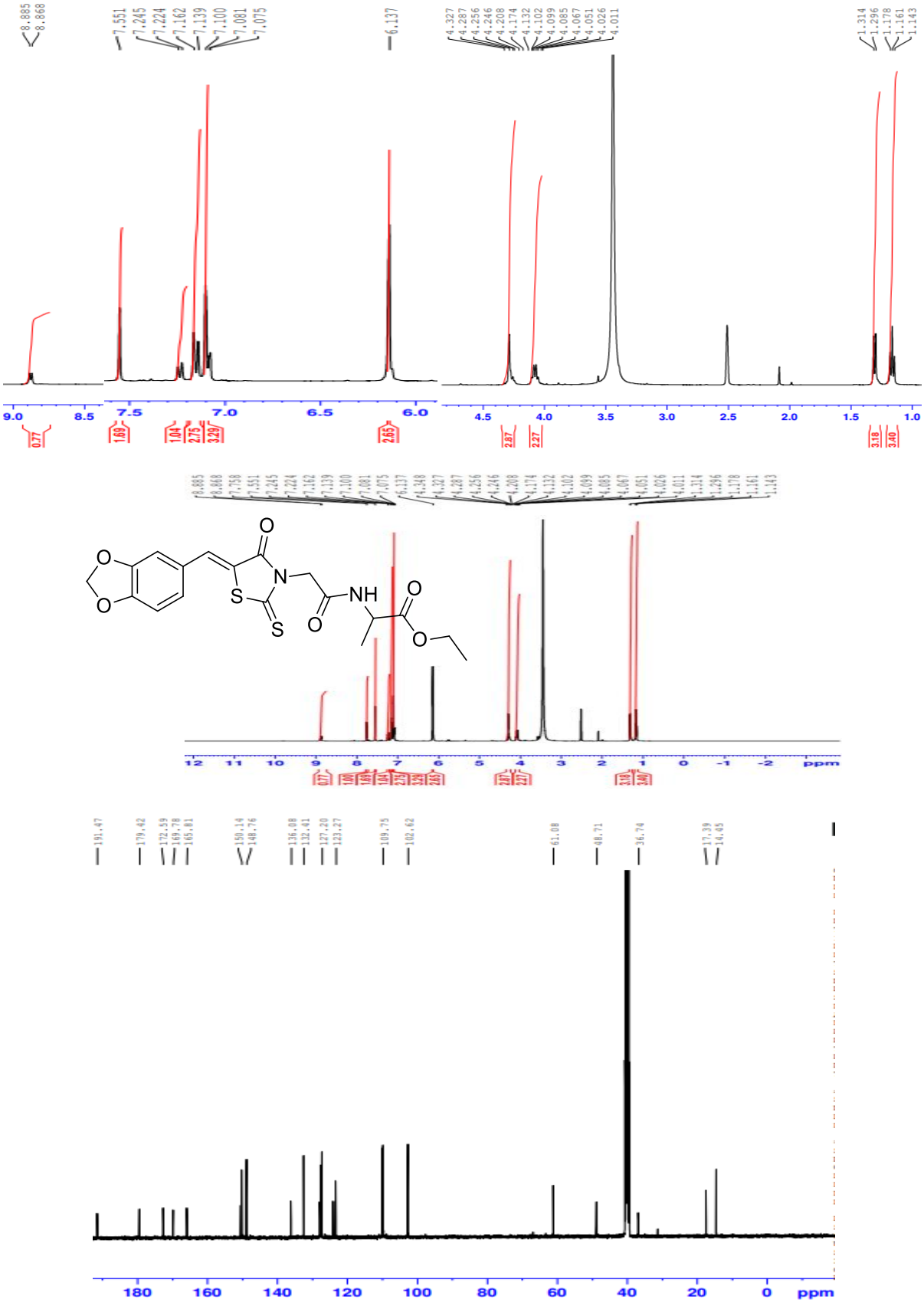




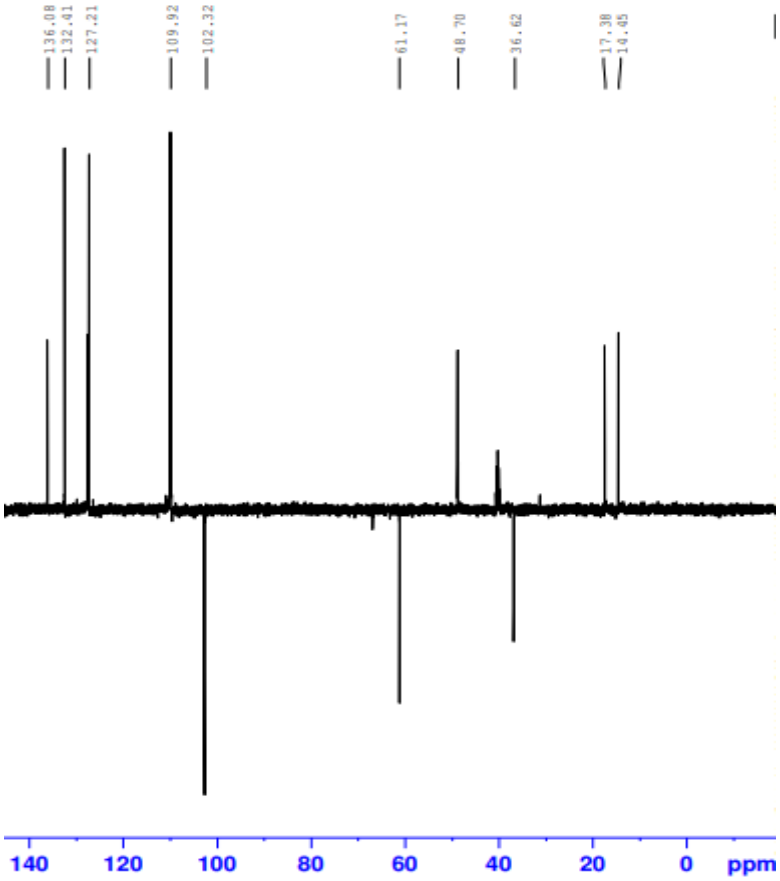
A22. ^{1}H, ^{13}C NMR, IR and dept spectra of compound **111c**

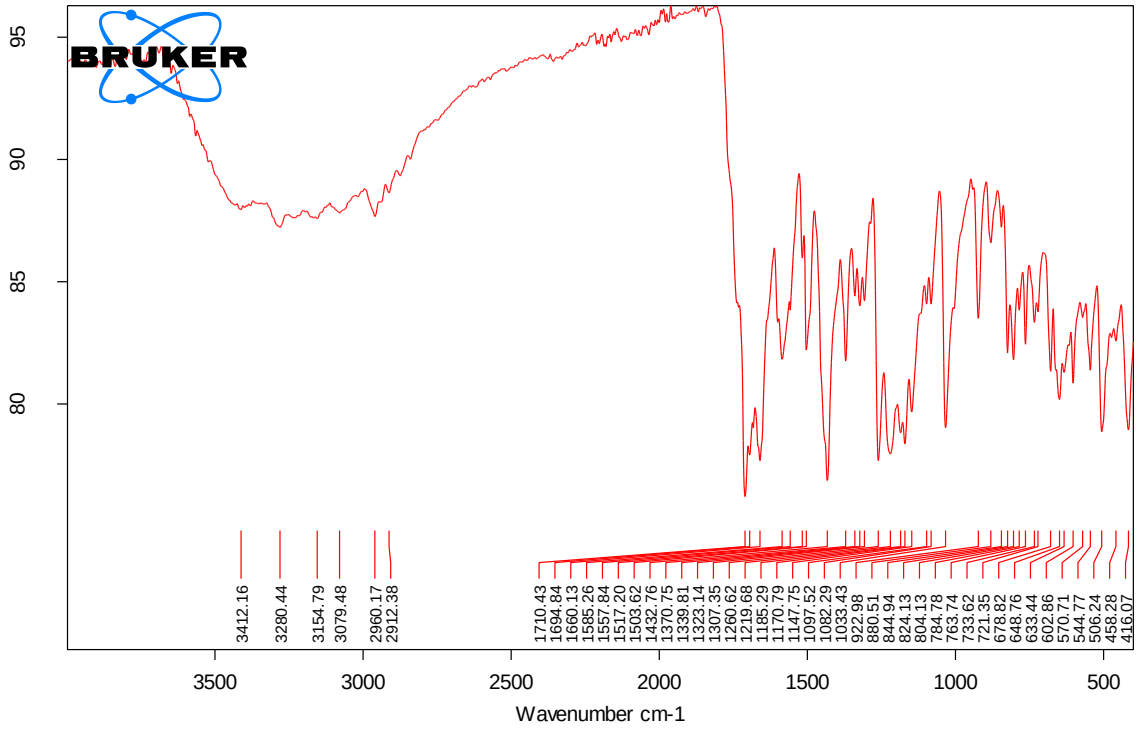


A23. ^{1}H and ^{13}C NMR spectra of compound **105d**

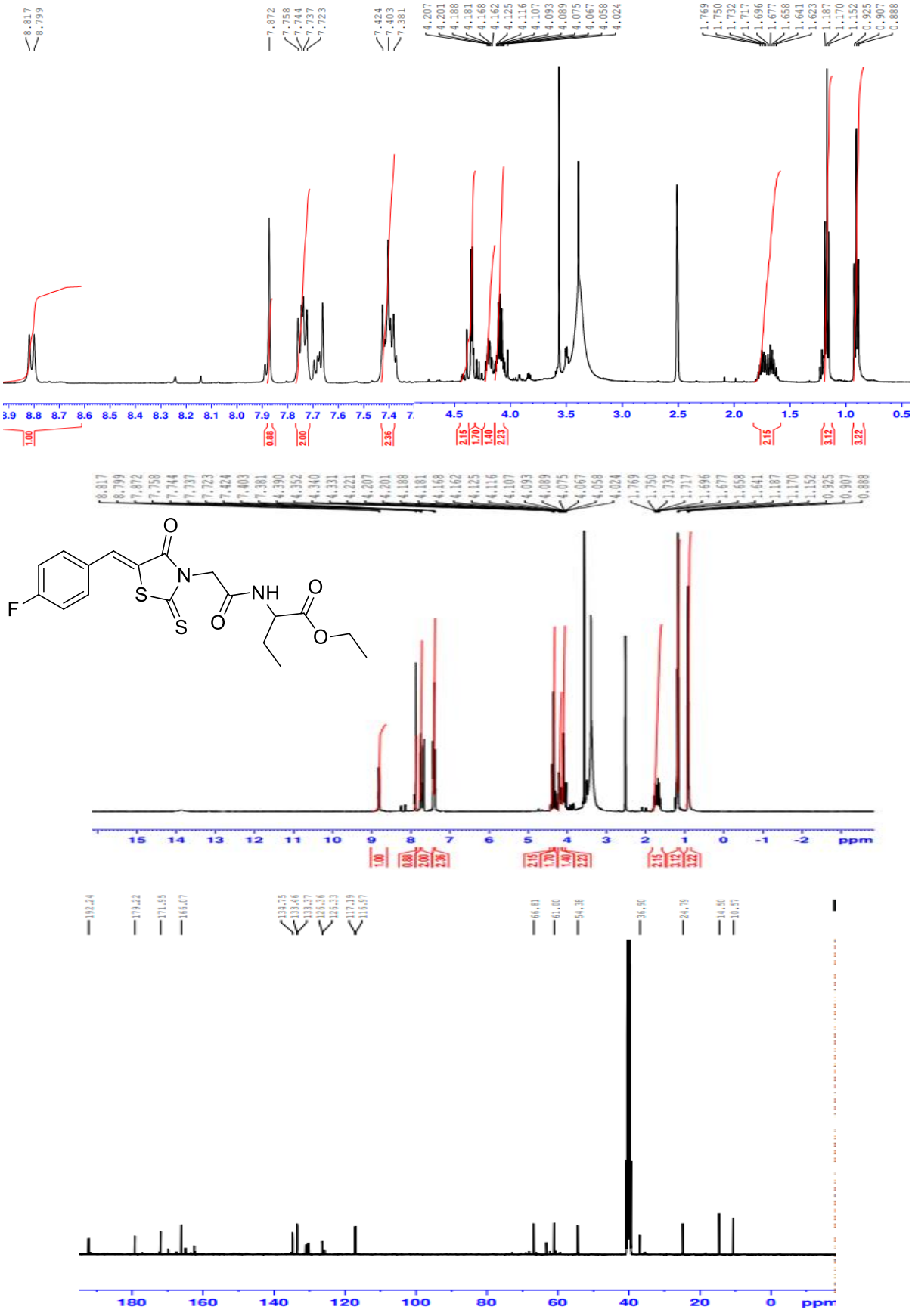


A24. IR spectrum of compound **105d**

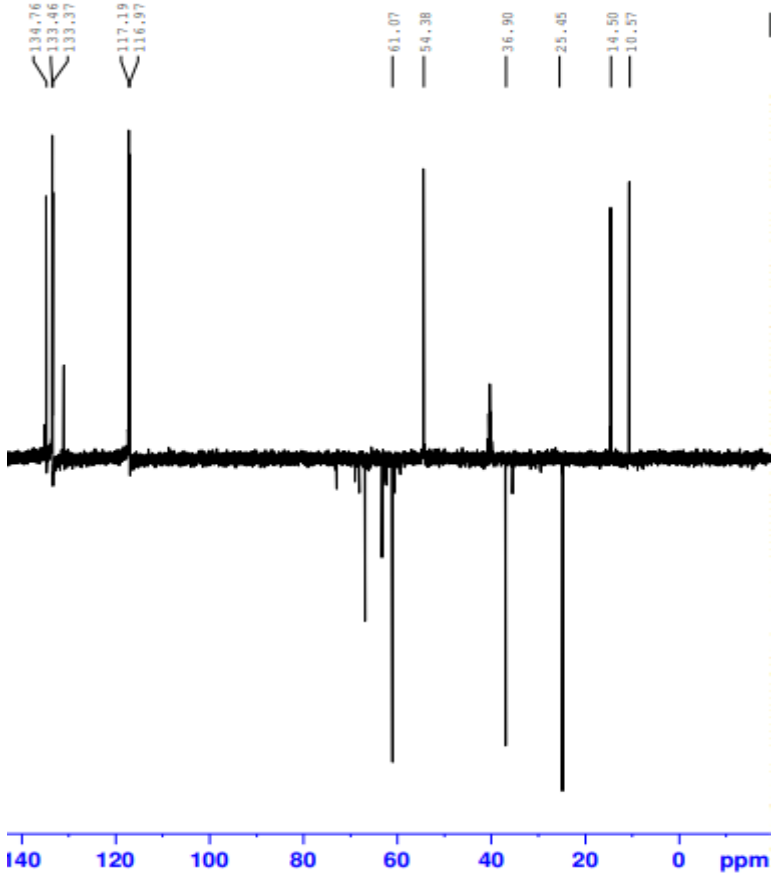


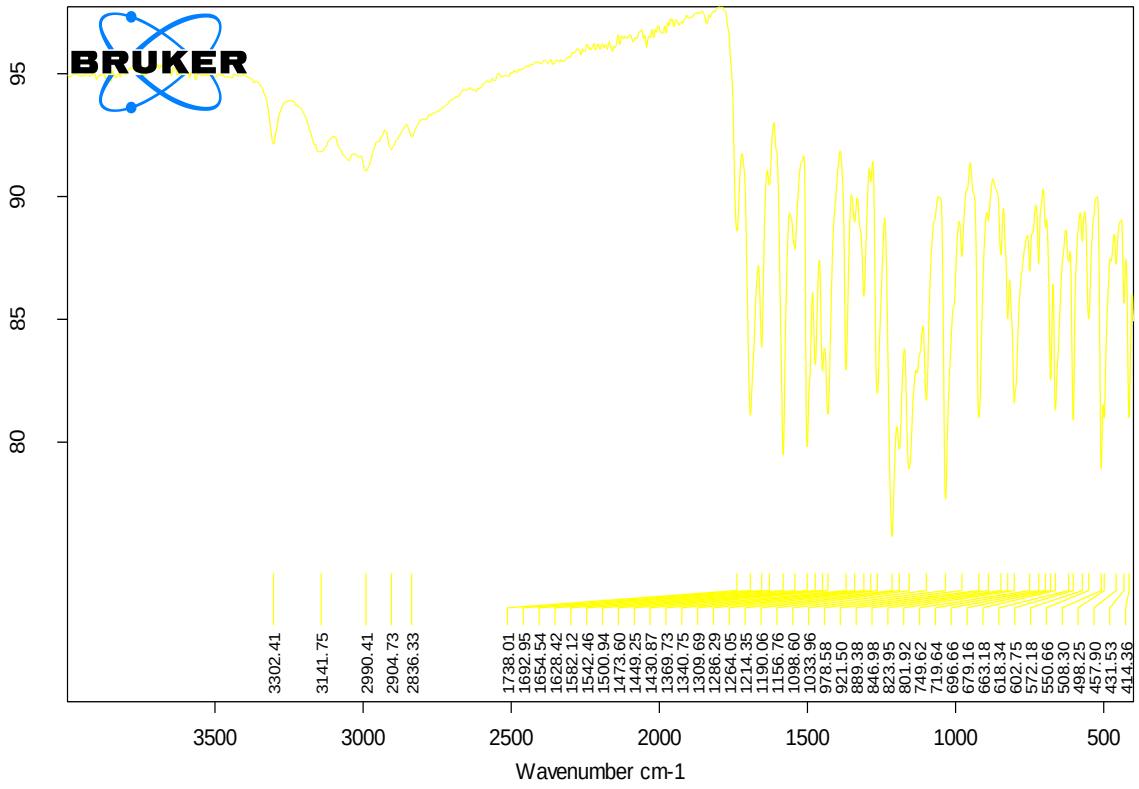


A25. ^{1}H and ^{13}C NMR spectra of compound **105g**

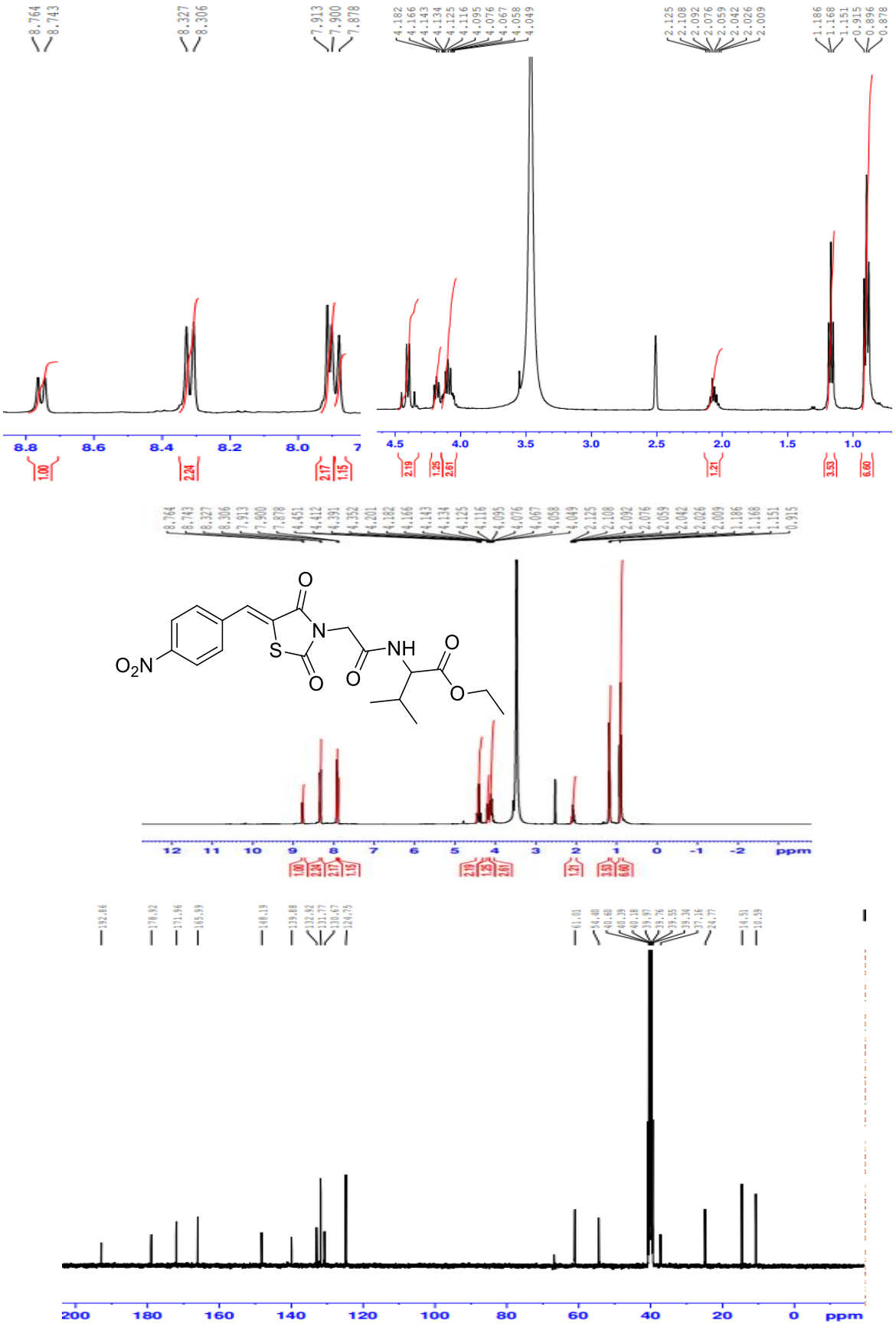


A26. ^{1}H and ^{13}C NMR spectra of compound **105g**

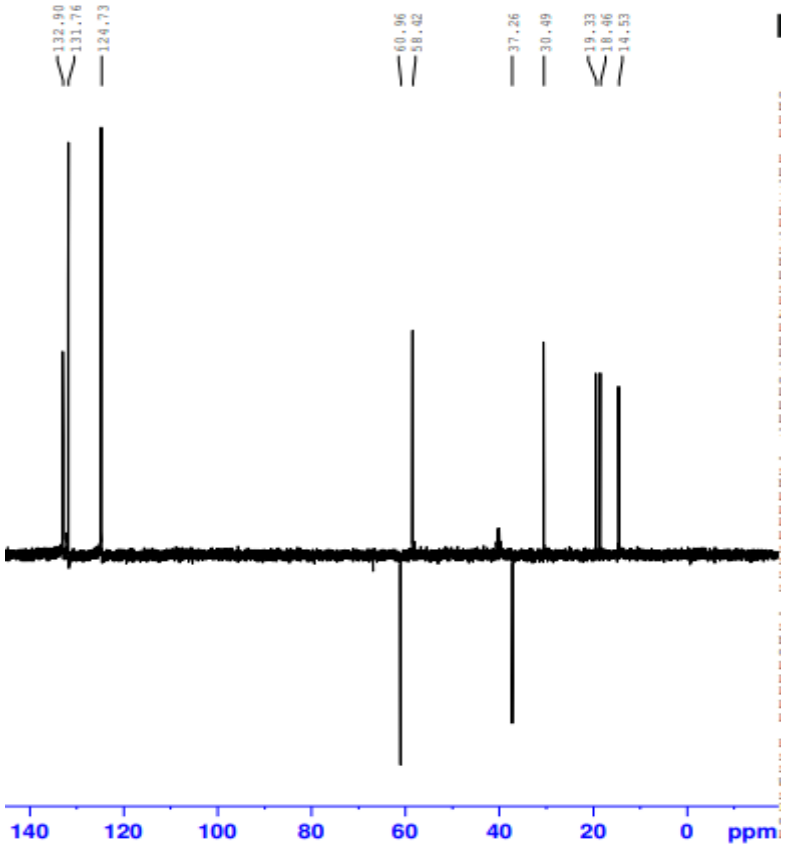


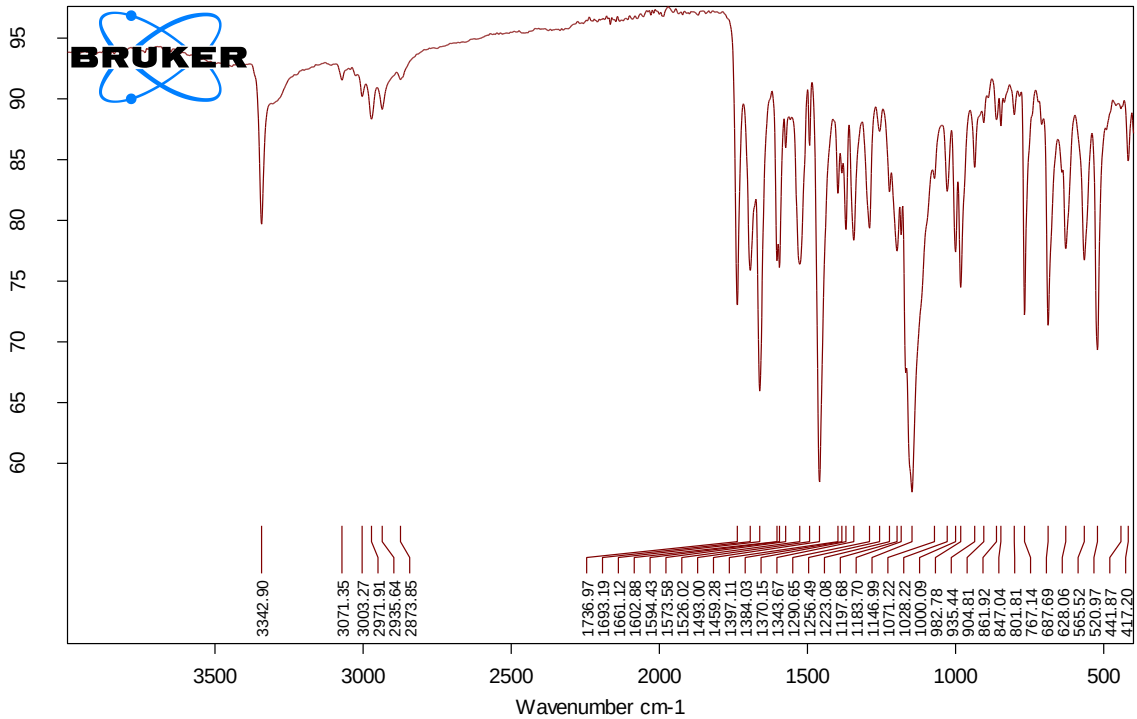


A27. ^{1}H and ^{13}C NMR spectra of compound **105i**

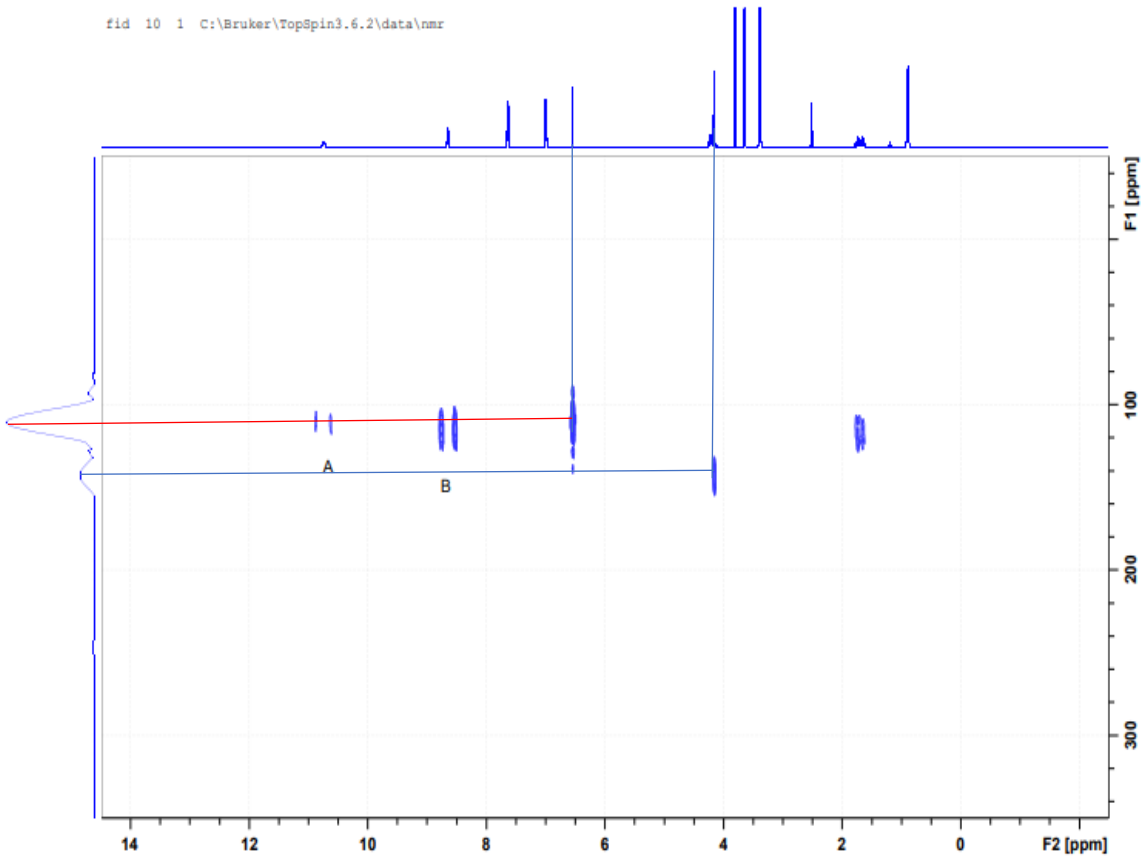


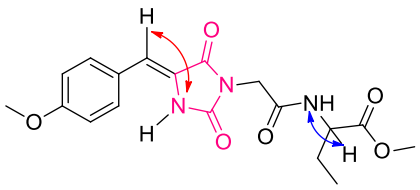
A28. Dept and IR spectrum of compound **105i**



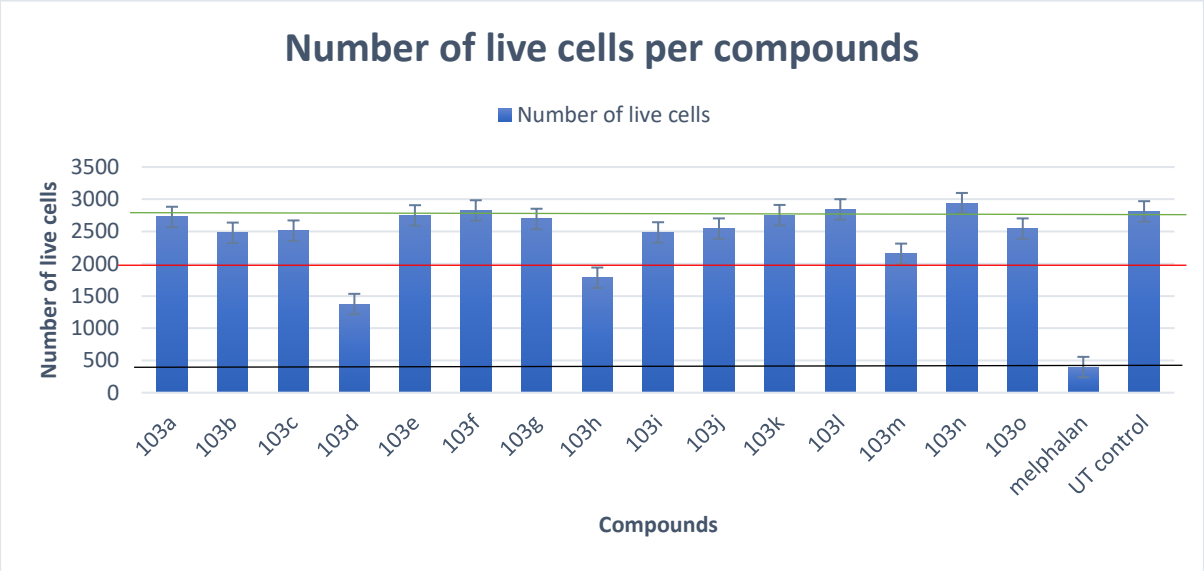


A29: ^{1}H-^{15}N HMBC spectrum of compound **104j**

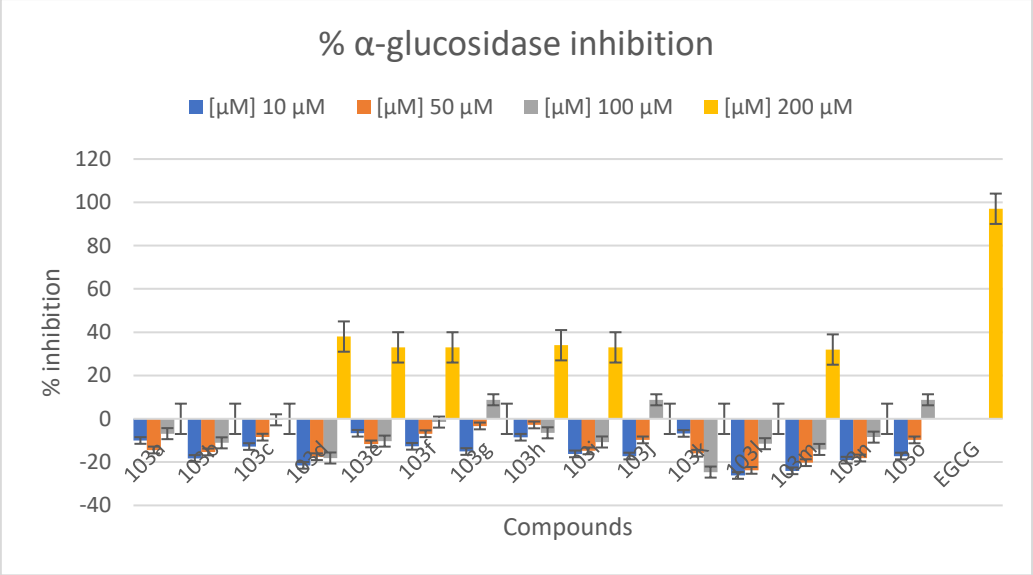




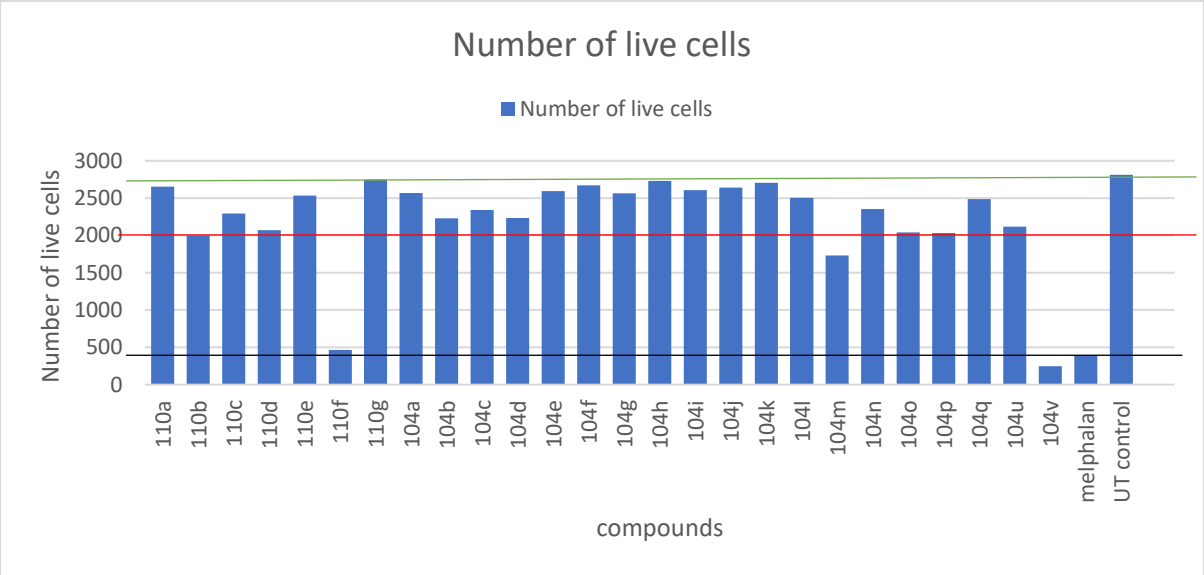
A30: Cytotoxicity chart of $Caco_2$ human cell line of glitazone containing compounds **(103a-o**)



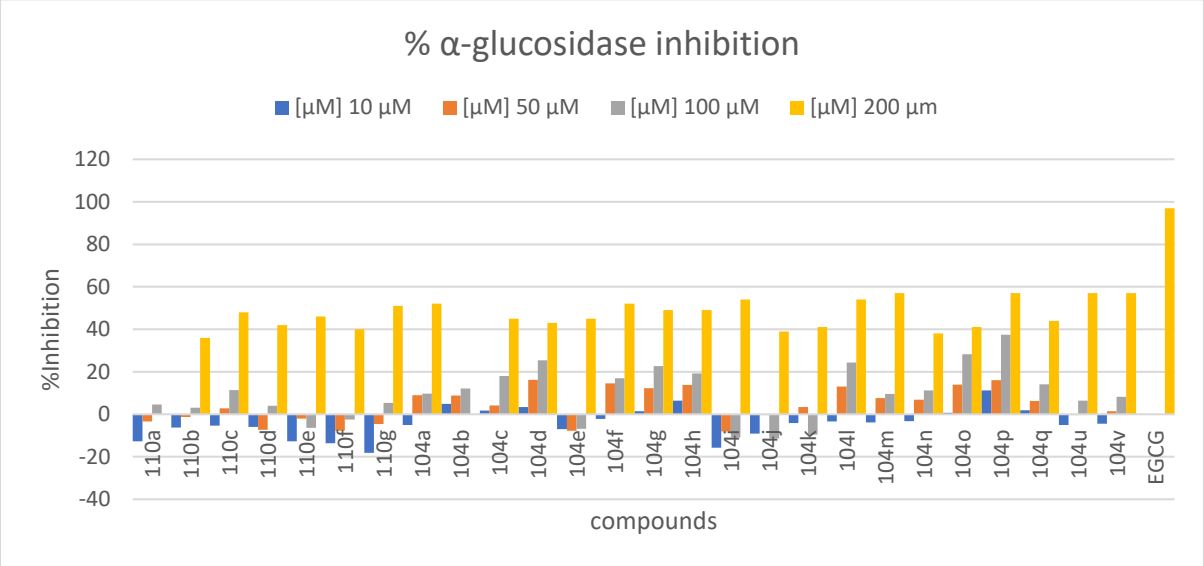
A31: % α-glucosidase chart of glitazone containing compounds **(103a-o**)



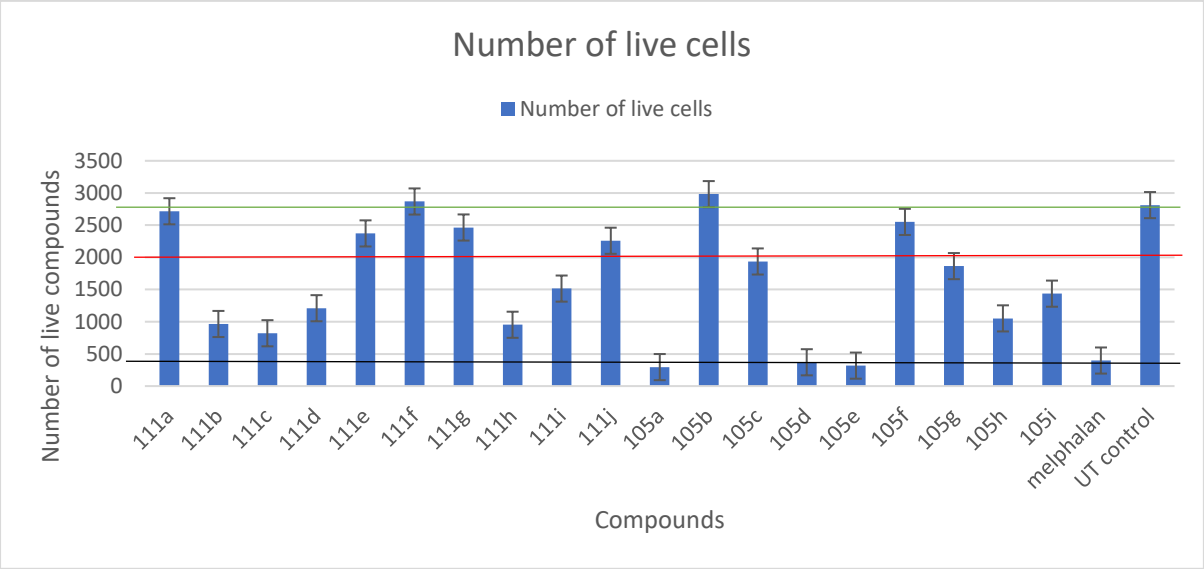
A32: Cytotoxicity chart of $Caco_2$ human cell line of hydantoin containing compounds **110a-f and 104a-v**



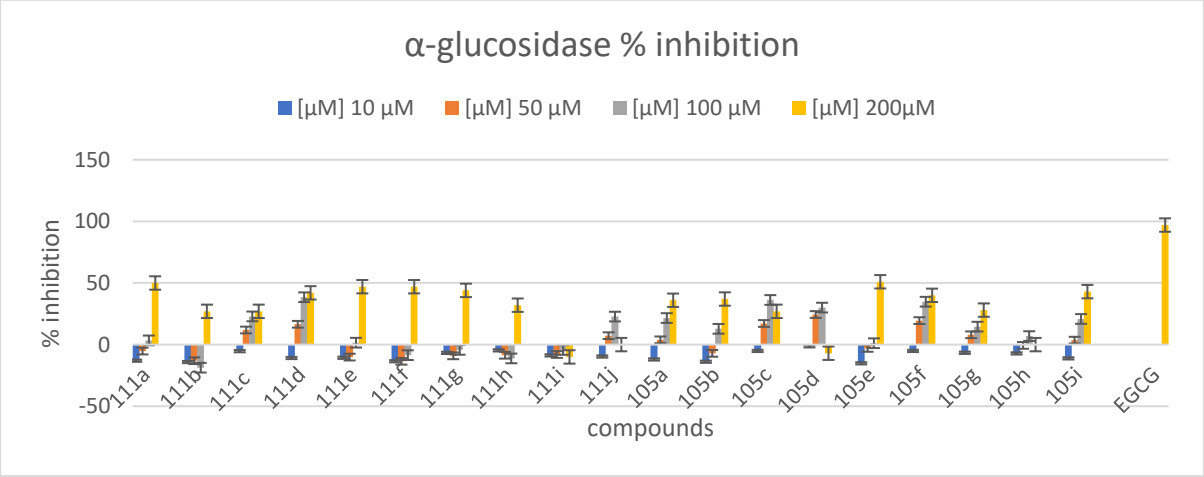
A33: % α-glucosidase chart of hydantoin containing compounds **110a-f and 104a-v**



A34: Cytotoxicity chart of $Caco_2$ human cell line of rhodanine containing compounds **110a-f and 104a-v**



A35: % α-glucosidase chart of rhodanine containing compounds **110a-f and 104a-v**



List of Publications, conference proceedings and student co-supervision/mentoring

➢ Publications

- Tshiluka, N. R.; Bvumbi, M. V; Ramaite, I. I.; Mnyakeni-moleele, S. 'Synthesis of Some New 5-Arylidene-2, 4-Thiazolidinedione' esters. *Arkivoc*, v, **2021**,161-175
- Tshiluka, N. R.; Bvumbi, M. V;Tshishonga U.; Mnyakeni-moleele, S.S 'Synthesis of new 5-benzylidene hydantoin esters. *J. Chem. Res.* 14 (6), **2022,** 1-7
- Tshiluka, N. R.; Bvumbi, M. V.; Mnyakeni-Moleele, S.S 'Synthesis, cytotoxic and α-glucosidase inhibition of N-substituted glitazone and rhodanine derivatives''. *submitted manuscript*, *O.C*., **2022**

➢ Conference preceedings

- Attended and presented oral presentation at 43rd national SACI Convention 2018
- Attended and presented oral presentation at Frank Warren Organic Chemistry 2019
- Attended and presented poster presentation at Traditional African medicine conference 2018
- Attended and presented poster presentation at SACI YCS symposium UI-2016
- Attended and presented poster presentation at SACI YCS symposium Univen 2019

➢ CO-Supervision and Mentoring

- Tshishonga Unarine
- Masia Rirhadzu
- Mbhokadzi Nyeleti
- Mlangeni Ntokozo
- Tshikudo Fhulufhelo
- Dube-Johnstone Michael