

Synthesis of Glitazone Analogues as Anti-Diabetic Drugs



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By

Ndivhuwo Raymond Tshiluka

11641072

Supervisor: Dr SS Mnyakeni-Moleele

Co-Supervisor: Prof. IDI Ramaite

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Declaration

I **Ndivhuwo Raymond Tshiluka** hereby declare that the work presented under this dissertation titled "Synthesis of Glitazones Analogues as Anti-Diabetic drugs" is my own and unaided work performed under the supervision of **Dr SS Mnyakeni Moleele and Prof I.D.I Ramaite** and has never been submitted before to this or any other University for any degree and it is presented for Master of Science degree at the University of Venda.

Candidate Signature

Date

.....

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Abstract

In this study, three series of novel glitazones (acetates, glycinates and alaninates) were designed and prepared by using appropriate synthetic methods to incorporate aromatic ring, alicyclic amines and alanine moiety instead of glycine moiety as a linker via two carbons. This was done over five reaction steps. Compounds were synthesized using conventional methods from step one with nucleophilic substitution to Knoevenagel condensation reaction as the final step and were characterized by using a combination of ^1H NMR, ^{13}C NMR, IR spectroscopies as well as HRMS analysis. Fourteen final compounds were successfully obtained in good to excellent yields. Furthermore, the same compounds were subjected to *in vitro* screening for their inhibitory activities against α -glucosidase and α -amylase.

Among all the synthesized compounds, **36f** exhibited excellent antidiabetic activities against α -glucosidase. Compounds **36a**, **36b**, **36i** and **36j** also displayed good activities and have potential to be further investigated whereas compounds **36c**, **36e**, **36g** and **36h** exhibited moderated activities against α -glucosidase. Only compounds **36a**, **36b**, **36f**, **36i** and **36j** displayed weak activities against α -amylase and the rest of the compounds were not active at all against α -amylase.

Dedication

I dedicate this MSc dissertation to **God** – You are my rock, You gave me life and opportunity to undertake this work when no one else would. Thank you, my Messiah, Dear Lord thank you indeed, You are the holy spirit who always keeps His promises. “For this thing, I besought the Lord thrice, that it might depart from me”. // *Cor 12: 6-16*.

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Abbreviations/ Acronyms

ATP: Adenosine Triphosphate

AIR2: Aldose reductase 2

ADP: Adenosine Diphosphate

AMPK: activated protein kinase

CSII: Continuous Subcutaneous insulin infusion

CGRP: Calcitonin-gene related peptide

¹³C NMR: Carbon 13 Nuclear Magnetic Resonance

DM: Diabetes mellitus

DPP-4: Dipeptidyl Peptidase-4

DPPH: 2,2-diphenyl-1-picrylhydrazyl

GLP-1: Glucogen-like peptide-1

DMF: N,N Dimethyl formamide

DCM: Dichloromethane

DMSO-d₆: Deuterated Dimethyl Sulfoxide

DMAA: Dimethoxymethylamphetamine

EtOH: Ethanol

Et₃N: Triethylamine

FP-2: Falcipain-2

FTIR: Fourier Transform Infrared Spectroscopy

FDA: Food and Drug Administration

GLUT1: Glucose transporter 1

GLUT2: Glucose transporter 2

GSIR: Glucose-Stimulated insulin release

IC₅₀: Half maximal inhibitory concentration

IDF: International Diabetes Federation

INS-1: Insulin-secreting cell lines 1

IR: Infrared

K_{atp}: Potassium Adenosine Tri-Phosphate

HRMS: High Resolution Mass Spectrometer

¹H NMR: Proton Nuclear Magnetic Resonance

LDL: Low density lipoprotein

MIC: Minimum Inhibitory Concentration

µm: Micrometer

m.p: Melting point

ml: Milliliter

NADP: Nicotinamide adenine dinucleotide phosphate

NAFLD: Non-alcoholic fatty liver disease

NAD⁺: Nicotinamide adenine dinucleotide

NIDDM: non-insulin-dependent diabetes mellitus

NMR: Nuclear Magnetic Resonance

PPARG or PPAR-γ: Peroxisome Proliferator-Activated Receptor Gamma

PPM: Parts per million

PI3K: Phosphatidylinositol-3-kinase

Pd/C: Palladium on carbon

SAR: Structure Activity Relationship

SGLT2-I: Sodium glucose co-transport 2 inhibitors

THF: Tetrahydrofuran

T1D: Type 1 diabetes

T2D: Type 2 diabetes

TZDS: thiazolidinediones

WHO: World Health Organization

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CHAPTER-ONE

1.1. Introduction and Literature Review

Diabetes mellitus (DM) is a group of metabolic diseases characterized by elevated blood glucose levels (hyperglycemia) resulting from defects in insulin secretion, insulin action or both. Insulin is a hormone manufactured by the beta cells of the pancreas, which is required to utilize glucose from digested food as an energy source.^[1] Diabetes results from an inability of the pancreas to produce insulin (type 1 diabetes) or the insufficient secretion of insulin or the insulin target tissues inability to respond to insulin effectively and efficiently (type 2 diabetes). Chronic hyperglycemia is associated with microvascular and macrovascular complications that can lead to visual impairment, blindness, kidney disease, nerve damage, amputations, heart disease, and stroke.

The epidemic of diabetes is rapidly increasing and diabetes has become a worldwide health threat in the past decades.^[2] According to the WHO the number of people with diabetes had risen from 108 million in 1980 to 422 million (4.5%) in the US population in 2014. Direct and indirect health care expenses were estimated at \$98 billion.^[2] According to one statistical report, it is estimated that 382 million people worldwide, or 8.3% adults, had diabetes in 2013, and if these trends continue, this number will rise to 592 million by 2035.^[3]

1.1.1. Diabetes in South Africa

According to recent statistics released by statistics South Africa in March 2018, diabetes mellitus is ranked the second killer in South Africa after tuberculosis followed by HIV/ AIDS accounting for 5.1% deaths in 2016.^[4] Recent data from the International Diabetes Federation (IDF) estimates that out of 54 million population in 2014,^[5] almost 2.3 million people had diabetes in South Africa with 79% of people living with diabetes in urban areas. It was estimated that 7% of South Africans between the ages of 21 and 79 years have diabetes based on the latest population survey or study of South Africa.^[4, 5]

Diabetes caused more than 68 thousand deaths in South Africa in 2013 with a prevalence of 70%.^[6] The highest prevalence of diabetes is among the Indian population (11-13%) as this group has a strong genetic predisposition for diabetes. This is followed by 8-10% in the coloured community, 5-8% among blacks and 4% among whites. Mortality estimates for the year 2000 found that within KwaZulu-Natal (KZN), diabetes was the fourth largest cause of death among adult women and ranked seventh in the leading causes of death among adult men.^[7] According to global estimations, developing countries are predicted to experience an increase of as much as 69% in the number of adults diagnosed with diabetes by 2030.^[8] The increase in the prevalence of the disease, especially in developing countries, is speculated to be due to a general increase in life expectancy, sedentary lifestyles, urbanisation and changes in dietary habits.^[9]

1.1.2. Main types of diabetes

Type 1 diabetes (T1D) also referred as insulin-dependent diabetes mellitus or juvenile-onset diabetes, accounts for 5-10% of all cases of diabetes.^[10] Historically, type 1 diabetes was found in children, teenagers and young adults. However, this view has changed over the past decade as type 1 diabetes can develop at any age.^[11] Type 1 diabetes is commonly caused by an auto-immune response of the body. In this condition, the body fails to recognise the β -cells in the pancreas and then destroys them using antibodies and white blood cells, resulting in pancreatic defects in insulin secretion.^[12] People with type 1 diabetes rely on insulin injections or a continuous infusion of insulin via an insulin pump. Recently, there has been a rapid evolution of the care and treatment for people with type 1 diabetes, such as genetically engineered insulin and glucose monitoring devices in order to control the blood glucose levels and to prevent or delay the diabetes-related complications.^[13] In addition, a pancreas transplantation procedure has been applied to type 1 diabetic people. However, this approach still holds many limitations and needs further investigation.

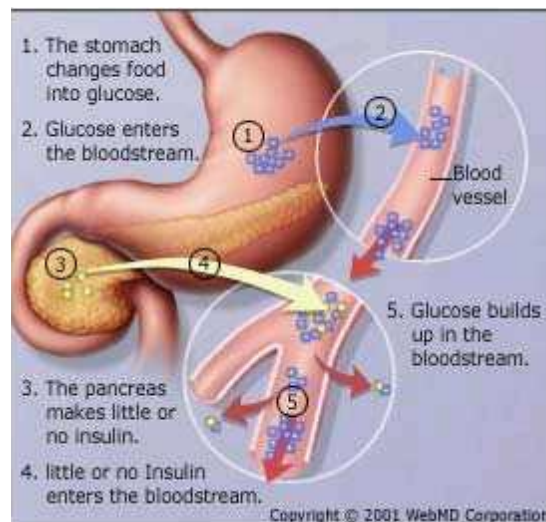


Figure 1: Carbohydrate digestion and Type 1 diabetes ^[14]

Carbohydrate digestion and Type 1 diabetes as shown in **Figure 1**, undergoes various processes (stages 1-5). The first stage involves conversion of dietary carbohydrate into glucose after ingestion of a meal (**stage 1**), the converted glucose is absorbed into the bloodstream through blood cells (**stage 2**). The problem arises when the pancreas is unable to produce a sufficient amount of insulin required by the body to enter the blood stream (**stage 3 and 4**). This leads to an increased glucose plasma level in the blood stream and causes type 1 diabetes (**stage 5**).

Type 2 diabetes (T2D), formerly known as non-insulin dependent diabetes mellitus or adult onset diabetes, is caused by metabolic disorders of insulin resistance which leads to the progression of high blood glucose levels. It accounts for 85-90% of all cases of diabetes.^[15] Type 2 diabetes is commonly diagnosed in the middle-aged population, over the age of 45 years.^[16] However, recently it is increasingly developing in younger people, even in children under 14 years of age.^[17] In most people diagnosed with type 2 diabetes, their insulin levels in the blood are found to be normal or elevated initially at early stages and in later stages the body fails to recognise the insulin and hence, the insulin levels increase. However, there are some people who can manage their condition to delay this disease and, in some cases, even prevent the disease completely by adopting a healthy lifestyle. This include regular physical activities, healthy food intake and losing excess body weight. However, at a later stage of the process, many people with type 2 diabetes become insulin deficient and may require oral medications and/or eventually need insulin injections when other

medications fail to control blood glucose levels adequately. Over 40% of people with type 2 diabetes require insulin as part of their diabetes management plan.^[18]

Gestational diabetes is another form of diabetes. It is characterised by high blood glucose levels during the late stage of pregnancy (usually between the 24th and 28th week).^[19] Gestational diabetes affects about 4% of pregnancies worldwide and is associated with complications to both mother and foetus.^[20] Gestational diabetes usually resolves after the delivery of the baby but women with gestational diabetes and their children are at a higher risk of developing type 2 diabetes.^[21] Approximately half of women with a history of gestational diabetes have developed type 2 diabetes within five to ten years after delivery of their babies.^[22] Since gestational diabetes can affect both mother and foetus, it is essential to treat it as early as possible. Treatments for gestational diabetes include special dietary plans and regular physical activity to keep normal blood glucose levels, which are similar to other pregnant women who do not have gestational diabetes.^[23]

1.1.3. Diabetes-associated complications

The mortality rate of diabetes varies sharply with the prosperity of a country. Overall, low- and middle- income countries have more than double the mortality rate compared to high-income countries. It is estimated that 4.0 million people aged between 20 and 79 years died worldwide from diabetes in 2017, which is equivalent to one death every eight seconds. Diabetes accounted for 10.7% of global all-cause mortality among people in this age group.^[24] The number of deaths caused by diabetes showed a 27% increase in 2013.^[25] The increasing mortality rate of diabetes is not only caused by diabetes itself but also largely due to its complications. People with diabetes are at a high risk of developing a number of life-threatening health problems like cardiovascular disease,^[26] retinopathy,^[27] nephropathy,^[28] neuropathy, and pregnancy complications.^[29] Diabetes can also interfere with wound healing.^[30] **Figure 2** presents the major complications caused by diabetes in different organs, including brain, eyes, heart and kidney.

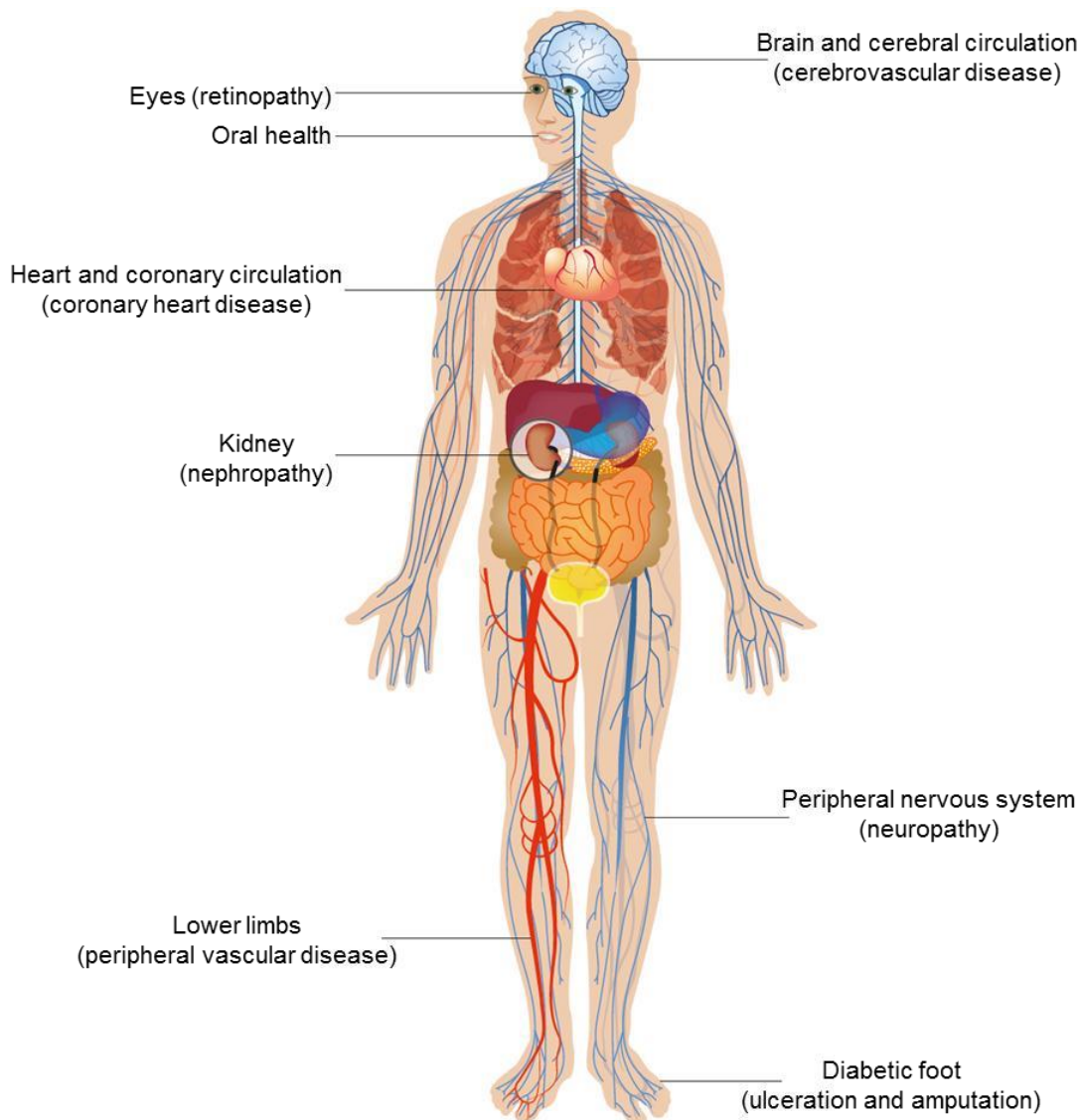


Figure 2: The major diabetes-associated complications^[6]

1.1.4. Insulin action

Insulin is a hormone made by the pancreas that allows your body to use sugar (glucose) from carbohydrates in the food that you eat for energy or to store glucose for future use. Insulin helps keeps your blood sugar level from getting too high (hyperglycemia) or too low (hypoglycemia).^[6]

1.1.4.1. The Metabolic Role of Insulin

Insulin is the principal hormone controlling blood glucose levels and is synthesised by the β -cells in the pancreas. The level of insulin is a central metabolic control mechanism. The immediate effect of insulin is to promote storage of dietary calories and thus lower the circulating levels of glucose coming from carbohydrate throughout the body. For example, after ingestion of a meal, dietary carbohydrates increase

plasma glucose level, which is sensed by pancreatic β -cells, promoting insulin secretion into the bloodstream. This suppresses the glucose production in the liver and stimulates the uptake of glucose by peripheral tissues (skeletal muscle and adipose tissue) (Figure 3).^[31]

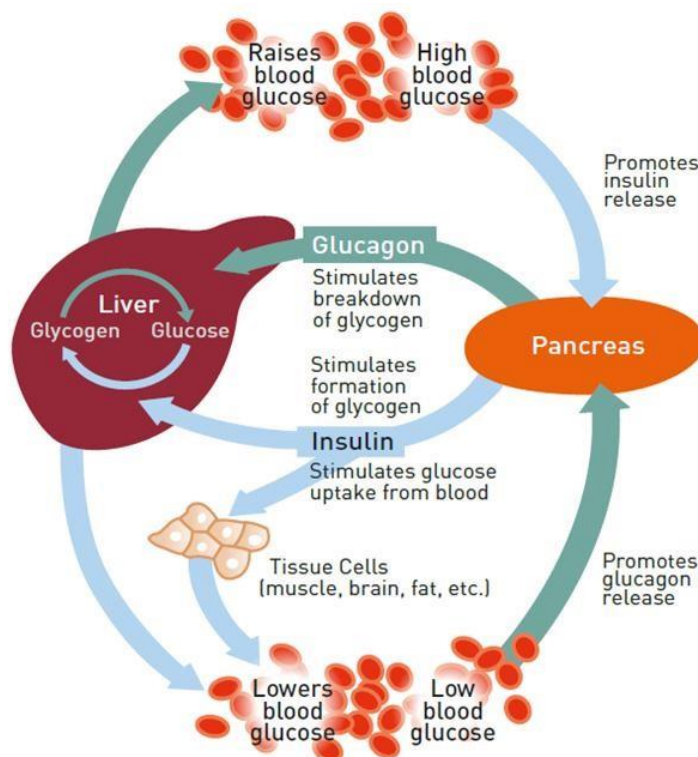


Figure 3: Metabolic role of insulin

Insulin production and action. Adapted from the International Diabetes Federation (IDF), Diabetes Atlas, 6th edition, 2013.^[3]

Therefore, liver, skeletal muscle and adipose tissue are the main target tissues of insulin. In the liver, insulin increases the activity and/or expression of enzymes that enhance glycogen, lipid and protein synthesis. At the same time, insulin suppresses the activity and/or expression of those enzymes that catalyse gluconeogenesis and glycogenolysis, which break down the glycogen stored in the liver and muscles into glucose.^[32] In the skeletal muscle, insulin stimulates the uptake of glucose, fatty acids and amino acids from the blood, permitting synthesis and storage of glycogen, carbohydrates, lipids, and proteins.^[33] In adipose tissue, insulin stimulates glucose uptake, promotes lipogenesis to store substrates and inhibits lipolysis. The majority of the glucose (80%) that is taken up by peripheral tissues is utilised by muscles with a relatively smaller amount being disposed into fat and liver.^[34]

1.1.4.2. Insulin Resistances

Insulin resistance is defined as reduced responsiveness to normal levels of insulin by target tissues (mainly liver and skeletal muscle).^[35] Insulin resistance plays a major role in the pathogenesis of type 2 diabetes, and it has also been recognised as a key link between obesity (particularly visceral adiposity) and type 2 diabetes.^[36] Insulin resistance does not appear suddenly, but is a result of a progressive deterioration of the insulin signalling cascades. At the pre-diabetic stage, which is the insulin resistance stage, the blood glucose level is maintained relatively normally via a compensatory increase in insulin secretion to counteract insulin resistance.^[37] In type 2 diabetes, ectopic lipid accumulation impairs insulin signalling. With the accumulation of intracellular lipids, insulin-mediated glucose uptake in the skeletal muscle is impaired resulting in the diversion of glucose to the liver. Increased lipid in the liver also impairs the ability of insulin to regulate gluconeogenesis and glycogenesis. In contrast, the unaffected lipogenesis together with the increased delivery of dietary glucose leads to increased glucose-stimulated insulin release (GSIR) and non-alcoholic fatty liver disease (NAFLD).^[38] Impaired insulin action in the adipose tissue causes increased lipolysis, thus promoting re-esterification of lipids in other tissues (such as liver) and further exacerbating insulin resistance. This prolonged insulin resistance may eventually result in pancreatic β -cell failure and the circulating insulin levels become insufficient to control blood glucose level, leading to hyperglycaemia. Therefore, improvements in insulin action and insulin secretion are important for the treatment of type 2 diabetes.

1.2. Genetics and symptoms

Diabetes is known to be a hereditary disease because it is known to run in families. If one's parent has diabetes, the chances of getting it immediately become significantly higher than those with a negative family history of diabetes. This is because people inherit the type of cells with diabetes from the parents. Currently 20 different chromosomal regions have been linked to type 1 diabetes (T1D) susceptibility in humans. Using genome screening, candidate gene testing, and studies of human homologues of mouse susceptibility gene found that is usually the one associated with inherited (genetic) factors.^[39] Type 2 diabetes mellitus is a heterogeneous disease that is caused by both genetic and environmental factors. Only a minority of cases of type 2 diabetes are caused by a single-gene defect, such as maturity onset diabetes of

youth (mutated MODY gene), syndrome of insulin resistance (insulin receptor defect), and maternally inherited diabetes and deafness (mitochondrial gene defect). The genetic component of the more common form of type 2 diabetes is complex and involves the interactions of multiple genes and environmental factors. The candidate gene approach has identified several genes that regulate insulin signalling and secretion, but their contributions to diabetes are small. Recent genome scan studies have been conducted to identify major susceptibility loci that are linked with type 2 diabetes.^[40] The symptoms of diabetes include excessive thirst, hunger and tiredness, urinating often, loss of weight, dry itchy skin and blurry eyesight.^[41]

1.3. Classes of antidiabetic drugs and their mode of action.

Antidiabetic drugs are medications that lower blood glucose concentrations or the amount of sugar in the blood. They are an important way of treating gestational, type 1 and type 2 diabetes mellitus. Herbal remedies and other forms of alternative medicines are also used for diabetes treatment, especially in areas where modern drugs are not easily accessible. Antidiabetic drugs exert their useful effects through: (1) increasing insulin levels in the body (secretagogues) or (2) increasing the body's sensitivity (or decreasing its resistance) to insulin, or (3) decreasing glucose absorption in the intestines. Antidiabetic drugs do not cure diabetes, but they help keep the condition under control and reduce the risk of serious complications. People with diabetes may need to take this medication for the rest of their lives.^[42] Different types of anti-diabetic drugs and their mode of action are summarized in **Table 1**.

Table 1: Antidiabetic medications against type 2 diabetes.^[42]

Antidiabetic agents		
Class	Examples	Principal mode of action
Sulphonylureas	Gliclazide, Tolbutamide, Glipizide	Secretagogues, stimulate insulin secretion
Biguanides	Metformin, Buformin, phenformin	Sensitizers, improve insulin action
Alpha-glucosidase inhibitors	Acarbose, miglitol, voglibose	Slow down the rate of carbohydrate ingestion.
Meglitinides	Nateglinide Repaglinide	Meglitinides increase the amount of insulin produced by the pancreas, which lowers blood sugar.
Amylin analogues	Pramlintide	Decrease glucose levels and reduce the re-introduction of glucose in the circulation.
Sodium Glucose co-Transporter 2 inhibitors	Remogliflozin, Dapagliflozin	Reduce glucose reabsorption in the proximal renal tubules and lower renal glucose threshold.
Thiazolidinediones	Pioglitazone Rosiglitazone	Increase insulin action

1.3.1. Biguanides

Biguanides are an interesting class of anti-diabetic drugs with many known or potential applications like anti-inflammatory and antiproliferative actions. The structure of biguanides continues to be commonly depicted in a manner that leads to misleading perception. X-ray crystallographic analysis, NMR spectroscopy, molecular modelling and tautomer stability studies have confirmed that biguanides should be represented with hydrogen on the bridging nitrogen.^[43]

Mechanism of action

Well known biguanides are buformin (**1**) and metformin (**2**) (**Figure 4**) which were introduced in 1957 as oral glucose-lowering agents to treat non-insulin-dependent diabetes mellitus (NIDDM). Another well known biguanide, phenformin (**3**), was withdrawn in many countries because of an association with lactic acidosis. On the other hand metformin and buformin do not have the same risk if appropriately prescribed.^[44] Hepatic sensitivity of these drugs to insulin is increased, thereby reducing gluconeogenesis as well as glycogenolysis, which contribute to the post-prandial plasma glucose lowering effects. Skeletal muscles and adipocytes undergo up-regulation of the insulin-sensitive GLUT-4 and GLUT-1 transporters to the cell membranes, thereby increasing glucose uptake.^[45] Glucose metabolism in the splanchnic bed also increases. Further metabolic effects include suppression of fatty acid oxidation as well as triglyceride lowering.^[46]

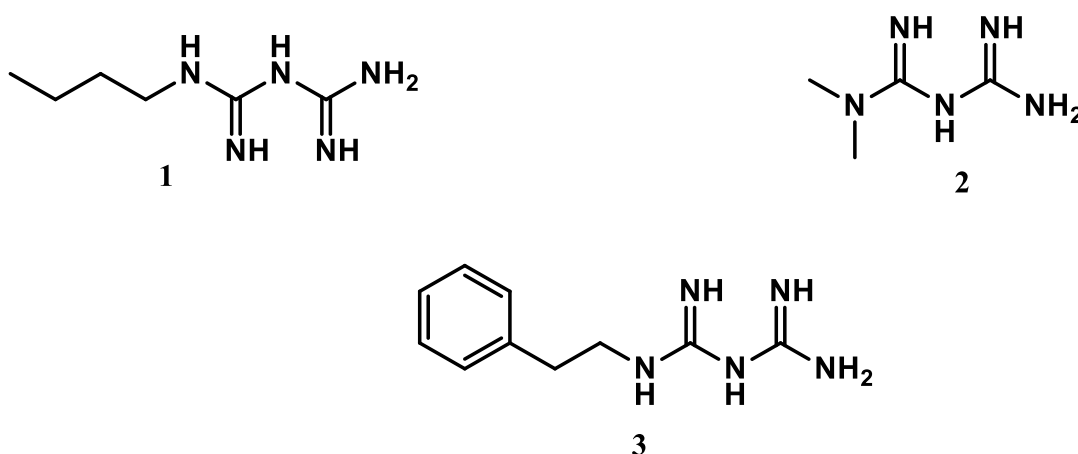


Figure 4: Examples of Biguanides

1.3.2. Sulphonylureas

Sulphonylureas constitute a well known class of compounds which exhibit a wide range of biological activities like antidiabetic and diuretic activities. They increase insulin production by shutting the potassium channels in the pancreatic β -cells and helping lower blood glucose levels. Unfortunately, this treatment is only effective when there is sufficient β -cell mass to provide enough insulin. This specific treatment is inefficient after several years due to the dramatic reduction of β -cells mass.^[47] These drugs can be prepared from various reactions starting from various substituted phenyl

sulphonamides, naphthylsulphonamides as well as heteroarylsulphonamides such as 2-ethenyl and benzo-2-sulphonamide.^[48]

Mechanism of action

In Pancreas: Sulphonylureas stimulate insulin secretion by beta cells of the pancreas by sensitising them to the action of glucose. Sulphonylureas bind to an ATP-dependent K^+ (K_{ATP}) channel on the cell membrane of pancreatic beta cells. This inhibits a tonic, hyperpolarizing outflux of potassium, which causes the electric potential over the membrane to become more positive. This depolarization opens voltage-gated Ca^{2+} channels. The rise in intracellular calcium leads to increased fusion of insulin granule within the cell membrane, and therefore increased secretion of (pro) insulin.^[49]

In Liver: There is some evidence that sulphonylureas also sensitise β -cells to glucose, that they limit glucose production in the liver, that they decrease lipolysis (breakdown and release of fatty acids by adipose tissue) and decrease the clearance of insulin by the liver.^[50] Moreover, they inhibit glucagon secretion and sensitise target tissues to the action of insulin. Several side effects such as hypoglycaemia, body weight gain, skin rashes, stomach upsets and jaundice are associated with sulphonylureas.^[51] Examples of sulphonylureas are tolbutamide (4) and glipizide (5) (Figure 5)

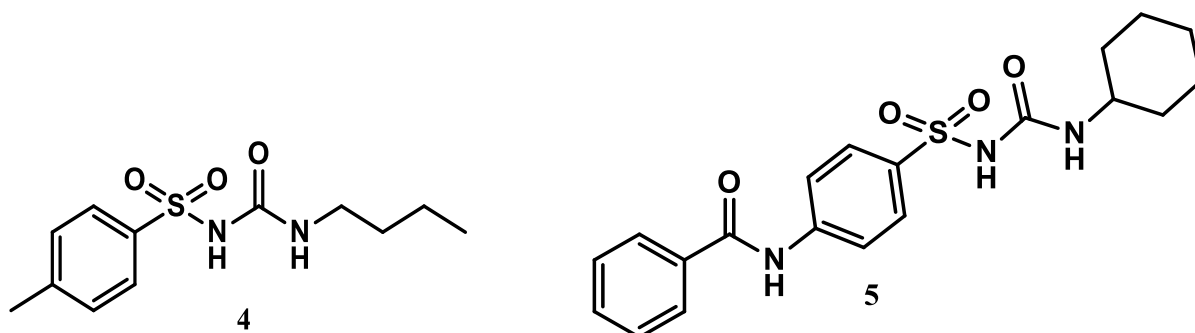


Figure 5: Examples of Sulphonylureas

1.3.3. Alpha Glucosidase Inhibitors

Alpha Glucosidase Inhibitors catalyse the liberation of alpha-glucose from the non-insulin reducing end of a substrate, including malt oligosaccharides, aryl- and alkyl-alpha-d-glucopyranosides. Besides this hydrolase activity, the enzyme also displays a

transferase activity (transglycosylation reaction), which results in the formation of various alpha glycosylated compounds. Alpha glucosidase inhibitors prevent the digestion and absorption of complex carbohydrates and the metabolism of polysaccharides to monosaccharides, which results in decreased glucose uptake through the intestine.^[52] Unlike the meglitinides (another class of anti-diabetic medications) or sulphonylureas, this class of medicines do not stimulate the pancreas to produce more insulin to prevent high levels of blood glucose after a meal. Resultantly, they do not cause hypoglycaemia unless they are used with other medicines for diabetes or with insulin. The effect of α -glucosidase inhibitors on reducing glucose levels is less than that achieved with biguanides or sulphonylureas.

Mechanism of action

The intestinal alpha glucosidases are membrane-bound enzymes located in the intestinal brush border that release glucose in the intestine by hydrolysis of starch residues, namely oligosaccharides and disaccharides. This hydrolysis is necessary for the digestive absorption of carbohydrates because only monosaccharides like glucose and fructose are absorbed. Inhibition of alpha-glucosidases by products such as acarbose, voglibose (**6**) and miglitol (**7**) (**Figure 6**), reduces and delays the digestive absorption of glucose and decreases postprandial hyperglycemia. Acarbose is a pseudo tetrasaccharide which binds with high affinity to alpha glucosidases and inhibits them. Its effect is reversible because it is hydrolyzed and inactivated by bacterial amylases and other enzymes. Acarbose is only slightly absorbed by the digestive tract. It has no inhibitory activity against lactase and consequently would not be expected to induce lactose intolerance body weight loss. Flatulence, bloating and diarrhoea are the common side effects associated with α -glucosidase inhibitors.^[53]

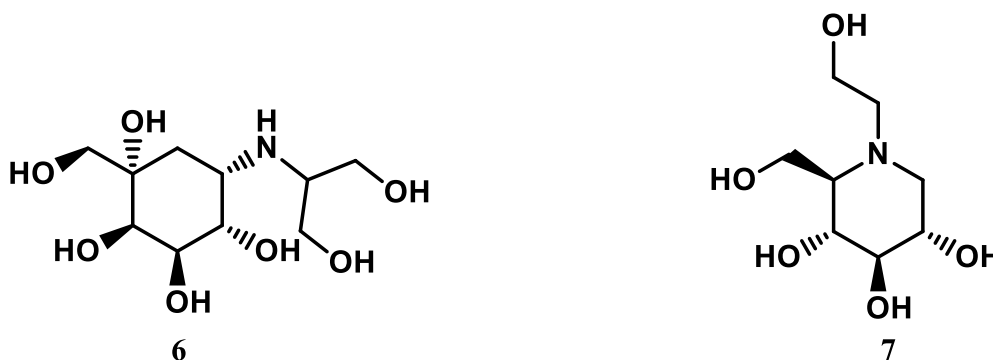


Figure 6: Examples of Alpha Glucosidase Inhibitors

1.3.4. Meglitinides

Meglitinide are benzamides belonging to the class of antidiabetic agents with hypoglycaemic activity. They are referred to as endogenous insulin secretagogues because they induce the pancreatic release of endogenous insulin. The activity of meglitinides is similar to sulphonylureas since they both bind to an ATP-dependent K^+ (KATP) channel on the cell membrane of pancreatic beta cells.^[54] Meglitinides are used to treat type 2 diabetes in people whose blood sugar levels have not stayed within a target range even though the people are being active and eating healthy foods.^[55]

Mechanism of action

The mechanism of action of meglitinides is similar to that of sulphonylureas. They stimulate first-phase insulin release in a glucose-sensitive manner from pancreatic beta cells reducing the risk of hypoglycaemic events.^[56] There are two major differences between these two classes, (1) meglitinides causes much faster insulin production than sulphonylureas, and (2) effects of meglitinides do not last as long as the effect of sulphonylureas. The meglitinides appear to last less than one hour while sulphonylureas continue to stimulate insulin production for several hours.^[57] Most of the commonly used meglitinides are metabolized in the liver and mainly excreted through the bile, except for a small proportion of the parent compound that has been found in the urine. Examples of meglitinides are netaglinide (**8**) and repaglinide (**9**) as shown below (**Figure 7**).

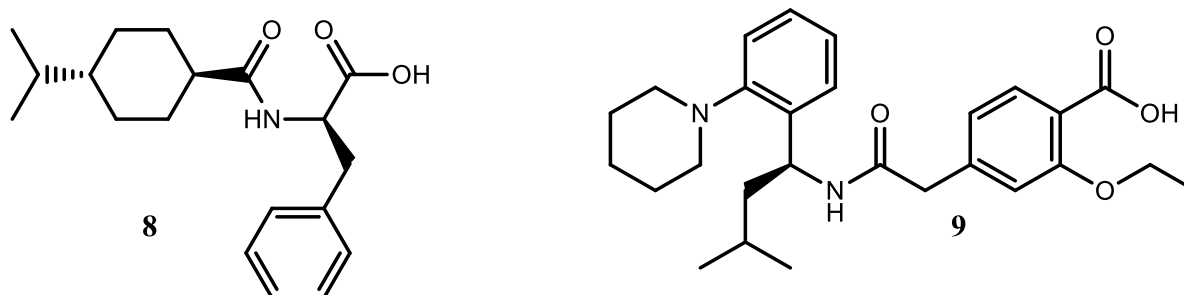


Figure 7: Examples of Meglitinides

1.3.5. Amylin Analogues

Amylin is a 37-amino acid peptide and insoluble protein that is stored in pancreatic beta cells and is co-secreted with insulin with a relatively low tissue concentration.^[58]

Since amylin is highly insoluble and even toxic to pancreatic beta cells because of deposition of fibrillary proteins, an aqueous, non-aggregating form of amylin was established by replacing three amino acid residues.^[59] Its amino acid sequence is 46% identical to that of human beta-CGRP (calcitonin-gene related peptide). This form of amylin was found to be effective in reducing blood glucose level when given subcutaneously.^[60]

Mechanism of action

Amylin analogues like pramlintide (**10**) affect glucose control through several mechanisms, including slowed gastric emptying, regulation of postprandial glucagon and reduction of food intake glucagon-like peptide 1 (GLP-1). They exhibit similar properties as amylin, with the exception of insulin secretory effects.^[61] They also partly regulate hyperglycemia through amelioration of inappropriate glucagon secretion and gastric emptying. GLP-1 and amylin appear to have differing magnitudes of physiologic effects and bind to different receptors in the area postrema, the part of the brain that may be key to their effects on satiety.^[62] An example of an amylin analogue is pramlintide (**10**) (**Figure 8**).

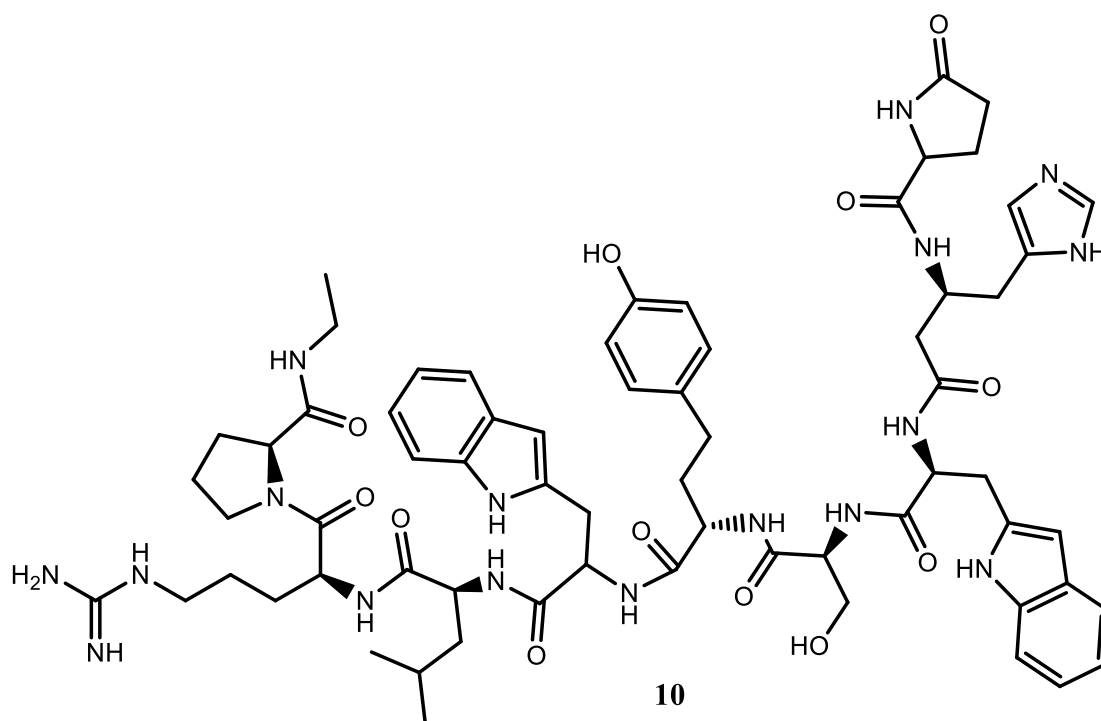


Figure 8: Example of amylin analogues

1.3.6. Sodium Glucose co-Transporter 2 inhibitors

Sodium glucose co-transporter 2 inhibitors (SGLT2-i) are a new class of anti-diabetic oral medications which were recently approved (2013) by the Food and Drug Administration (FDA) for the treatment of diabetes. In October 2017, dapagliflozin was the first of this class to be introduced in South Africa.^[63]

Mechanism of action

SGLT2 inhibitors block glucose reabsorption in the kidneys, which prompts urinary excretion of glucose and results in lowering of its plasma levels. The mechanism of action of the SGLT2 inhibitors is independent of insulin and their effect is diminished as plasma glucose concentrations decrease. They are associated with a low risk of hypoglycaemia.^[64] Furthermore, caloric loss associated with increased glucose excretion leads to a reduction in body weight and fat mass. SGLT2 inhibitors have a mild diuretic effect, hence they are associated with a modest reduction in blood pressure.^[65] Examples of sodium glucose co-transporter 2 inhibitors are dapagliflozin (**11**) and empagliflozin (**12**) (Figure 9).

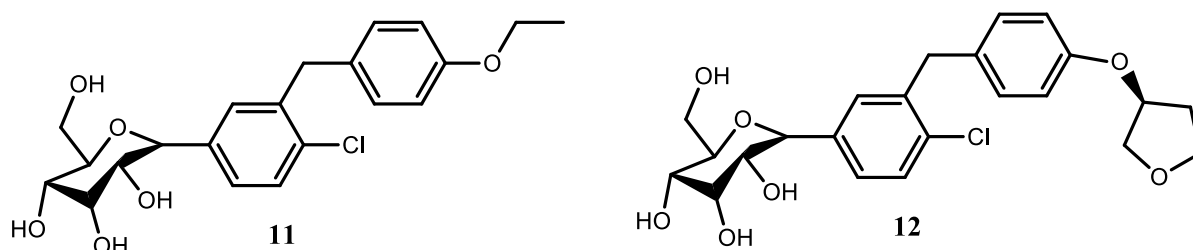


Figure 9: Examples of sodium glucose co-transporter 2 inhibitors

1.3.7. Thiazolidinediones (TZDs) or glitazones

1,3-Thiazolidine-2,4-diones are derivatives of thiazolidine with two carbonyl groups at the 2- and 4-position. The substituents in the 3- and 5-position may be varied. A significant difference in structure and properties is exerted by (1) attachment of various groups to the carbon atom in the 5-position or (2) by replacing an oxo group and (3) by replacing the thio group at 1- position. Also, variations in the substituents attached to the nitrogen atom and the methylene carbon atom are possible variations for the structures.

Mechanism of action

The hypoglycaemic effect of the TZDs is related to their ability to increase insulin sensitivity and consequently increase peripheral glucose utilisation. Although the exact mechanism of action is not completely understood, the most widely accepted hypothesis is that their effect on insulin sensitivity is related to their well-known ability to bind and activate the nuclear peroxisomal proliferator-activated receptors gamma (PPAR- γ).^[66] The beneficial effects of TZDs for the treatment of type 2 diabetes are due to their ability to stimulate activated protein kinase (AMPK) activity which promotes an improvement in glucose tolerance and whole-body insulin sensitivity.^[67] TZDs can effectively improve insulin action by normalising blood glucose levels. Undesirable side effects such as body weight gain, increased risk of cardiovascular disease and bladder cancer are associated with these drugs.^[68] 1,3 Thiazolidinediones have a wide range of biological activities such as anti-inflammatory,^[69] anti-tubercular,^[70] anti-microbial,^[71] cytotoxic^[72] and anti-oxidant activities.^[93] Examples of thiazolidinediones are rosiglitazone (**13**) and pioglitazone (**14**) (**Figure 10**).

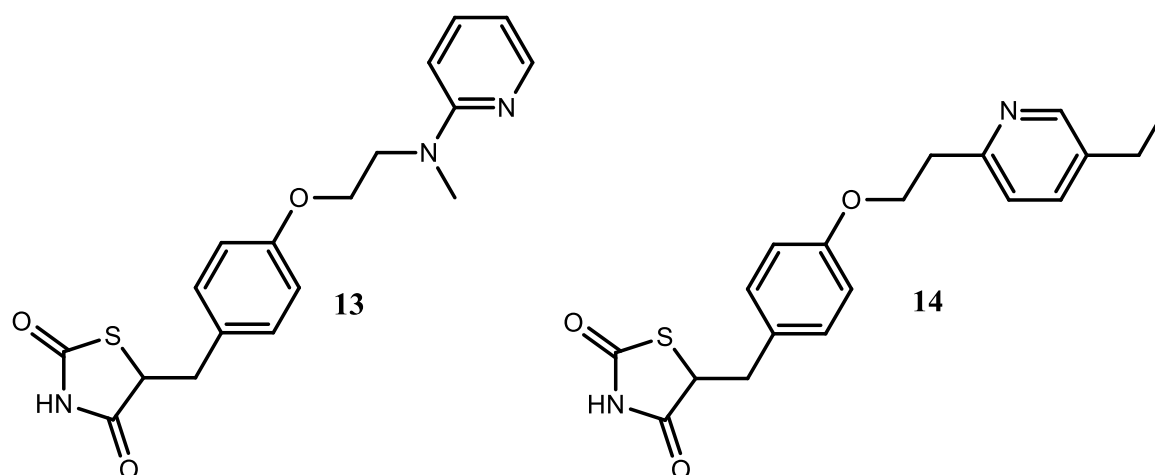


Figure 10: Examples of thiazolidinediones.

1.4. Pharmacological developments in thiazolidinediones

In recent times 2,4-thiazolidinediones have gained more importance as antidiabetic agents and their mechanism of action has been thoroughly investigated. The main representatives of this group are rosiglitazone (**13**) (trade name Avandia), pioglitazone (**14**) (trade name Actos), troglitazone (**15**) (trade name Rezulin) and ciglitazone (**16**), (**Figure 11**) which are found to display significant antidiabetic activity.

Troglitazone (**15**) was the first glitazone to be launched in the market in the early 1990s but was withdrawn in 1997 because of liver toxicity and related deaths associated with the drug. Among these four anti-diabetic drugs rosiglitazone (**13**) displays superior antihyperglycemic activity. The prototypical 2,4-thiazolidinedione, ciglitazone (**16**) (**Figure 11**) was discovered by Takeda Chemical Industries, Ltd., Japan, and has antihyperglycemic activity in insulin-resistant animal models, KKAY mice and wistar fatty rats but no effect in insulin-deficient animal models of diabetes.

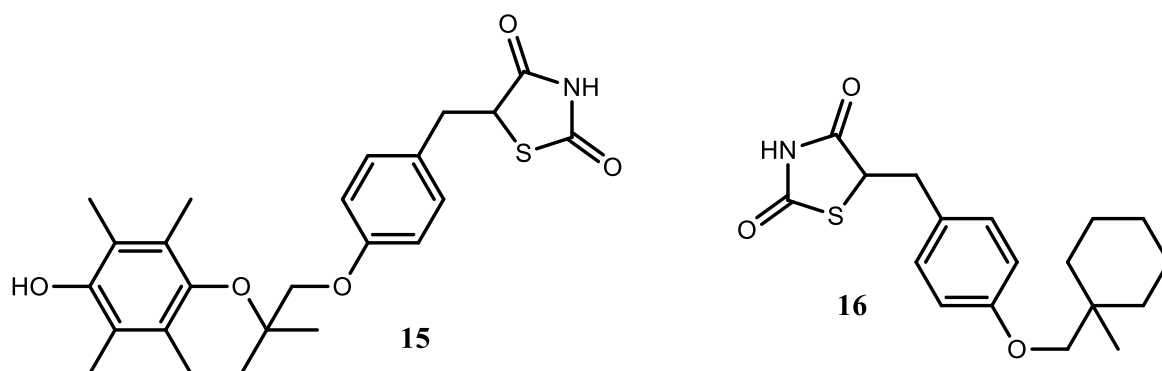


Figure 11: Biologically active compounds with a 2,4-thiazolidinedione moiety

TZDs are one of the significant heterocyclic ring systems that have therapeutic importance when combined with other heterocyclic rings like thiazole, phenyl, triazole, and oxadiazole. For the exploration of novel and highly active therapeutic compounds the combination of two pharmacophores into a single molecule is an interesting, effective and much used direction in modern medicinal chemistry.

Mohammed Iqbal *et al.* synthesized a series of thiazolidinedione derivatives by incorporating pharmacologically significant heterocycles like substituted triazole (**17**), oxadiazole (**18**) and thiazole (**19**) (**Figure 12**) moieties linked to the central phenyl ring via a heteroatom linkage with a one or two carbon spacer as the structural analogues of pioglitazone by employing multistep synthetic protocols.^[73] These synthesized compounds were screened for their *in vivo* hypoglycemic and hypolipidemic activities in male wistar rats and showed interesting insulin sensitizing properties.

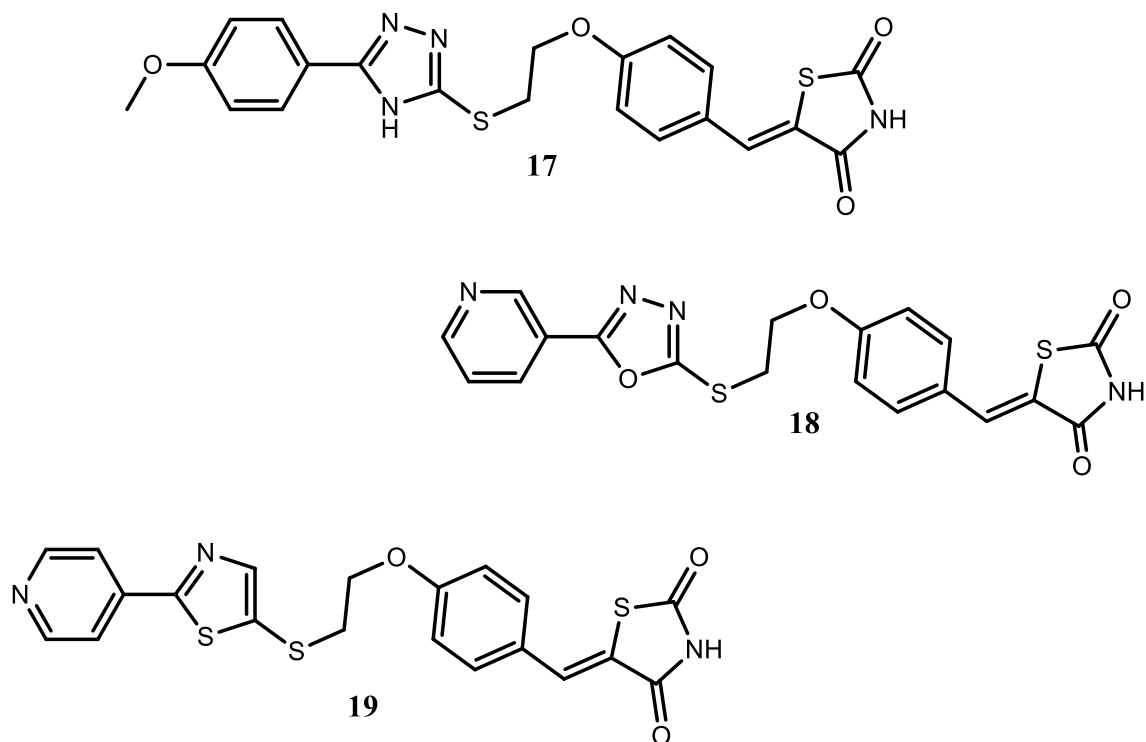


Figure 12: Biologically active compounds against diabetes.

Recently a library of conjugates of chromones and 2,4-thiazolidinedione were synthesized by Nazreen *et al* using a Knoevenagel condensation reaction followed by reduction using hydrogen gas and Pd/C.^[74] Compounds **20** and **21** (**Figure 13**) displayed comparable hypoglycaemic and hypolipidemic efficacy to that of the standard and significantly decreased plasma glucose levels and lowered the triglyceride level, which is preferable for treatment of both hyperglycaemia and cardiovascular complications. In addition, these compounds did not cause any damage to the liver and may be considered as capable candidates for the development of new antidiabetic agents.

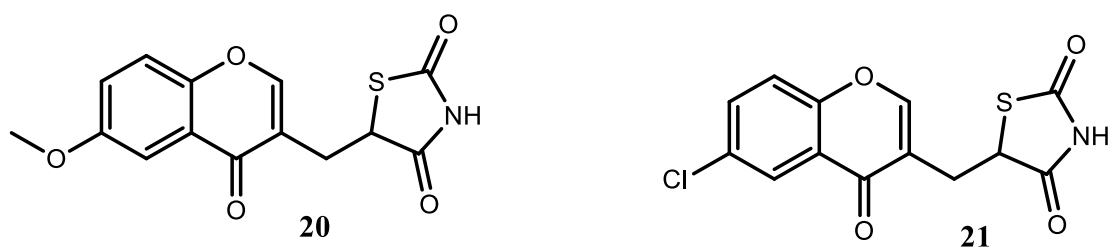


Figure 13: Biologically active compounds against diabetes with less side effects.

In addition to the above, the different pharmacological activities of 2,4-thiazolidinedione derivatives are shown in **Figure 14**.^[75]

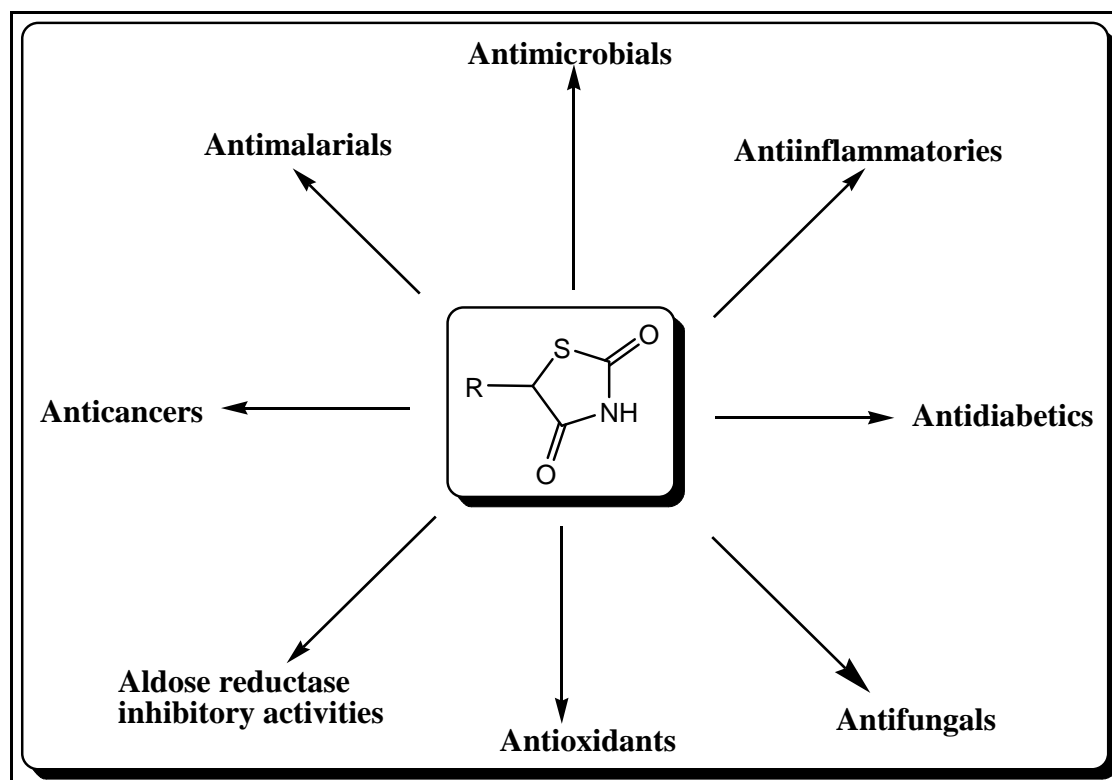


Figure 14: Pharmacological activities of 2,4-thiazolidinediones.

1.4.1. Aldose reductase inhibitory activities:

Bozdag-Dundar *et al.* synthesized a series of chromonyl and flavonyl-2,4-thiazolidinediones using a Knoevenagel condensation reaction and screened them for their ability to inhibit rat kidney aldose reductase (AR) and for their insulinotropic activities in INS-1 cells. Compound **22** was able to increase insulin release in the presence of 5.6 mmol/l glucose. Some of the compounds displayed moderate to high AR inhibitory activity levels. Particularly, compound **23 (Figure 15)** showed the highest AR inhibitory activity (86.57%).^[76]

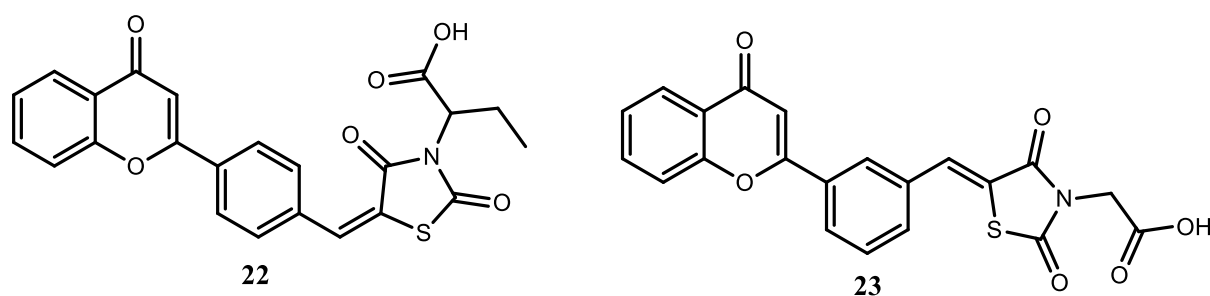


Figure 15: Biologically active compounds against aldose reductase.

1.4.2. Antimicrobial activities:

Thiazolidinediones are known to have anti-bacterial and antiviral^[77] potential. Anti-bacterial activity of these compounds is of particular importance given the dramatic rise of drug-resistant bacteria and the paucity of new agents currently in development. A series of 5-[(3-(aryl)-1-phenyl-1*H*-pyrazol-4-yl) methylene]-3-phenyl thiazolidine-2,4-diones was synthesized by Prakash *et al.* using Knoevenagel condensation of various 3-(aryl)-1-phenyl-1*H*-pyrazole-4-carbaldehydes with 3-phenylthiazolidine-2,4-dione in ethanol in the presence of piperidine as a catalyst. After being screened for their *in vitro* antibacterial (*Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*) and antifungal (*Aspergillus niger* and *Aspergillus flavus*) activity, the biological activities of two compounds **24** and **25** (**Figure 16**) were compared with those of commercially available antibiotics, ciprofloxacin and antifungal agent fluconazole and were found to be most effective against *S. aureus* and *B. subtilis*.^[78]

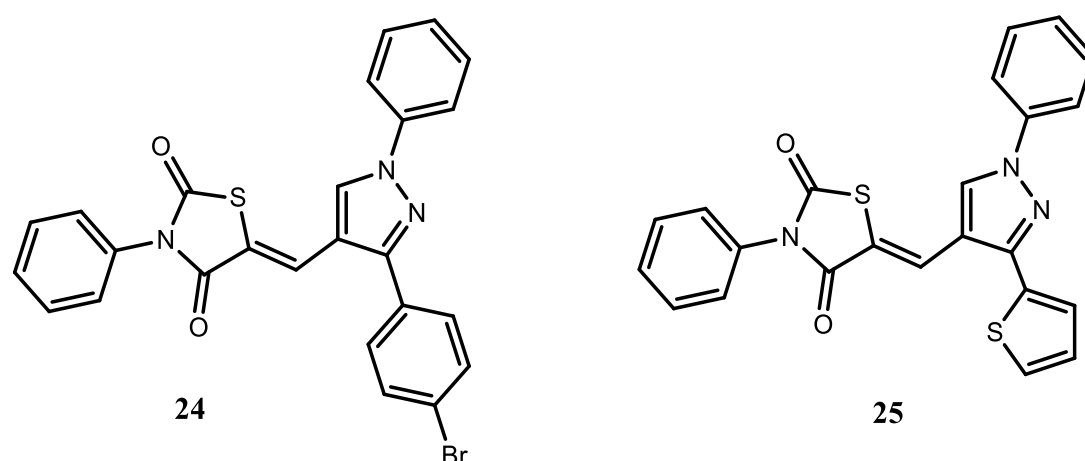


Figure 16: Glitazones with antimicrobial activities.

1.4.3. Anticancer activities:

Cancer is the second leading cause of death after heart disease across the globe which affected 8.2 million lives in the year 2012.^[79] Colorectal cancer is the third leading cause of death in the United States with 50% of patients losing their lives in the year 2010.^[80] Patil *et al.* have explored the anti-cancer activity of the derivatives of 5-benzylidene-2,4-thiazolidinediones synthesized and evaluated for their anti-proliferative activity in a panel of 7 cancer cell lines using four concentrations at 10-fold dilutions. although the compounds showed varying degrees of cytotoxicity in the tested cell lines,^[81] the most marked effect was observed from compound **26**, in MCF-7 (breast cancer), K562 (leukaemia) and GURAV (nasopharyngeal cancer) cell lines with $\log_{10}GI_{50}$ values of -6.7, -6.72 and -6.73, respectively. Also, a series of novel hybrid (*Z*)-5-(acridin-9-ylmethylene)-3-(4-chlorobenzyl) thiazolidine-2,4-diones (compounds **27**, **Figure 17**), were synthesized via *N*-alkylation and Michael reaction. These compounds were evaluated against two human cancer cell lines; breast carcinoma (MCF-7) and non-small cell lung cancer (A549) and showed promising cytotoxic activity.^[82]

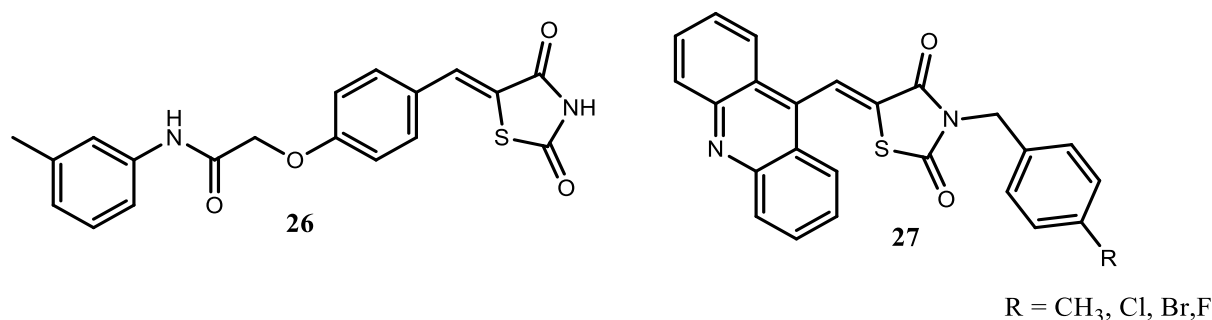


Figure 17: Glitazones with anti-cancer activities

1.4.4. Anti-inflammatory activities:

Garg *et al.* synthesized 5-substituted arylidene-2,4-thiazolidinediones derivatives and evaluated them for their *in vivo* anti-inflammatory and analgesic activities. The 3-Cl derivative, compound **28**,^[83] (**Figure 18**), gave the best anti-inflammatory activities (71.63%) which is comparable to that of indomethacin analgesic and anti-inflammatory activities^[84] (IC_{50} : 7.73 μ g/ml). Pomel *et al.*^[85] synthesized a series of furan-2-ylmethylene thiazolidinediones as selective ATP competitive PI3K α inhibitors. They identified the key pharmacophoric features for potency and selectivity. They found that an acidic NH group on the thiazolidinedione moiety and a hydroxy group on the furan-

2-yl-phenyl part of the molecule play crucial roles in binding to PI3K and contribute to PI3K α selectivity. Compound **29**, (**Figure 18**) was identified as a potent and selective small-molecule PI3K α inhibitor (IC_{50} : 33 ± 10 nM) and its interactions with the key residues at the active site were studied providing insights into its binding mode.

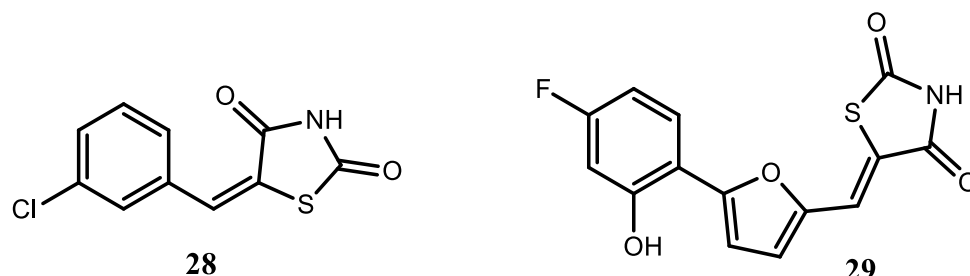


Figure 18: Glitazones with anti-inflammatory activities

1.4.5. Antimalarial activities:

Malaria is a major cause of morbidity and mortality. In light of established and continuing development of resistance of *Plasmodium falciparum* to most antimalarial drugs, the identification and characterization of novel antimalarial chemotypes is an important priority.^[86] Recently in 2015, Sharma *et al.*^[87] have recently synthesized a series of 2,4- thiazolidinediones following a structure-based virtual screening in order to explore structure activity relationships for inhibition of the *P. falciparum* cysteine protease falcipain-2 (FP-2) and of whole cell antiparasitic activity. Most compounds displayed low micromolar antiplasmodial activities against the *P. falciparum* drug resistant W2 strain.^[88]

The most active compounds of the series were tested for *in vitro* microsomal metabolic stability and found to be susceptible to hepatic metabolism.^[89] Molecular docking studies of a frontrunner inhibitor were carried out to determine the probable binding mode of this class of inhibitors in the active site of FP-2. The activities of all synthesized compounds were determined in *in vitro* against FP-2 and the chloroquine resistant W2 strain of *P. falciparum*. It was revealed that the *para* position of the phenyl ring resulted in improved enzyme inhibitory activity for compound **30**, ($IC_{50} = 13.44$ mM) and compound **31** (**Figure 19**), ($IC_{50} = 18.04$ mM).

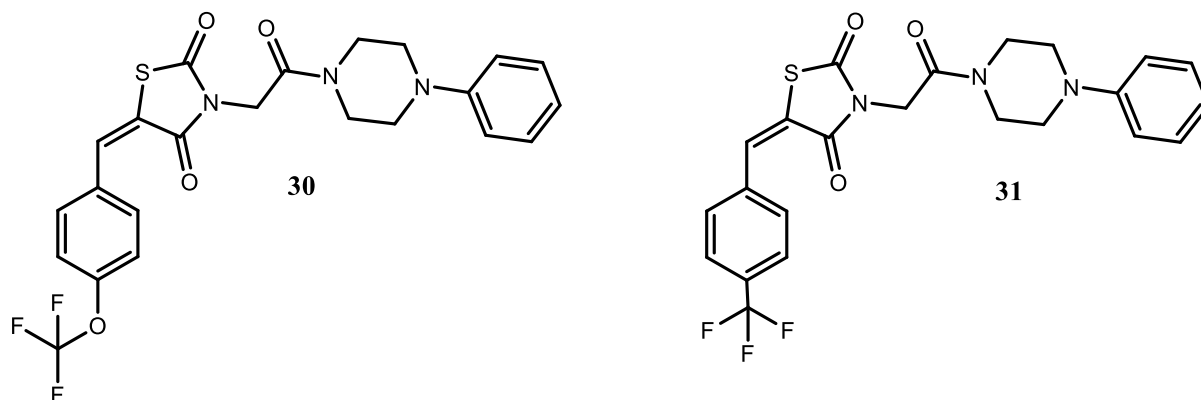


Figure 19: Glitazones with anti-malarial activities

1.4.6. Antifungal activities:

Alagawadi and Alegaon reported the synthesis and *in vitro* antifungal activity of 5-substituted-2,4-thiazolidinedione derivatives. Their synthesized compounds were found to be active against tested fungal strains at 1–64 $\mu\text{g/mL}$ concentration. In addition, compounds **32** and **33** (**Figure 20**) showed good antifungal activity against *C. albicans* at 1–4 $\mu\text{g/mL}$ and *C. neoformans*, *A. flavus*, *A. niger* at 2–8 $\mu\text{g/mL}$ concentrations.^[90] These compounds were also evaluated and showed good *in vitro* activity against *S. aureus* and *E. faecalis* with minimum inhibitory concentration (MIC) values between 4 and 32 $\mu\text{g/mL}$.

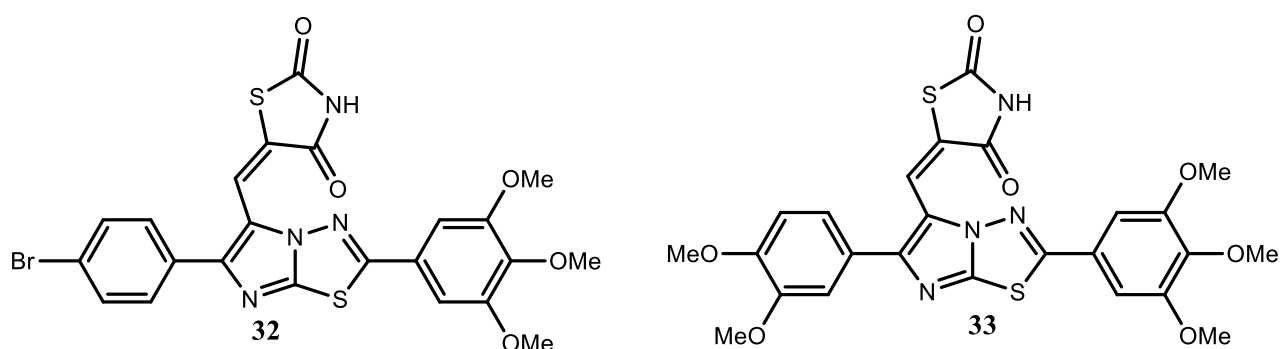


Figure 20: Glitazones with anti-fungal activities

1.4.7. Antioxidative activities:

2,2-Diphenyl-1-picrylhydrazyl (DPPH) is usually used as a substrate to evaluate antioxidant activity. The DPPH assay is based on the measurement of the scavenging ability of antioxidants towards the stable DPPH radical. The method is based on the reduction of purple coloured methanol solution of DPPH in the presence of hydrogen

donating antioxidants and by the formation of the yellow coloured non-radical form of DPPH.^[91] A series of 2,4-dichlorothiazolyl thiazolidine-2,4-diones and 4-chloro-2-benzylsulphonylthiazolyl-thiazolidine-2,4-dione derivatives were tested for their antioxidant properties by determining their effects on superoxide anion formation, and the DPPH stable free radical. Compounds **34** and **35** (**Figure 21**), showed the best superoxide anion scavenging activity at the 10^{-3} M and 10^{-4} M concentrations respectively.^[92]

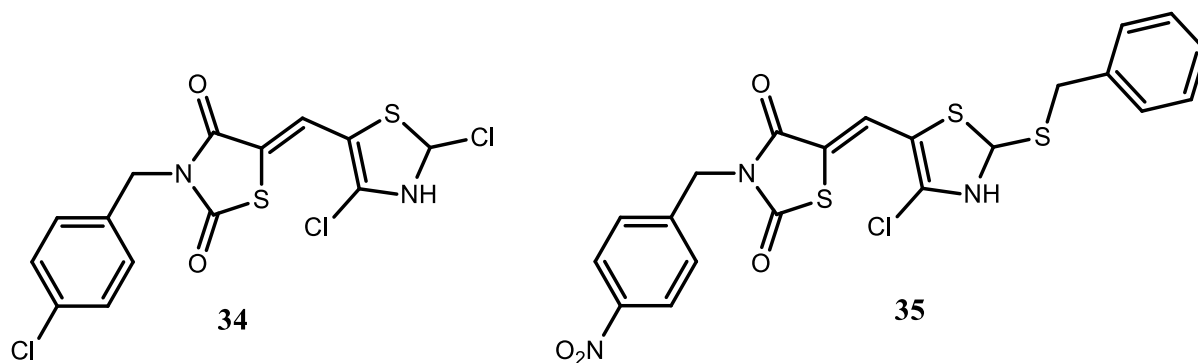


Figure 21: Glitazones with anti-oxidative activities

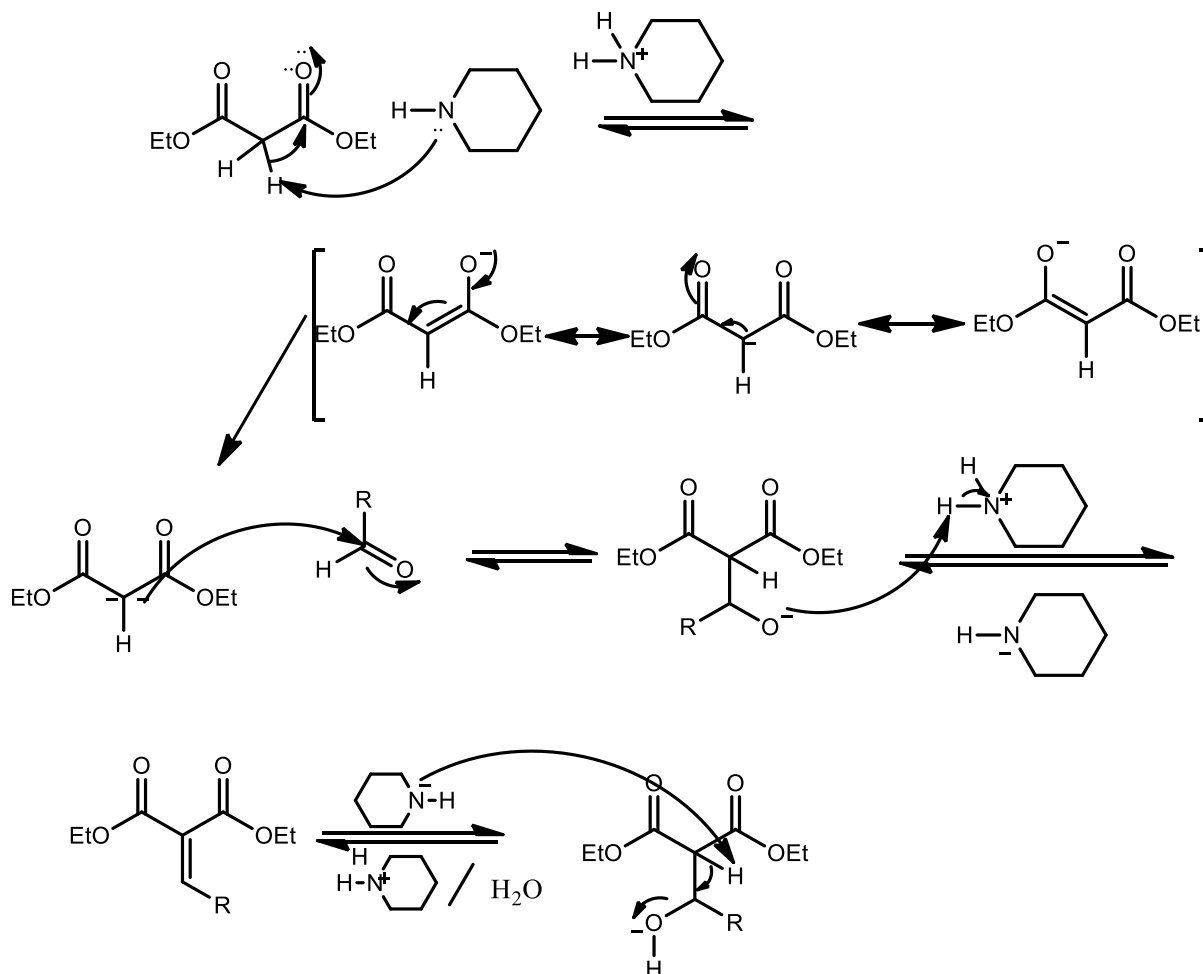
1.5. General synthetic method towards substituted glitazones: Knoevenagel Condensation Reaction

The Knoevenagel condensation reaction^[93] is one of the most useful C=C bond forming reactions in organic synthesis.^[94] The α,β -unsaturated products obtained have been widely used as intermediates in the synthesis of therapeutic drugs,^[95] natural products,^[96] functional polymers,^[97] and fine chemicals.^[98] It is generally performed in organic solvents and catalysed by organic bases such as piperidine or pyridine. Many of these conditions are associated with disadvantages such as hazardous and carcinogenic solvents and unrecoverability of the catalysts, which limit the use of these reactions in industrial processes.^[99]

1.6. General reaction Mechanism of Knoevenagel condensation reaction

In the first step the base piperidine will attack the carbonyl group to form an enol. The enol formed reacts with an aldehyde forming aldol and further undergo subsequent base induced elimination. The intermediate compound formed gets deprotonated by the base to give another enolate while the amine of the intermediate gets protonated.

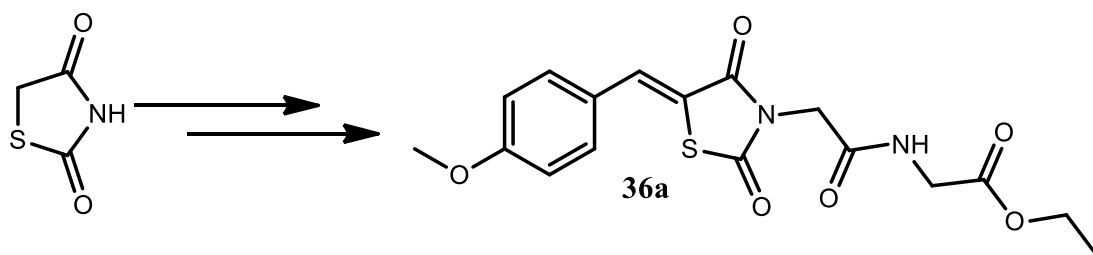
A rearrangement then ensues which releases the amine base, regenerates the catalyst, and yields the final olefin product (**scheme 1**).^[99]



Scheme 1: Knoevenagel condensation reaction mechanism

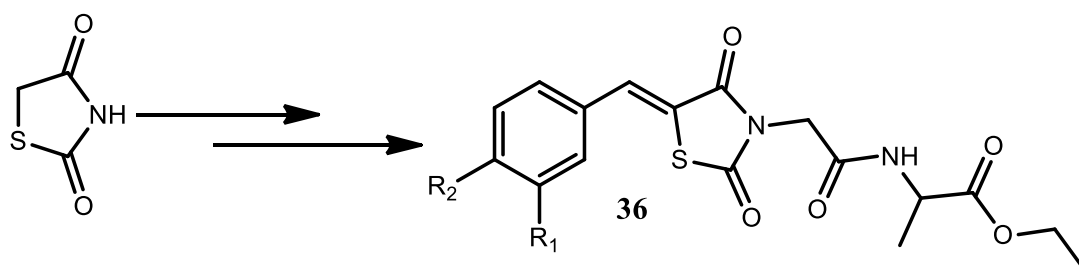
1.7. Origins and aims of this project

Owing to the biological activity of thiazolidinedione-containing compounds as discussed above, synthesis of this type of compounds, particularly as antidiabetic drugs, is always intriguing. What mostly caught our eye was the work done by Kumar and co-workers^[100] where compound **36a** was synthesized over five reaction steps starting from glitazone (**Scheme 1**). This compound was tested against diabetes and showed interesting activity.



Scheme 2: *Synthesized anti-diabetic drug*

Taking a closer look at compound **36a**, we realized that it contains a glycine moiety protected as an ester. This gave us an idea to probe biological activity of compounds containing alanine protected as an ester. Hence the aim of this project was to synthesize analogues of compound **36** by (1) varying the phenyl ring of compound **36** and (2) using the alanine moiety protected as an ester instead of glycine as depicted in **Scheme 2**. These compounds would then be tested against diabetes to probe their biological activity.



Scheme 3: *Envisaged anti-diabetic drugs*

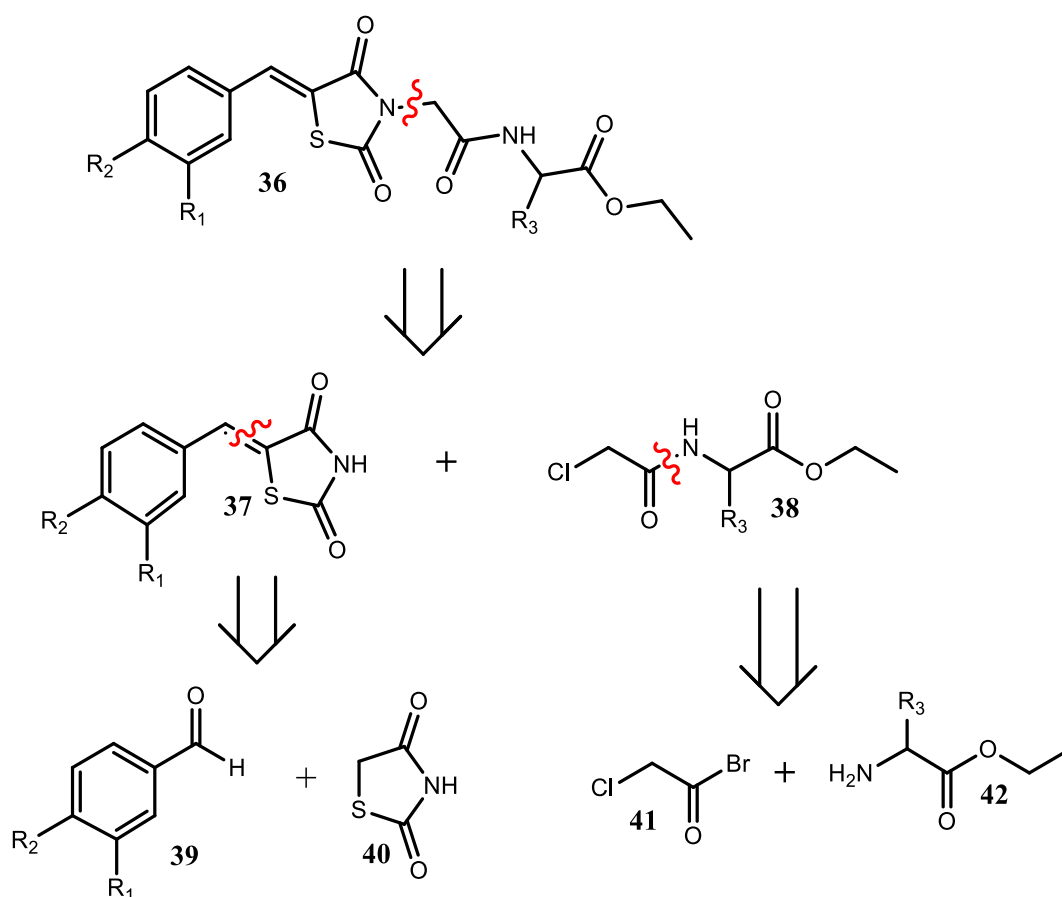
CHAPTER-TWO

2.0. Results and Discussion

This chapter looks at the detailed results and discussion obtained from different characterization techniques used during the study of this project.

2.1. Chemistry

The general synthetic method used by Kumar *et al*^[100] provides the most versatility for preparing the 2,4-thiazolidinediones analogues needed for this study. The analogues were thus prepared following the general retrosynthetic scheme shown in **Scheme 3**.

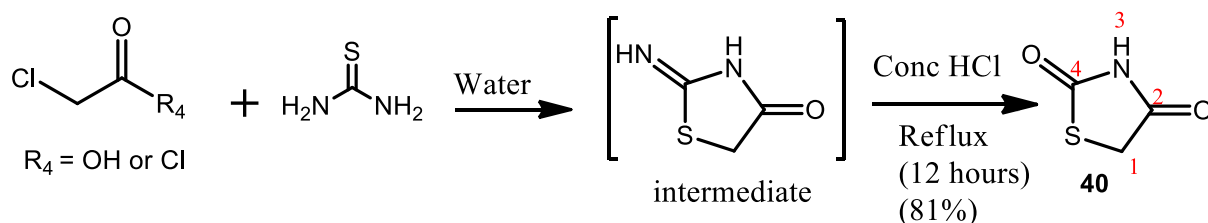


Scheme 4: A retrosynthetic analysis of the target anti-diabetic compounds.

Target compounds **36** could be retrosynthesized to 5-benzylidene thiazolidinediones **37** and ethyl (chloroacetamide) esters **38** by cleavage of the bond between the nitrogen and the carbon atoms as depicted in **Scheme 3**. Compounds **37** would in turn be retrosynthesized to commercially available benzaldehydes **39** and 2,4-thiazolidinedione **40** by the cleavage of double bond between the phenyl ring and the

thiazolidinedione. On the other hand, compounds **38** could be retrosynthesized to commercially available bromo acetyl chloride **41** and ester protected amino acid **42** by cleavage of the amide moiety of compounds **38**. Ester protected amino acids **42** could be retrosynthesized to amino acids alanine and glycine.

2.1.1. Approach towards the synthesis of 2,4-thiazolidinediones



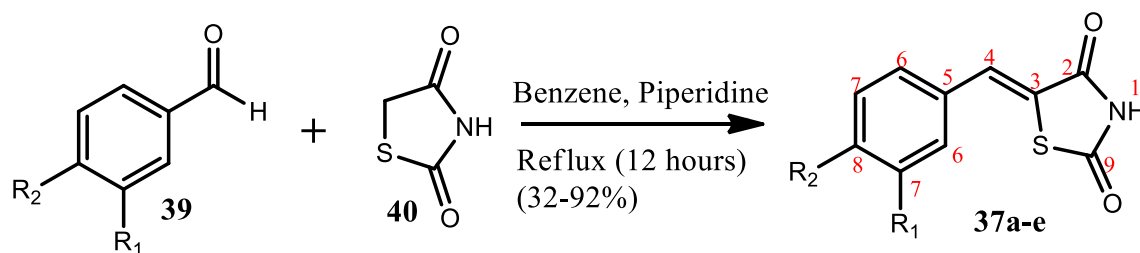
Scheme 5: Synthesis of Glitazone/ thiazolidinedione

Due to the significant expense of 2,4-thiazolidinedione **40**, this study began with its one-step synthesis which was achieved by treating commercially available chloroacetyl chloride or chloroacetic acid with thiourea by employing water as solvent under reflux to form 4-iminothiazolidin-2-one as an intermediate (**Scheme 4**). This was followed by addition of concentrated HCl to give the desired compound **40** in 81% yield which was used without further purification.

The product was confirmed by ^1H NMR, ^{13}C NMR as well as IR spectroscopy. The ^1H NMR spectrum revealed a singlet integrating for 2Hs at 4.14 ppm, confirming the presence of methylene protons of compound **40**. Also, there was a broad singlet integrating for 1H at 12.03 ppm confirming the N-H proton. There were 3 signals in the ^{13}C NMR spectrum of compound **40**, as expected. The spectrum was characterized by a CH_2 peak at 36.26 ppm and two carbonyl peaks observed at 173.59 and 174.34 ppm.

The IR spectrum of compound **40** was characterized by a peak at 3123 cm^{-1} confirming the presence of an N-H. Also, two carbonyl peaks were observed at 1615 and 1645 cm^{-1} . The melting point of the product was found to be $123.7\text{-}125.5\text{ }^\circ\text{C}$ which is consistent with the reported literature value of $125\text{-}126\text{ }^\circ\text{C}$ ^[112] confirming the formation of product **40**.

2.1.2. Knoevenagel condensation reaction



Scheme 6: Knoevenagel Condensation Reaction

The next step of our synthesis involved condensation of selected commercially available substituted benzaldehydes (vanillin, anisaldehyde, benzaldehyde, nitro benzaldehyde and piperonal) and the thiazolidinedione (**40**). These Knoevenagel condensation reactions were done by employing benzene as a solvent using a catalytic amount of piperidine to afford the corresponding 5-substituted benzylidene-2,4-thiazolidinediones^[101] (**37a-e**) as depicted in **Scheme 5**. Compounds **37a-e** were obtained in unoptimized yields ranging from poor to excellent (due to the recrystallization of some compounds using water which led to loss of mass), with melting points ranging from 224-247 °C (**Table 5**).

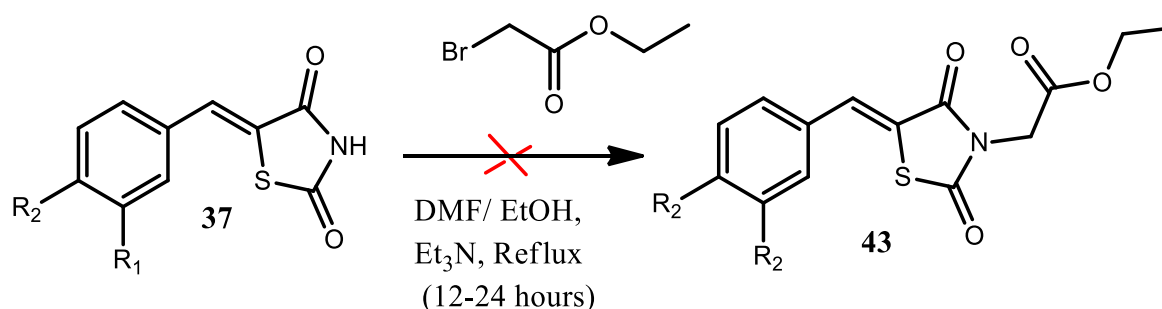
Table 5: Percentage yields and melting points of 5-substituted benzylidene-2,4-thiazolidinediones (37)

Compound	R ₁	R ₂	% Yield	Melting Point (°C)	Literature melting Point (°C)
37a	H	H	38	230.3-236.4	240 ^[113]
37b	H	MeO	36	247.2-249.9	215-217 ^[114]
37c	H	NO ₂	87	246.7-248.7	235-238 ^[115]
37d	OH	MeO	92	224.8-225.7	194-196 ^[116]
37e	OCH ₂ O		32	244.7-245.9	246-247 ^[117]

The ^1H NMR spectra of products **37a-e** were characterized by the absence of an aldehydic proton peak at ~ 9 ppm, confirming the consumption of benzaldehydes. Importantly all the spectra indicated the appearance of a new methine proton (**H-4**) appearing as a singlet in the aromatic region, confirming that the condensation reaction had taken place. Also, all ^1H NMR spectra of our products confirmed the presence of the N-H proton, which was observed at 11-12 ppm and appeared as a broad singlet accounting for one proton.

The ^{13}C NMR spectra of our compounds were characterized by the absence of an aldehydic carbon peak at ~ 190 ppm confirming the consumption of benzaldehydes. Moreover, the spectra of the products indicated an extra C-H peak (**C-4**) in the aromatic region confirming the condensation products **37**. The IR spectra of compounds **37a-e** showed N-H stretches peaks at $3100\text{-}3390\text{ cm}^{-1}$, aromatic C=C stretch at $2700\text{-}2900\text{ cm}^{-1}$, and C=O stretch at $1600\text{-}1760\text{ cm}^{-1}$.

2.1.3. Attempted synthesis of ethyl-2-(5-substituted benzylidene)-2,4-dioxothiazolidin-3-yl) acetate

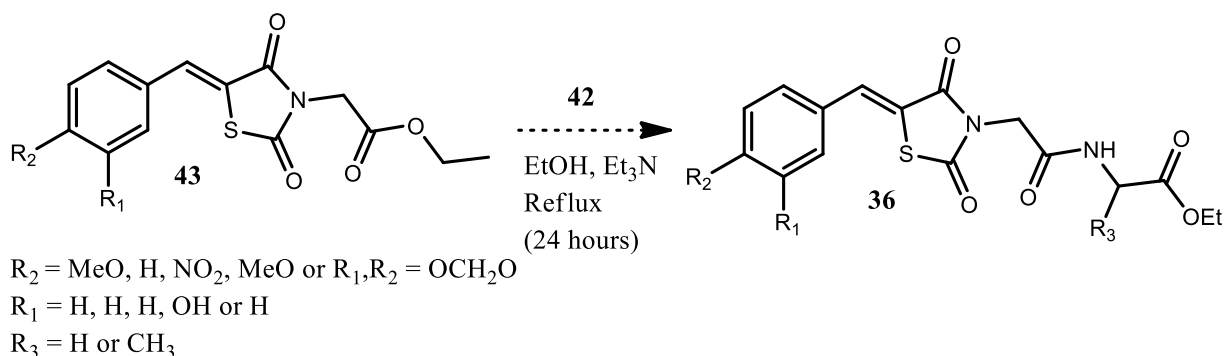


R₁	H	H	H	OH	H
R₂	H	Meo	NO ₂	MeO	OCH ₂ O

Scheme 7: Attempted synthesis of ethyl-2-(5-substituted benzylidene)-2,4-dioxothiazolidin-3-yl) acetate

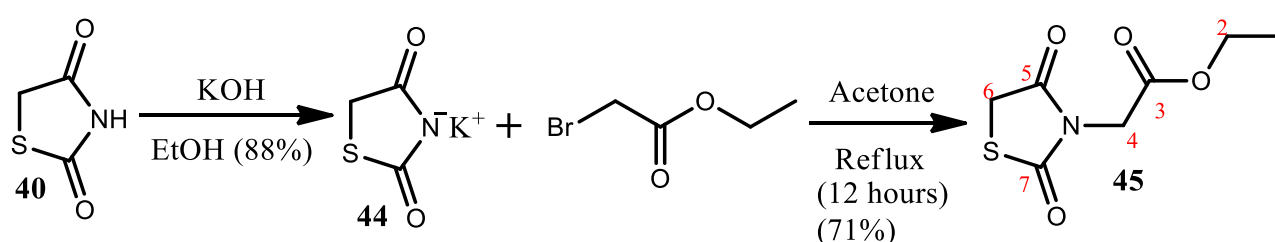
After preparing compounds **37**, the next step was to add ethyl bromoacetate in a nucleophilic substitution reaction to afford the first set of these [dioxothiazolidine] compounds (**43**). The reaction was carried out in the presence of ethanol or DMF as a solvent and a base (Et_3N) and refluxed for 12-24 hours monitored by TLC. (**Scheme 6**)

Surprisingly, the reaction was not successful. The failure of this reaction was confirmed by TLC which revealed that both compounds **37** and ethyl bromo acetate were not consumed even after 24 hours of reaction time. The plan here was to subsequently react the desired product **43** with protected amino acids (**42**) in ethanol to afford compounds **36** according to **Scheme 7**



Scheme 8: *Synthesis of envisaged anti-diabetic drugs route*

2.1.4. Synthesis of ethyl (2,4-dioxo-1,3-thiazolidin-3-yl) acetate



Scheme 9: Synthesis of *ethyl (2,4-dioxo-1,3-thiazolidin-3-yl) acetate*

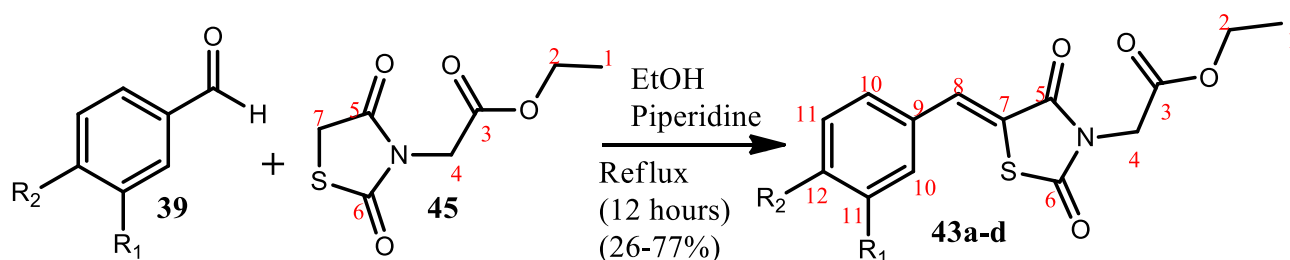
Resultantly an alternative method was sought and included the treatment of glitazones (**40**) with potassium hydroxide as strong base in ethanol to give the potassium salt of glitazone **44**. Compound **44** was subjected to *N*-alkylation with ethyl bromoacetate in acetone to give the desired product (**45**) as a colourless oil in a yield of 71 % [102, 103] (**Scheme 8**).

Compound **44** was characterized by NMR and IR spectroscopy. The ^1H NMR spectrum showed the absence of N-H peak at 12.03 ppm. Furthermore, the IR spectrum showed absence of a characteristic N-H stretch at 3100 cm^{-1} confirming that the proton was indeed abstracted. The melting point of compound **44** was found to be $260.0\text{-}266.6\text{ }^\circ\text{C}$ which is consistent with a literature value of $247\text{-}250\text{ }^\circ\text{C}$ [118] confirming that product **44** was formed.

Product **45** was also characterized by NMR and IR spectroscopy. In addition to the singlet methylene CH₂ peak integrating for 2H (**H-6**) confirmed for compound **44**, the ¹H NMR spectrum of compound **45** was characterized by the presence of two extra methylene protons (**H-2**) appearing as a quartet and **H-4** appearing as singlet at 4.17 ppm and 4.01 ppm respectively. These methylene protons for the starting material ethylbromoacetate were reported at 4.19 ppm and 3.87 ppm,^[104] respectively, confirming the consumption of this starting material.

The ¹³C NMR spectrum showed four extra aliphatic signals (C-1, C-2, C-4, and C-6) and the carbonyl carbon signals (C-3, C-5, and C-7). In addition to the IR spectrum of **44**, the IR spectroscopic analysis of compound **45** showed four signals which included a C-H stretch at 2971 cm⁻¹, carbonyl stretches at 1778 cm⁻¹ assigned for (C-5), 1775 cm⁻¹ (C-6) and 1609 cm⁻¹ for (C-3) together with a C-O stretch at 1597 cm⁻¹.

2.1.5. Synthesis of ethyl 2-(5-(substituted benzylidene)-2,4-dioxothiazolidin-3-yl) acetates.



Scheme 10: Synthesis of ethyl 2-(5-(substituted benzylidene)-2,4-dioxothiazolidin-3-yl) acetates

The next step of the synthesis involved reacting selected substituted benzaldehydes with ethyl (2,4-dioxo-1,3-thiazolidin-3-yl) acetate (**45**) to afford the benzylidenes (**43**) (**Scheme 9**). These reactions were done by the application of the Knoevenagel condensation reaction with catalytic amounts of piperidine in ethanol as a solvent.^[105] The solid products were obtained in low to fairly good yields and their melting points are shown in **Table 5**.

Bhat *et al.*^[113] had previously synthesized and studied the biological activity of compound **43a** against diabetes. Their results indicated that compound **43a** was been active in different pharmacological activities, especially promising was an increase in the glucose activity in the absence of insulin. Even though compound **43a** was not part of the retrosynthetic **Scheme 3**, we decided to synthesize it together with its three derivatives (**43b-d**) due to its variety of pharmacological activity, especially the anti-diabetic activity.

Table 5: Percentage yields and melting points of ethyl-2-(5-substituted benzylidene)-2,4- dioxothiazolidin-3-yl acetates

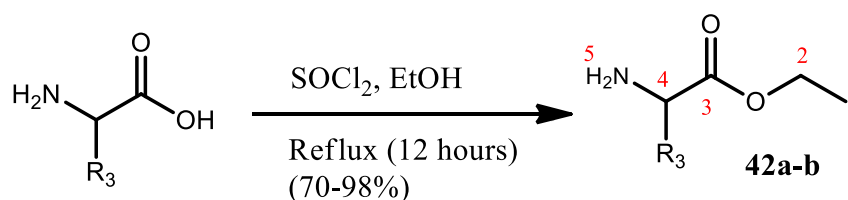
Compound	R ₂	R ₁	% Yield	Melting point (°C)	Literature Melting point (°C)
43a	MeO	H	77	134.5-136.6	129-131 ^[120]
43b	MeO	OH	24	139.9-141.5	Novel compound
43c	H	H	88	89.9-91.7	75-77 ^[120]
43d	OCH ₂ O		26	131.5-133.4	Novel compound

¹H NMR spectra of compounds **43a-d** were characterized by the absence of the characteristic aldehydic proton peak at ~ 9 ppm confirming that benzaldehydes were consumed in the reaction. Also, the spectra were characterized by the absence of a singlet methylene protons (**H-7**) from the starting material **45**, confirming that the reaction had taken place. ¹H NMR spectra of compounds **43a-d** also indicated the appearance of a new methine peak (**H-8**) integrating for 1H appearing as a singlet at ~ 7.90-8.85 ppm confirming that the condensation reaction had indeed taken place. Furthermore, ¹H NMR spectra of compounds **43a-d** showed a characteristic singlet methylene proton integrating for 2H at ~ 4.5 ppm which represent **H-4**, a quartet integrating for 2s at ~ 4.2 ppm (**H-2**) and a triplet integrating for 3Hs at ~1 ppm (**H-1**) integrating for 3H.

The ^{13}C NMR spectra of compounds **43a-d** were characterized by the absence of a signal indicating the characteristic aldehydic carbon peak at ~ 190 ppm. However, there was an appearance of a new benzylic carbon (**C-8**) peak at ~ 132 ppm confirming the formation of the condensation product, the appearance of two carbonyl carbon peaks at 168-169 ppm and lastly an appearance of a new quaternary carbon peak (**C-7**) at ~ 129 ppm also confirming the formation of the double bond. Furthermore, ^{13}C NMR spectra were characterized by three aliphatic carbon signals which are denoted as C-1, C-2 and C-4.

The IR spectra of compounds **43a-d** showed the absence of an N-H peak at ~ 3123 cm^{-1} confirming the attachment of ethyl bromoacetate to the product from the N-alkylation reaction with glitazone and a peak at ~ 2820 cm^{-1} for a C-H stretch was also observed. Furthermore, two carbonyl peaks at 1615-1645 cm^{-1} and the C-O stretch at 1100-1190 cm^{-1} were observed in the spectra of compounds **43a-d**. The melting points of compounds **43a** and **43c** recorded were found to be in agreement with the literature values as indicated in **Table 5**. Compounds **43b** and **43d** were found to be novel since they have not yet been reported in the literature. **HRMS** (ES-TOF) of all compounds gave further evidence for the successful synthesis of these compounds as they were consistent with the expected calculated values.

2.1.6. Synthesis of ester protected amino acids



Scheme 11: Synthesis of ester protected amino acids

Ester protected amino acids **42a-42b** were achieved by reacting amino acids glycine and alanine with thionyl chloride in refluxing ethanol (**Scheme 10**). Compound **42a** was obtained as a white solid in 98 % yield whereas compound **42b** was obtained as a colourless oil in 78 % yield as depicted in **Table 6**. The choice of the amino acids was motivated by the fact that in the retrosynthesis (**Scheme 3**) our desired compound **36** would be synthesized by using glycine moiety as linker.

We thought it would be interesting to synthesize its novel derivatives by using alanine instead of glycine as a linker.

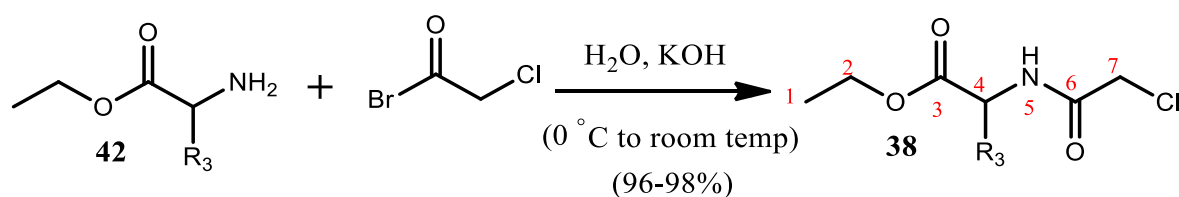
Table 6: Percentage yields and melting points of ester protected amino acids compounds

Product	R ₃	% yield	Melting point (°C)	Literature Melting point (°C)
42a	H	98	88-90	91-95 ^[121]
42b	CH ₃	70	-	-

Compounds **42a-b** were confirmed using ¹H NMR, ¹³C NMR and IR spectroscopy. ¹H NMR spectra of compounds **42a-b** showed a new signal at of 3.56 ppm for compound **42a** and 3.74 ppm for compound **42b** indicating the presence of the methylene protons (**H-2**) appearing as a quartet and another new signal at 1.06 ppm for compound **42a** and 1.05 ppm for compound **42b**, indicating methyl protons (**H-1**) appearing as a triplet and confirming the successful attachment of the ethoxy group to the amino acids.

The ¹³C NMR spectra of both compounds **42a** and **42b** were characterized by two extra peaks at 13 ppm and 41 ppm which are indicative of the methyl carbon (**C-1**) and the methylene carbon (**C-2**) signals respectively. In addition, in the ¹³C NMR spectrum of compound **42b**, another methyl carbon peak was observed at 16.17 ppm confirming the presence of the alanine moiety. The IR spectra of the two compounds were characterized by the presence of an N-H peak at ~ 3381 cm⁻¹ with a carbonyl peak at 1720 cm⁻¹ for compound **42a** and 3385 cm⁻¹ with a carbonyl peak at 1741 cm⁻¹ for compound **42b**.

2.1.7. Synthesis of ethyl 2-(2-chloroacetamide) esters



Scheme 12: Synthesis of *ethyl 2-(2-chloroacetamide) esters*

Following the successful protection of glycine and alanine as esters, the next step involved nucleophilic substitution of the resultant compounds **42** with bromoacetyl chloride in water as depicted in **Scheme 11**. The reactions were done at zero degrees Celsius and allowed to warm to room temperature. The compounds were obtained in excellent yields (**Table 7**).

Table 7: Percentage yields and melting points of ethyl 2-(2-chloroacetamide) esters

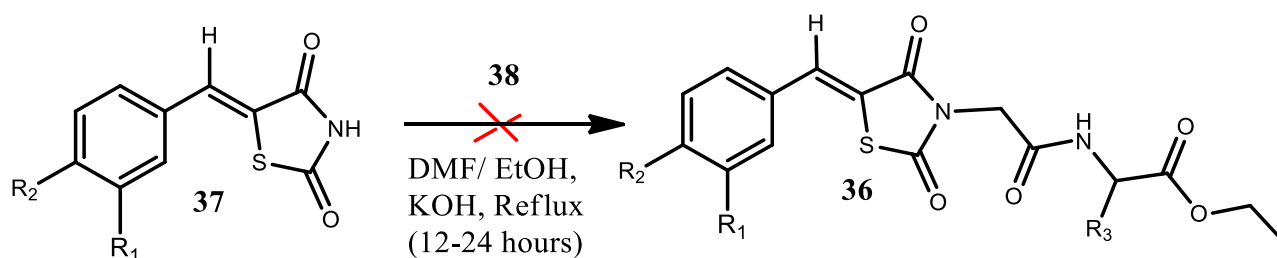
Product	R ₃	% Yield	Melting point (°C)	Literature melting point (°C)
38a	H	96	75.8-76.5	77.5 ^[123]
38b	CH ₃	98	82.2-83.6	71-74 ^[100]

¹H NMR spectra of both compound **38a** and **38b** were characterized by a singlet methylene proton peak (**H-7**, **Scheme 11**) at 3.93 ppm for compound **38a** and at 3.89 ppm for compound **38b**. This methylene proton peak is observed at 4.37 ppm^[106] for unreacted bromo acetyl chloride. Furthermore, both the ¹H NMR spectra revealed a quartet proton integrating for 2H (**H-2**) at 4.26 ppm for compound **38a** and at 4.22 ppm for compound **38b** and a triplet integrating for 3H (**H-1**) at 1.31 ppm for compound **38a** and 1.29 ppm for compound **38b**. In addition, a doublet integrating for 2Hs (**H-4**) was observed at 4.10 ppm for compound **38a** and a quintet at 4.56 ppm (**H-4**) for compound **38b** was also observed. Furthermore, the ¹H NMR spectrum of compound **38b** indicated a doublet methyl peak at 1.45 ppm. Finally, a broad triplet peak (**H-5**) integrating for 1H assigned to the N-H proton at 7.15 ppm for compound **38a** and at 7.19 ppm for compound **38b** appearing as a doublet due to coupling with **H-4** was observed.

The ¹³C NMR spectrum of compound **38a** was characterized by a total of 7 peaks whereas the spectrum of compound **38b** had 8 peaks in total. Prominently the two spectra confirmed the appearance of an extra methylene carbon peak at 28.57 ppm (**C-7**) for compound **38a** and at 28.72 ppm for compound **38b**. This carbon peak is observed at 34.11 ppm^[107] for the unreacted bromoacetyl chloride. The IR spectra of the two compounds were characterized by the presence of an N-H peak at a 3381 cm⁻¹ for compound **38a** and at 3385 cm⁻¹ for compound **38b**, respectively, with carbonyl

peaks at 1720 cm^{-1} for compound **38a** and 1741 cm^{-1} for compound **38b** confirming the presence of the ethoxy groups on the amino acids and attachment of the bromoacetyl chloride moiety. Melting points obtained were found to be in agreement with the literature values (**Table 7**)

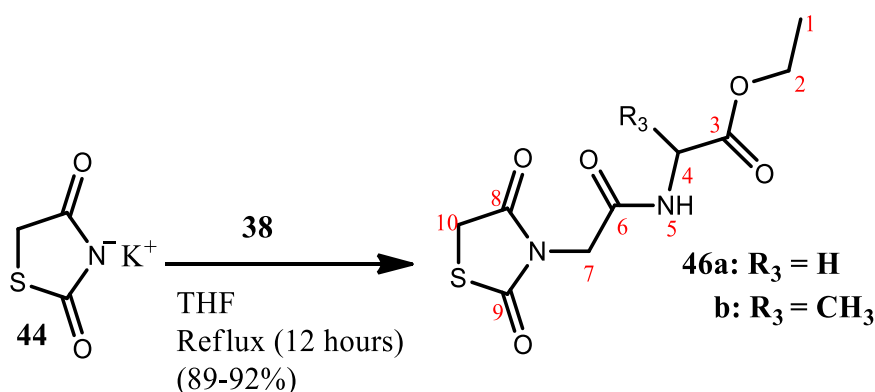
2.1.8. Attempted synthesis of analogues of anti-diabetic drugs



Scheme 13: Attempted synthesis of anti-diabetic drugs

As it is clear from the general retrosynthetic scheme (**Scheme 3**), the next step was to synthesize compounds **36** using *N*-alkylation of compounds **37** by compounds **38** employing either DMF or ethanol as a solvent in the presence of a base (KOH) (**Scheme 12**). The reactions were refluxed for 12-24 hours while being monitored by TLC. Unfortunately, the reactions did not take place. The failure of the reactions was confirmed by a combination of ^1H NMR and TLC which revealed that both compounds **37** and **38** were not consumed. As a result, other alternative methods were sought.

2.1.9. Synthesis of ethyl 2-(2-(2,4-dioxothiazolidin-3-yl) acetamido) esters



Scheme 14: Synthesis of ethyl 2-(2-(2,4-dioxothiazolidin-3-yl) acetamides esters

Resultantly, an alternative method was sought, and it included the reaction of the potassium salt of glitazone **44** with subsequent nucleophilic substitution with alkylation of compounds **38** in an effort to afford the desired ethyl 2-(2-(2,4-dioxothiazolidin-3-yl)

acetamides esters **46** by employing THF as the solvent (**Scheme 13**). Envisaged compounds were obtained in excellent yields (**Table 8**)

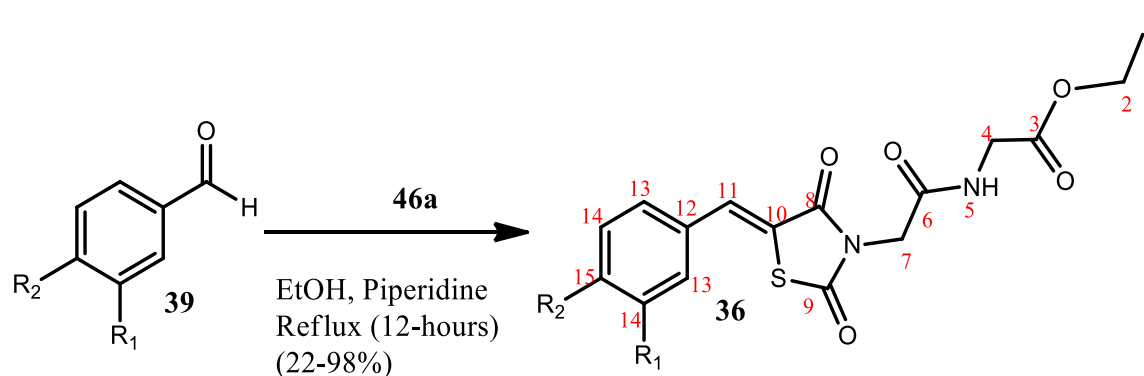
Table 8: Percentage yields and melting points of ethyl 2-(2-chloro acetamide) esters

Product	R ₃	% yield	Melting point (°C)	Literature melting point (°C)
46a	H	89	110-112.8	Novel compounds
46b	CH ₃	92	100.6-101.9	

The ¹H NMR spectrum of compound **46a** exhibited 6 signals whereas that of compound **46b** exhibited 7 signals. Prominently among these signals was the methylene proton (**H-7**) peak which appeared as a singlet at 4.15 ppm in both the spectra of compound **46a** and **46b**. These peaks were observed at 3.93 ppm and 3.89 ppm for the starting materials **38a** and **38b** respectively.

The ¹³C NMR spectrum of compound **46a** showed 6 new signals whereas that of compound **46b** exhibited 7 new signals. These signals were denoted as C-1, C-2, C-3, C-4, C-6 and C-7 (**Scheme 13**) in addition to C-8, C-9 and C-10 from the glitazone. The IR spectra of both compounds **46a** and **46b** were characterized by the presence of an N-H peak at 3294 cm⁻¹ for compound **46a** and 3288 cm⁻¹ for compound **46b**, C-O stretch at 1100 for compound **46a** and 1250 cm⁻¹ for compound **46b** and four carbonyl stretches (C=O) at 1600-1750 cm⁻¹ for compound **46a** and 1610-1790 cm⁻¹ for compound **46b**.

2.1.10. Synthesis of analogues of anti-diabetic drugs having glycine moiety as a linker (36a-e)



Scheme 15: Synthesis of anti-diabetic drugs containing glycine moiety

In the final step of the synthesis, compound **46a** was subjected to the Knoevenagel condensation reaction with five various benzaldehydes to get compounds **36a-e**. The reactions were performed under conventional methods using ethanol as the solvent and catalytic amounts of piperidine (**Scheme 14**)

Table 9: Percentage yields and melting points of anti-diabetic drugs having glycine as a linker

Product	R ₂	R ₁	% Yield	Melting point (°C)	Literature melting point (°C)
36a	MeO	H	50	204.9-208.8	217 ^[100]
36b	H	H	22	185.7-187.2	Novel compounds
36c	MeO	OH	97	233.4-234.8	
36d	NO ₂	H	34	208.9-211.3	
36e	OCH ₂ O		98	211.6-212.9	

Compounds **36a-e** were successfully synthesized with unoptimized poor to excellent yield (**Table 9**). Structures of synthesized compounds were confirmed by IR, ¹H NMR and ¹³C NMR spectroscopy as well as mass spectrometric analysis.

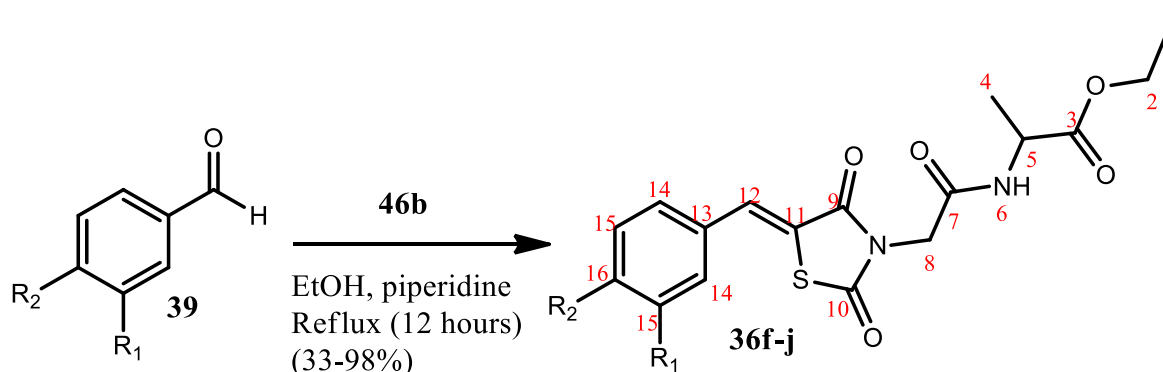
^1H NMR spectra of compounds **36a-e** were characterized by the absence of the aldehydic proton peak at ~ 9 ppm confirming that benzaldehydes had been consumed. Furthermore, the spectra were characterized by the presence of a downfield N-H proton at ~ 8.8 ppm (**H-5**, **Scheme 14**) appearing as a triplet arising due to coupling to the adjacent methylene protons (**H-4**). In addition, ^1H NMR spectra of all our compounds indicated the appearance of a new methine proton (**H-11**) as a singlet at ~ 7.9 ppm confirming the formation of a condensation product.

The heteroatomic region of compounds **36a-e** were characterized by three methylene protons at ~ 4.4 ppm (**H-7**, appearing as singlets), ~ 4.2 ppm (**H-2**, appearing as quartets) and at ~ 3.9 ppm (**H-4**, appearing as doublets). Spectra of compounds **36a** and **36c** were further characterized by methoxy protons (OCH_3) appearing as a singlet at 3.89 and 3.82 ppm, respectively. The aliphatic region of the spectra was characterized by methyl protons (**H-1**) appearing at ~ 1.2 ppm.

^{13}C NMR spectra of compound **36a-e** were characterized by the absence of the aldehydic carbon peak at ~ 190 ppm confirming consumption of benzaldehydes during the reactions according to **Scheme 14**. Spectra were further characterized by appearance of an extra methine carbon (**C-11**) signal in the aromatic region at ~ 134 ppm confirming that the condensation reaction had taken place. Also, all ^{13}C NMR spectra indicated four carbonyl carbon peaks at ~ 170 ppm, ~ 167 ppm, ~ 166 ppm and ~ 165 ppm indicating **C-9**, **C-8**, **C-6** and **C-3** respectively (**Scheme 14**). Furthermore, spectra were characterized by three methylene carbons peaks at ~ 61 ppm (**C-2**), ~ 44 ppm (**C-7**) and at ~ 41 ppm (**C-4**) (**Scheme 14**). The Spectrum of compound **36d** showed another methylene carbon peak at 102.56 ppm confirming the presence of the dioxolane group in the benzaldehyde. All the spectra were characterized by a methyl carbon signal at ~ 14 ppm representing **C-1** (**Scheme 14**). Furthermore, spectra of compounds **36a** and **36c** showed extra methyl carbon peaks at 55.99 ppm and 56.09 ppm, respectively, confirming the presence of methoxy carbon atoms.

The IR spectra of all the final compounds **36a-e** showed characteristic peaks for N-H stretching in the range of $3000\text{-}3400\text{ cm}^{-1}$ and C=O stretching in the range of $1685\text{-}1740\text{ cm}^{-1}$. The melting point of the one known compound, **36a**, was recorded to be $204.9\text{-}208.8\text{ }^\circ\text{C}$ which is in accordance with the literature value of $217\text{ }^\circ\text{C}$.

2.1.11. Synthesis of novel analogues of anti-diabetic drugs having alanine moiety as a linker (36f-j)



Scheme 16: Synthesis of anti-diabetic drugs containing alanine moiety

In the successful synthesis of these novel analogues of anti-diabetic drugs, alanine was previously acylated with an acyl carbon linker to form ethyl 2-(2-(5-(4-substituted benzylidene)-2,4-dioxothiazolidin-3-yl) acetamido) propanoate (**46b**). In the final step compound **46b** was subjected to Knoevenagel condensation reaction under conventional method with different benzaldehydes. The reactions were heated under reflux for 12 hours using ethanol as solvent and a catalytic amount of piperidine (**Scheme 15**). Novel compounds **36f-j** were obtained as solids in unoptimized poor to excellent yields (**Table 10**)

Table 10: Characterization techniques of anti-diabetic drugs containing alanine as a linker

Product	R ₂	R ₁	% Yield	Melting point (°C)	Literature melting point (°C)
36f	MeO	H	98	208.9-209.6	Novel compounds
36g	H	H	50	166.8-167.4	
36h	MeO	OH	61	183.8-184.9	
36i	OCH ₂ O		33	173.8-174.9	
36j	NO ₂	H	90	223.9-224.7	

All compounds were characterized by using ^1H NMR, ^{13}C NMR, and IR spectroscopy and mass spectrometric analysis. ^1H NMR spectra of compounds **36f-j** showed the absence of aldehydic protons at ~ 9 ppm confirming the consumption of benzaldehydes. The spectra were further characterized by the presence of an N-H peak (**H-6**) integrating for 1H appearing as a doublet at ~ 8.8 ppm due to coupling with **H-5**. Importantly all the spectra indicated the appearance of a new methine proton (**H-12**) appearing as a singlet at ~ 7.4 ppm confirming the formation of a condensation product.

The heteroatomic region of compounds **36f-j** were characterized by two methylene protons at ~ 4.08 ppm (**H-2**, appearing as quartet) and 4.27 ppm (**H-8**, appearing as a singlet) as well as a methine proton **H-5** appearing as a quintet. The aliphatic regions of the spectra of compounds **36f-j** (**Scheme 15**) were characterized by two sets of methyl protons at ~ 1.19 ppm (**H-1**, appearing as triplet) and at ~ 1.30 ppm (**H-4**, appearing as doublet).

Lending further credence to the successful preparation of these compounds was that ^{13}C NMR spectra of compound **36f-j** were characterized by the absence of an aldehydic carbon peak at ~ 190 ppm confirming the consumption of benzaldehydes during reactions according to **Scheme 15**. Spectra were also characterized by a presence of the presence of new extra methine carbon atom signal at ~ 134 ppm assigned to **C-12**, confirming the formation of a condensation product. Also, all the ^{13}C NMR spectra indicated four carbonyl carbon peaks at ~ 172 ppm, ~ 167 ppm, ~ 166 , and ~ 165 ppm indicating **C-10**, **C-9**, **C-7** and **C-3** respectively (**Scheme 15**). Furthermore, the spectra were characterized by two methylene carbon peaks at ~ 61 ppm (**C-2**), and at ~ 43 ppm (**C-8**) with an extra methine peak (**C-5**) observed at ~ 48 ppm. The spectrum of compound **36i** showed a methylene carbon peak at 102.57 ppm confirming the presence of the dioxolane group. All the spectra **36f-j** were characterized by the two methyl carbon signals at ~ 17 ppm (**C-4**) and ~ 14 ppm (**C-1**). Compounds **36a** and **36c** showed an extra methyl carbon peak at 55.98 ppm and 56.10 ppm respectively confirming the presence of methoxy carbon atoms.

The IR spectra of all the final compounds **36f-j**, showed characteristic peaks for N-H stretching in the range of $3000\text{-}3400\text{ cm}^{-1}$ and C=O stretching in the range of $1685\text{-}1740\text{ cm}^{-1}$. In addition, The calculated masses of compounds **36f-j** were also found to

be consistent with the **HRMS** found values. The melting point of these compounds were observed to be ranging from 174 to 224 °C. However, there was no literature melting point recorded since none of this compound were reported in literature yet.

CHAPTER-THREE

3.0. Pharmacology of final compounds as anti-diabetic drugs

3.1. Background

This chapter briefly describes the detailed biological evaluation of our final synthesized compounds. The biological testing against glucose activity was done using α -glucosidase and α -amylase assay methods. These screening tests were performed at CSIR by Dr Malefa Tselanyane.

3.1.1. α -Glucosidase

α -Glucosidase is a carbohydrate-hydrolase that liberates α -glucose from the non-reducing terminal of the substrate. It is widely distributed in microorganisms, plants, and animal tissues.^[108] In animal tissues, it is membrane bound and is present in the epithelium of the small intestine, facilitating the absorption of glucose by the small intestine. Inhibiting α -glucosidase slows the elevation of blood sugar by delaying the overall absorption rate of glucose into the blood.^[109] The α -Glucosidase inhibition assay is a colorimetric assay which measures the hydrolysis of *p*-nitrophenyl- α -D-glucopyranoside to glucose and *p*-nitrophenyl. The resulting yellow-coloured product is detected at 405 nm on a Tecan Infinite 500 spectrophotometer.

3.1.2. α -Amylase

α -Amylase is an enzyme that can be found in microorganisms, plants and higher organisms. There are two types of amylases, namely salivary amylase and pancreatic amylase.^[110] The α -Amylase hydrolyzes starch to glucose therefore its inhibition is a therapeutic strategy for disorders of carbohydrate uptake such as diabetes and obesity.^[111] α -Amylase inhibition assay is a colorimetric assay which measures the hydrolysis of starch to glucose by using a 3,5-dinitrosalicylic acid (DNS) to give a brown-coloured product which is detected at 540 nm on a Tecan Infinite 500 spectrophotometer.

3.2. ASSAY METHOD

3.2.1. α -Glucosidase inhibition Assay

In a 96-well plate, 30 μ l phosphate buffer (20 mM, pH 6.9) and 10 μ l α -glucosidase (100 μ g/ml)(from *Saccharomyces cerevisiae*) were added for the control wells. For the blank wells 40 μ l phosphate buffer was added. To the inhibitor wells, 20 μ l dimethoxymethylamphetamine (DMAA) (1 mg/ml), 10 μ l phosphate buffer and 10 μ l α -glucosidase were mixed added. For the test samples, 20 μ l extract (1 mg/ml), 10 μ l phosphate buffer and 10 μ l α -glucosidase were added. The plate was Incubated at 37°C for 30 min. 60 μ l substrate was added to each well and the plate was incubated further for 15 min. All samples were done in triplicate at a final concentration of 200 μ g/ml. The Absorbance was read at 405 nm on a Tecan micro-plate reader. In all the test 10% DMSO had no effect on glucosidase and amylase

3.2.2. α -Amylase inhibition Assay

The assay was carried out with 1% soluble starch as the substrate (Sigma, S2004), human salivary α -amylase (1 mg/ml)(Sigma, A1031), 20 mM phosphate buffer (pH 6.9)(Sigma, S0751), standard inhibitors, acarbose (1 mg/ml)(Sigma A8980) and DNS reagent (1% 3, 5-dinitrosalicylic acid (Sigma, D0550) and 12% potassium sodium tartrate (S6170) in 0.4 M NaOH).

In control tubes (2 ml), 40 μ l phosphate buffer and 20 μ l α -amylase were added. For the blank add 60 μ l of phosphate buffer without the enzyme and to the inhibitor tubes 10 μ l phosphate buffer was added, 20 μ l α -amylase and 30 μ l acarbose or α -amylase inhibitor from wheat seed. In solvent control tubes add 10 μ l phosphate buffer, 20 μ l α -amylase and 30 μ l solvent (10% ethanol). For the test samples, 10 μ l phosphate buffer as added, 20 μ l α -amylase and 30 μ l test sample.

The tubes were incubated in a water bath at 37°C for 30 min. To all the tubes 90 μ l of 1% starch was added and the tubes were incubated further for 20 min in a water bath at 37°C. 50 μ l of DNS reagent were added and the tubes were placed in a boiling water bath for 5 min. The tubes were left to cool down and 500 μ l of water to each tube was added. All the samples were done in triplicate at a final concentration of 200 μ g/ml.

α -Amylase activity is recognized by the formation of the brown product while the blank and the inhibitors show no colour change. The samples that show inhibition on α -amylase are recognized by a pale brown colour as compared to the control or no colour change, which in turn determines the extent of inhibition. Transfer the contents in the tubes to the respective wells in a 48 well plate. Measure the absorbance at 540 nm on a Tecan-Infinite 500 multiwell spectrophotometer (Tecan Group Ltd., Switzerland). In all the test 10% DMSO had no effect on glucosidase and amylase.

3.3. Results

In order to investigate the inhibitory effect of the synthesized compounds **43a-d** and **36a-j**, *in vitro* α -glucosidase and α -amylase tests were performed, and the results obtained are summarized in **Table 11**. Only compounds **43b**, **43d** and **36a** had been reported in the literature with only compound **36a** tested against diabetes and the rest of the compounds were novel and were tested for the first time. The control DMAA showed a percentage inhibition of $99.85 \pm 0.093\%$ and compound **36f** was the most potent exhibiting an artificially high result with the percentage inhibition of $113.46 \pm 0.52\%$ against α -glucosidase followed by compounds **36b**, **36j**, **36i** and **36a** exhibiting $96.15 \pm 0.90\%$, $93.37 \pm 0.4\%$, $87.44 \pm 0.45\%$ and $80.91 \pm 0.30\%$ respectively. Compounds **36g**, **36e**, **36h** and **36c** exhibited moderate activity against α -glucosidase giving $66.07 \pm 1.05\%$, $64.31 \pm 1.98\%$, $60.71 \pm 1.25\%$ and $53.85 \pm 1.37\%$ respectively whereas the rest of the compounds **43d**, **36d** and **43a** showed weak activity against α -glucosidase giving $30.88 \pm 2.86\%$, $26.29 \pm 1.77\%$ and $15.11 \pm 4.57\%$ respectively. Compounds **43b** and **43c** were not active at all against α -glucosidase.

In the α -amylase assay, only compounds **36a**, **36f**, **36j**, **36i** and **36b** displayed weak activities against α -amylase giving values of giving $35.03 \pm 3.34\%$, $25.66 \pm 2.22\%$, $24.92 \pm 2.70\%$, $14.58 \pm 1.62\%$ and $2.03 \pm 0.99\%$ respectively and the control acarbose displayed with the highest activity ($80.18 \pm 1.18\%$) as shown in **Table 11**. Furthermore, compounds **43a**, **43b**, **43c**, **43d**, **36c**, **36d**, **36e**, **36g** and **36h** failed to produce activities against α -amylase.

Table 11: % Inhibition of compounds on α -glucosidase.

Compound	% Inhibition \pm SD α -Glucosidase Assay	% Inhibition \pm SD α -Amylase Assay
DMAA (glucosidase)	99.85 \pm 0.093%	
Acarbose (Amylase)	-	80.18 \pm 1.18%
43a	15.11 \pm 4.57	-
43b	-6.38596 (0)	-
43c	-0.01685(0)	-
43d	30.88 \pm 2.86	-
36a	80.91 \pm 0.30	35.03 \pm 3.34
36b	96.15 \pm 0.90	2.03 \pm 0.99
36c	53.85 \pm 1.37	-
36d	26.29 \pm 1.77	-
36e	64.31 \pm 1.98	-
36f	113.46 \pm 0.52	25.66 \pm 2.22
36g	66.07 \pm 1.05	-
36h	60.71 \pm 1.25	-
36i	87.44 \pm 0.45	14.58 \pm 1.62
36j	93.37 \pm 0.42	24.92 \pm 2.70

3.4. Discussion

3.4.1. α -Glucosidase inhibition

Since diabetes is characterized by high concentrations of blood sugar levels which can cause serious complication. Treatment methods focus mainly on reducing fluctuations in sugar levels and complications. The testing of compounds **43a-d** and **36a-j** was done to investigate the therapeutic treatment approaches in order to decrease the reabsorption of glucose through the inhibition of carbohydrate hydrolyzing enzymes which are alpha amylase and alpha glucosidase.

Compounds **43a-d** (acetates) were found to be very weak against α -glucosidase because of the attached ethyl bromoacetate which seems to decrease the activity against α -glucosidase. Novel compounds **36f**, **36i**, **36h** and **36j** having alanine moiety (Alaninates) were found to be more active against α -glucosidase than compounds **36a**, **36h** and **36e** having glycine moiety (glycinate) as a linker and vice versa for

compound **36b** and **36g**. Structurally connected *para* 4-methoxy benzylidene ring attached to the 5th position of the thiazolidinediones with alaninate connected to the third position exhibited the highest activity against α -glucosidase than where glycinate is connected to the third position of the thiazolidinediones (**Figure 22**). Connecting the *para* 4-nitro benzylidene ring attached to the 5th position of the thiazolidinediones with alaninate connected to the third position decreases the activity against α -glucosidase. Surprisingly, combining the methoxy group at the *para* position and the hydroxy group at the *meta* position also reduces the α -glucosidase activity. In general, this implies that our active compounds **36a**, **36b**, **36c**, **36e**, **36g**, **36h**, **36f**, **36i** and **36j** have the ability to reduce the rate of carbohydrate digestion and delay the carbohydrate absorption from the digestive tract, therefore, they have the potential to treat type 2 diabetes by lowering the glucose levels after a meal.

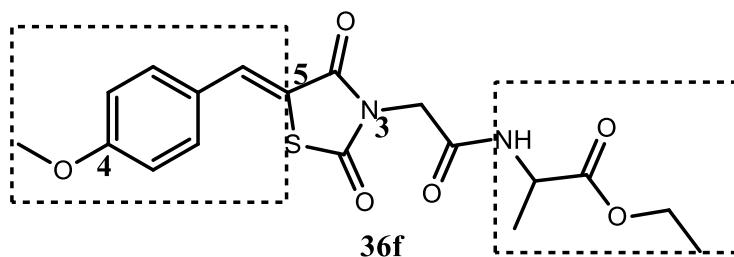


Figure 22: Schematic activity of synthesized antidiabetic compounds

3.4.2. α -Amylase Inhibition

Compounds **43a-d** (acetates) failed to show any activity against α -Amylase. Only compounds **36a** and **36b** of the glycinate showed weak activities and only compound **36f**, **36i** and **36j** of the alaninate also displayed weak activities against α -Amylase. This implies that compounds **43a-d** and **36a-j** do not have the potential to delay carbohydrate hydrolysis rate and maintains the serum blood glucose in hyperglycaemic individuals.

3.5. Conclusion

The antidiabetic activities of compounds **43a-d** and **36a-j** were evaluated *in vitro* against α -glucosidase and α -amylase and showed activity against these enzymes. Compounds **43a-d** showed very weak or no anti-diabetic activity whereas compounds **36a**, **36b**, **36f**, **36i** and **36j** showed excellent antidiabetic activity in contrast to **36c**, **36e**, **36g** and **36h** which exhibited moderate activity. This suggest that compounds

36a, 36b, 36f, 36i and **36j** may be powerful potent against α -glucosidase and need to be further studied.

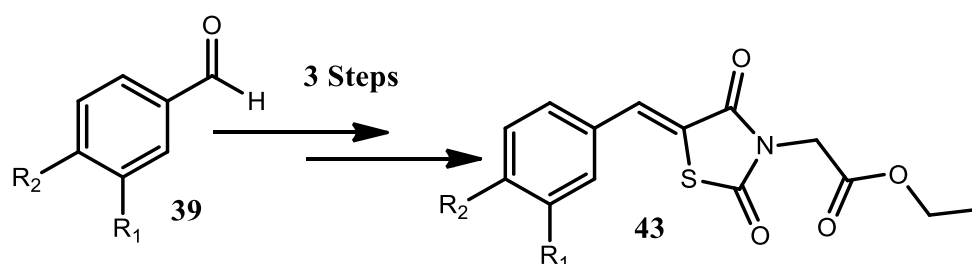
CHAPTER-FOUR

4.0. Conclusion and Future work

This chapter looks at the general conclusions of this project. This project has successfully explored a series of novel acetates, glycinate and alaninate as antidiabetic drugs. Biological properties of the synthesized compounds were studied and gave interesting results.

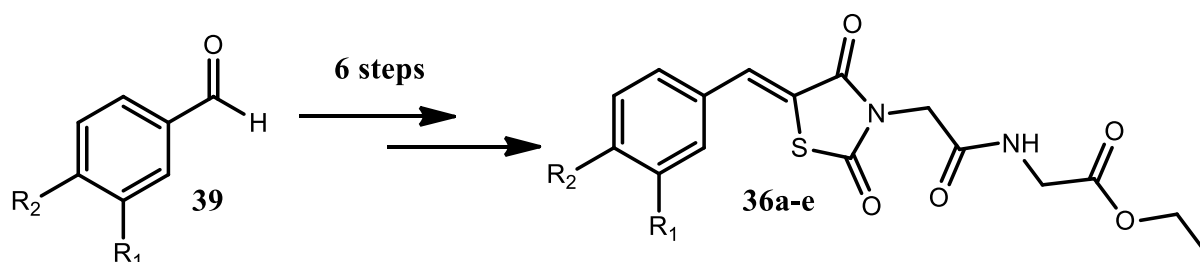
4.1. Synthesis of glitazones analogues

Generally, the application of the Knoevenagel condensation reaction was used throughout the project to afford the successful synthesis of the desired compounds. Acetates and acetamide derivatives were used as part of the synthesized compounds. Firstly, four compounds **43a**, **43b**, **43c** and **43d** (ethyl 2-(5-(substituted benzylidene)-2,4-dioxothiazolidin-3-yl acetates) with the yields of 77%, 24%, 88% and 26% respectively were successfully synthesized in three reaction steps starting from benzaldehyde **39** using known methods (**Scheme 16**)



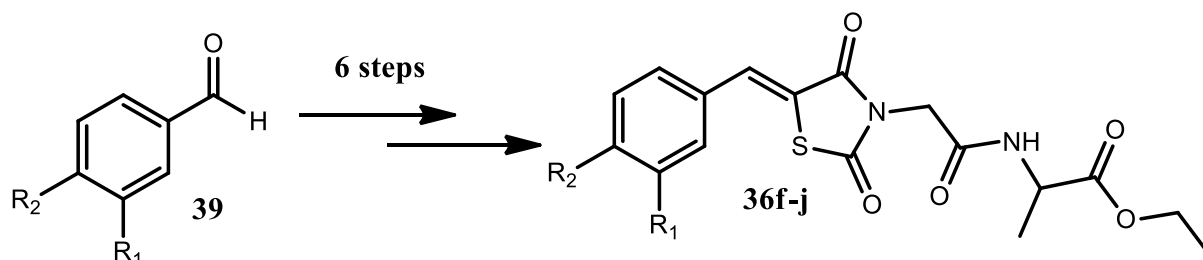
Scheme 17: *Synthesis of acetate derivatives*

Secondly, five envisaged anti-diabetic compounds **36a**, **36b**, **36c**, **36d** and **36e** having a glycine moiety as a linker with the yields 50%, 22%, 97%, 34% and 98% respectively were successfully synthesized in six reaction steps starting from benzaldehyde **39** using known methods (**Scheme 17**).



Scheme 18: *Synthesis of envisaged antidiabetic drugs containing glycine moiety*

After preparing compounds **36a-e** (**Scheme 17**), we then successfully synthesized five antidiabetic drugs **36f**, **36g**, **36h**, **36i** and **36j** having the alanine moiety as a linker in six reaction steps with the yields 98%, 50%, 61%, 33% and 90%, respectively, starting from benzaldehydes by using conventional methods (**Scheme 18**)



Scheme 19: Synthesis of antidiabetic drugs with alanine moiety

4.2. Biological Evaluation

A series of three novel compounds containing acetates, ester protected glycine and ester protected alanine synthesized in this study as mentioned above were successfully tested against diabetes. Novel compounds with the alanine moiety showed very good activity against α -glucosidase and weak activity against α -amylase followed by compounds with the glycine moiety which exhibited moderate activity against α -glucosidase and weak activity against α -amylase. Finally, synthesized acetates showed no activity against both α -glucosidase and α -amylase.

4.3. Future Work

This project has the capability to be expanded by synthesizing alanine containing compounds **36a**, **36b**, **36f**, **36i** and **36j** and their derivatives as a powerful potent inhibitor of α -glucosidase and α -amylase in future studies. This may be achieved by varying the phenyl ring of compound **36f**.

CHAPTER – FIVE

Experimental Procedures

All reagents used were analytical grade from Fluka and Sigma Aldrich and were used as supplied without further purification.

5.0. General procedures

5.0.1. Spectroscopic Techniques

^1H 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 were 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) is measured in Hertz (Hz).

All 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). Furthermore, abbreviations such as Ar = Aromatic and Ali = Aliphatic has been used to assign some signals observed.

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. The 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 $^\circ\text{C}$. The source temperature was 100 $^\circ\text{C}$ and the desolvation temperature was set at 300 $^\circ\text{C}$. 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).

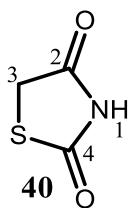
5.0.2. Thin Layer Chromatography

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.

5.0.3. Nomenclature of compounds

The 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.

5.1. Synthesis of thiazolidine-2,4-dione/glitazone (40)

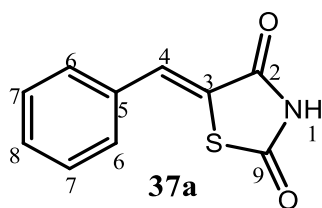


2-Chloroacetic acid (10.0 g, 106.0 mmol) and thiourea (8.05 g, 105.7 mmol) were mixed together in a flask to which water (50 ml) was added and the reaction mixture was stirred for 20 minutes at room temperature until the reagents completely reacted to form a white precipitate of 2-imino-thiazolidine-4-one. Concentrated HCl (5 ml) was then added to the reaction mixture which was then refluxed for 12 hours. After this reaction time the mixture was allowed to cool down to room temperature over which time a white precipitate was formed. This precipitate was filtered, washed with a small volume of ice water to give the desired product as a white solid (8.01 g, 81%) m.p = 123.7-125.2 °C (lit m.p = 126-127 °C), ^[112] ¹H NMR (400 MHz, DMSO-d₆) δ_H (ppm) 12,03 (br.s,1H, H-1), 4.14 (s, 2H, H-3), ¹³C NMR (100 MHz, DMSO-d₆), δ_C (ppm) 174.34 (C-2), 173.59 (C-4), 36.26 (C-3), IR (KBr cm⁻¹): 3123 (N-H), 2820 (C-H), 1645 (C=O), 1615 (C=O)

5.2. General synthetic procedure for the Knoevenagel condensation reaction.

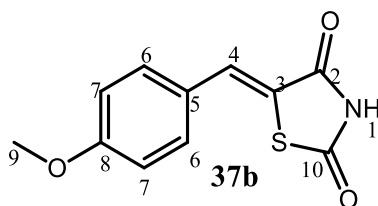
Benzaldehydes (1 mmol) were added to a suspension of thiazolidinedione (**40**) (1 mmol) in benzene followed by catalytic amounts of piperidine (10%) dropwise and acetic acid (10%) dropwise. The reaction mixture was stirred for 5 mins at room temperature and then refluxed for 12 hrs. After being allowed to cool to room temperature, a precipitate was obtained via filtration and was washed with diethyl ether to afford the expected thiazolidine-2,4-diones (**37a-e**)

5.2.1. 5-Benzylidene thiazolidine-2,4-dione (37a).



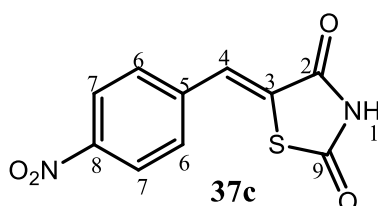
A mixture of benzaldehyde (4.84 ml, 42.7 mmol) and thiazolidine-2,4-diones (**40**) (5.04 g, 42.7 mmol) in benzene (50 ml) gave compound **37a** as a white solid (3.30 g, 38%) m.p = 230.2-236.4 °C (lit m.p = 240 °C),^[113] **¹H NMR (400 MHz, DMSO-d₆)**, δ_{H} (ppm) 12.64 (br.s, 1H, H-1), 7.77 (s, 1H, H-4), 7.57 (d, 2H, $J = 7.2$ Hz, H-6), 7.53-7.45 (m, 3H, H-7+H-8), **¹³C NMR (100 MHz, DMSO-d₆)**, δ_{C} (ppm) 168.34 (C-2), 167.76 (C-9), 133.45 (C-5), 132.23 (C-4), 130.84 (C-6), 130.44 (C-7), 129.74 (C-8), 123.97 (C-3); **IR (KBr cm⁻¹)**: 3313 (N-H), 2779 (ArC-H), 1775 (C=O), 1736 (C=O), 1682 (C=C).

5.2.2. 5-(4-Methoxy-benzylidene) thiazolidine-2,4-dione (37b)



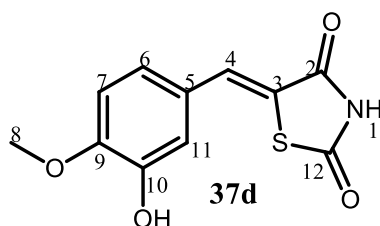
A mixture of anisaldehyde (5.19 ml, 42.7 mmol) and thiazolidine-2,4-diones (**40**) (5.02 g, 42.7 mmol) in benzene (50 ml) gave compound **37b** as a brown solid (3.90 g, 36%), m.p = 247.7-249.4 °C, (lit mp = 215-217 °C),^[114] **¹H NMR (400 MHz, DMSO-d₆)**, δ_{H} (ppm) 12.55 (br.s, 1H, H-1), 7.71 (s, 1H, H-4), 7.52 (d, 2H, $J = 8.8$ Hz, H-6), 7.06 (d, 2H, $J = 8.8$ Hz, H-7), 3.83 (s, 3H, H-9); **¹³C NMR (100 MHz, DMSO-d₆)** δ_{C} (ppm) 168.44 (C-10), 167.90 (C-2), 161.40 (C-8), 133.51 (C-6), 132.26 (C-4), 125.90 (C-5), 120.68 (C-3), 115.29 (C-7), 55.88 (C-9) ppm; **IR (KBr cm⁻¹)**: 2965 (N-H), 2928 (ArC-H), 1777 (C=O), 1747 (C=O), 1668 (C=C).

5.2.3. 5-(4-Nitro-benzylidene) thiazolidine-2,4-dione (37c)



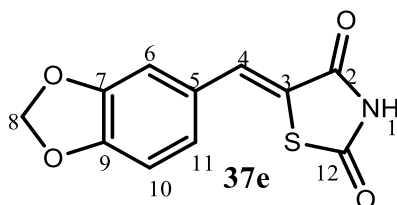
A mixture of 4-nitrobenzaldehyde (6.45 g, 42.7 mmol) and thiazolidine-2,4-diones (**40**) (5.03 g, 42.7 mmol) in benzene (50 ml) gave compound **37c** as a cream white solid (8.80 g, 87%), m.p = 246.7-248.7 °C, (lit m.p = 235-238 °C),^[115] **¹H NMR (400 MHz, DMSO-d₆)**, δ_{H} (ppm) 12.01 (br.s, 1H, H-1), 8.30 (d, 2H, $J = 7.6$ Hz, H-7), 8.10 (s, 1H, H-4), 7.78 (d, 2H, $J = 8.4$ Hz, H-6), **¹³C NMR (100 MHz, DMSO-d₆)** δ_{C} (ppm) 169.96 (C-9), 168.35 (C-2) 147.14 (C-8) 139.55 (C-6), 130.56 (C-4) 127.6 (C-5), 124.79 (C-7), 122.23 (C-3); **IR** (KBr cm⁻¹): 3382 (N-H), 2981 (ArC-H), 1730 (C=O), 1690 (C=O), 1622, (C=C).

5.2.4 5-(4-Hydroxy-3-methoxybenzylidene) thiazolidine-2,4-dione (**37d**)



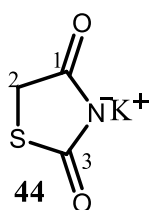
A mixture of vanillin (6.50 g, 42.7 mmol) and thiazolidine-2,4-diones (**40**) (5.04 g, 42.7 mmol) in benzene (50 ml) gave compound **37d** as a yellow solid (13.03 g, 92 %) m.p = 224.8-225.7 °C (lit m.p = 194-196 °C),^[116] **¹H NMR (400 MHz, DMSO-d₆)**, δ_{H} (ppm) 10.08 (br.s, 1H, H-1), 7.69 (s, 1H, H-4), 7.15 (s, 1H, H-11), 7.05 (d, 1H, $J = 8.4$ Hz, H-7), 6.92 (d, 1H, $J = 8.0$ Hz, H-6), 3.82 (s, 3H, H-8); **¹³C NMR (100 MHz), DMSO-d₆** δ_{C} (ppm) 168.65 (C-2), 168.16 (C-12) 149.85 (C-10) 148.42 (C-9), 132.91 (C-4) 124.90 (C-5), 124.59 (C-11), 119.88 (C-3), 116.62 (C-6), 114.53 (C-7), 56.06 (C-8) ; **IR** (KBr cm⁻¹): 3457 (O-H), 3176 (N-H), 3014 (ArC-H), 1773 (C=O), 1673 (C=O), 1598 (C=C)

5.2.5. 5-(Benzo[d][1,3] dioxol-5-ylmethylene) thiazolidine-2,4-dione (**37e**)



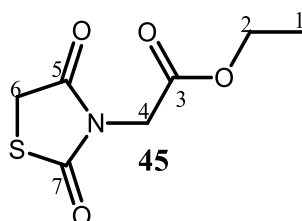
A mixture of piperonal (6.41 g, 42.7 mmol) and thiazolidine-2,4-diones (**40**) (5.01 g, 42.7 mmol) in benzene (50 ml) gave compound **37e** as a yellow solid (0.36 g, 32 %) m.p = 244.7-245.9 °C, (lit m.p = 246-247 °C),^[117] **¹H NMR (400 MHz, DMSO-d₆)**, δ_{H} (ppm) 9.80 (s, 1H, H-1), 7.70 (s, 1H, H-4), 7.15-7.12 (m, 2H, H-11+H-6) 7,07 (d, 1H, J = 8.0 Hz, H-10), 6.13 (s, 2H, H-8), **¹³C NMR (100 MHz, DMSO-d₆)** δ_{C} (ppm) 168.42 (C-2), 168.08 (C-12), 149.68 (C-9), 148.60 (C-7), 132.20 (C-4), 127.68 (C-5), 126.29 (C-6), 121.62 (C-3), 109.58 (C-10), 109.64 (C-11), 102.47 (C-8), **IR (KBr cm⁻¹):** 3105 (N-H), 2999 (ArC-H), 1771 (C=O), 1604 (C=O), 1598 (C=C)

5.3. Synthesis of the potassium salt of thiazolidine-2,4-dione (**44**)



To a hot solution of 1,3 thiazolidine-2,4-dione (5.04 g, 42,7 mmol) in ethanol (50 ml) was added a solution of KOH (2.40 g, 42,7 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-diones (**44**) as a white solid (7.80 g, 78%) m.p = 260-266.6 °C (lit m.p = 247-250 °C,^[118] **¹H NMR (400 MHz, DMSO-d₆)** δ_{H} (ppm) 4.14 (s, 2H, H-2), **¹³C NMR (100 MHz, DMSO-d₆)**, δ_{C} (ppm) 174.23 (C-1), 173.40 (C-3), 36.12 (C-2), **IR (KBr cm⁻¹):** 2972 (C-H), 1653 (C=O), 1602 (C=O)

5.4. Synthesis of ethyl (2,4-dioxo-1,3-thiazolidin-3-yl) acetate (**45**)

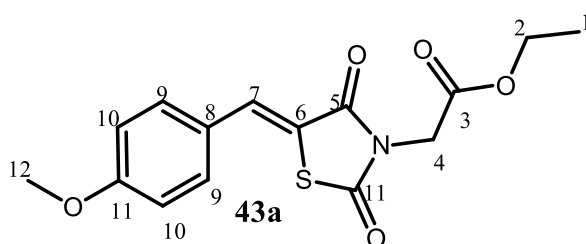


A mixture of the potassium salt of thiazolidine-2,4-dione (**44**) (5.02 g, 42.7 mmol) and ethyl bromoacetate (4.72 ml, 42.7 mmol) in acetone was heated under reflux for 6 hours. After being allowed to cool down to room temperature, the excess potassium hydroxide salt was solid filtered off and the filtrate concentrated using a rotary vapour to provide ethyl (2,4-dioxo-1,3-thiazolidin-3-yl) acetate (**45**)^[119] as a colourless oil (7.05 g, 71%), ¹H NMR (400 MHz, CDCl₃) δ_H (ppm) 4.25 (s, 2H, H-4), 4.17 (q, 2H, *J* = 3.8 Hz, H-2), 4.01 (s, 2H, H-6), 1.20 (t, 3H, *J* = 4.9 Hz, H-1), ¹³C NMR (100 MHz, CDCl₃), δ_C (ppm) 172.33 (C-7), 171.76 (C-5), 167.90 (C-3), 62.23 (C-2), 42.01 (C-4), 33.89 (C-6), 13.91 (C-1), IR (KBr cm⁻¹): 3208 (C-H), 2981, (1778 (C=O), 1609 (C=O)

5.5 General procedure for synthesis of ethyl 2-(5-(substituted benzylidene)-2,4-dioxothiazolidin-3-yl)acetates (**43**) using Knoevenagel Condensation reaction.

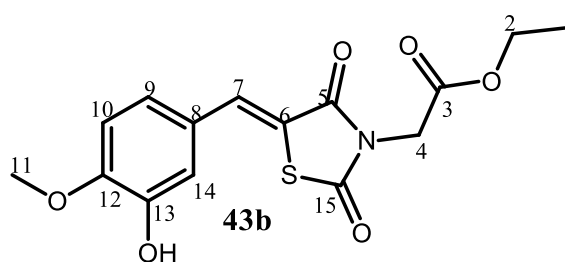
An appropriate benzaldehyde (1 mmol) and ethyl (2,4-dioxo-1,3-thiazolidin-3-yl) (**45**) acetate (1 mmol), piperidine (cat.) and were taken in ethanol (50 ml) and the mixture was heated under reflux for 12 hours. After being allowed to cool down to room temperature, the mixture was poured into ice water and the resultant precipitate was collected by filtration. The resultant solid was washed with distilled water and diethyl ether. The solids obtained were dried in a fume hood at room temperature for 24 hours to obtain the expected products (**43a-d**).

5.5.1. Ethyl 2-(5-(4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)acetate (**43a**)



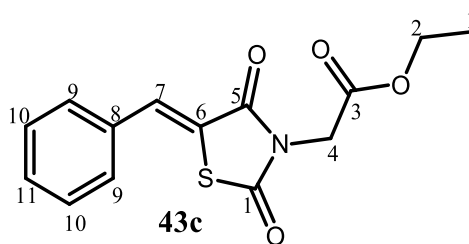
A mixture of anisaldehyde (0.64 ml, 5.03 mmol) and ethyl (2,4-dioxo-1,3-thiazolidin-3-yl) acetate (**45**) (1.02 g, 5.03 mmol) in ethanol (20 ml) gave compound **43a** as a shiny yellowish solid (1.03 g, 77 %) m.p = 134.5-136.6 °C (lit m.p = 129-131 °C),^[120] **¹H NMR (400 MHz, DMSO-d₆)**, δ_{H} (ppm) 7.96 (s, 1H, H-7), 7.62 (d, 2H, $J = 8.8$ Hz, H-9), 7.12 (d, 2H, $J = 8.8$ Hz, H-10), 4.49 (s, 2H, H-4), 4.18 (q, 2H, $J = 7.0$ Hz, H-2) 3.84 (s, 3H, H-12), 1.22 (t, 3H, $J = 7.0$, H-1), **¹³C NMR (100 MHz, DMSO-d₆)**, δ_{C} (ppm) 167.41 (C-11), 167.21 (C-5), 165.50 (C-3), 161.88 (C-11), 134.59 (C-7), 132.94 (C-9) 125.64 (C-8), 117.58 (C-3), 115.48 (C-10), 62.10 (C-2), 56.00 (C-12), 43.61 (C-4), 14.40 (C-1), **IR** (KBr cm⁻¹): 3016 (ArC-H), 2941, 1734 (C=O), 1677 (C=O), 1606 (C=O), 1177 (C-O). **HRMS** (ESI-TOF) m/z ; calculated C₁₅H₁₅NO₅S: 321.3483, found (M+H)⁺: 322.9745.

5.5.2. Ethyl 2-(5-(3-hydroxy-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl) acetate (**43b**)



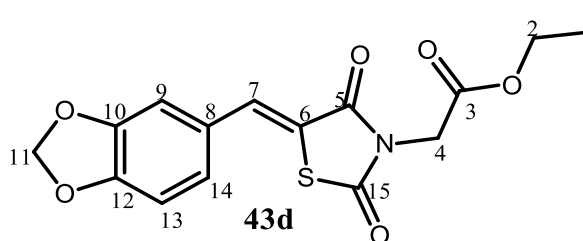
A mixture of vanillin (0.76 g, 4.97 mmol) and ethyl (2,4-dioxo-1,3-thiazolidin-3-yl) acetate (**45**) (1.04 g, 4.97 mmol) in ethanol (20 ml) gave compound **43b** as a yellow solid (0.39 g, 24 %) m.p = 139.9-141.5 °C, **¹H NMR (400 MHz, DMSO-d₆)**, δ_{H} (ppm) 7.91 (s, 1H, H-7), 7.22 (s, 1H, H-14), 7.13 (d, 1H, $J = 7.9$ Hz, H-10), 6.95 (d, 1H, $J = 8.2$, H-9), 4.48 (s, 2H, H-4), 4.17 (q, 2H, $J = 7.0$ Hz, H-2) 3.84 (s, 3H, H-11), 1.22 (t, 3H, $J = 7.0$ Hz, H-1), **¹³C NMR (100 MHz, DMSO-d₆)**, δ_{C} (ppm) 167.48 (C-15), 167.21 (C-5), 165.50 (C-3), 150.45 (C-12), 148.51 (C-13), 135.30 (C-7) 124.99 (C-8), 124.55 (C-6), 116.72 (C-14), 116.52 (C-9), 114.91 (C-10), 62.08 (C-2), 56.11 (C-11), 42.56 (C-4), 14.40 (C-1), **IR** (KBr cm⁻¹): 3318 (N-H), 2994 (ArC-H), 1746 (C=O), 1723 (C=O), 1668 (C=O), 1147 (C-O). **HRMS** (ESI-TOF) m/z ; calculated for C₁₅H₁₅NO₆S: 337.3447, found (M)⁺: 337.0611

5.5.3. Ethyl 2-(5-benzylidene-2,4-dioxothiazolidin-3-yl) acetate (43c)



A mixture of benzaldehyde (0.51 ml, 5.03 mmol) and ethyl (2,4-dioxo-1,3-thiazolidin-3-yl) acetate (**45**) (1.02g, 5.03 mmol) in ethanol (20 ml) gave compound **43c** as a pale yellow solid (1.28 g, 88 %) m.p = 89.9-91.7 °C (lit m.p = 75-77 °C),^[120] **¹H NMR (400 MHz, DMSO-*d*₆)**, δ_{H} (ppm) 8.00 (s, 1H, H-7), 7.66 (d, 2H, $J = 7.2$ Hz, H-9) 7.58-7.52 (m, 3H, H-11+H-11), 4.50 (s, 2H, H-4) 4.18 (q, 2H, $J = 7.1$ Hz, H-2) 1.22 (t, 3H, $J = 7.1$ Hz, H-1), **¹³C NMR (100 MHz, DMSO-*d*₆)**, δ_{C} (ppm) 167.35 (C-5), 167.16 (C-1), 165.39 (C-3), 134.60 (C-7), 133.14 (C-9), 131.43 (C-8) 130.72 (C-9), 129.60 (C-10), 120.95 (C-6), 62.17 (C-2), 42.67 (C-4), 14.39 (C-1), **IR (KBr cm^{-1})**: 2987 (ArC-H), 1736 (C=O), 1676 (C=O), 1605 (C=O), 1149 (C-O). **HRMS (ESI-TOF) m/z** , calculated for C₁₄H₁₃NO₄S: 291,3223 found (M+H)⁺: 292.1371

5.5.4. Ethyl 2-(5-(Benzo[*d*][1,3] dioxol-5-ylmethylene)-2,4-dioxothiazolidin-3-yl) acetate (43d)



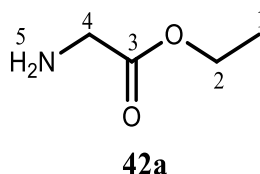
A mixture of piperonal (0.49 g, 4.97 mmol) and ethyl (2,4-dioxo-1,3-thiazolidin-3-yl) acetate (**45**) (1.01 g, 4.97 mmol) in ethanol (50ml) gave compound **43d** as a yellow solid (0.43 g, 26 %) m.p = 131.5-133.4 °C, **¹H NMR (400 MHz, DMSO-*d*₆)**, δ_{H} (ppm) 7.92 (s, 1H, H-7), 7.23-7.18 (m, 2H, H-13) 7.11 (d, 1H, $J = 8.0$ Hz, H-9), 6.15 (s, 2H, H-11), 4.48 (s, 2H, H-4), 4.17 (q, 2H, $J = 6.8$ Hz, H-2), 1.21 (t, 3H, $J = 6.4$ Hz, H-1), **¹³C NMR (100 MHz, DMSO-*d*₆)**, δ_{C} (ppm) 167.31 (C-5), 167.19 (C-15), 165.43 (C-3), 150.21 (C-12), 148.71 (C-10), 134.66 (C-7) 127.30 (C-8), 126.88 (C-9), 118.32 (C-6), 109.88 (C-13), 109.71 (C-14), 102.60 (C-11), 62.13 (C-2), 42.62 (C-4), 14.39 (C-1),

IR (KBr cm^{-1}): 2994 (ArC-H), 2952 (Ar-OCH₂O), 1730 (C=O), 1688 (C=O), 1608 (C=O), 1150 (C-O). **HRMS** (ESI-TOF) m/z , calculated for C₁₅H₁₃NO₆S: 335,3318 found (M+H)⁺: 385.1242

5.6. General procedure for synthesis of ester protected amino acids (42)

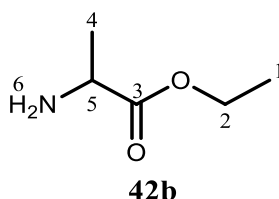
A solution of an amino acid (1 mmol) in ethanol was cooled to 0-5 °C in an ice bath. To this reaction mixture was added thionyl chloride (1 mmol) dropwise. The mixture was then allowed to warm up to room temperature before being refluxed for 24 hours. The resulting mixture was allowed to cool down to room temperature over which time a white solid was formed and obtained via filtration (**42a**) and also a colourless liquid was obtained at reduced pressure (**42b**). The desired products obtained were used without further purification.

5.6.1. Ethyl 2-aminoacetate (42a)



A mixture of glycine (5.01 g, 66.0 mmol) and thionyl chloride (4.80 ml, 66.0 mmol) in ethanol (50 ml) gave **42a** as a white solid (6.72 g, 98 %). m.p = 88-90 °C, (lit 91-95 °C),^[121] **¹H NMR (400 MHz, D₂O)** δ_{H} (ppm) 7.19 (br.s, 2H, H-5), 4.72 (s, 2H, H-4), 3.56 (q, 2H, J = 6.8 Hz, H-2), 1.06 (t, 3H, J = 7.2 Hz, H-1) ; **¹³C NMR (100 Hz, D₂O)** δ_{C} (ppm) 171.84 (C-3), 57.48 (C-2), 41.30 (C-4), 17.00 (C-1) ; **IR** (KBr cm^{-1}): 3381 (N-H), 1741 (C=O).

5.6.2. Ethyl 2-amino propanoate (42b)

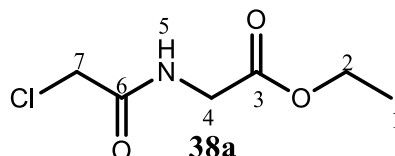


A mixture of alanine (5.03g, 56.1 mmol) and thionyl chloride (4.09 ml, 56.1 mmol) in ethanol (50 ml) gave compound **42b** as a colourless liquid^[122] (4.80 g, 70 %), **¹H NMR (400 MHz, D₂O)** δ_{H} (ppm) 7.58 (d, 2H, $J = 6.8$ Hz, H-6), 4.19-4.16 (m, 1H, H-5) 3.74 (q, 2H, $J = 5.6$ Hz, H-2), 1.10 (d, 3H, $J = 7.2$ Hz, H-4), 1.05 (t, 3H, $J = 7.0$ Hz, H-1); **¹³C NMR (100 MHz, D₂O)** δ_{C} (ppm) 170.27 (C-3), 62.14 (C-2), 58.3 (C-5), 16.17 (C-4), 14.36 (C-1) : **IR** (KBr cm^{-1}): 3385 (N-H), 2978 (C-H), 1720 (C=O).

5.7. General procedure for synthesis of ethyl -2-(2-chloroacetamido) esters (**38**)

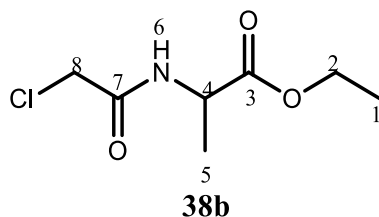
A solution of bromoacetyl chloride (1 mmol) in a mixture of dichloromethane and water was added to a cooled solution (-10 °C) of the corresponding protected amino acids (1 mmol) and potassium carbonate (3 mmol). The resulting solution was allowed to warm up to room temperature before being stirred for 16 hours at the same temperature. After the completion of the reaction the organic layer was extracted and washed with water (2 x 30ml) and brine (1 x 30ml), dried with MgSO₄ and concentrated in a rotary vapour. The crude product was recrystallized with ethyl acetate to afford the corresponding chloro acetamide derivatives (**38a-b**).

5.7.1. Ethyl 2-(2-chloroacetomido) acetate (**38a**)



A mixture of ethyl-2-amino acetate **42a** (2.03 g, 17.0 mmol) and bromoacetyl chloride (1.42 ml, 17.0 mmol) in a mixture of DCM (20 ml) and water (20 ml) gave compound **38a** as a white solid (4.90 g, 96 %) m.p = 75.8-76.5 °C (lit m.p = 77.5 °C),^[123] **¹H NMR (400 MHz, CDCl₃)** δ_{H} (ppm) 7.15 (t, 1H, $J = 8.0$ Hz, H-5), 4.26 (q, 2H, $J = 7.2$ Hz, H-2) 4.10 (d, 2H, $J = 5.2$ Hz, H-4), 3.93 (s, 2H, H-7), 1.31 (t, 3H, $J = 7.1$ Hz, H-1) ; **¹³C NMR (100 MHz, CDCl₃)** δ_{C} (ppm) 169.22 (C-6), 165.74 (C-3), 61.79 (C-2), 41.92 (C-4), 28.57 (C-7) 14.12 (C-1) : **IR** (KBr cm^{-1}): 3396 (N-H), 1723 (C=O), 1646 (C=O), 1548 (C-H).

5.7.2. Ethyl 2-(2-chloroacetomido) propanoate (38b)

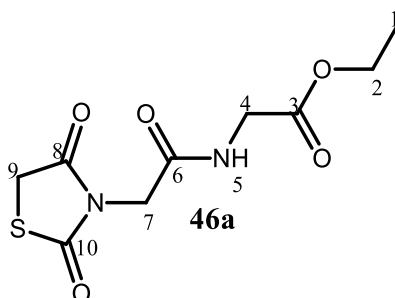


A mixture of ethyl-2-amino propanoate **42b** (5.05 g, 42.7 mmol) and bromo acetylchloride (3.56 ml, 42.7 mmol) in a mixture of DCM (20 ml) and water (20 ml) gave compound **38b** as a white solid (7.60 g, 98 %), mp = 82.2-83.6 °C (lit mp = 71-74 °C),^[105] **¹H NMR (400 MHz, CDCl₃)** δ_{H} (ppm) 7.19 (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) ; **¹³C NMR (100 MHz, CDCl₃)** 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 ν_{max} / (cm^{-1}) : 3396 (N-H), 1723 (C=O), 1646 (C=O): IR (KBr cm^{-1}) : 3396 (N-H), 1746 (C=O), 1670 (C=O), 1548 (C-O)

5.8. General procedure for synthesis of ethyl 2-(2,4-dioxothiazolidin-3-yl) acetamido esters (46)

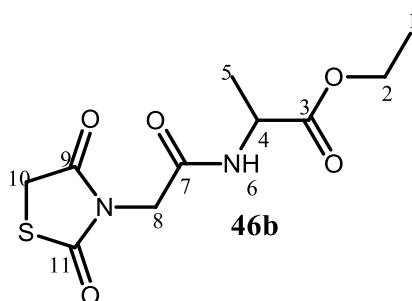
A mixture of the potassium salt of thiazolidine-2,4-dione (**44**) (1 mmol) and ethyl -2-(2-chloro acetamide) derivatives (**38a-b**) (1 mmol) in tetrahydrofuran was refluxed for 12 hours. After cooling to room temperature, the mixture was filtered to remove excess potassium hydroxide, and the filtrate was concentrated on a rotary vapour to provide ethyl 2-(2-(2,4-dioxothiazolidin-3-yl) acetamide derivatives (**46a-b**) which were used without any further purification.

5.8.1. Ethyl 2-(2-(2,4-dioxothiazolidin-3-yl) acetamido) acetate (46a)



A mixture of potassium salt of thiazolidine-2,4-dione (**44**) (4.50 g, 38.3 mmol) and ethyl-2-(2-chloroacetomido) acetate (**38a**) (6.90 g, 38.3 mmol) in THF (50 ml) gave compound **46a** as a white solid (8.50 g, 89%), m.p = 110.0-112.8 °C, **¹H NMR (400 MHz, DMSO-d₆)** δ_{H} (ppm) 8.72 (t, 1H, $J = 5.7$ Hz, H-5), 4.30 (s, 2H, H-7), 4.15 (s, 2H, H-9), 3.88 (d, 2H, $J = 5.6$ Hz, H-4), 4.11 (q, 2H, $J = 2.1$ Hz, H-2), 1.20 (t, 3H, $J = 2.9$ Hz, H-1); **¹³C NMR (100 MHz, DMSO-d₆)** δ_{C} (ppm) 172.35 (C-10), 171.97 (C-8), 169.91 (C-6), 166.21 (C-3), 60.97 (C-2), 43.35 (C-4), 41.54 (C-7), 34.46 (C-9), 14.48 (C-1), **IR** (KBr cm⁻¹): 3294 (N-H), 3108 (ArC-H), 1615 (C=O), 1678 (C=O), 1738 (C=O), 1652 (C=O).

5.8.2. Ethyl 2-(2-(2,4-dioxothiazolidin-3-yl) acetamido) propanoate (**46b**)

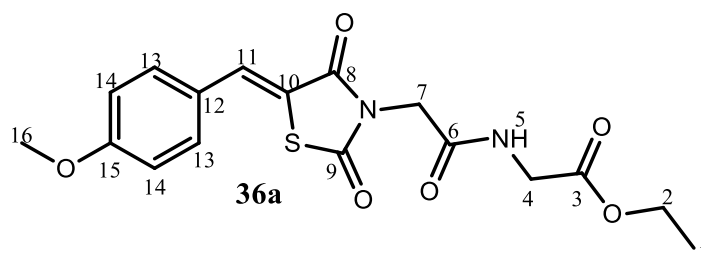


A mixture of A mixture of potassium salt of thiazolidine-2,4-dione (**44**) (3.55 g, 30.3 mmol) and ethyl-2-(2-chloroacetomido) propanoate (**38b**) (5.87 g, 30.3mmol) in THF (50 ml) gave compound **46b** as a white solid (9.70 g, 92%), m.p = 100.6-101.9 °C, **¹H NMR (400 MHz, DMSO-d₆)** δ_{H} (ppm) 8.67 (d, 1H, $J = 6.8$ Hz, H-6), 4.27 (s, 2H, H-8), 4.24 (quint, 1H, $J = 7.2$ Hz, H-4), 4.15 (s, 2H, H-10), 4.09 (q, 2H, $J = 5.6$ Hz, H-2), 1.28 (d, 3H, $J = 4.0$ Hz, H-5), 1.18 (t, 3H, $J = 7.1$, H-1), **¹³C NMR (100 MHz, DMSO-d₆)**, δ_{C} (ppm) 172.65 (C-11), 172.32 (C-9), 171.96 (C-7), 165.49 (C-3), 61.06 (C-2), 48.47 (C-4), 43.47 (C-8), 34.39(C-10), 17.46 (C-5), 13.91 (C-1), **IR** (KBr cm⁻¹): 3288 (N-H), 3078 (ArC-H), 1635 (C=O), 1688 (C=O), 1735 (C=O), 1687 (C=O).

5.9. General procedure for synthesis of anti-diabetic drugs (36) having glycine as a linker via Knoevenagel condensation reaction

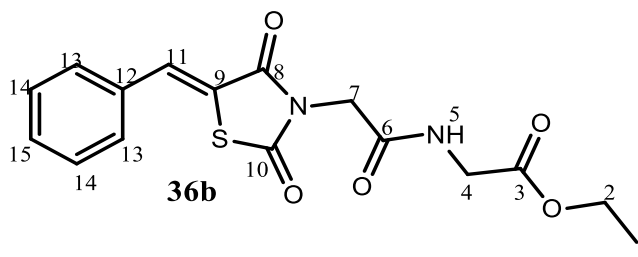
To a suspension of ethyl 2-(2-(2,4-dioxothiazolidin-3-yl) acetamido) acetate (**46a**) (1 mmol) was added appropriate benzaldehydes (1 mmol) in ethanol (20 ml) followed by catalytic amount of piperidine. The resultant mixture was heated under reflux for 12 hours. After being allowed to cool down to room temperature, the mixture was poured into ice water and the resultant precipitate was collected by filtration. The solids obtained were further recrystallized from methanol to afford the desired anti-diabetic drugs (**36a-e**).

5.9.1. Ethyl 2-(2-(5-(4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl) acetamido) acetate (**36a**)



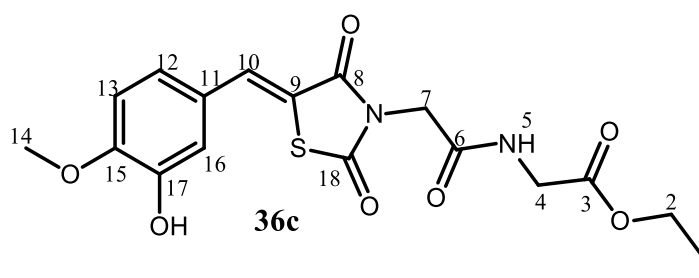
A mixture of anisaldehyde (0.47 ml, 3.84 mmol) and ethyl 2-(2-(2,4-dioxothiazolidin-3-yl) acetamido) acetate (**46a**) (1.05 g, 3.84 mmol) in ethanol (20 ml) gave compound **36a** as a white amorphous solid (0.72 g, 50%), m.p = 204.9-208.8 °C (lit m.p = 217 °C),^[105] **¹H NMR (400 MHz, DMSO-*d*₆)** δ_{H} (ppm) 8.82 (t, 1H, J = 5.7 Hz, H-5), 7.96 (s, 1H, H-11), 7.67 (d, 2H, J = 8.8 Hz, H-13), 7.18 (d, 2H, J = 8.8 Hz, H-14), 4.39 (s, 2H, H-7), 4.16 (q, 2H, J = 7.2 Hz, H-2), 3.93 (d, 2H, J = 6.0 Hz, H-4), 3.89 (s, 3H, H-16), 1.24 (t, 3H, J = 7.1 Hz, H-1), **¹³C NMR (100 MHz, DMSO-*d*₆)** δ_{C} (ppm) 169.91 (C-9), 167.63 (C-8), 166.28 (C-6), 165.81 (C-3), 161.73 (C-15), 133.85 (C-11), 132.80 (C-14), 125.76 (C-10), 118.25 (C-12), 115.49 (C-13), 61.05 (C-2), 55.99 (C-16), 43.58 (C-7), 41.25 (C-4), 14.47 (C-1), **IR (KBr cm^{-1}):** 3293 (N-H), 3107 (ArC-H), 1760 (C=O), 1736 (C=O), 1677 (C=O), 1663 (C=O), 1180 (C-O). **HRMS (ESI-TOF) m/z ,** calculated for C₁₇H₁₈N₂O₆S: 378.3996 found (M)⁺: 378.0542

5.9.2. Ethyl 2-(2-(5-benzylidene-2,4-dioxothiazolidin-3-yl) acetamido) acetate (36b)



A mixture of benzaldehyde (0.40 ml, 3.84 mmol) and ethyl 2-(2-(2,4-dioxothiazolidin-3-yl) acetamido) acetate (**46a**) (1.03 g, 3.84 mmol) in ethanol (20 ml) gave compound **36b** as a brown amorphous solid (0.30 g, 22%), m.p = 185.7-187.2 °C, **¹H NMR (400 MHz, DMSO-*d*₆)** δ_{H} (ppm) 8.78 (t, 1H, J = 5.6 Hz, H-5), 7.97 (s, 1H, H-11), 7.66 (d, 2H, J = 6.8 Hz, H-13), 7.56-7.53 (m, 3H, H-14+H-15), 4.35 (s, 2H, H-7), 4.09 (q, 2H, J = 6.8 Hz, H-2), 3.89 (d, 2H, J = 4.8 Hz, H-4), 1.20 (t, 3H, J = 6.8 Hz, H-1), **¹³C NMR (100 MHz, DMSO-*d*₆)** δ_{C} (ppm) 169.89 (C-10), 167.50 (C-8), 166.16 (C-6), 165.68 (C-3), 133.83 (C-11), 133.33 (C-15), 131.24 (C-12), 130.62 (C-14), 129.89 (C-13), 121.59 (C-9), 61.01 (C-2), 43.69 (C-7), 41.25 (C-4), 14.49 (C-1), **IR** (KBr cm^{-1}): 3210 (N-H), 3076 (ArC-H), 2983, 1745 (C=O), 1737 (C=O), 1724 (C=O), 1684 (C=O), 1186 (C-O). **HRMS** (ESI-TOF) m/z ; calculated for $\text{C}_{16}\text{H}_{16}\text{N}_2\text{O}_5\text{S}$: 348.3736, found (M)⁺: 347.9888.

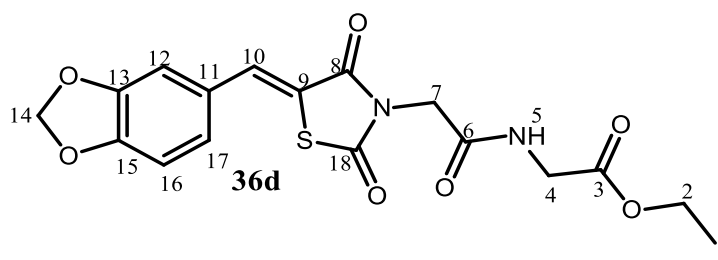
5.9.3. Ethyl 2-(2-(5-(3-hydroxy-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl) acetamido) acetate (36c)



A mixture of vanillin (0.58 g, 3.84 mmol) and ethyl 2-(2-(2,4-dioxothiazolidin-3-yl) acetamido) acetate (**46a**) (1.02 g, 3.84 mmol) in ethanol (20 ml) gave compound **36c** as a yellow amorphous solid (1.47 g, 97%), m.p = 233.4-234.8 °C, **¹H NMR (400 MHz, DMSO-*d*₆)** δ_{H} (ppm) 8.78 (t, 1H, J = 6.4 Hz, H-5), 7.86 (s, 1H, H-10), 7.20 (s, 1H, H-16), 7.12 (d, 1H, J = 8.0 Hz, H-13), 6.92 (d, 1H, J = 8.1 Hz, H-12), 4.33 (s, 2H, H-7), 4.09 (q, 2H, J = 6.8 Hz, H-2), 3.88 (d, 2H, J = 5.2 Hz, H-4), 3.82 (s, 3H, H-14), 1.20 (t,

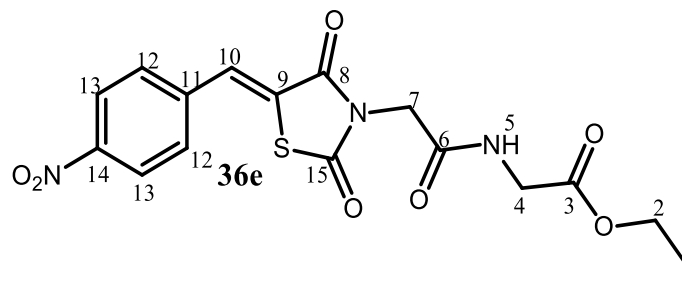
3H, $J = 7.2$ Hz, H-1), ^{13}C NMR (100 MHz, DMSO- d_6), δ_c (ppm) 169.92 (C-18), 167.70 (C-8), 166.28 (C-6), 165.81 (C-3), 151.14 (C-15), 148.67 (C-17), 134.67 (C-10), 125.04 (C-16), 124.16 (C-9), 116.85 (C-13), 116.60 (C-11), 114.73 (C-12), 61.01 (C-2), 56.07 (C-14), 43.54 (C-7), 41.25 (C-4), 14.48 (C-1), IR (KBr cm^{-1}): 3378 (O-H), 3294 (N-H), 2987 (ArC-H), 1742 (C=O), 1727 (C=O), 1677 (C=O), 1657 (C=O), 1168 (C-O), HRMS (ESI-TOF) m/z ; calculated for $\text{C}_{17}\text{H}_{18}\text{N}_2\text{O}_7\text{S}$: 394.3990, found (M) $^+$: 394.0719

5.9.4. Ethyl 2-(2-(5-(Benzo[*d*][1,3] dioxol-5-ylmethylene)-2,4-dioxothiazolidin-3-yl)acetamido)acetate (36d)



A mixture of piperonal (0.58 g, 3.84 mmol) and ethyl-2-(2-(2,4-dioxothiazolidin-3-yl)acetamido) acetate (**46a**) (1.01 g, 3.84 mmol) in ethanol (20 ml) gave compound **36d** as a yellow amorphous solid (0.51 g, 34%), m.p = 211.6-212.9 °C, ^1H NMR (400 MHz, DMSO- d_6) δ_H (ppm) 8.82 (t, 1H, $J = 5.7$ Hz, H-5), 7.94 (s, 1H, H-10), 7.28-7.25 (m, 2H, H-12+H-17), 7.17 (d, 1H, $J = 8.0$ Hz, H-16), 6.20 (s, 1H, H-14), 4.39 (s, 2H, H-7), 4.15 (q, 2H, $J = 7.1$ Hz, H-2), 3.93 (d, 2H, $J = 5.8$ Hz, H-4), 1.25 (t, 3H, $J = 7.1$ Hz, H-1), ^{13}C NMR (100 MHz, DMSO- d_6), δ_c (ppm) 169.90 (C-18), 167.46 (C-8), 166.20 (C-6), 165.73 (C-3), 150.05 (C-13), 133.91 (C-10), 127.47 (C-11), 126.64 (C-12), 118.97 (C-9), 109.80 (C-17), 109.70 (C-16), 102.56 (C-14), 61.00 (C-2), 43.63 (C-7), 41.25 (C-4), 14.48 (C-1), IR (KBr cm^{-1}): 3287 (N-H), 3094 (ArC-H), 1730 (C=O), 1682 (C=O), 1658 (C=O), 1623 (C=O), 1155 (C-O), HRMS (ESI-TOF) m/z ; calculated for $\text{C}_{17}\text{H}_{16}\text{N}_2\text{O}_7\text{S}$: 392.3831, found (M) $^+$: 392.0414

5.9.5. Ethyl 2-(2-(5-(4-nitrobenzylidene)-2,4-dioxothiazolidin-3-yl) acetamido) acetate (**36e**)

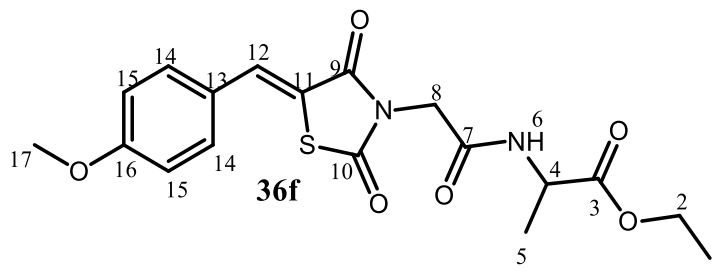


A mixture of nitrobenzaldehyde (0.58 g, 3.84 mmol) and ethyl-2-(2-(2,4-dioxothiazolidin-3-yl) acetamido) acetate (**46a**) (1.04g, 3.84 mmol) in ethanol (20 ml) gave compound **36e** as a white amorphous solid (1.57 g, 98%), m.p = 208.9-211.3 °C, $^1\text{H NMR}$ (400 MHz, DMSO-d_6) δ_{H} (ppm) 8.80 (t, 1H, $J = 6.0$ Hz, H-5), 8.36 (d, 2H, $J = 8.4$ Hz, H-13), 8.10 (s, 1H, H-10), 7.93 (d, 2H, $J = 8.4$ Hz, H-12), 4.37 (s, 2H, H-7), 4.10 (q, 2H, $J = 7.2$ Hz, H-7), 3.90 (d, 2H, $J = 5.2$ Hz, H-4), 1.20 (t, 3H, $J = 7.2$ Hz, H-1), $^{13}\text{C NMR}$ (100 MHz, DMSO-d_6) δ_{C} (ppm) 169.87 (C-15), 166.98 (C-8), 166.01 (C-6), 165.36 (C-3), 148.15 (C-14), 139.56 (C-11), 131.55 (C-13), 131.27 (C-10), 125.88 (C-9), 124.79 (C-12), 61.01 (C-2), 43.85 (C-7), 41.27 (C-4), 14.49 (C-1), IR (KBr cm^{-1}): 3291 (N-H), 3084 (ArC-H), 1744 (C=O), 1695 (C=O), 1661 (C=O), 1612 (C=O), 1154 (C-O), HRMS (ESI-TOF) m/z ; calculated for $\text{C}_{16}\text{H}_{15}\text{N}_3\text{O}_7\text{S}$: 393.3712, found $(\text{M}+\text{H})^+$: 394.6368

5.10. General procedure for synthesis of anti-diabetic drugs (**36**) having alanine as a linker via Knoevenagel condensation reaction

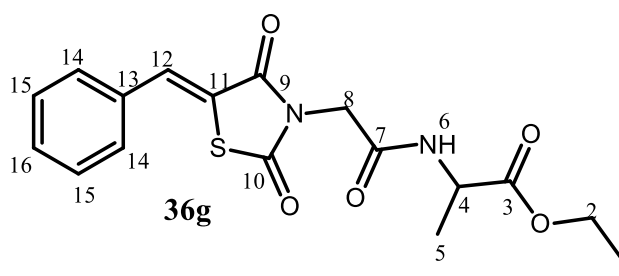
To a suspension of ethyl 2-(2-(2,4-dioxothiazolidin-3-yl) acetamido) propanoate (**46b**) (1 mmol), was added appropriate benzaldehyde (1 mmol) in ethanol followed by catalytic amounts of piperidine. The resultant mixture was heated under reflux for 12 hours. After being allowed to cool down to room temperature, the mixture was into ice water and the resultant precipitate was collected by filtration. The solids obtained were further recrystallized from water to afford the desired anti-diabetic drugs (**36f-j**).

5.10.1. Ethyl 2-(2-(5-(4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl) acetamido) propanoate (36f)



A mixture of anisaldehyde (0.45 ml, 3.65 mmol) and ethyl 2-(2-(2,4-dioxothiazolidin-3-yl) acetamido) propanoate (**46b**) (1.02 g, 3.65 mmol) in ethanol (20 ml) gave compound **36f** as a brown amorphous solid (1.34 g, 98%), m.p = 208.9-209.6 °C, **¹H NMR (400 MHz, DMSO-*d*₆)** δ_{H} (ppm) 8.77 (d, 1H, $J = 6.6$ Hz, H-6), 7.92 (s, 1H, H-12), 7.62 (d, 2H, $J = 8.3$ Hz, H-14), 7.12 (d, 2H, $J = 8.3$ Hz, H-15), 4.32 (s, 2H, H-8), 4.25 (quint, 1H, $J = 6.8$ Hz, H-4), 4.08 (q, 2H, $J = 7.8$ Hz, H-2), 3.84 (s, 3H, H-17), 1.30 (d, 3H, $J = 7.0$ Hz, H-5) 1.19 (t, 3H, $J = 6.9$ Hz, H-1), **¹³C NMR (100 MHz, DMSO-*d*₆)**, δ_{C} (ppm) 172.64 (C-10), 167.53 (C-9), 165.78 (C-7), 165.50 (C-3), 161.73 (C-16), 133.84 (C-12), 132.79 (C-15), 125.80 (C-13), 118.22 (C-9), 115.48 (C-14), 61.02 (C-2), 55.98 (C-17), 48.40 (C-4), 45.54 (C-8), 17.47 (C-5), 14.45 (C-1), **IR (KBr cm^{-1}):** 3296 (N-H), 3085 (ArC-H), 1733 (C=O), 1681 (C=O), 1659 (C=O), 1591 (C=O), 1183 (C-O), **HRMS (ESI-TOF) m/z;** calculated for C₁₈H₂₀N₂O₆S: 392.0720, found (M)⁺: 392.4262

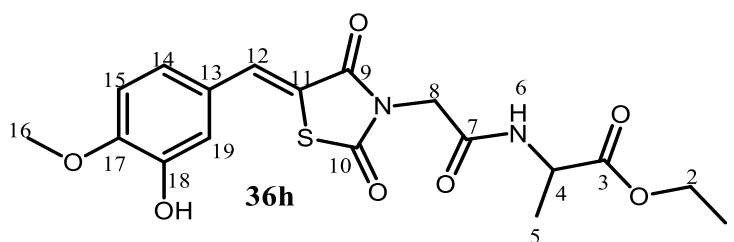
5.10.2. Ethyl 2-(2-(5-benzylidene-2,4-dioxothiazolidin-3-yl) acetamido) propanoate (36g)



A mixture of benzaldehyde (0.40 ml, 3.65 mmol) and ethyl 2-(2-(2,4-dioxothiazolidin-3-yl) acetamido) propanoate (**46b**) (1.05g, 3.65 mmol) in ethanol (20 ml) gave compound **36g** as a white amorphous solid (0.64 g, 50%), m.p = 166.8-167.4 °C, **¹H NMR (400 MHz, DMSO-*d*₆)** δ_{H} (ppm) 8.79 (d, 1H, $J = 6.8$ Hz, H-6), 7.96 (s, 1H, H-12), 7.65 (d, 2H, $J = 7.2$ Hz, H-14), 7.58-7.5 (m, 3H, H-15+H-16), 4.29 (s, 2H, H-8), 4.26

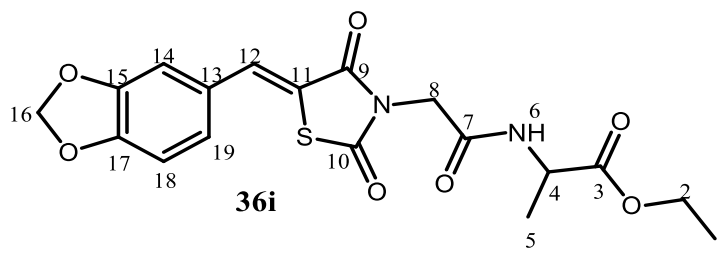
(quint, 2H, $J = 7.2$ Hz, H-4), 4.08 (q, 2H, $J = 6.2$ Hz, H-2), 1.30 (d, 3H, $J = 7.2$ Hz, CH₃) 1.18 (t, 3H, $J = 7.2$ Hz, CH₃), ¹³C NMR (100 MHz, DMSO-d₆), δ_c (ppm) 172.65 (C-10), 167.49 (C-9), 165.67 (C-7), 165.46 (C-3), 133.86 (C-12), 133.30 (C-13), 131.26 (C-16), 130.62 (C-15), 129.89 (C-14), 121.52 (C-11), 61.06 (C-2), 48.42 (C-4), 43.59 (C-8), 17.43 (C-5), 14.44 (C-1), IR (KBr cm⁻¹): 3287 (N-H), 3087 (ArC-H), 1734 (C=O), 1687 (C=O), 1660 (C=O), 1608 (C=O), 1149 (C-O), HRMS (ESI-TOF) m/z; calculated for C₁₇H₁₈N₂O₅S: 362.4002, found (M)⁺: 362.0108

5.10.3. Ethyl 2-(2-(5-(3-hydroxy-4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl) acetamido) propanoate (36h)



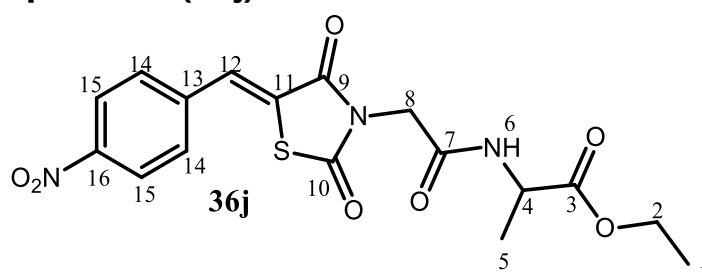
A mixture of vanillin (0.55g, 3.65 mmol) and ethyl 2-(2-(2,4-dioxothiazolidin-3-yl) acetamido) propanoate (**46b**) (1.03 g, 3.65 mmol) in ethanol (20 ml) gave compound **36h** as a yellow amorphous solid (0.88 g, 61%), m.p = 183.8-184.9 °C, ¹H NMR (400 MHz, DMSO-d₆) δ_H (ppm) 8.76 (d, 1H, $J = 6.9$ Hz, H-6), 7.87 (s, 1H, H-12), 7.22 (s, 1H, H-19), 7.12 (d, 1H, $J = 8.4$ Hz, H-15), 6.94 (d, 1H, $J = 8.0$ Hz, H-14), 4.25 (s, 2H, H-8), 4.24 (quint, 1H, $J = 7.2$ Hz, H-4), 4.07 (q, 2H, $J = 6.0$ Hz, H-2), 3.83 (s, 3H, H-16), 1.30 (d, 3H, $J = 7.6$ Hz, H-5) 1.18 (t, 3H, $J = 7.2$ Hz, H-1), ¹³C NMR (100 MHz, DMSO-d₆), δ_c (ppm) 172.66 (C-10), 167.64 (C-9), 165.81 (C-7), 165.56 (C-3), 150.25 (C-17), 148.49 (C-18), 134.58 (C-12), 124.77 (C-19), 124.69 (C-9), 117.14 (C-13), 116.71 (C-15), 114.71 (C-14), 61.04 (C-2), 56.10 (C-16), 48.40 (C-4), 43.47 (C-8), 17.45 (C-5), 14.44 (C-1), IR (KBr cm⁻¹): 3294 (N-H), 2954 (ArC-H), 1737 (C=O), 1720 (C=O), 1682 (C=O), 1654 (C=O), 1168 (C-O), HRMS (ESI-TOF) m/z; calculated for C₁₆H₂₀N₂O₇S: 408.4256, found (M)⁺: 408.1403

5.10.4. Ethyl 2-(2-(5-(benzo[d][1,3] dioxol-5-ylmethylene)-2,4-dioxothiazolidin-3-yl) acetamido) propanoate (36i)



A mixture of piperonal (0.55 g, 3.65 mmol) and ethyl 2-(2-(2,4-dioxothiazolidin-3-yl) acetamido) propanoate (**46b**) (1.01 g, 3.65 mmol) in ethanol (20 ml) gave compound **36i** as a yellow amorphous solid (0.49 g, 33%), m.p = 173.8-174.9 °C, **¹H NMR (400 MHz, DMSO-*d*₆)** δ_{H} (ppm) 8.77 (d, 1H, $J = 7.0$ Hz, H-6), 7.88 (s, 1H, H-12), 7.23-7.19 (m, 2H, H-19+H-14), 7.11 (d, 1H, $J = 8.0$ Hz, H-18), 6.15 (s, 2H, H-16), 4.31 (s, 2H, H-8), 4.25 (quint, 1H, $J = 7.2$, H-4), 4.08 (q, 2H, $J = 6.4$ Hz, H-2), 1.29 (d, 3H, $J = 7.2$, H-5) 1.18 (t, 3H, $J = 6.8$ Hz, H-1), **¹³C NMR (100 MHz, DMSO-*d*₆)**, δ_{C} (ppm) 172.64 (C-10), 167.43 (C-9), 165.72 (C-7), 165.49 (C-3), 150.05 (C-17), 148.70 (C-15), 133.93 (C-12), 127.46 (C), 126.65 (C-14), 118.93 (C), 109.80 (C-19), 109.71 (C-18), 102.56 (C-16), 61.04 (C-2), 48.41 (C-4), 43.54 (C-8), 17.44 (C-5), 14.44 (C-1), **IR (KBr cm^{-1}):** 3315 (N-H), 2998 (ArC-H), 1736 (C=O), 1680 (C=O), 1654 (C=O), 1610 (C=O), 1156 (C-O), **HRMS (ESI-TOF) m/z :** calculated for $\text{C}_{18}\text{H}_{18}\text{N}_2\text{O}_7\text{S}$: 406.4097, found (M)⁺: 406.0552

5.10.5. Ethyl 2-(2-(5-(4-nitrobenzylidene)-2,4-dioxothiazolidin-3-yl) acetamido) propanoate (36j)



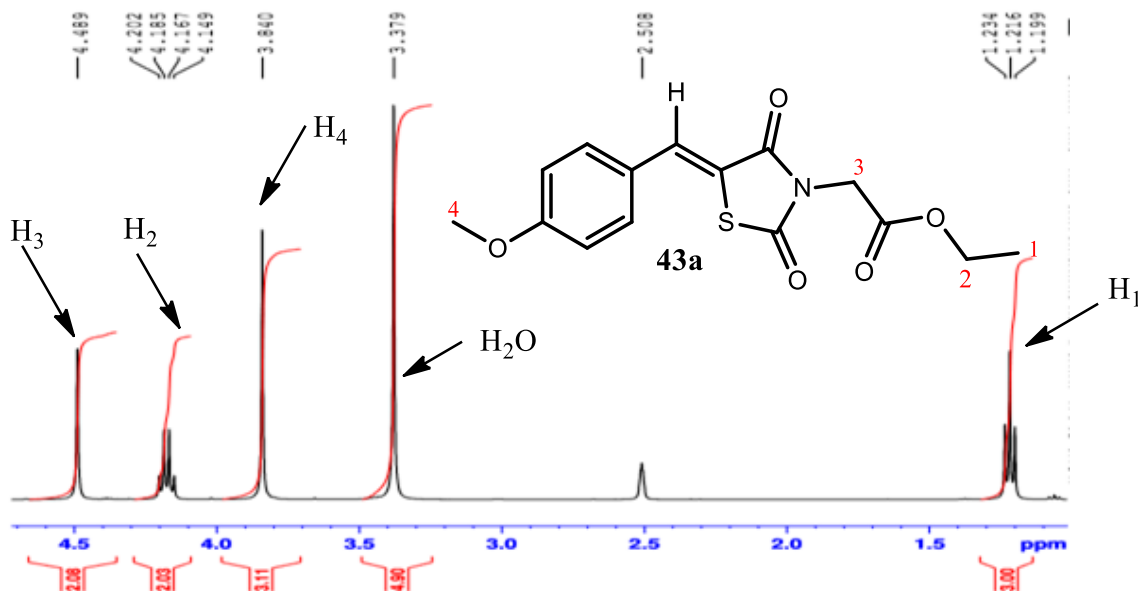
A mixture of nitrobenzaldehyde (0.55 g, 3.65 mmol) and ethyl 2-(2-(2,4-dioxothiazolidin-3-yl) acetamido) propanoate (**46b**) (1.02 g, 3.65 mmol) in ethanol (20 ml) gave compound **36j** as a brown amorphous solid (1.31 g, 90%), m.p = 223.9-224.7 °C, **¹H NMR (400 MHz, DMSO-*d*₆)** δ_{H} (ppm) 8.88 (d, 1H, $J = 6.9$ Hz, H-6), 8.43 (d, 2H, $J = 8.8$ Hz, H-15), 8.16 (s, 1H, H-12), 7.99 (d, 2H, $J = 8.4$ Hz, H-14), 4.42 (s, 2H, H-8),

4.34 (quint, 1H, $J = 7.2$ Hz, H-4), 4.16 (q, 2H, $J = 6.0$ Hz, H-2), 1.38 (d, 3H, $J = 7.3$ Hz, H-5), 1.29 (t, 3H, $J = 7.1$ Hz, H-1), **^{13}C NMR (100 MHz, DMSO- d_6)**, δ_{C} (ppm) 172.61 (C-10), 166.97 (C-9), 165.35 (C-7), 165.31 (C-3), 148.14 (C-16), 139.55 (C-13), 131.56 (C-15), 131.30 (C-12), 126 (C-11), 125.84 (C-14), 61.05 (C-2), 48.43 (C-4), 43.77 (C-8), 17.45 (C-5), 14.44 (C-1), **IR** (KBr cm^{-1}): 3325 (N-H), 3035 (ArC-H), 1760 (C=O), 1737 (C=O), 1687 (C=O), 1671 (C=O), 1159 (C-O), **HRMS** (ESI-TOF) m/z ; calculated for $\text{C}_{17}\text{H}_{17}\text{N}_3\text{O}_7\text{S}$: 407.3978, found (M) $^+$: 407.078

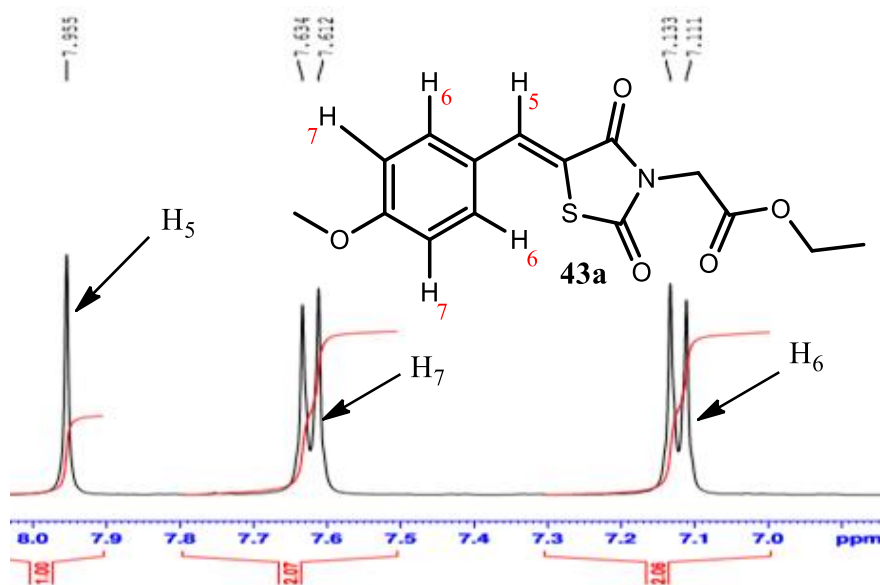
CHAPTER-SIX

6.1. Appendix: Selected NMR Spectra

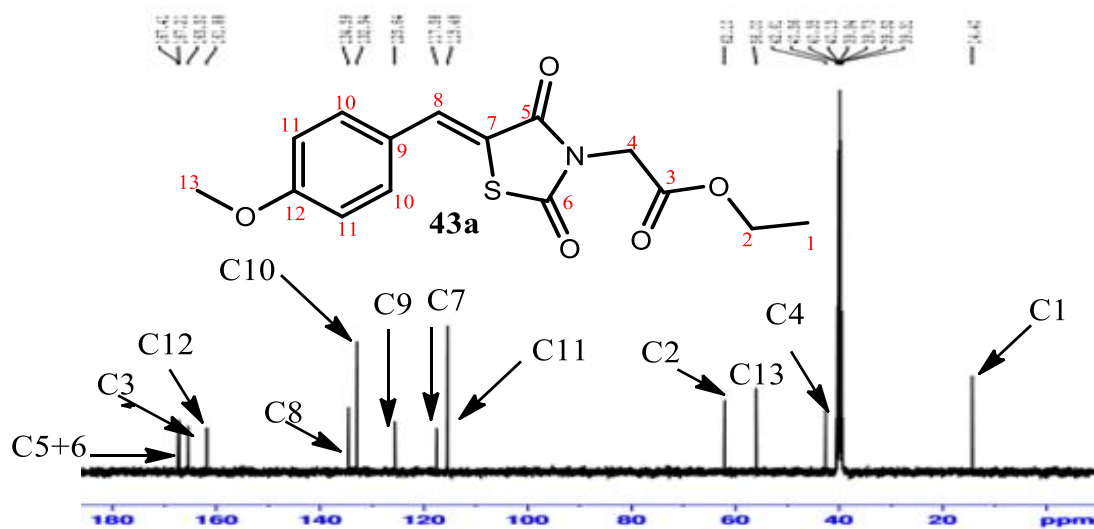
6.1.1. ¹H NMR spectrum (aliphatic expansion) of ethyl 2-(5-(4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl) acetate (43a)



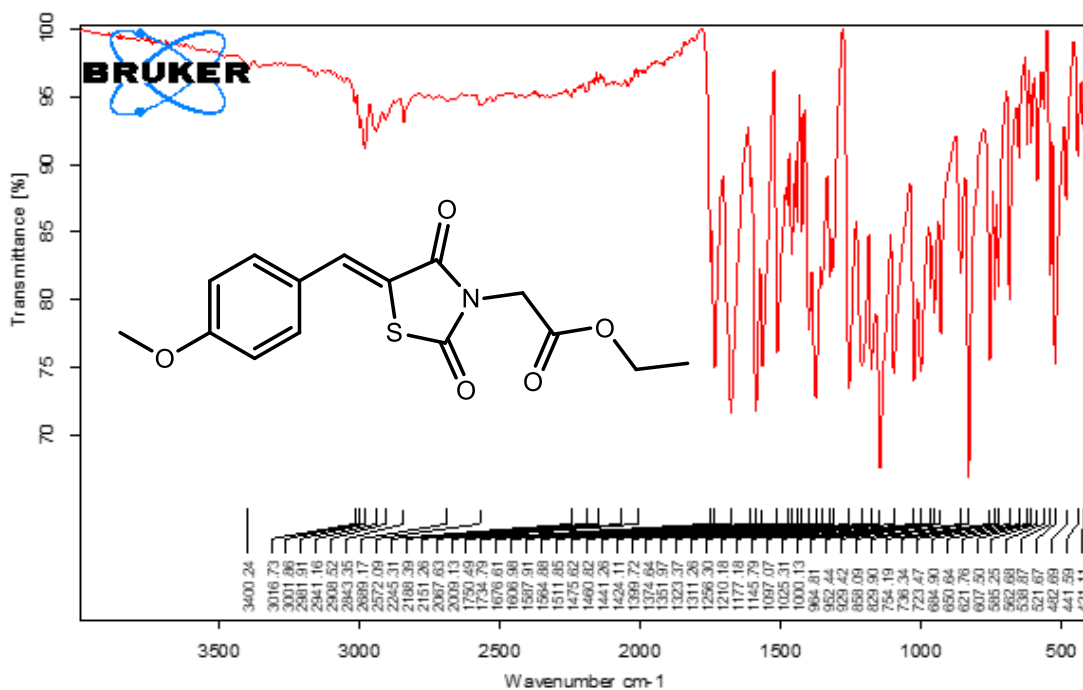
6.1.2. ¹H NMR spectrum (aromatic expansion) of ethyl 2-(5-(4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl) acetate (43a)



6.1.3. ¹³C NMR spectrum of ethyl 2-(5-(4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl) acetate (43a)

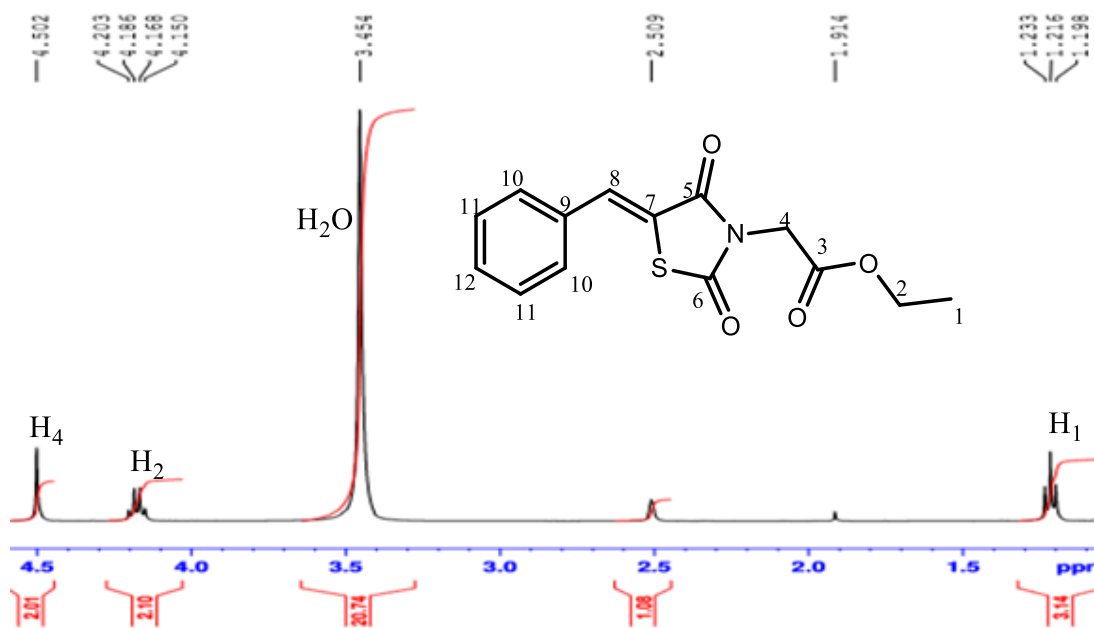


6.1.4. IR spectrum of ethyl 2-(5-(4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl) acetate (43a)

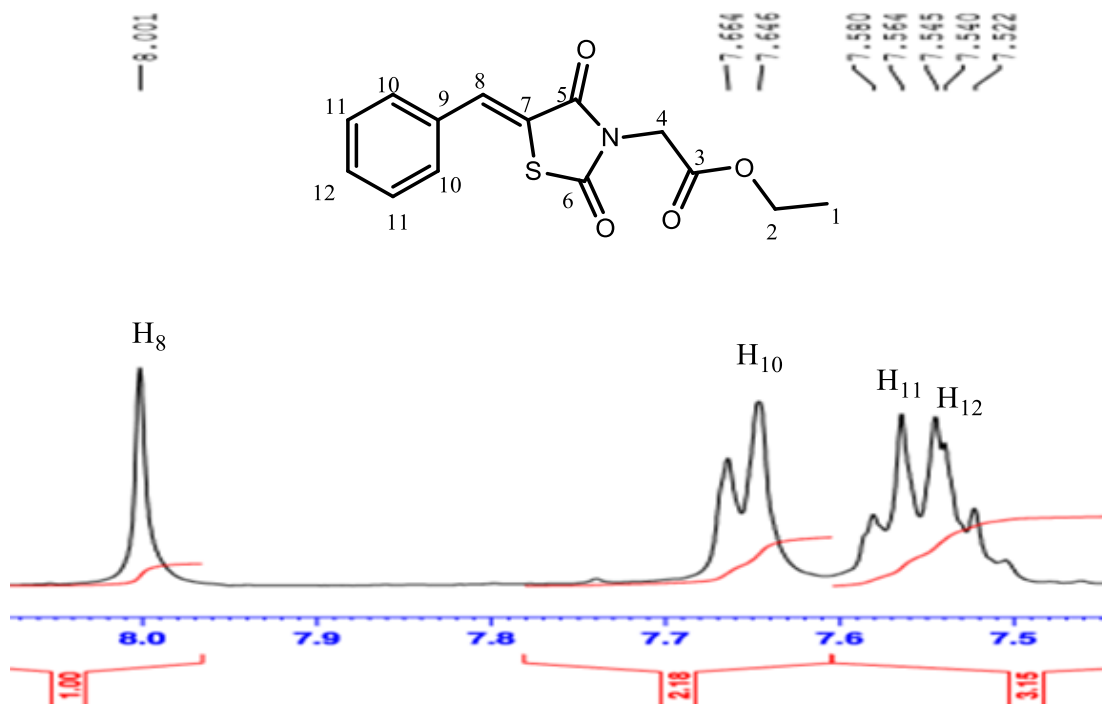


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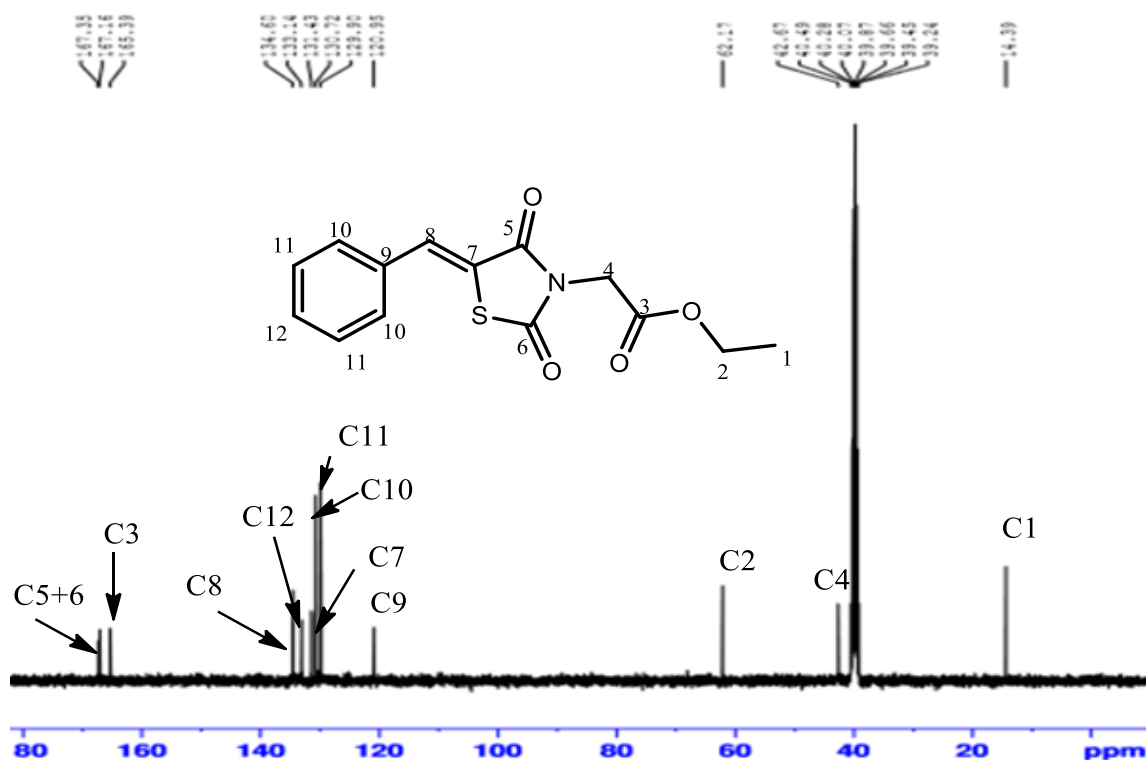
6.1.5. ¹H NMR spectrum (aliphatic expansion) of ethyl 2-(5-(benzylidene)-2,4-dioxothiazolidin-3-yl) acetate (43c)



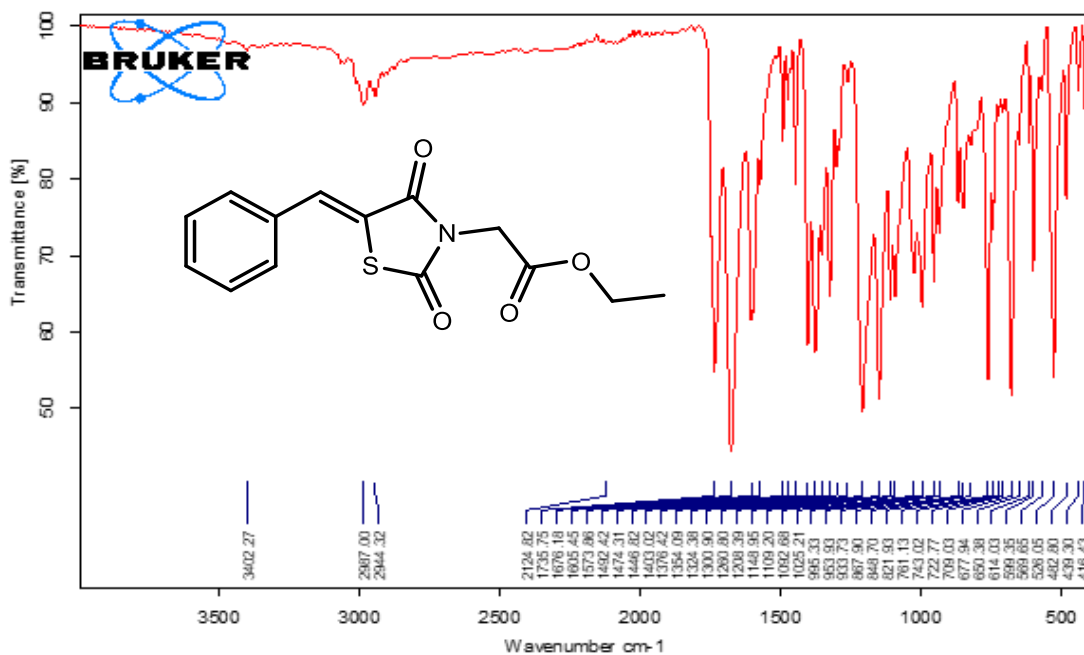
6.1.6. ¹H NMR (aromatic expansion) of ethyl 2-(5-(benzylidene)-2,4-dioxothiazolidin-3-yl) acetate (43c)



6.1.7. ¹³C NMR spectrum of ethyl 2-(5-(benzylidene)-2,4-dioxothiazolidin-3-yl) acetate (43c)

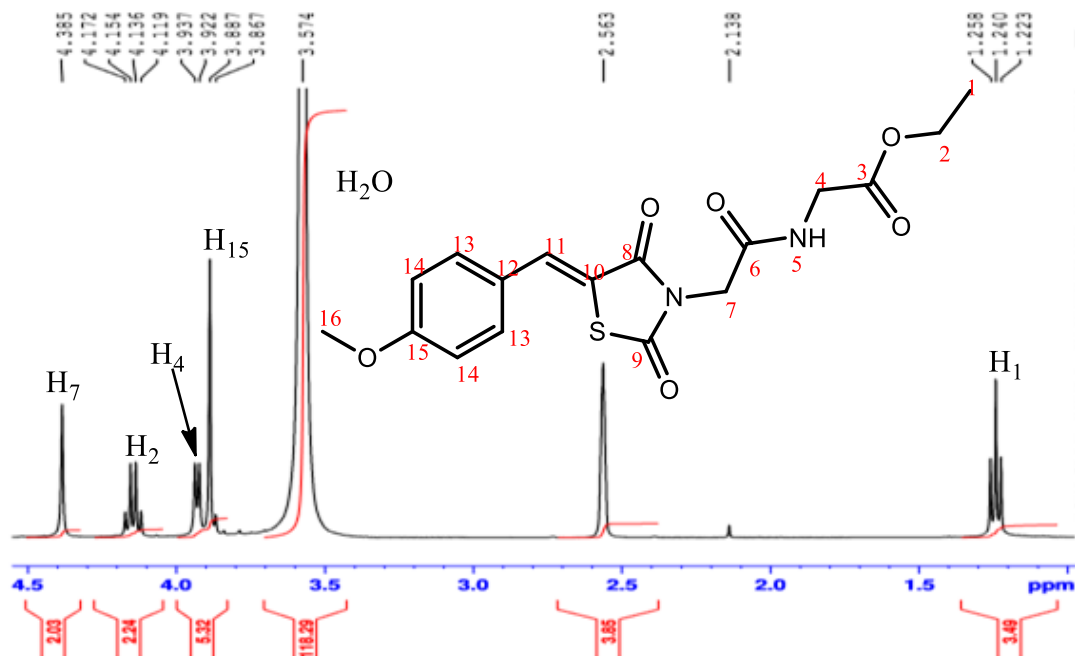


6.1.8. IR of spectrum of ethyl 2-(5-(benzylidene)-2,4-dioxothiazolidin-3-yl) acetate (43c)

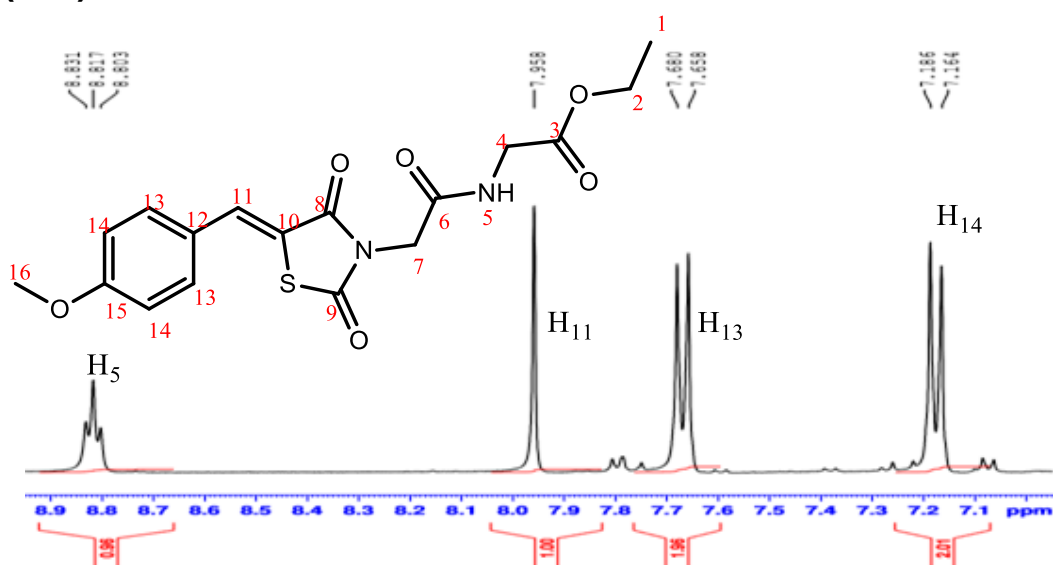


C:\OPUS_7.0.129\MEAS\NR 23A.0	NR 23A	Instrument type and / or accessory	8/18/2017
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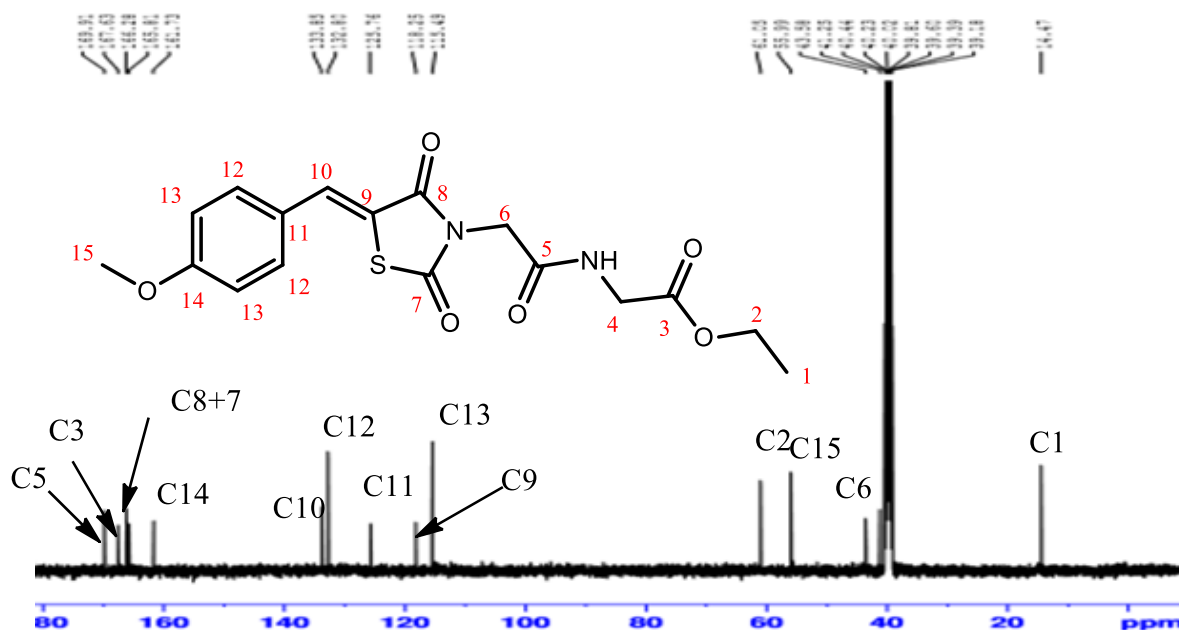
6.1.9. ¹H NMR (aliphatic expansion) of ethyl 2-(2-(5-(4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl) acetamido) acetate (36a)



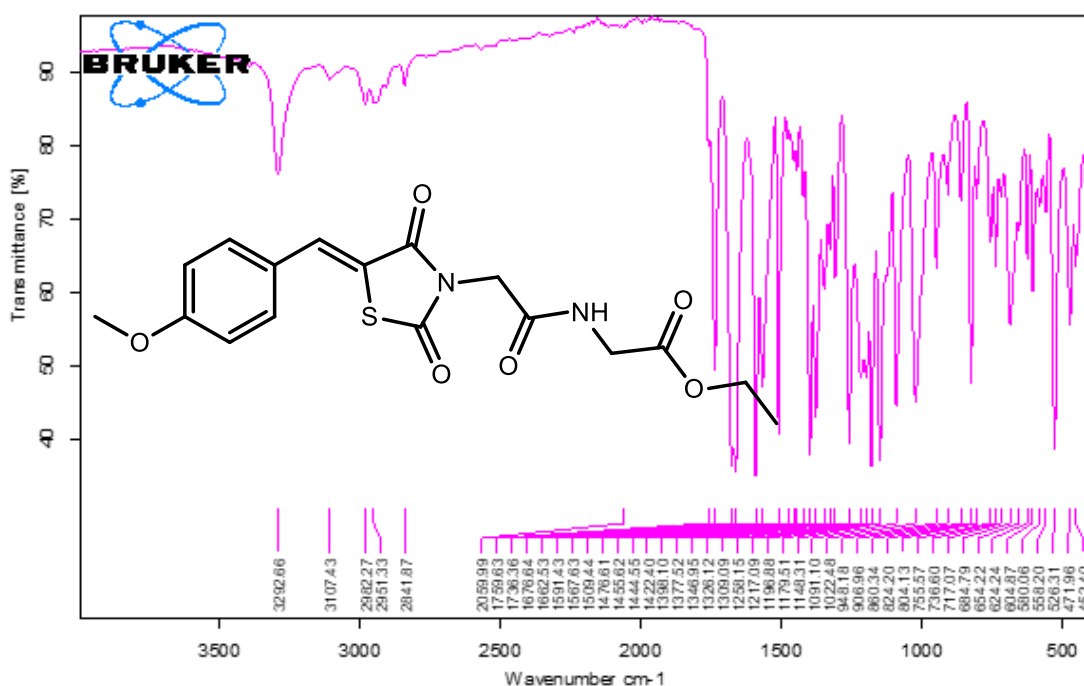
6.1.10. ¹H NMR (aromatic expansion) of ethyl 2-(2-(5-(4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl) acetamido) acetate (36a)



6.1.11. ¹³C NMR spectrum of ethyl 2-(2-(5-(4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl) acetamido) acetate (36a)



6.1.12. IR spectrum of ethyl 2-(2-(5-(4-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl) acetamido) acetate (36a)



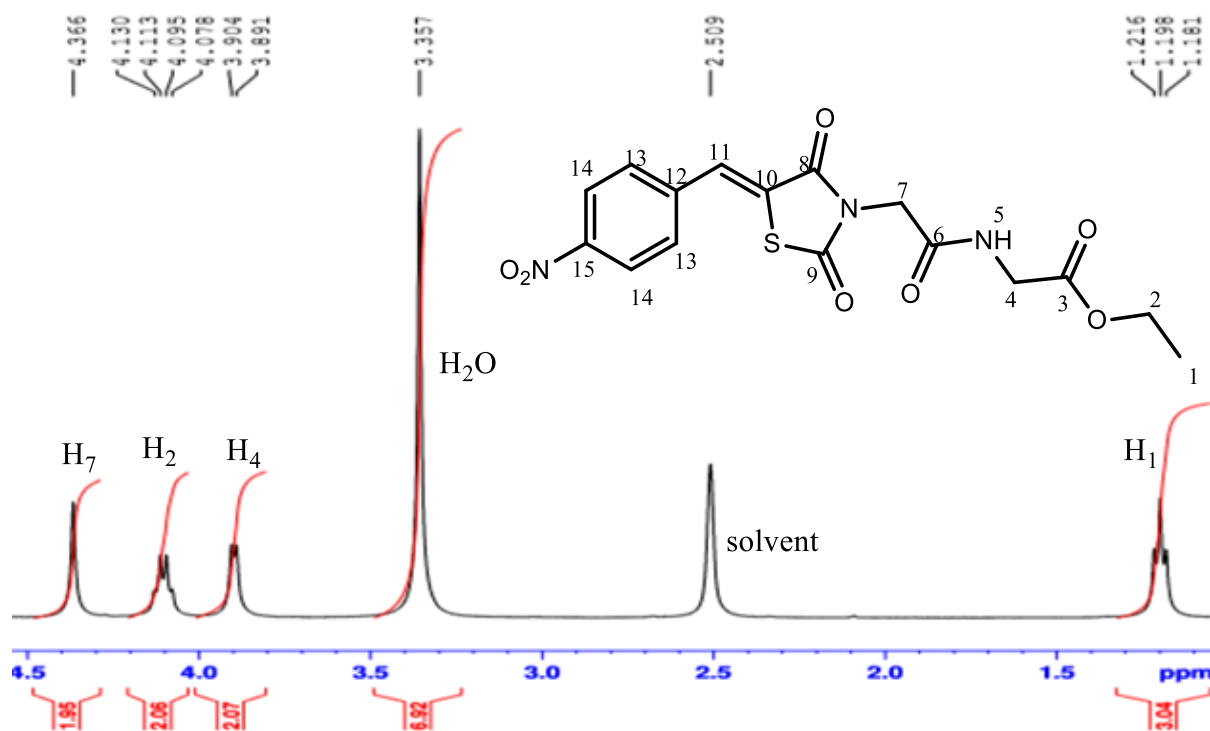
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NR 41

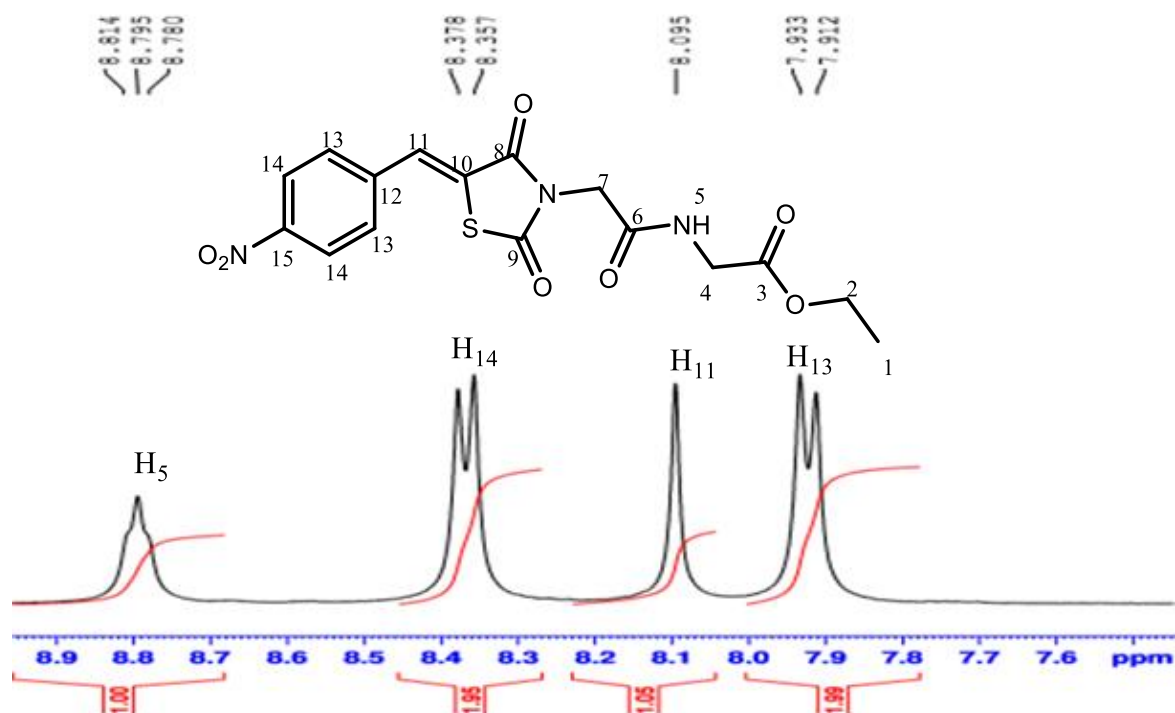
Instrument type and / or accessory

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6.1.13. ¹H NMR spectrum (aliphatic expansion) of ethyl 2-(2-(5-(4-nitro benzylidene)-2,4-dioxothiazolidin-3-yl) acetamido) acetate (36e)

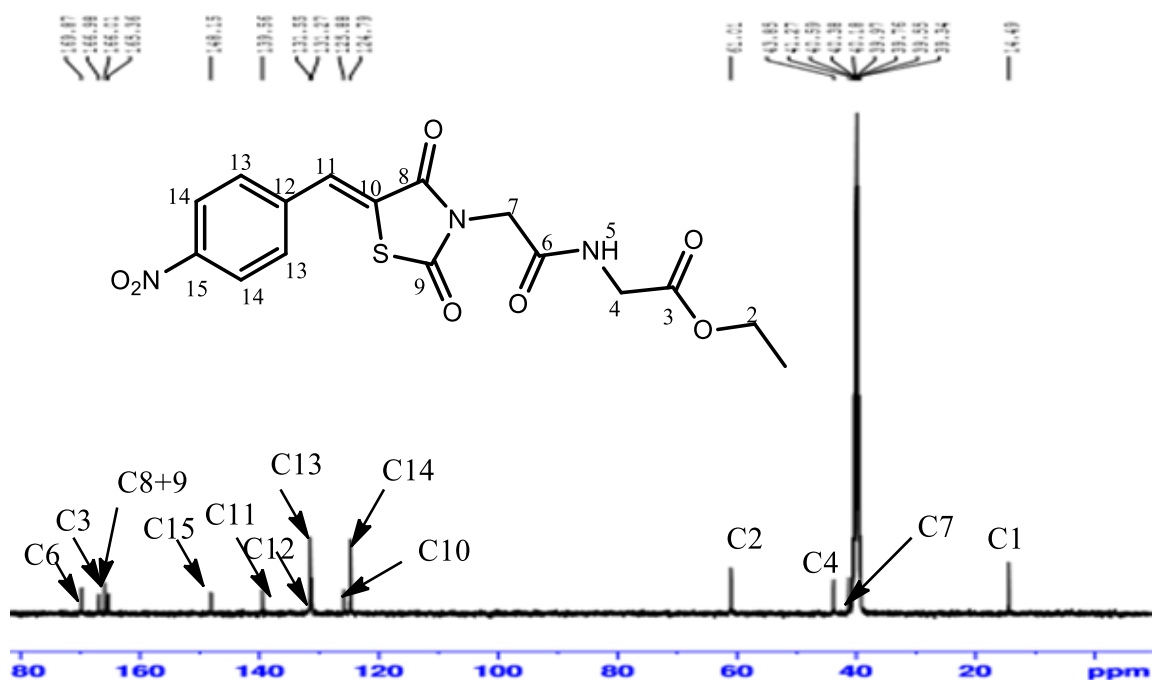


6.1.14. ¹H NMR spectrum (aromatic expansion) of ethyl 2-(2-(5-(4-nitrobenzylidene)-2,4-dioxothiazolidin-3-yl) acetamido) acetate (36e)

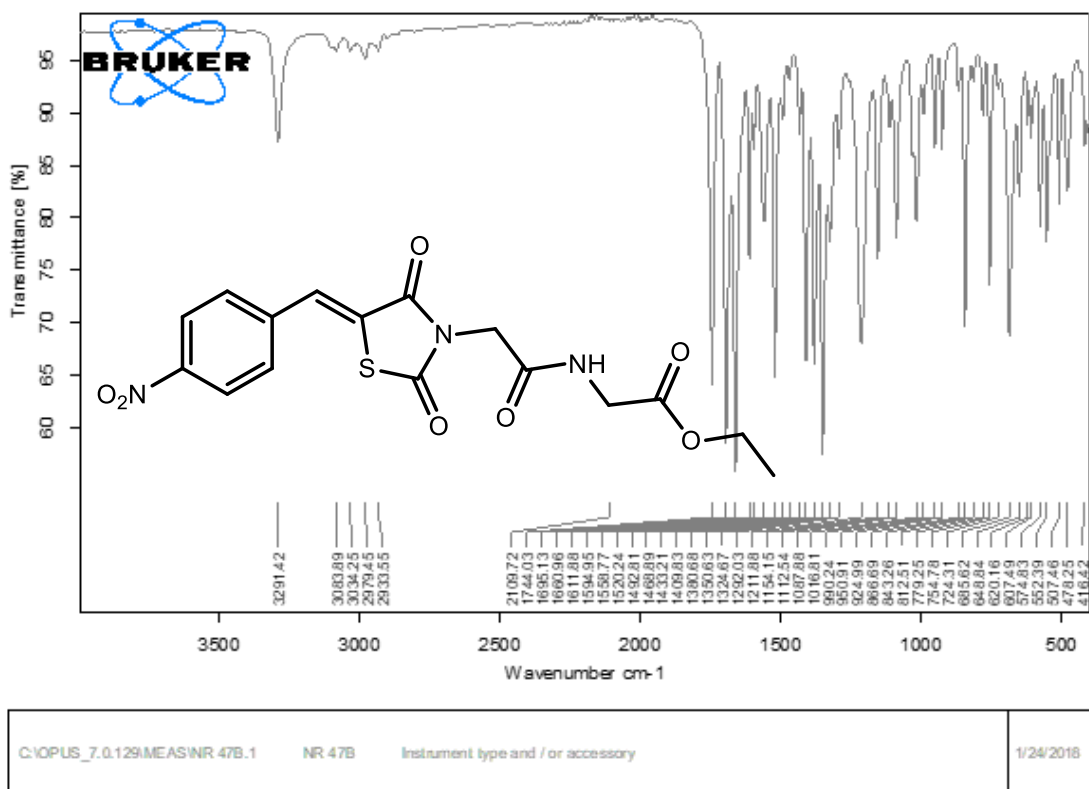


6.1.15. ¹³C NMR spectrum of ethyl 2-(2-(5-(4-nitrobenzylidene)-2,4-dioxothiazolidin-3-yl) acetamido) acetate (36e)

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6.1.16. IR spectrum of ethyl 2-(2-(5-(4-nitrobenzylidene)-2,4-dioxothiazolidin-3-yl) acetamido) acetate (36e)



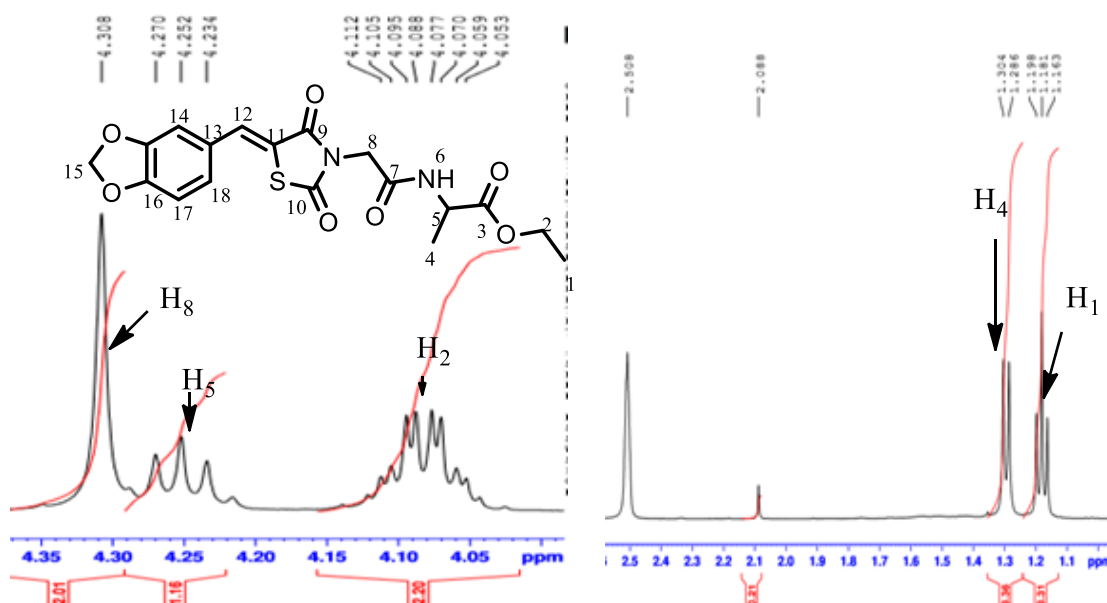
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NR 47B

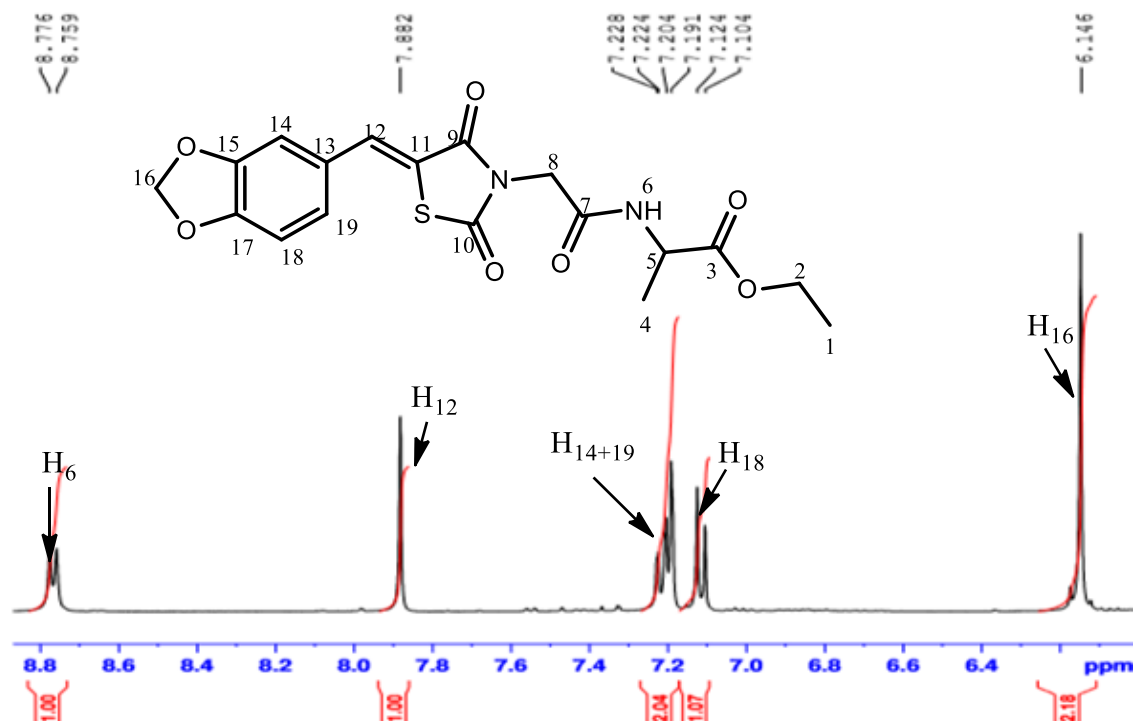
Instrument type and / or accessory

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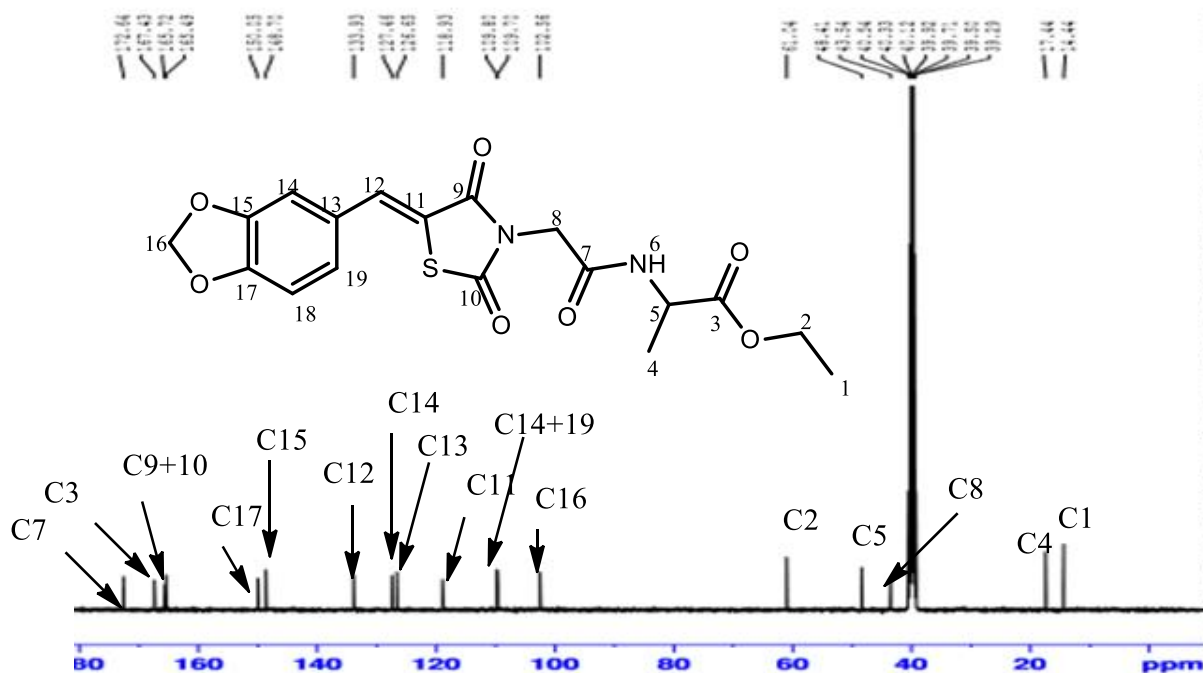
6.1.17. ¹H NMR spectrum (aliphatic expansion) of ethyl 2-(2-(5-(benzo[d][1,3]dioxol-5-ylmethylene)-2,4-dioxothiazolidin-3-yl)acetamido)propanoate (36i)



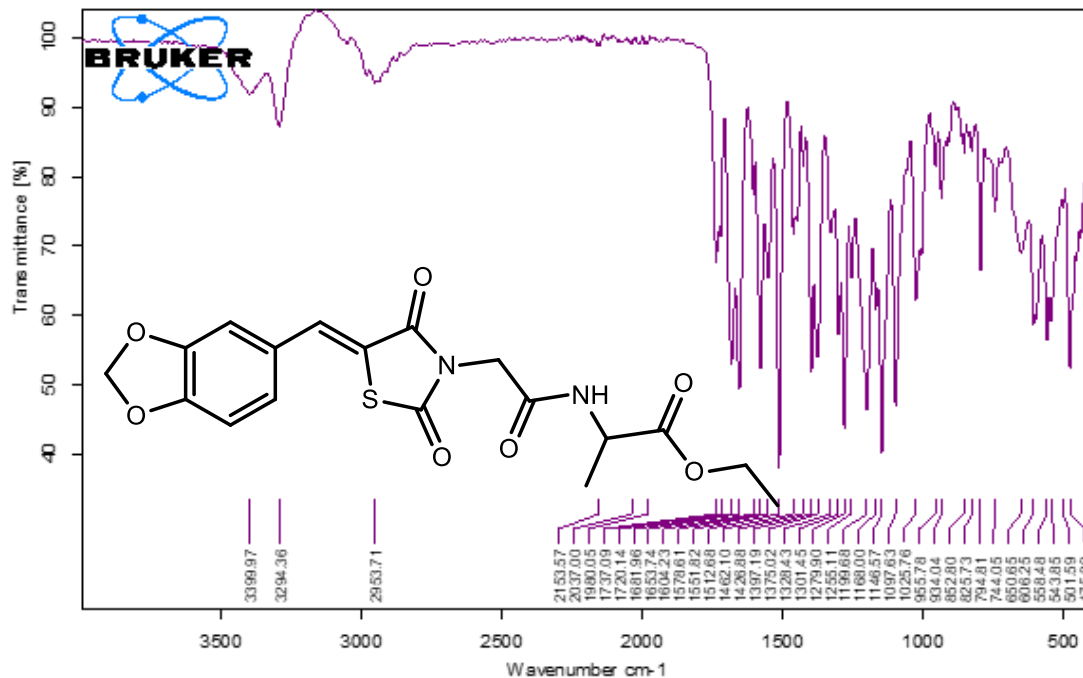
6.1.18. ¹H NMR spectrum (aromatic expansion) of ethyl 2-(2-(5-(benzo[d][1,3]dioxol-5-ylmethylene)-2,4-dioxothiazolidin-3-yl)acetamido)propanoate (36i)



6.1.19. ¹³C NMR spectrum of ethyl 2-(2-(5-(benzo[d][1,3]dioxol-5-ylmethylene)-2,4-dioxothiazolidin-3-yl)acetamido)propanoate (36i)

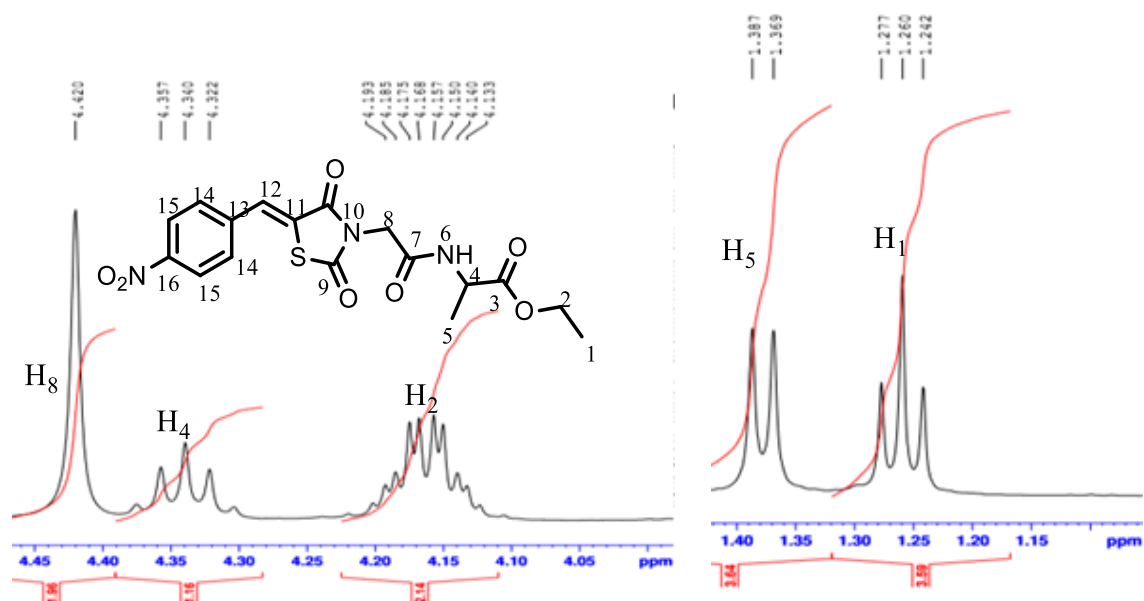


6.1.20. IR spectrum of ethyl 2-(2-(5-(benzo[d][1,3]dioxol-5-ylmethylene)-2,4-dioxothiazolidin-3-yl)acetamido)propanoate (36i)

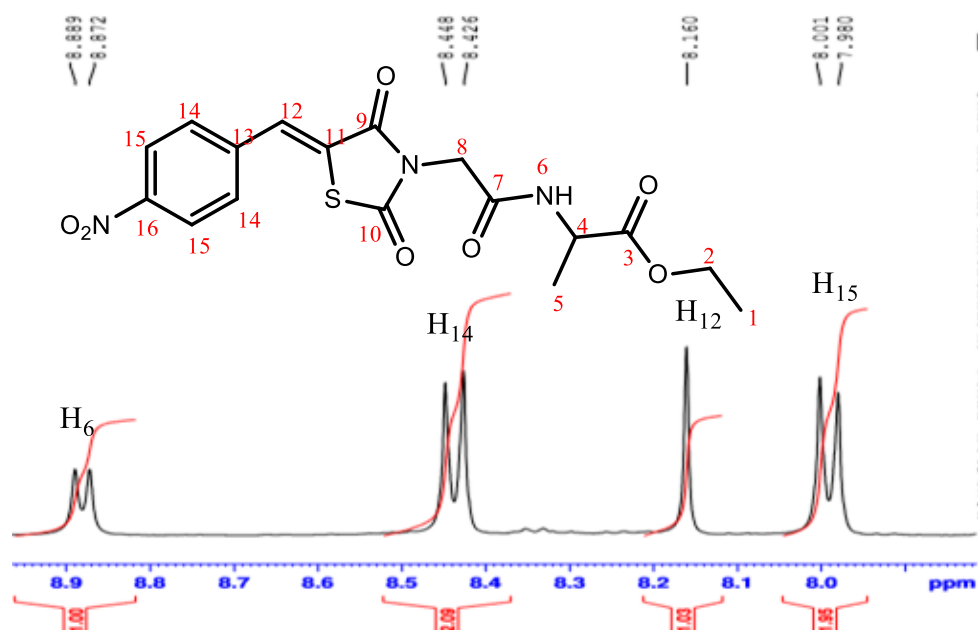


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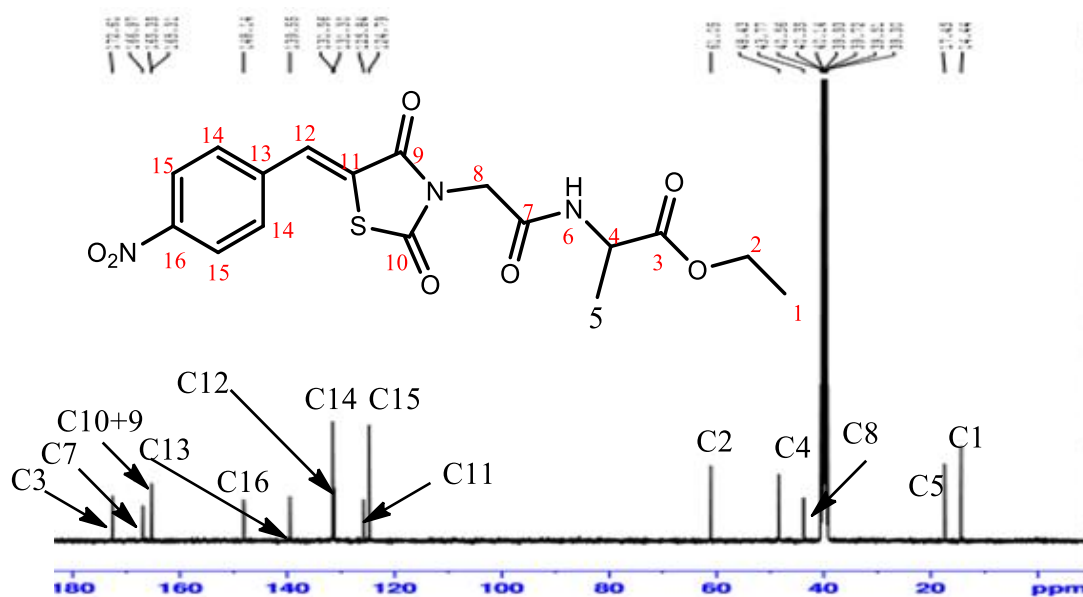
6.1.21. ¹H NMR spectrum (aliphatic expansion) of ethyl 2-(2-(5-(4-nitrobenzylidene)-2,4-dioxothiazolidin-3-yl) acetamido) propanoate (36j)



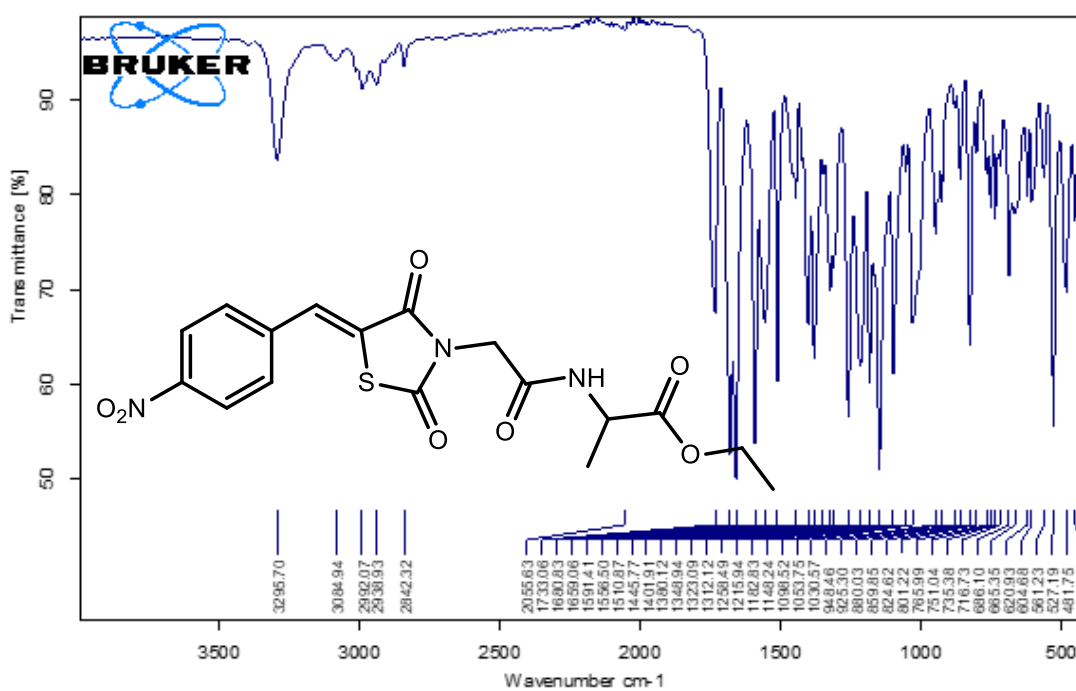
6.1.22. ¹H NMR spectrum (aromatic expansion) of ethyl 2-(2-(5-(4-nitrobenzylidene)-2,4-dioxothiazolidin-3-yl) acetamido) propanoate (36j)



6.1.23. ¹³C NMR spectrum of ethyl 2-(2-(5-(4-nitrobenzylidene)-2,4-dioxothiazolidin-3-yl) acetamido) propanoate (36j)



6.1.24. IR spectrum of ethyl 2-(2-(5-(4-nitrobenzylidene)-2,4-dioxothiazolidin-3-yl) acetamido) propanoate (36j)



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