

**DESIGN, SYNTHESIS AND EVALUATION OF HETEROCYCLIC
COMPOUNDS AS POTENTIAL ANTIDIABETIC AGENTS**

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

**in
Pharmaceutical Chemistry**

**By
Anuradha**

Registration Number. 12208835

**Supervised by
Dr. Amit Mittal (31641)
(Professor)**

**Department of Pharmaceutical Chemistry
School of Pharmaceutical Sciences**



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DECLARATION

I, hereby declared that the presented work in the thesis entitled **“Design, Synthesis and Evaluation of Heterocyclic Compounds as Potential Antidiabetic Agents”** In fulfilment of degree of **Doctor Of the Philosophy (Ph.D.)** is outcome of research work carried out by me under the supervision Dr. Amit Mittal, working as Professor, in the Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other university or institute for the award of any degree.

Anuradha

(Signature of Scholar)

Anuradha (12208835)

Department of Pharmaceutical Chemistry

School of Pharmaceutical Sciences,

Lovely Professional University,

Punjab, India

CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled **“Design, Synthesis and Evaluation of Heterocyclic Compounds as Potential Antidiabetic Agents”** submitted in fulfillment of the requirement for the award of degree of the **Doctor of Philosophy (Ph.D.)** in the Department of Pharmaceutical chemistry, School of Pharmaceutical sciences, is a research work carried out by Anuradha (12208835), is bonafide record of her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.



(Signature of Supervisor)

Dr. Amit Mittal (31641)

Professor

Department of Pharmaceutical Chemistry

School of Pharmaceutical Sciences

Lovely Professional University

Punjab-144411, India

ABSTRACT

Type 2 diabetes mellitus (T2DM) is a significant, progressive metabolic disorder triggered by impaired β -cell glucose sensing, insulin secretion, and defects in hepatic glucose regulation. Modern treatments improve glycemia but often fail to restore physiological glucose sensing or are limited by safety, endurance, and off-target liabilities. Glucokinase (GK), a liver/cell glucose sensor, is a desirable target; allosteric stabilization of the active form of GK can enhance insulin secretion in response to glucose and hepatic glucose metabolism. However, many published GKAs (Glucokinase activators) are associated with hypoglycemia, metabolic instability, or lack of specificity. In this study, we aimed to synthesize a targeted library of coumarin-based heterocycles (AM1-AM28) to identify potent and safe GK activators capable of being shown to have *in vitro* and *in vivo* activities.

Crystallographic and pharmacophore studies have revealed that the GK allosteric pocket interacts with the amino acid residues Arg63, Tyr61, Thr65, and Tyr214. We used a privileged-fragment-merging strategy to generate two complementary structural series and attempted to develop a structure-activity relationship (SAR) based on open chain, cyclic linker, electronic factors, steric factors, lipophilicity, and hydrogen bonding. Series I, hydrazide linker (AM1-AM14) and Series II, oxadiazole linker (AM15-AM28) to permit a conformational change into the allosteric cleft and to impose the extent of substituent positional effects and lipophilicity on the engagement and pharmacology of GK. These were mapped in each series by systematically varying aryl tail substituents (*ortho/meta/para*; electron-donating and electron-withdrawing groups).

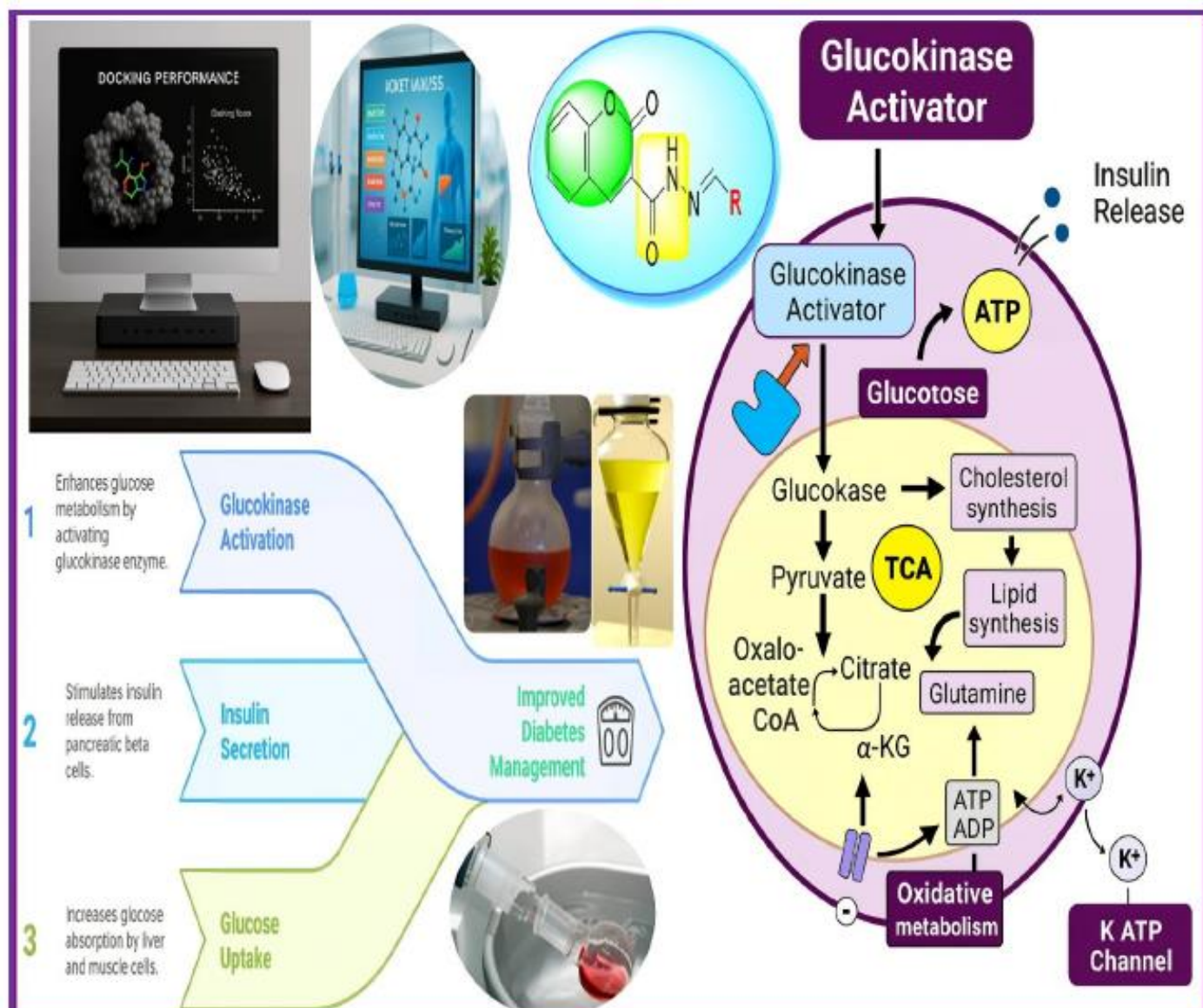
Both series were designed using concise, common coumarin substrates: a linker (hydrazide condensation) to form AM1-AM14 and oxadiazole analogues AM15-AM28 and the tail part. All molecules were characterized using spectroscopic techniques (FT-IR, ^1H -NMR, ^{13}C -NMR, and high-resolution mass spectrometry (HRMS)). The EC_{50} and % activation of all synthesized compounds were determined using a standardized coupled enzyme reaction (GK + G6PDH; NADPH readout at 340 nm). In the entire dataset, it was evident that there was a clear differentiation throughout the library; many compounds exhibited single-digit EC_{50} and good percent activation when compared with the standard. It is noteworthy, AM18 with EC_{50} value and % enzyme activation (3.51 μM ; 156.18%), AM21 (3.63 μM , 148.72%), AM22 (3.77 μM , 151.74%), AM10 (4.08 μM , 137.46%), and AM16 (4.56 μM , 144.93%) were the potential

activators. A reference, Dorzagliatin, was used under identical assay conditions had a significantly larger EC_{50} (19.82 μ M) and reduced the percentage activation (120.11%), which is an indication of the better activation strength of the synthesized molecules.

The top ten shortlisted compounds, selected based on their low EC_{50} values and high % activation, were subjected to the MTT assay to determine their cell viability. The results revealed acceptable safety margins for the synthesized molecules. AM18 had a relatively high safety profile in the MTT assay (IC_{50} AM18 $\approx$ 97.9 μ g/mL), implying low cytotoxicity, and AM20 exhibited the same level of safety margin (IC_{50} AM20 $\approx$ 104.5 μ g/mL). AM18 and AM20 were subjected to *in vivo* testing based on the collective evaluation of the potency and safety of the cells. STZ-induced diabetic rats were orally (100 mg/kg) using an oral glucose tolerance test (OGTT) was performed. The two compounds achieved time-dependent and statistically significant decreases in blood glucose levels at 30, 60, 90, and 120 min compared to the reference and performed better under the same conditions. *In vivo* results reflected the *in vitro* potency of AM18, and AM20 elicited an antihyperglycemic effect, which coincided with its higher docking energy and better activation of the enzyme. A comparative analysis of the SAR in the two series showed that oxadiazole linkers exhibited lower EC_{50} and higher percent activation than the corresponding hydrazide linkers, indicating that tail orientation is beneficial to the allosteric pocket. *Ortho*-substitution (small polar or halogen groups) generally led to an increase in the potency of the lead, suggesting that the directional dipole/H-bonding of the ligand to the allosteric pocket is driven by a favorable orientation/interaction pose.

The top-ranking compounds and rationalization of the trends of activities were performed using molecular docking (AutoDock Vina with PDB:1V4S). The most favorable predicted free energies were found in Series II, with the best docking score (-11.0 kcal/mol) in AM18 and productive polar and hydrophobic interactions with active allosteric residues. However, the binding affinities of the other oxadiazoles (AM21 and AM22) and hydrazides (AM10 and AM4) were strongly predicted. DFT and ΔE analyses showed that compounds with moderate electronic softness (e.g., AM14, AM18, and AM25) have good charge transfer properties for stabilizing the active state of GK. The experimental results were then compared with the consequent *in silico* analysis, which aided in the exploration of SAR. However, it is noteworthy that the *in silico* predictions matched well with the *in vitro* results, demonstrating that the experimental and computational results were synchronized.

GRAPHICAL ABSTRACT



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LIST OF ABBREVIATIONS

Abbreviation/Symbol	Description
ACC	Acetyl-CoA Carboxylase
ADME	Absorption, Distribution, Metabolism, Excretion
ADP	Adenosine Diphosphate
AMP	Adenosine Monophosphate
AMPK	AMP-Activated Protein Kinase
ANOVA	Analysis of Variance
ARG	Arginine
AUC	Area Under Curve
Å	Angstrom
β	Beta
β -cells	Beta cells of pancreas
°C	Degree Centigrade
BBB	Blood Brain Barrier
BAX	BCL2-Associated X Protein
BG	Blood Glucose
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
CSIR	Council of Scientific & Industrial Research
DFT	Density Functional Theory
DGAT	Diacylglycerol O-Acyltransferase
DIO	Diet-Induced Obesity
DM	Diabetes Mellitus
DMSO	Dimethyl Sulfoxide
DPP	Dipeptidyl Peptidase
EC ₅₀	Half-Maximal Effective Concentration
EDG/EWG	Electron-donating group/Electron-withdrawing group
EMAX	Maximum Effect
Et ₃ N	Triethylamine
FT-IR	Fourier Transform Infrared Spectroscopy

g/mol	Gram per Mole
GI	Glycemic Index
GK	Glucokinase
GKA	Glucokinase Activator
GKRP	Glucokinase Regulatory Protein
GLP-1	Glucagon-Like Peptide 1
GI	Glycemic Index
HBA	Hydrogen Bond Acceptor
HBD	Hydrogen Bond Donor
HOMO	Highest Occupied Molecular Orbital
H-bond	Hydrogen Bond
HRMS	High-Resolution Mass Spectrometry
IC ₅₀	Half-Maximal Inhibitory Concentration
IGF-1	Insulin-Like Growth Factor-1
IR	Insulin Resistance
IRB	Institutional Review Board
IV	Intravenous
KATP	ATP-Sensitive Potassium Channel
LD ₅₀	Lethal Dose 50
Log S	Logarithmic Solubility
LUMO	Lowest Unoccupied Molecular Orbital
MD	Molecular Dynamics
MP	Melting Point
MS	Mass Spectrometry
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
MTD	Maximum Tolerated Dose (used in toxicity table)
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NMR	Nuclear Magnetic Resonance

OGTT	Oral Glucose Tolerance Test
ORAT	Oral Rat Acute Toxicity
ppm	Parts Per Million
R_f	Retention Factor
RMSD	Root Mean Square Deviation
STZ	Streptozotocin
SAR	Structure-Activity Relationship
SEM	Standard Error of the Mean
$T_{1/2}$	Half-Life
TLC	Thin-Layer Chromatography
UV	Ultraviolet Spectroscopy
V _{max}	Maximum Velocity
XRD	X-Ray Diffraction

CHAPTER 1

INTRODUCTION

“Understanding the Challenge,
Exploring the Need,
Paving the Way for Innovative
Solutions in Diabetes Care.”

Type 2 Diabetes Mellitus (T2DM) is a multifactorial metabolic disorder characterized by hyperglycemia, insulin resistance, and impaired insulin secretion. Despite the availability of many therapeutic agents, there is an ongoing need for safer and more effective drugs. This work explores diverse molecular targets and privileged scaffolds for the design and discovery of novel antidiabetic agents.

KEY THEMES OF THIS CHAPTER



The Growing
Burden of Diabetes



Complex
Pathophysiology



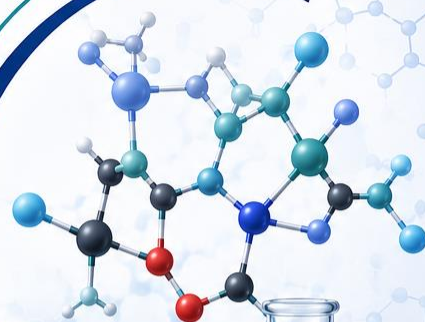
Need for Novel
Therapeutic Targets



Heterocyclic Chemistry
for Drug Discovery



Towards Better
Diabetes Care



1. Introduction

Diabetes is a persistent disease globally, which occurs either due to dysfunction of pancreatic β -cells, leading to a reduction in the production of insulin, or due to the inability of body cells to use the insulin produced efficiently [1-3]. Insulin regulates blood sugar levels by helping body cells absorb glucose for the generation of ATP [4, 5]. Diabetes severely damages the body's systems, including the nerves and blood vessels, due to hyperglycemia [6-8]. The types of diabetes are shown in Figure 1.

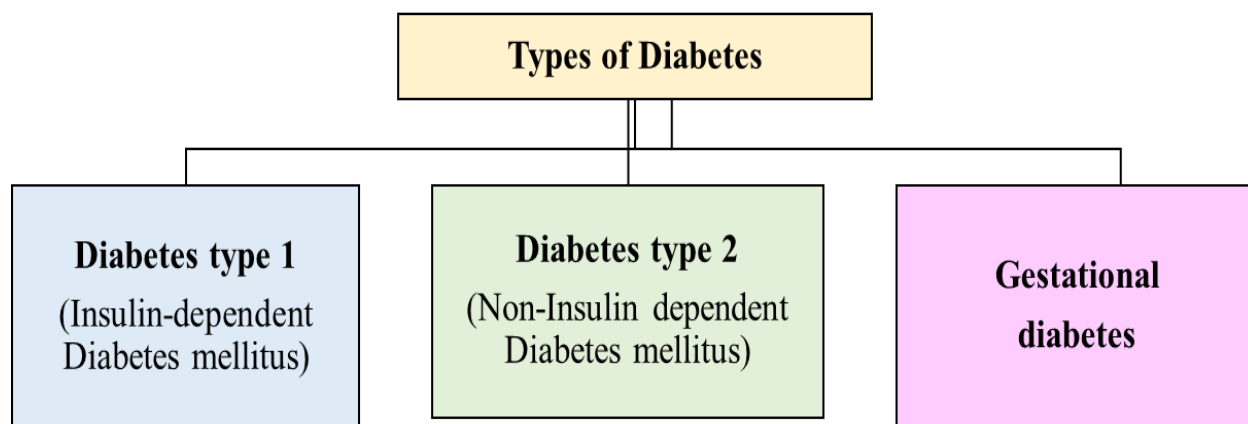


Figure 1. Different types of Diabetes

Prediabetes. An elevated upper threshold compared to normal but below the threshold for consideration as diabetes is an indicator of a persistent metabolic condition [9, 10]. This state is manifested by blood sugar levels rising greater than normal (70 mg/dL to 100 mg/dL); however, not to a level considered diabetes-level rising [11, 12]. Prediabetes is a precursor to diabetes mellitus. It is asymptomatic and is typically not recognized by most people [13, 14]. In this condition, blood glucose levels rise after an oral glucose tolerance test (140–199 mg/dL) and during fasting (100–125 mg/dL) (Table 1), according to the American Diabetes Association [15, 16]. Changing the lifestyle, such as changing the diet, increasing physical activity, and weight loss, can alter this reversible state (prediabetes) back to normal [17-19].

1.1. Diabetes type 2. A multifactorial pathophysiology associated with type 2 diabetes (T2D) consists of defective insulin secretion, insulin resistance, adiposity, increased glucagon secretion, decreased incretin effect and dyslipidemia [20]. Preventing macrovascular, microvascular

complications and mortality burden is dependent on early detection and proactive management. T2D denotes non-insulin-dependent diabetes or insulin resistance that can progress to absolute resistance over time [21]. T2D manifested excessive urination, hyperglycemia, increased fluid intake, increased thirst, blurry vision, lethargy and unreasonable weight loss. As a result of impaired glucose tolerance, pancreatic β -cells continuously upregulate insulin synthesis. Adipocytes and skeletal muscle cells take up glucose from the blood under insulin stimulation. Insulin promotes the conversion of glucose to glycogen in the liver and skeletal muscles by activating enzymes such as glucokinase and glycogen synthase [22]. Dropping blood glucose levels decreases insulin secretion and increases glucagon secretion, which in turn promotes glycogen conversion to glucose, further modulating the balance between insulin glucagon release. Overnight fasting results in gluconeogenesis and glycogenolysis, supplying most of the glucose. The hepatic glucose production occurs at an increased rate in T2D, and insulin action is impaired, resulting in hyperglycemia. Insulin resistance is characterized by the suppression or deceleration of the insulin response [23]. Elevated blood glucose levels are the principal manifestations of this disease. Most individuals with diabetes fall into this category. Insulin resistance occurs when the body does not respond or responds less efficiently to insulin [24]. This condition is a non-insulin-dependent form of diabetes. Older and middle-aged people are mostly affected by T2D [25]. Several factors contribute to insulin resistance, including obesity, genetics, and impaired communication between cells [26]. The risk of T2D is greatly influenced by several factors, such as depression, prediabetes, gestational diabetes, high blood pressure, and polycystic ovary syndrome [27, 28].

1.2. Diabetes type 1. A chronic metabolic disorder in which the β -cells of the pancreas are destroyed, resulting in either a severe lack of insulin production or none at all [29, 30]. A lack of insulin leads to increased blood glucose levels (hyperglycemia). Also known as juvenile or insulin-dependent diabetes, it affects the body's response to insulin. Since insulin lowers blood glucose levels by enhancing glucose uptake by cells, it plays an imperative role in lowering blood sugar levels. Children, adults, and adolescents are most likely to be affected [33-35]. It occurs in 5-10% of people, and T-cells mediate this process. An exogenous insulin injection is required to sustain the life of patients with this autoimmune disorder. The cause of type 1 diabetes can be attributed to viral exposure, genetics, or environmental factors [36, 37].

1.3. Gestational diabetes. Gestational diabetes manifests in pregnant women as increased blood sugar levels arising from causes such as insulin resistance and autoimmune disorders [38-40]. Obesity is a major cause of gestational diabetes mellitus. This leads to an increase in fetal and maternal complications [41-43]. It is observed in 5% of the gestation cases, but this number is continuously increasing due to unhealthy lifestyles such as obesity, junk foods, lack of physical exercise, and increased sweet food intake [44-46].

Table 1. Blood glucose level in different conditions.

Condition	Fasting Blood Glucose Levels	Post-meal Blood Glucose Levels
Normal	70-100 mg/dl	70-140 mg/dl
Prediabetes	101-125 mg/dl	141-200 mg/dl
Diabetes	Above 125 mg/dl	Above 200 mg/dl

1.4. Prevalence of diabetes type 2. The spread of this disease across the globe affects both developed and developing countries [47, 48]. The IDF Diabetes Atlas 2025, the 11th edition, reports 537 million adults with diabetes aged 20-79 years [49, 50]. Consequently, diabetes is estimated to have caused 6.7 million deaths in 2021. The number of patients with diabetes is expected to increase from 643 million in 2030 to 783 million in 2045, according to estimates [51, 52]. Diabetes affects more than four in five adults (81%) living in middle- and low-income countries [53, 54]. There have been at least USD 966 billion in health expenditures associated with diabetes in the past 15 years (an increase of 316%). Diabetes remains largely undiagnosed in approximately 46.5% of adults. Globally, there are 240 million undiagnosed diabetics, of whom 46 million live in Southeast Asia. In 2021, global health expenditures on diabetes totalled USD 966 billion. Over the past 15 years, this has been increased by 316%. Southeast Asia accounts for 1% (10 billion USD) of global spending. Impaired of glucose tolerance is a condition resulting in the risk of developing T2D in 541 million adults worldwide (10.6% of all adults). A total of 47 million people in Southeast Asia suffer from impaired glucose tolerance. In the last survey conducted by the IDF in 2021, more than 74 million adults lived with diabetes in India, making it the world's second largest diabetic population after China. Diabetes is undiagnosed in

over half (53.1%) of Indian. The presence of Impaired Glucose Tolerance places 40 million people in India at risk of developing T2D. Individuals and families living in India face significant challenges related to diabetes because of the ever-expanding number of people suffering from the disease and at risk of developing it. Untreated or undetected diabetes can cause serious and life-threatening complications, including stroke, heart attack, kidney failure, lower limb amputation, and blindness. High healthcare costs and reduced quality of life are consequences of these factors, which increase the need for access to care [55-57].

1.5. Pathophysiology. Risk factors such as metabolic, genetic, and environmental factors contribute to the rise in the prevalence of diabetes. Increased physical activity and avoidance of obesity and unhealthy eating habits can help prevent T2D [58, 59]. The pathogenesis of the disease involves a malfunction between insulin action and insulin secretion, resulting in abnormally high blood glucose levels [60]. In the event of β -cell malfunction, insulin production is impaired, further reducing the ability of the body to maintain physiological glucose levels. As a result of insulin resistance, the liver produces more glucose, whereas the liver, fat cells, and muscles absorb less glucose (Figure 2) [61, 62]. Insulin resistance and β -cell dysfunction often co-occur in the pathogenesis of the disease, though β -cell dysfunction tends to be more clinically severe. Therefore, both insulin resistance and β -cell dysfunction lead to the development of T2D [63]. Although a number of classes of drugs exist to treat diabetes, maintaining acceptable long-term glycaemic control in T2D is still a challenge. These traditional therapies have a number of drawbacks such as gastrointestinal (GI) side effects, hypoglycemia, weight gain, loss of efficacy with time, cardiovascular issues, and lack of patient compliance.

In addition, many of these therapies act on insulin secretion or peripheral glucose utilization, but have no direct effect on the defect in glucose-sensing that occurs in T2D. As the disease progresses, it also leads to progressive β -cell dysfunction, resulting in progressive loss of efficacy of currently available treatments. Glucokinase (GK) is an emerging target for the potential development of a new drug to treat T2D due to its role in glucose homeostasis. GK is mainly found in pancreatic β -cells and hepatocytes and acts as a glucose sensor, catalyzing the phosphorylation of glucose to glucose-6-phosphate, the initial and rate-limiting step of glycolysis.

The activation of GK in pancreatic β -cells stimulates insulin secretion from the cells, while in the

liver, the activation of GK stimulates glycogen formation and glucose consumption, both of which decrease hepatic glucose production. GK has a high K_m and sigmoidal kinetic behaviour which allows it to be very responsive to changes in blood glucose in physiology. GKAs have been much discussed due to their ability to simultaneously enhance both the secretion of insulin by the pancreas and glucose metabolism in the liver. It is a dual mechanism that has been better than currently known targets, such as GLP-1 receptor agonists and SGLT2 inhibitors that have been used so far, with its primary mechanisms being the enhancement of incretins and renal glucose excretion, respectively. In T2D, the fundamental defect in glucose sensing and regulation is addressed directly by a direct action of GKAs. Also, GKAs can offer better glycemic control independently of insulin sensitization pathways. Some of the previously identified GKAs, however, were also linked to hypoglycaemia, hypertriglyceridaemia and reduced efficacy with long-term use, and ongoing research has led to the development of safer, more selective glucokinase activators.

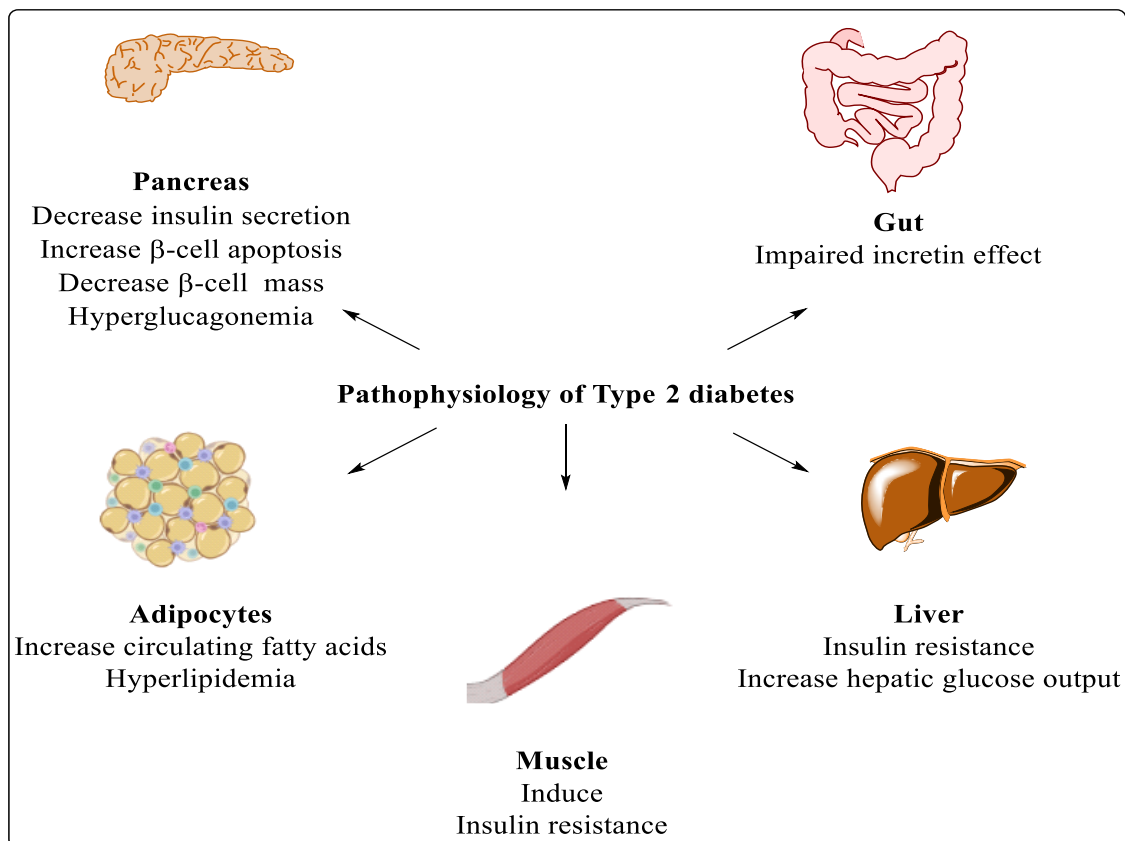


Figure 2. The pathophysiology of T2D



CHAPTER 2

LITERATURE REVIEW

*“ Building on Existing Knowledge,
Exploring the Pathway
to Innovative Discoveries. ”*



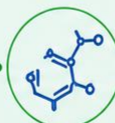
Exploring
Previous Research



Identifying
Key Findings



Understanding
Research Gaps



Connecting Concepts
and Theories



Driving Future
Research

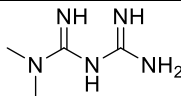
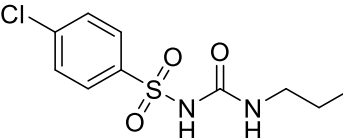
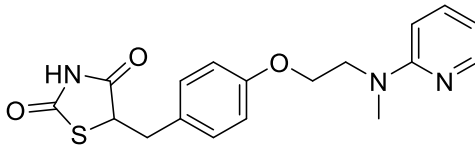
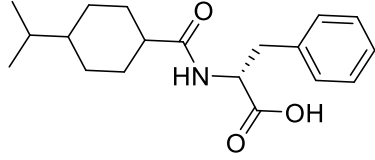
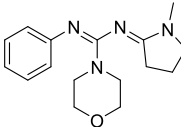


2. Literature Review

2.1 Therapeutic Targets in T2DM

Antidiabetic agents currently used in the management of T2DM can be broadly categorized based on their primary pharmacological targets (**Table 2**).

Table 2. Chemical classification of Antidiabetic drugs.

Sr. No.	Name of Class	Examples	Structure
1.	Biguanide compounds	Phenformin, Metformin	 Metformin
2.	Sulfonylurea derivatives	Chlorpropamide, Tolbutamide, Tolazamide, Glimepiride, Glyburide, Glipizide	 Chlorpropamide
3.	Thiazolidinedione analogues	Pioglitazone, Rosiglitazone, Ciglitazone	 Rosiglitazone
4.	Benzoic acid-based insulin secretagogues	Nateglinide, Meglitinide, Repaglinide	 Nateglinide
5.	Miscellaneous Agent	Linoglriride	 Linoglriride

2.2 Receptor and Enzyme Oriented Therapeutic Approaches

Several molecular receptors and metabolic enzymes play crucial roles in glucose homeostasis and insulin sensitivity. The major molecular targets and their corresponding modulators are listed in Table 3.

Table 3. Key molecular targets and modulators employed in T2D therapy

Sr No.	Target	Examples
1.	DPP4-Inhibitor	Saxagliptin, Linagliptin
2.	GLP-1 receptor agonists	Dulaglutide, Semaglutide
3.	GPR40/FFAR1 agonist	TAK-875 (reported clinical candidate)
4.	Pyruvate dehydrogenase kinase 4 Inhibitor	AZD7545
5.	SGLT2 Inhibitors	Empagliflozin, Ertugliflozin
6.	Protein tyrosine phosphatase 1B (PTP1B) Inhibitor	Trodusquemine (MSI-1436)
7.	11- β Hydroxysteroid dehydrogenase type 1 inhibitor	MK-0916
8.	Fibroblast growth factor 21 (FGF21) Inhibitor	Pegbelfermin
9.	Diacylglycerol Acyl transferase 1 Inhibitors	Pradigastat
10.	Fructose 1,6-bisphosphatase 1 Inhibitors	MB06322 (CS-917)
11.	Glucagon receptor antagonist	LY2409021
12.	Acetyl-CoA carboxylase	ND-630
13.	Glycogen phosphorylase inhibitor	CP-91149
14.	Farnesoid X receptor modulators	Obeticholic acid
15.	Glucokinase activator	Dorzagliatin
16.	AMPK activator	A-769662

2.2. Possible Therapeutic Targets in the management of Type 2 Diabetes.

2.2.1. GLP-1 Receptor Agonists

GLP-1 receptor agonists are a valuable form of antidiabetic medication employed in the management of high blood glucose levels. As glucose-dependent agents, they control glycemic balance by affecting the secretion of pancreatic hormones, stimulating insulin secretion, and inhibiting glucagon production. Due to this mechanism, GLP-1 receptor agonists are often called incretin analogs. Their major activity includes the stimulation of insulin secretion from pancreatic β -cells, which helps lower the levels of glucose in the circulation effectively [64].

GLP-1 receptor agonists administered exogenously to patients with T2D have been found to normalize fasting plasma glucose levels. In addition to glycemic control, these agents have numerous other metabolic benefits, including increased insulin responsiveness, decreased gastric emptying, decreased secretion of glucagon, diminished levels of free fatty acids, and weight loss. The most commonly prescribed drugs in this group are lixisenatide, oral semaglutide, exenatide (Figure 3), dulaglutide, and liraglutide. Although GLP-1 receptor agonists have clinical advantages, they have been associated with some adverse effects. The frequently reported symptoms are early satiety, nausea, vomiting, loss of appetite, diarrhea, and abdominal pain. Additionally, there are reports of a risk of pancreatitis, chronic effects of long-term use, and some evidence of a possible association with thyroid C-cell carcinoma with long-term therapy use [65].

H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH₂

Figure 3. Molecular structure of exenatide, a GLP-1 receptor agonist.

2.2.2. DPP-4 Inhibitors

DPP-4 inhibitors are a group of orally delivered antihyperglycemic medications, usually prescribed as supplements or substitutes for the treatment of T2DM. This category of drugs is also referred to as the gliptin family and has a therapeutic influence on the inhibition of the dipeptidyl peptidase-4 enzyme. In patients with T2D, poor insulin activity,

Excessive glucagon and insulin secretion are traits of disease progression. The treatment of these abnormalities is the core of contemporary therapeutic measures.

These agents halt the action of DPP-4 to inhibit the enzymatic breakdown of incretin hormones, particularly glucagon-like peptide-1 (GLP-1). High levels of GLP-1 increase the secretion of glucose-dependent insulin and reduce the secretion of glucagon, thus restoring the fasting plasma glucose to normal levels. Although effective, DPP-4 inhibitors can be regarded as second-line therapy and are often used along with metformin, which is often the first-choice agent. Nevertheless, when metformin is contraindicated or not well tolerated, DPP-4 inhibitors may be used as appropriate alternatives. Drugs commonly used in this category include vildagliptin, alogliptin, sitagliptin, linagliptin, teneligliptin, saxagliptin, and anagliptin [66].

Physiologically, the DPP-4 enzyme rapidly inactivates endogenous incretin hormones in non-diabetic individuals. Therefore, the discovery of molecular structures that can selectively block DPP-4 has become a promising strategy for treating T2D. In this respect, Jung et al. presented the discovery of evogliptin (DA-1229) (1) (Figure 4), a new orally active and highly specific DPP-4 inhibitor [67]. The application of evogliptin at a dose of 5 mg led to a major decrease in the levels of glycated albumin and HbA1c.

Omarigliptin (MK-3102), (2), is another DPP-4 long-acting drug that has recently been approved by Japanese regulators. Goldenberg et al. compared the effectiveness and safety of semaglutide in patients with T2DM in a randomized clinical trial. The study showed that once-weekly omarigliptin (25 mg) had equivalent glycemic control to daily sitagliptin (100 mg), without producing any change in body weight [68]. Maezaki et al. discovered that compound 3 had outstanding inhibitory power, with an IC_{50} of 0.38 nM [69]. Similarly, Wang et al. examined a series of pyrrolidine-2-carbonitrile analogs and found that compound 4 (IC_{50} = 0.017 μ M) exhibited modest potentiation compared to the reference drug vildagliptin (IC_{50} = 0.020 μ M) [70].

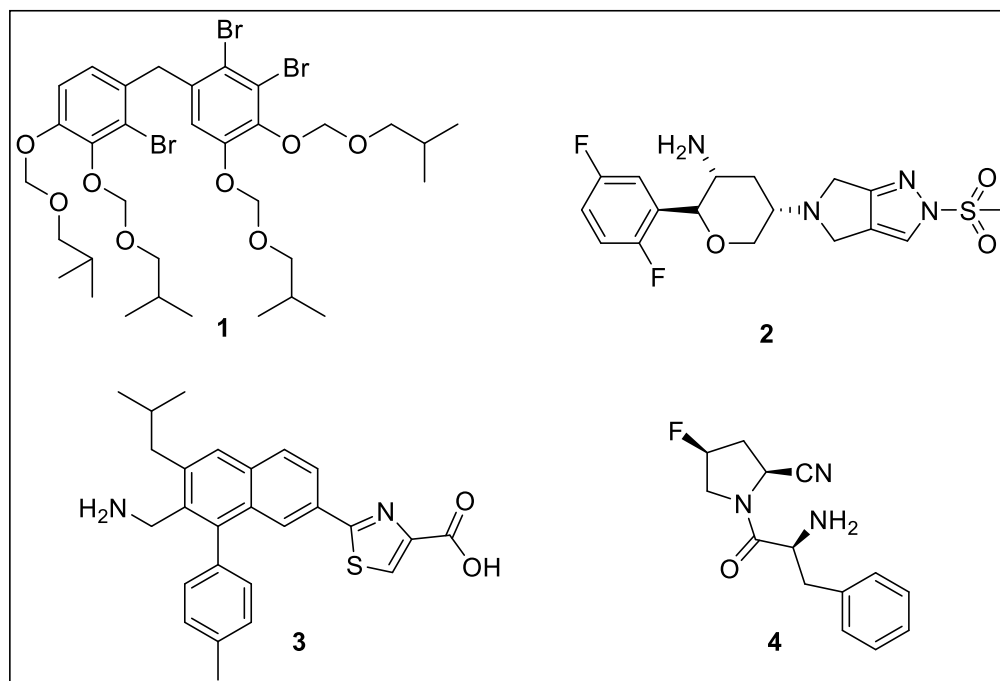


Figure 4. Molecular framework of DPP-4 inhibitory agents.

2.2.3. GPR40 / FFAR1 Agonists

GPR40 belongs to the G protein-coupled receptor family and is critical for the regulation of glucose in the body. It has emerged as a promising molecular marker for the treatment of hyperglycemia in patients with T2DM. GPR40 is also known as free fatty acid receptor-1 (FFAR1) and is mostly expressed in pancreatic β -cells, intestinal tissues, and fat-linked immune cells.

FFAR1, stimulated by medium- and long-chain fatty acids in pancreatic β -cells, causes intracellular signaling in pancreatic β -cells, activating insulin secretion in a glucose-dependent manner. This mechanism helps to effectively decrease high blood glucose levels without excessive insulin release during normoglycemic conditions. Accordingly, GPR40 agonists are associated with enhanced glucose homeostasis, recovery of impaired glucose tolerance, and enhanced insulin secretion in relation to the existing glucose levels in the blood. Owing to these positive results, FFAR1 agonists have attracted much interest as effective therapeutic tools for treating T2D [71,72]. In this regard, Li et al. presented a pyrrole-based analog that acts as a strong FFAR1 agonist in the management of T2D. One of the tested compounds (5, Figure 5)

showed the highest receptor stimulation, with an EC₅₀ of 34.7 nM. Similarly, screening of thiazole-based derivatives revealed that compounds 6 and 7 were viable FFAR1 agonists with EC₅₀ values of 77.3 nM and 48.7 nM, respectively. Moreover, Li et al. obtained an array of phenoxy-substituted derivatives, of which the 3-methyl analog (compound 8) exhibited the greatest FFAR1 activity, with an EC₅₀ of 108.2 nM [73].

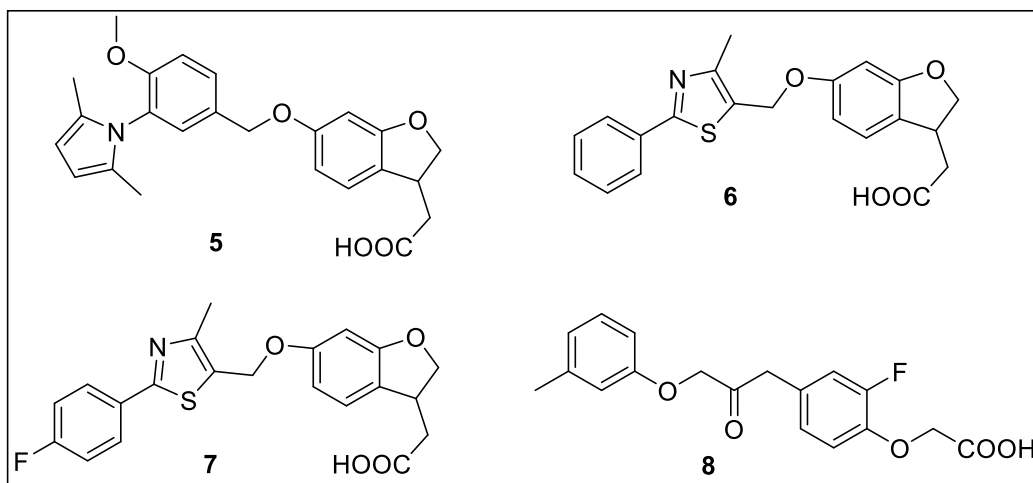


Figure 5. Chemical structures of GPR40/FFAR1 antagonist compounds.

2.2.4. SGLT2 Inhibitors

Sodium-glucose cotransporter-2 (SGLT2) inhibitors are a widely used category of drugs for the treatment of T2DM [74,75]. Popularly known as gliflozins, these drugs reduce blood glucose levels by inhibiting the reabsorption of glucose by the renal system, thus facilitating the excretion of glucose in the urine [76]. SGLT2 inhibitors are a promising strategy for glycemic control by adjusting nephron function through the regulation of renal glucose reabsorption instead of insulin signaling pathways [77]. Therefore, they are generally used as second- or third-line methods of treatment for diabetes [78, 79].

The SGLT2 transporter is highly expressed in the proximal convoluted tubules of the kidney, and it is at this location that it becomes central in the process of reclaiming filtered glucose [80, 81]. SGLT2 is expressed in organs other than the kidneys, including the brain, liver, skeletal muscle, and heart [82, 83]. SGLT2 pharmacological inhibition fosters a decrease in glucose reentry into the bloodstream and boosts its output through urine, with the final effect being long-lasting decrease in plasma glucose [84, 85].

Several clinically approved SGLT2 inhibitors are already in circulation, including dapagliflozin, ertugliflozin, canagliflozin, empagliflozin, sotagliflozin, and ertugliflozin [86, 87]. In recent medicinal chemistry studies, Ohtake et al. constructed carbasugar-based D-glucopyranose analogs of carbasugars that can inhibit hSGLT1 and hSGLT2 [88, 89]. The most potent of them were the ethoxy-substituted derivatives 9 and 10 (Figure 6), which showed high levels of inhibition of human SGLT2 with IC_{50} values of 19 nM and 9.9 nM, respectively [90]. Imamura et al. described the production of benzothiophene-based C-glucoside analogs, one of which, 11, displayed significant SGLT2 inhibitory activity, with an IC_{50} of 7.4 nM [91]. In addition, Yao et al. examined a system of C-indolylxyloside scaffolds and found compound 12 to be the most active analog, with an EC_{50} of 47 nM [92].

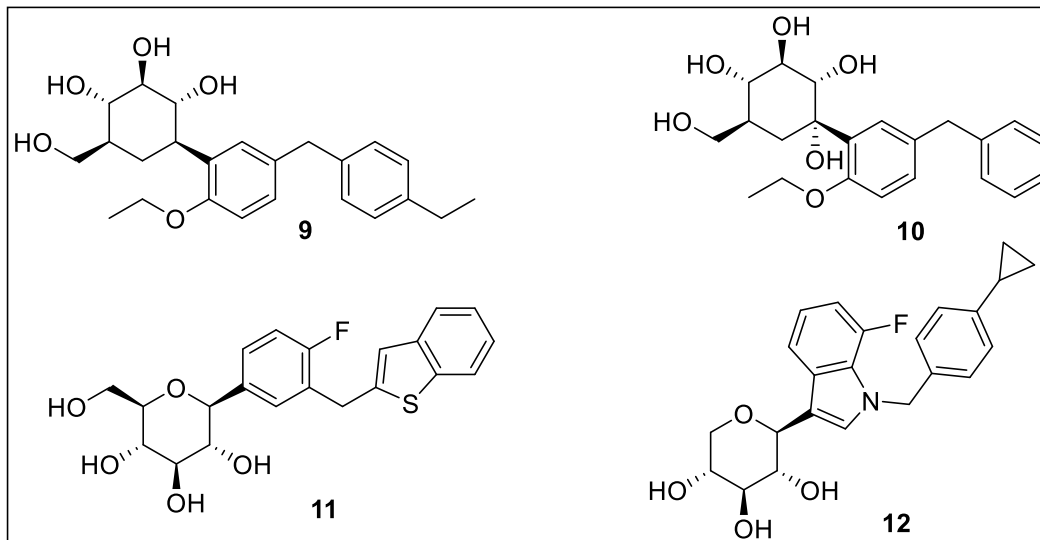


Figure 6. Structural representation of compounds acting as SGLT2 inhibitors.

2.2.5. PDK4 Inhibitors Pyruvate Dehydrogenase Kinase 4.

The pyruvate dehydrogenase complex (PDC) is a regulator of metabolic homeostasis and is important for the maintenance of metabolic disorders. The PDC plays a crucial role in the coordination of glucose and free fatty acid usage in maintaining adenosine triphosphate (ATP) at the required level to keep cellular power at a consistent level. PDK4 inhibition improves the pyruvate to acetyl-CoA ratio in response to pyruvate in β -cells, which increases its transfer to the tricarboxylic acid (TCA) cycle.

This metabolic change enhances fatty acid metabolism and simultaneously suppresses glycolytic flux, shifting glycolytic intermediates to other biochemical pathways. This energy metabolism reprogramming contributes to increased glucose regulation and an alleviated hyperglycemic state in metabolic disease [93-95]. To create PDK inhibition, Tso and co-workers designed new inhibitors by structurally modifying a dihydroxyphenyl sulfonyl isoindoline structure. Analogues 13 (Figure 7) and 14 (Figure 7) exhibited good inhibitory properties against PDK, with IC_{50} values of 58 nM and 456 nM, respectively [96]. These compounds have a significant effect on glucose tolerance that is measurable when assessed in diet-induced obese mouse models, where they greatly improve hepatic pyruvate dehydrogenase complex activity.

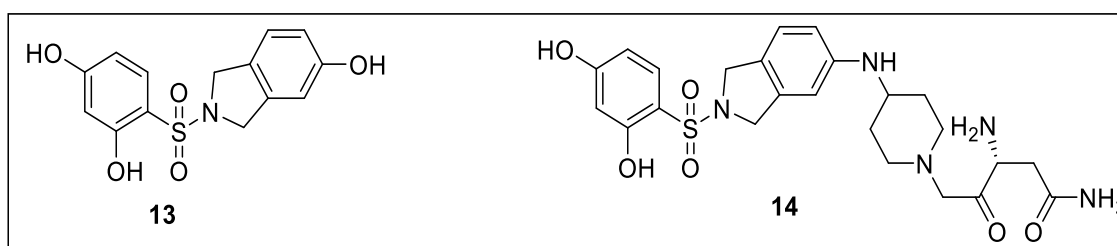


Figure 7. Molecular framework of compounds targeting PDK4 inhibition.

2.2.6. The PTP1B (protein Tyrosine Phosphatase 1B)-Inhibitors.

Insulin is the most important factor in maintaining glucose balance, and one of the critical attributes of T2DM is the interference of insulin signaling. Reinforcement of insulin-mediated metabolic responses Inhibition of protein tyrosine phosphatase 1B (PTP1B), a negative regulator of insulin signaling, stimulates insulin receptor phosphorylation and glucose uptake in insulin-sensitive tissues [97]. These effects make PTP1B a promising molecular target for designing antidiabetic therapeutic molecules.

Many chemical entities have been studied for their PTP1B inhibitory properties. According to Zhang et al., bromophenol glycosylate conjugates are highly selective PTP1B inhibitors. The most active compound, with two hydroxyl substituents in the bromophenol moiety, was compound 15, which exhibited the strongest activity with an IC_{50} of 199 nM and a selectivity of approximately 32-fold against PTP1B [98].

Marine natural products have also played a significant role in the discovery of PTP1B inhibitors. Abdju et al. identified multiple furanoterpenes (compounds 15-20) (Figure 8) in two Indonesian marine sponges, which exhibited dose-dependent inhibitory effects against PTP1B with IC_{50} values of 1.5, 7.1, and 9.9 μM , respectively [99]. Similarly, Yamasaki et al. discovered a group of N-methyladenine-containing terpenoids in the Okinawan marine sponge *Agelas nakamurai*. One of them was agelasine G (19), which inhibited PTP1B with an IC_{50} value of 15 μM [100]. Moreover, a synthetic analog of 2 (4-fluorophenylimino)-4-thiazolidinedione was identified as a good PTP1B inhibitor with an IC_{50} of 10 μM . Compound 20 also increased the uptake of 2-deoxyglucose in skeletal muscle cells, indicating increased cellular glucose uptake [101].

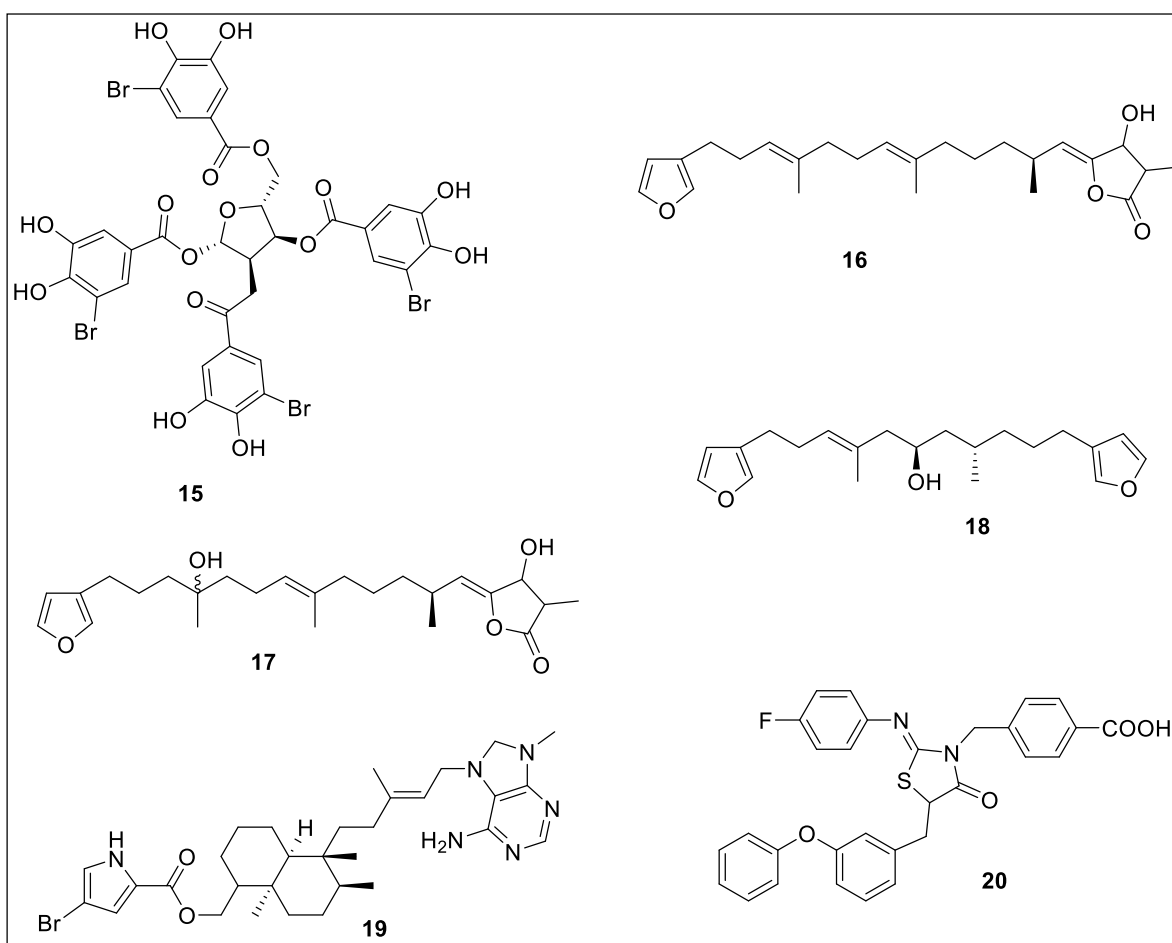


Figure 8. Molecular structures of Protein Tyrosine Phosphatase 1B (PTP1B) inhibitors

2.2.7. Fibroblast growth factor 21 (FGF 21) Inhibitors

Fibroblast growth factor 21 (FGF21) has been found to be very important in regulating lipid and carbohydrate metabolism. High levels of FGF21 in the blood circulation are common in patients with diabetes and correlate with abnormal glucose metabolism and the onset of insulin resistance. By inhibiting FGF21 pharmacologically, the overactivity of this molecule is inhibited by a feedback mechanism, leading to metabolic balance.

Inhibition of FGF21 activity improves insulin signaling and results in improved glucose uptake and insulin resistance, which together help improve glycemic control. Nevertheless, the use of FGF21 inhibitors has been associated with some side effects, such as fatigue, gastrointestinal-related complications such as diarrhea and abdominal discomfort, xerostomia, musculoskeletal pain, and elevated serum phosphate levels [102,103]. Mo et al. prepared a new family of 2-aminopyridines-based molecules as selective FGF21 inhibitors. Compound 21 (Figure 9) was the top candidate with a high level of inhibition, characterized by an IC_{50} of 2.6 nM [104].

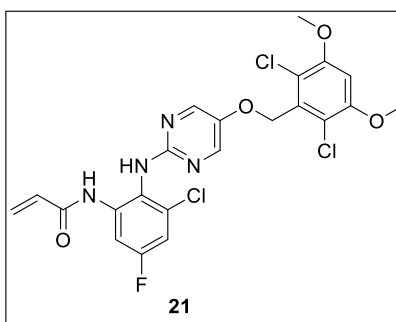


Figure 9. Compounds acting as inhibitors of Fibroblast Growth Factor 21

2.2.8. 11 β -Hydroxysteroid Dehydrogenase Type 1 (11 β -HSD1) Inhibitors

11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) is an intracellular enzyme that converts inactive glucocorticoids into active glucocorticoids in major metabolically active tissues, such as the liver, adipose tissue, and skeletal muscle. Overexpression of this enzyme raises the concentration of glucocorticoids in the area, which contributes to metabolic derangements, including poor glucose tolerance, low insulin sensitivity, and weight gain. Blocking 11 β -HSD1 reduces glucocorticoid concentration in the cell, thereby enhancing the responsiveness to insulin, glycemic regulation, and diet-related weight gain [105].

Researchers at Biovitrum were the first to report some of the first small-molecule 11 β -HSD1-inhibitors. BVT-2733 (Compound 22) and BVT-14225 (Compound 23) were the first compounds to showed inhibitory activity in the micromolar range, with IC₅₀ values (Figure 10). It was further optimized to demonstrated increased potency, whereby compound 22 had an IC₅₀ of 96 nM and compound 23 possessed an IC₅₀ of 52 nM [107]. Furthermore, Merck identified a group of azacyclic sulfonamide-based compounds as effective 11 β -HSD1 inhibitors. In this series, compounds 24 and 25 exhibited high enzyme inhibition, with IC₅₀ values of 40 nM and 37 nM, respectively [108].

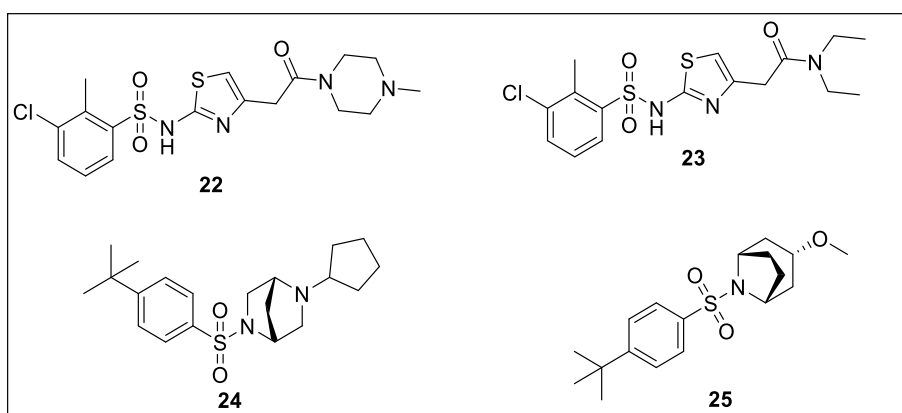


Figure 10. Molecular framework of 11 β -Hydroxysteroid Dehydrogenase Type 1 inhibitors

2.2.9. Diacylglycerol Acyltransferase A

Diacylglycerol acyltransferase 1 (DGAT1) is important in lipid metabolism, and its inhibition affects glucose metabolism via incretin-mediated processes. Inhibition of DGAT1 leads to an increase in glucagon-like peptide-1 (GLP-1) concentrations in the intestines, which is an incretin hormone that promotes glucose homeostasis and increases insulin sensitivity. The metabolic advantages of DGAT1 inhibitors have made them interesting therapeutic agents that can be used alongside other drugs that treat T2DM [109]. Sato et al. announced the production of N-phenylsulfonamide analogs as potential DGAT1 blockers as part of medicinal chemistry studies. The most active molecules observed in the evaluation were phenylalanine amino acids 26 and 27 (Figure 11), with IC₅₀ values of 130 nM and 3.4 nM [110]. Similarly, Hong et al. investigated benzene-based thieno [3, 2- d] pyrimidines as DGAT1 inhibitors. A biological assay in SF9 cells

indicated that the cis-configured analog (compound 28) inhibited DGAT1 with high potency and selectivity, with an IC₅₀ value of 74 nM [111].

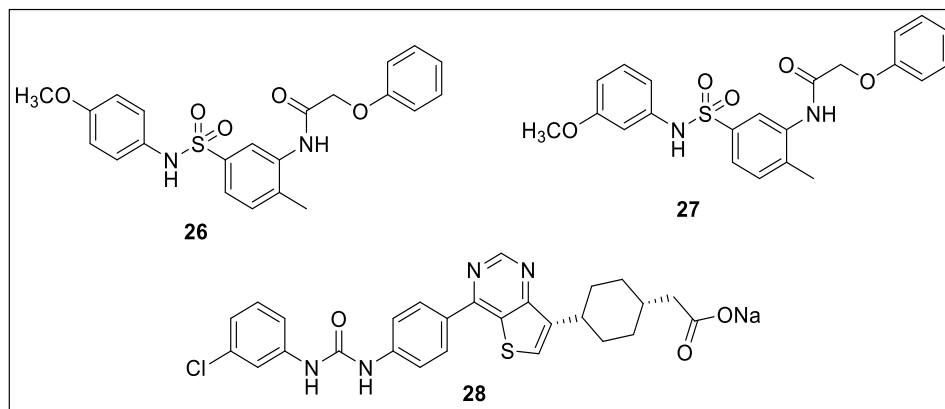


Figure 11. Chemical structures of compounds acting as DGAT1 inhibitors.

2.2.10. Glucagon receptor antagonist

GRAs are used as agents to inhibit glucagon receptors within the body, thereby reducing blood glucose levels. Glucagon is a peptide hormone secreted by pancreatic islet α -cells, and it is necessary for the regulation of blood glucose, especially during the fasting state. Oversecretion or maladjustment of glucagon secretion in patients with T2DM is associated with incessant hyperglycemia. This has made targeting glucagon signaling a promising approach for managing high blood glucose levels.

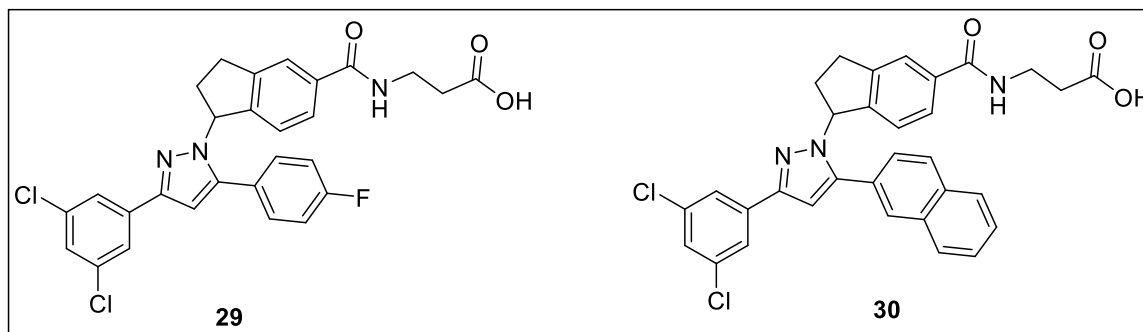


Figure 12. Molecules acting as glucagon receptor antagonists.

The antidiabetic effect of glucagon receptor antagonists is due to their ability to inhibit glucagon-stimulated production of hepatic glucose, thereby lowering the amount of glucose released into the circulation and restoring normal levels of glucose in the blood [112,113]. In this respect, Kerru et al. found a sequence of indazole- and indole-based compounds as potential glucagon

receptor antagonists. One of these, compounds 29 and 30 (Figure 12), showed significant antidiabetic potential, with IC_{50} values of 3.6 μ M and 1.2 μ M, respectively [114].

2.2.11. Fructose-1,6-bisphosphatase-1 (FBPase-1) Inhibitors

Another type of therapeutic agent that can be used to manage T2DM is fructose-1, 6-bisphosphatase-1 (FBPase-1) inhibitors, which may be used individually or with other therapies. FBPase-1 is an enzyme that plays a major role in hepatic gluconeogenesis and is involved in the irreversible conversion of fructose-1,6-bisphosphate to fructose-6-phosphate. This response is rate-limiting in the gluconeogenic process. Inhibition of FBPase-1 activity interferes with the endogenous glucose production process, leading to a decrease in the level of circulating blood glucose [115,116]. Bie et al. reported a series of indole-based compounds and tested their potential as FBPase-1 inhibitors. The most potent enzyme inhibitors were compounds 31 and 32 (Figure 13), which exhibited IC_{50} values of 0.10 μ M and 3.2 μ M, respectively [117,118]. In addition, a series of 1,3,4-oxadiazole analogs were prepared and evaluated for FBPase-1 inhibitory activity. Compounds 33 and 34 exhibited strong activity, with IC_{50} values of 1.32 μ M and 2.98 μ M, respectively [119,120].

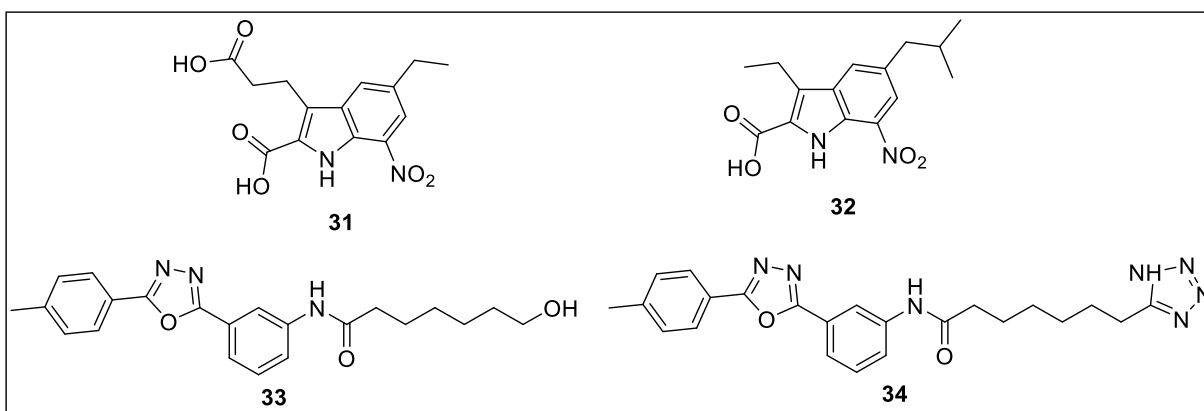


Figure 13. Structural representation of compounds acting as fructose 1,6-bisphosphatase inhibitors

2.2.12. Acetyl-CoA Carboxylase (ACC)

Acetyl-CoA carboxylase (ACC) is a crucial metabolic enzyme that is actively expressed in pancreatic β -cells and makes a significant contribution to glucose-stimulated insulin secretion. ACC activity has been modulated as a therapeutic approach to the management of T2DM. Some pharmacological agents have been studied with regard to their antidiabetic properties through

their interactions with this enzyme (such as avasimibe and pactimibe; Figure 14). ACC stimulation facilitates the augmented release of insulin, in contrast to the current blood glucose concentrations, and thus better glycemic regulation. In addition to the insulin secretion-stimulating effect, ACC-targeting agents also play a role in increased insulin sensitivity, making them ideal for the treatment of insulin resistance-induced T2D [121,122].

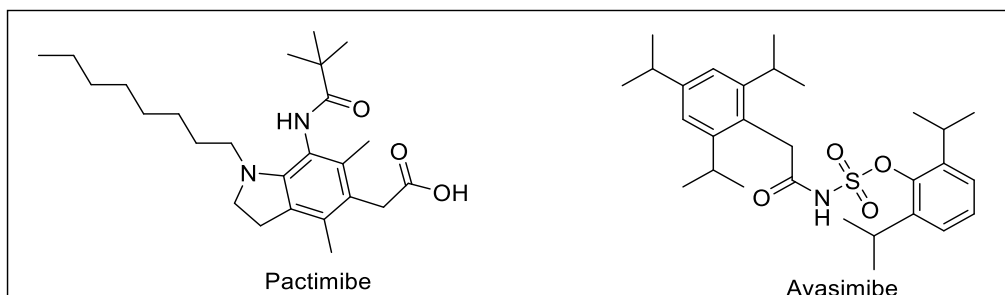


Figure 14. FDA-approved compounds with ACC activation activity.

2.2.13. Farnesoid X Receptor (FXR) Modulators

Farnesoid X receptor FXR (is a nuclear receptor that requires activation by bile acids and is key to metabolic control. FXR modulators have been of interest as possible therapeutic agents for the treatment of T2DM. Compounds that have been studied for their effects on FXR signaling pathways include fexaramine (Figure 15). FXR activation helps regulate lipid metabolism and maintain glucose homeostasis in the body. FXR has been found in pancreatic β -cells, the regulation of which may positively influence insulin-related metabolic processes. Following their ability to enhance metabolic homeostasis, FXR-targeting compounds are also under consideration as alternative systems for the management of T2D [123,124].

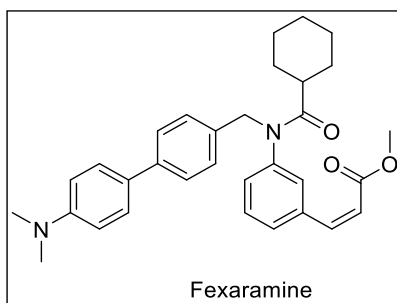


Figure 15. Molecular structure of an FDA-approved drug acting as a farnesoid X receptor (FXR) modulator.

2.2.14. Glycogen Phosphorylase (GP) Inhibitors

Glycogen phosphorylase (GP): This is a central enzyme of metabolism involved in catalyzing the breakdown (glycolysis) of glycogen in the liver, generating glucose in the blood. Overactivity of GP helps to increase the blood glucose level, especially in patients with T2DM. Blocking GP, which regulates the rate-limiting stage of glycogenolysis, is therefore an effective approach for reducing hepatic glucose production and sustaining glycemic homeostasis [125,126]. GP inhibitors also lower the release of glucose in hepatic stores by inhibiting the activities of glycogen phosphorylase, which results in better glycogen regulation of blood glucose levels and contributes to the general homeostasis of glucose levels.

Schweiker et al. tested a set of morpholine-substituted amino acid analogs as GP inhibitors and discovered a good candidate compound 35 with an IC_{50} of 6.5 μM [127]. Similarly, Zhang et al. described the production of new GP-targeting molecules, one of which, compound 36 (Figure 16), showed great inhibitory potency with an IC_{50} of 6.39 μM [128]. In addition, Loughlin et al. reported a series of 2-oxo-1,2-dihydropyridin-3-yl amide analogs, with one (compound 37), which contained a dichloro substitution, having good GP activity, with an IC_{50} of 1.92 μM [129].

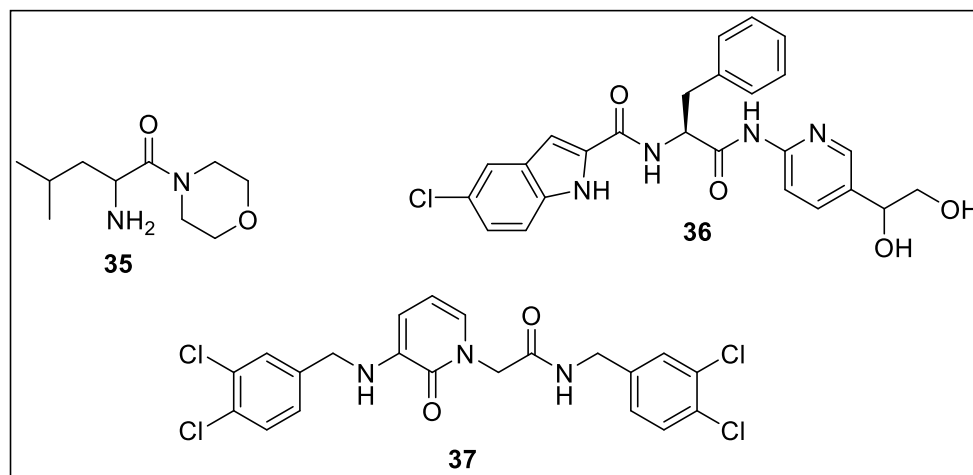


Figure 16. Chemical structures of GP inhibitor compounds.

2.2.15. Activated Protein Kinase (AMPK) Activators

AMP-activated protein kinase (AMPK) activators are considered effective supplements for the treatment of T2DM. These antidiabetic agents act by activating AMPK, an essential cellular energy sensor that regulates lipid and glucose metabolism. AMPK stimulation stimulates the

uptake of glucose in skeletal muscle tissue and the oxidation of fatty acids in the liver, which contributes to better metabolic utilization [130,131].

AMPK promotes a decrease in blood glucose levels and reverses hyperglycemia related to diabetes through enhanced muscular glucose metabolism and decreased hepatic lipid storage. Consequently, AMPK activators are significant in the reestablishment of metabolism and glycemic regulation in patients with T2D [132]. Zheng et al. synthesized and tested a family of pyrazolo[3,4-b]pyridine analogs owing to their AMPK-stimulating activity. Among the synthesized compounds, analogs 38 and 39 (Figure 17) exhibited significant AMPK activation, with EC₅₀ values of 0.42 μ M and 1.83 μ M, respectively [133].

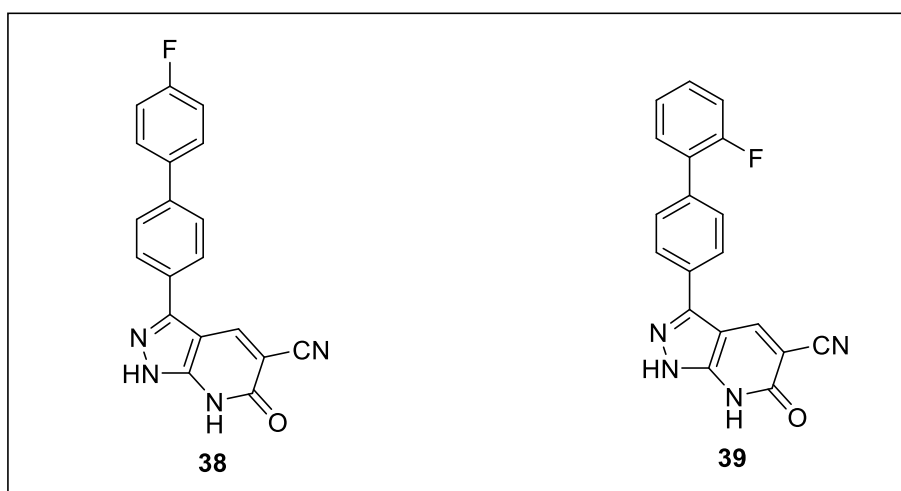


Figure 17. Molecular framework of compounds with AMPK activation activity

2.2.16. Glucokinase Activators

Glucokinase is an enzyme of the hexokinase family that is vital in glucose metabolism because it catalyzes the conversion of glucose to glucose-6-phosphate, which is a rate-limiting pathway in metabolism. This phosphorylation leads to the synthesis of glycogen and simultaneously activates pancreatic β -cells to secrete insulin [134,135].

Glucokinase activators are glucose sensors that stimulate insulin secretion at high glucose levels. They also enhance the production and storage of glycogen in hepatocytes, ensuring normal blood glucose levels. Due to these two effects on insulin release and hepatic glucose use, glucokinase activators are regarded as potential therapeutic agents for the control of T2D [136]. In their work on drug development, Khadse et al. produced hetero-substituted sulfonamide-benzamide analogs

as prospective glucokinase activators. In vitro (with human GK activation assays) and in vivo (with oral glucose tolerance tests) studies found that compounds 40 and 41 (Figure 18) were effective activators, with EC₅₀ values of 495 nM and 522 nM, respectively [137,138]. Similarly, Grewal et al. synthesized a group of 3,5-bis-substituted benzamide analogs and tested their glucokinase-activating and antihyperglycemic effects in animals. In the above experiments, compounds 42 and 43 demonstrated the strongest activity in oral glucose tolerance tests, with an EC₅₀ of 2.11 ± 0.05 μM and 2.11 ± 0.09 μM respectively [139].

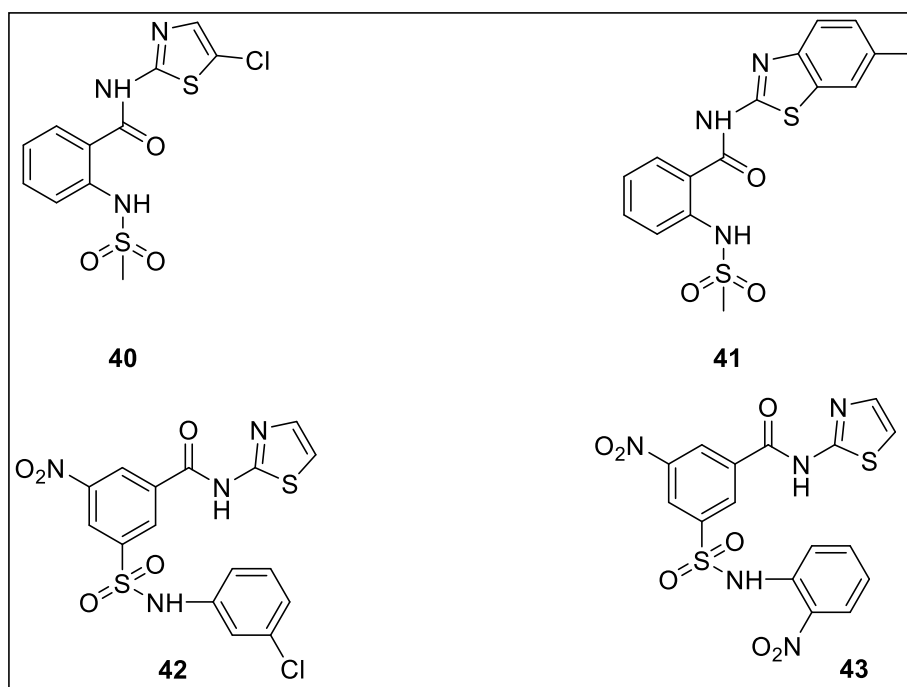


Figure 18. Structural representation of glucokinase-activating compounds.

2.2.17. Glucokinase activator for treatment of T2D

Glucokinase is a member of the hexokinase family and is involved in the conversion of glucose to glucose-6-phosphate, which is the rate-limiting step in glucose metabolism. This leads to the synthesis of glycogen and stimulation of insulin secretion by β-cells [140]. Glucokinase activators act as glucose sensors, promoting glycogen synthesis and storage in liver cells and stimulating insulin release in response to glucose levels [141]. These compounds have the potential to be innovative targets for the control of T2D by adapting normal blood glucose levels [142].

2.2.17.1. Role of Glucokinase in management of T2D

The cytoplasmic enzyme glucokinase is responsible for phosphorylating glucose to glucose-6-phosphate in the body. GK expression is primarily found in the β -cells of the pancreas and hepatocytes of the liver [143, 144]. Glucose-stimulated insulin release (GSIR) is elicited by GK in the β -cells of the pancreas [145]. The frequency of glucose phosphorylation in β -pancreatic cells is directly related to the glucose level in the tissues. The glucose phosphorylation rate constrains insulin secretion [146]. To maintain a balanced ATP/ADP ratio, glucokinase regulates oxidative ATP production and glycolysis (Figure 19). An increase in membrane potential stimulates the opening of the L-type calcium channel mediated by GK. It has been observed that Ca^{2+} facilitates the release of insulin in this process [147]. Pancreatic islets can recover their calcium response to glucose and insulin secretion by activating the GK. Hepatocyte glucose metabolism is dependent on GK.

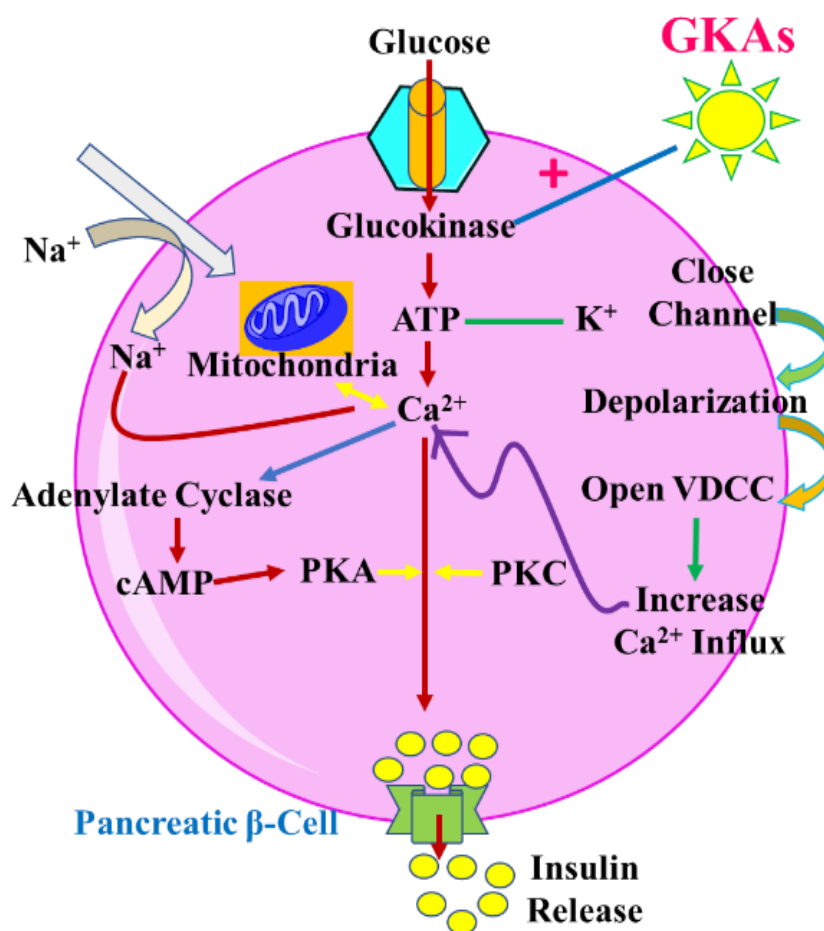


Figure 19. Mechanism of action of Glucokinase Activator

GKRP (Glucokinase regulatory protein) acts as an endogenous inhibitor that modulates hepatic glucokinase activity, which provokes insulin release [148]. The inactive complex of glucokinase with GKRP is found in low glucose concentrations in the nucleus. Allosteric sites on glucokinase are bound by GKAs. Directly or by destabilizing the complex between glucokinase and GKRP, GKAs could activate glucokinase [149]. A rise in blood glucose level dissociates glucokinase from the GKRP complex and translocates it to the cytoplasm. Insulin secretion and glycolysis (Figure 20) were stimulated by GKAs, which also improved glucose tolerance. RO-281675 was first identified as GKAs in 2003 by Hoffmann-La Roche AG [150]. The maximum metabolic rate ($V_{\max}$) of GK was 1.5-fold increased by RO-281675 at 3 μM . Furthermore, glucose was fall down from 86 mM to 20 mmol/L at half of $V_{\max}$ via a decrease in substrate concentration [$S_{0.5}$] [151].

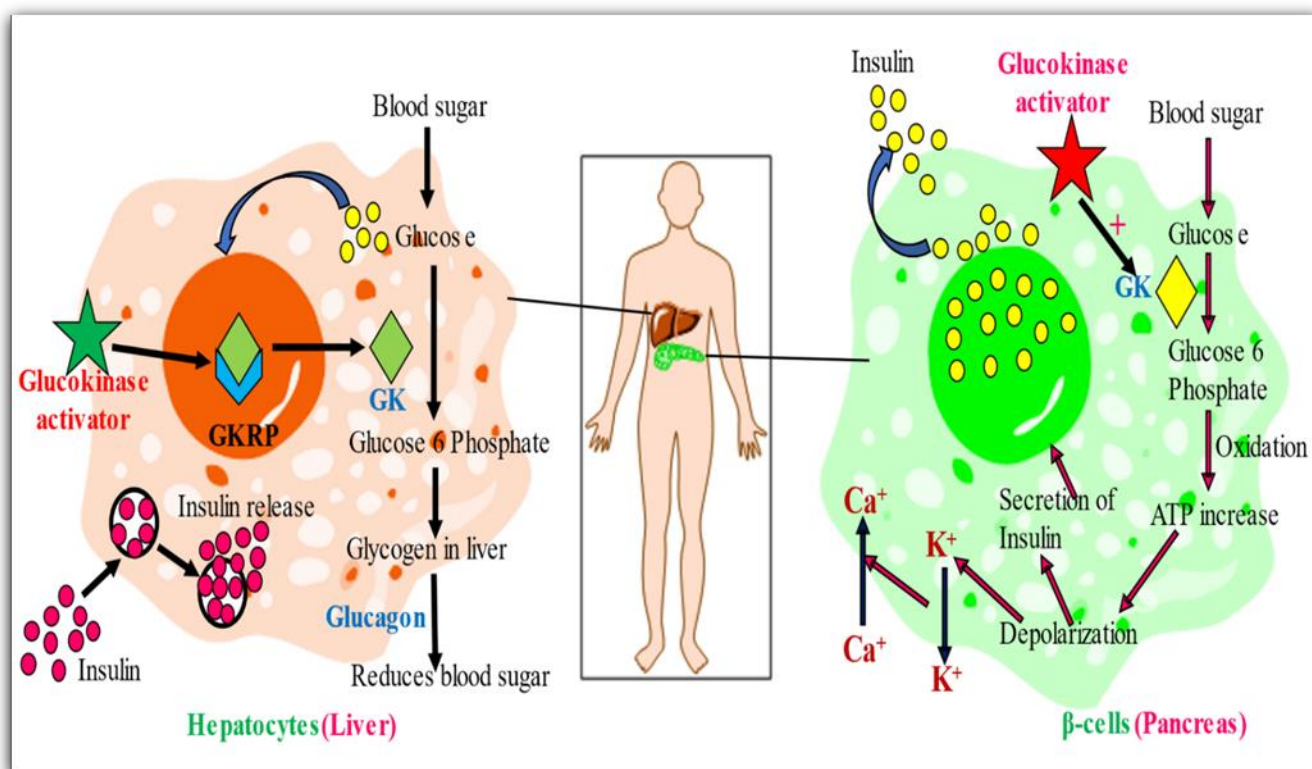


Figure 20. Insulin secretion is aided by the activation of the glucokinase enzyme to regulate blood glucose levels in the body.

Thus, using GK to manage diabetes appears to be a viable strategy. The cardiovascular risks associated with this compound have prevented it from undergoing clinical trials. Although this compound has not yet reached clinical trials, it paves the way for pharmaceutical companies to

develop safe and efficient GKAs [152]. An extensive diversity of molecules, including benzamides [153], acrylamides, propenamide, carboxamides, acetamides, benzimidazoles, quinazolines, urea, pyrimidone, and pyridines [154-155] derivatives, have been described in the preceding years to act as potential GK activators. The allosteric sites of GK are activated by ligands containing nitrogen, sulfur, oxygen, and amide linkages. Researchers have explored different core nuclei to develop GKA with improved efficacy, potency, reduced toxicity, and improved physicochemical properties by considering ligand-GK interaction data.

The patent review of GKAs is structured in sections based on the parent nucleus of the patented ligands 2.2.18.

2.2.18. Literature insights from patents

2.2.18.1 Advinus Therapeutics Limited. US9340506B2

In 2016, Advinus Therapeutics Limited, claimed acetamide derivatives as potent glucokinase activators. Most of the synthesized compounds showed less than 0.5 μM EC_{50} value against the GK enzyme, out of which ten compounds **1a-j** (Figure 21) were claimed to showed above 90% raise in the glucokinase activity (% E_{max}). The glycogen synthesis data of compounds **1a-e** exhibited activity more than 2.5-fold at 10 μM . These compounds act as glucokinase activator in a cellular atmosphere as it reveals a momentous rise in glycogen synthesis as compared to DMSO control in the hepatocytes with potent GK activity. The addition of the *c*-propyl group and 2-substituted phenyl-methanol give rise to the highly potent compound **1a** of the series with EC_{50} values of 0.064 μM having 93% E_{max} . In compound **1b** replacement with 2-methylbenzoic acid exhibits raised GK activity as EC_{50} value of 0.087 μM having 71% E_{max} . Compounds **1c** and **1d** were also found to illustrate good GK agonism as both contain *c*-pentyl and 3-substituted benzoic acid, just replacement of phenyl ring **1d** with pyridine ring **1c** leads to enhancement of potency as EC_{50} value 0.13 μM and E_{max} 95%. Compound **1e** contains 3-substituted benzoic acid substitution showed EC_{50} values of 0.19 μM having 91% E_{max} . In compound **1f**, replacement of tetrahydro-2H-pyran of compound **1b** with 2,4-difluorophenyl resulted in reduced potency an EC_{50} value 0.093 μM and E_{max} of 89%. Compound **1g** displayed an increase in potency and GK activity at EC_{50} of 0.094 μM having 91% E_{max} is observed with substitution of 2-methyl-2-phenylpropanoic acid. Compound **1h** contained 4-benzaldehyde, and compound **1i** contained 4-ethyl benzoate.

Compounds **1h**, **1i** showed equipotent activity with EC₅₀ values 0.1 μM and having E_{max} 91% and 95%, respectively. In compound **1j** addition of N-c-propyl benzamide exhibited EC₅₀ value of 0.13 μM and E_{max} 94% [156].

2.2.18.2 Takeda Pharmaceutical Company Limited. US8957070B2

Takeda Pharmaceutical Company Limited has continued to be active in the field of GKA research, advancing their work on GKAs and submitting a patent application in 2015. A series of substituted indole derivatives were discovered using a GK enzyme-based assay that is exemplified as compounds **2a-j** (Figure 21). The substitution with methyl and thiazol-5-ylmethanol led to the existence of a highly potent compound of the series, compound **2a** with EC₅₀ value is 0.019 μM. The replacement with the trifluoro(methoxy) group gives rise to compound **2b** that illustrates enhanced potency as the EC₅₀ value is 0.022 μM. Compound **2c** contains 4,5-dihydrothiazole exhibits an EC₅₀ value of 0.027 μM. Compound **2d** holds 4,5-dihydrothiazole and along with an attachment of fluoro at 5th position of indole ring that shows almost equipotent activity as that of compound **2c** as EC₅₀ of 0.028 μM. In compound **2e**, there is substitution at the 5th position of the thiophene ring with oxazole which leads to reduce in potency as the EC₅₀ value is 0.029 μM. The replacement with ethyl escalates compound **2f** results in reduced potency as EC₅₀ value 0.034 μM. Compound **2g** holds ethyl and 4-(thiazol-5-ylmethoxy) benzoic acid along with substitution at 4th position of indole ring with chloro exhibits reduced potency with an EC₅₀ value is 0.035 μM. Compound **2h** contains replacement with ethyl 2-(thiazol-5-yl)acetate showed EC₅₀ value of 0.048 μM. In compound **2i** substitution with 2-ethoxy-ethane and 5-(chloromethyl)thiazole, EC₅₀ value is 0.077 μM. While substituting with 3-(thiazol-5-yl)propanenitrile in compound **2j**, a vast increase in potency occurs as EC₅₀ value is 0.08 μM [157].

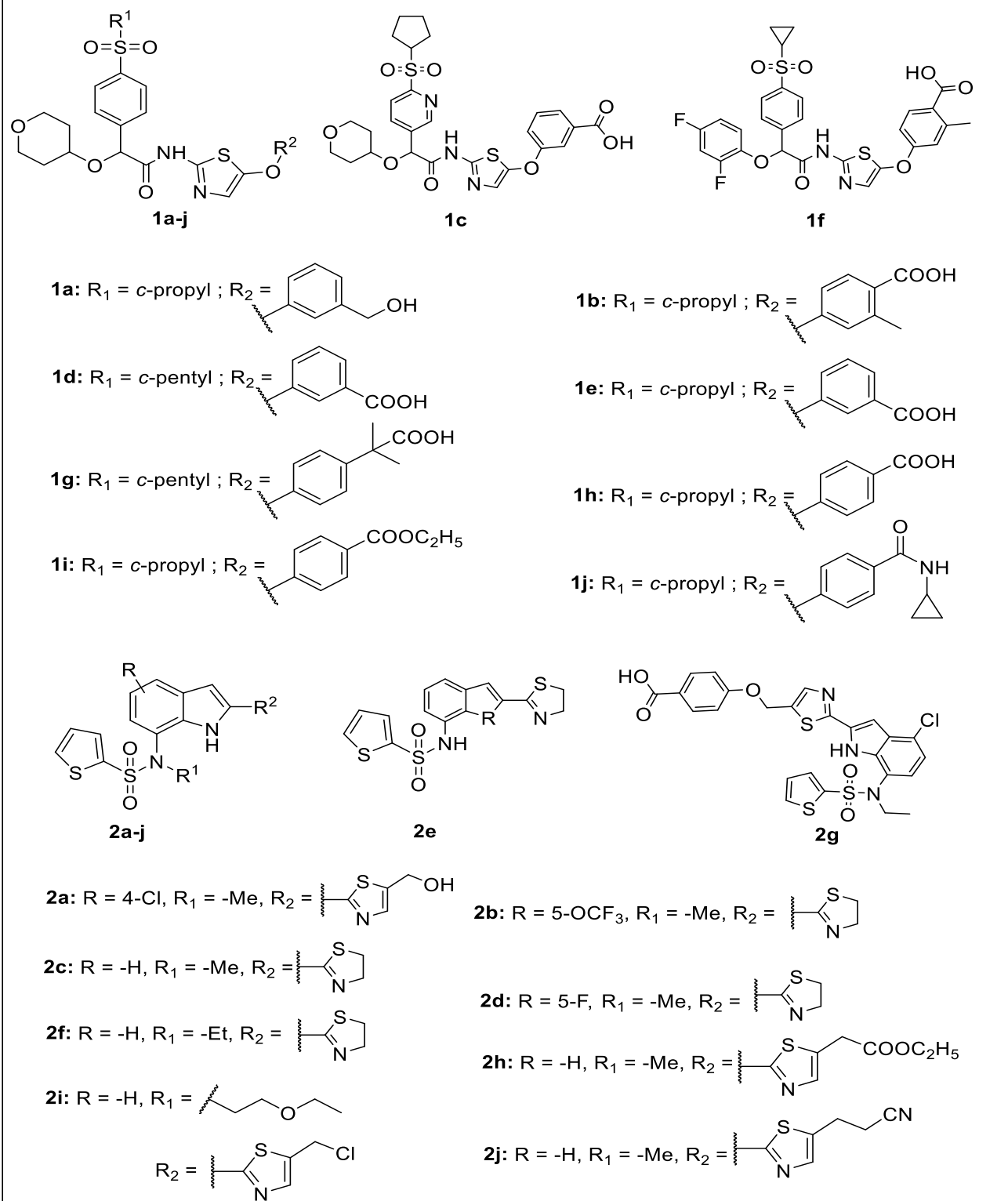


Figure 21. Acetamide derivatives **1a-j** and indole derivatives **2a-j**.

2.2.18.3 F. Hoffmann-La Roche Ag. AU2009237792B2

A patent has been filed for pyridazinone derivatives as GK activators for the treatment of T2D. *In vivo* glucokinase activity of the synthesized derivatives was performed, and compounds **3a-c** (Figure 22) exhibited higher % glucose-lowering activity in lean C57BL/6J mice at 4 h post 50 mg/kg dose along with good potency. Compound **3a** contains methyl and 2-methyl-1-(1H-pyrazol-1-yl)propan-2-ol, which exhibited an EC₅₀ value of 0.174 μ M (62.7 % glucose-lowering). In compound **3b**, substitution with *c*-pentyl and nicotinic acid resulted in elevated potency, with an EC₅₀ value of 0.063 μ M (60.9 % glucose-lowering). The substitution with fluoro and 2-methyl-1-(1H-pyrazol-1-yl)propan-2-ol resulted in compound **3c** which exhibited lower potency (0.312 μ M (EC₅₀ value) and increased

% glucose-lowering as 61.7%. *In vitro* GK enzyme assay revealed that compounds **3d-g** (Figure 22) exhibited potent antidiabetic activity by increasing the flux of glucose metabolism, resulting in augmented insulin secretion. SC_{1.5} was calculated for compounds with the aid of *in vitro* GK enzyme assay, and it is defined as the concentration of activator at which there is a 50% increase in the activity of GK. Compound **3d** comprehends difluoro groups and methyl nicotinate substitution, exhibited the most potent activity, with an EC₅₀ value of 0.008 μ M. In compound **S-3d** replacement of the fluoro group at R₁ and R₂ and methyl nicotinate exhibited potency as EC₅₀ values 0.013 μ M. The substitution with fluoro and nicotinic acid escalates compound **3e** with EC₅₀ value 0.026 μ M. In compound **3f**, removal of the fluoro group at R₂ resulted in decreased potency, with an EC₅₀ value of 0.033 μ M. The removal of the fluoro moiety at R₁ escalated compound **3h**, which exhibited reduced potency with an EC₅₀ value of 0.034 μ M. The replacement of the methyl group with a methyl group gave rise to compound **3g** showing equipotent activity with an EC₅₀ value of 0.033 μ M. Replacement with 2-methyl-1-(1H-pyrazol-1-yl) propan-2-ol gives rise to **S-3c**, resulting in decreased potency with an EC₅₀ value of 0.036 μ M [158].

2.2.18.4 Merck Sharp & Dohme Corp. WO2014099578A1

A patent application was filed for glucokinase activators useful for T2D, obesity, and metabolic syndrome. A series of substituted pyridine derivatives were produced using an enzyme-based assay, as exemplified by **4a-h** (Figure 22). Compound **4a**, contains sulfonyl-methane and methyl propionate, exhibited an EC₅₀ of 3.8 nM. In compound **4b** increase in potency was observed,

with an EC₅₀ value of 1.9 nM when replaced with sulfonyl ethane. The replacement of the pyridyl moiety of compound **4h** with pyrazine yielded compound **4c** with enhanced potency with an EC₅₀ value of 2.1 nM [159]. While the substitution of compound **4f** with methyl pentanoate (longer alkyl chain ester), escalates compound **4d** with enhanced potency, with an EC₅₀ value of 4.3 nM. In contrast, substitution with acetic acid generated compound **4g** with a reduced potency (EC₅₀ = 23.8 nM), whereas substitution with propionic acid yielded compound **4e** with an enhanced potency (EC₅₀ = 15.1 nM). Compound **4h** comprehends methyl propionate as a substituent, exhibited an EC₅₀ value of 39.1 nM. When a lower homologue, methyl acetate, was used, compound **4f** increased potency at an EC₅₀ value of 21 nM [160].

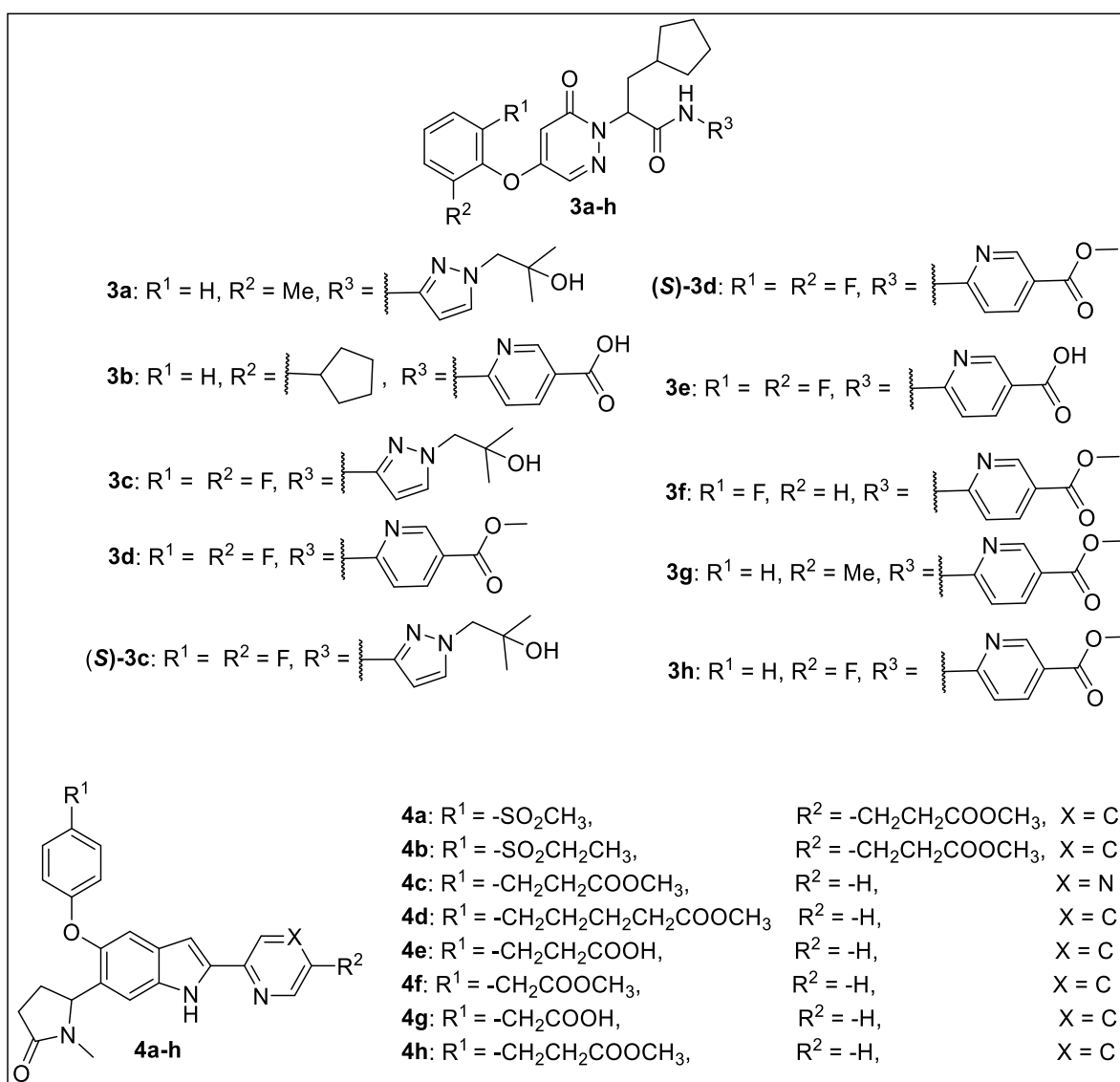


Figure 22. Pyridazinone derivatives **3a-h** and substituted pyridine derivatives **4a-h**.

2.2.18.5 Pfizer Inc. CA2754681C

In 2014, Pfizer Inc. claimed that benzofuranyl derivatives are potent glucokinase activators. Seven compounds (**5a-g**) (Figure 23) out of the synthesized compounds showed potent EC₅₀ values and were claimed to possess above 50% increase in the glucokinase enzyme activity (% E_{max}). The substitution with 5-methylpyrazine and methyl resulted rise to compound **5b** exhibiting an EC₅₀ value of 0.555 µM and E_{max} 54.7 %. Currently, compound **5b** (PB-201) is in phase 3 of clinical trials [161]. The alteration of an oxy-pyrimidine moiety of compound **5b** with oxy-pyrazine escalated compound **5a** shows enhanced potency EC₅₀ value 0.412 µM and E_{max} 60.8 %. Compound **5c** contains 5-methoxypyrazine, resulting in increased potency as well % glucokinase activity an EC₅₀ value 0.462 µM and E_{max} 69.5%. The substitution 5-methylpyrazine and ethyl resulted in the existence of compound **5e** possessed almost equipotent activity as that of compound **5b** with EC₅₀ value 0.546 µM and E_{max} 57.6%. The alteration of the oxy-pyrimidine moiety of compound **5c** with oxy-pyrazine resulted rise to compound **5g** exhibiting elevated potency an EC₅₀ value 0.474 µM and E_{max} 63.4%. Compound **5d** comprehend 1-methyl-1H-pyrazole and N, N-dimethyl substitutions, exhibited EC₅₀ value 0.629 µM and E_{max} 63.9%. The replacement of one methyl at R² position of compound **5d** with ethyl produced compound **5f** with improved activity EC₅₀ value of 0.382 µM and E_{max} 54.1%. The replacement of one methyl group at the R² position of compound **5a** with ethyl generated compound **5g** with almost equipotent activity an EC₅₀ value of 0.474 nM and E_{max} 63.4% [162].

2.2.18.6 Bristol-Myers Squibb. EP2059522B1

A patent application had been filed for the preparation of phosphinate and phosphonate compounds as GK activators for T2D treatment. *In vivo* glucokinase activity of the synthesized derivatives was done, compound **6a-f** (Figure 23) exhibits a higher % drop in glucose at a 30 mg/kg dose in male DIO (diet-induced obese) C57BL/6J mice, along with good potency. In compound **6a**, substitution with diethyl(thiazol-4-ylmethyl) phosphonate exhibited EC₅₀ value of 34 nM (88% glucose-lowering). The replacement with ethyl methyl(thiazol-4-ylmethyl) phosphinate gives rise to compound **6b** with reduced potency as EC₅₀ value of 49 nM (62-82% glucose-lowering). Compound **6d** contain diethyl (pyrazin-2-yl methyl) phosphonate position showed EC₅₀ value of 564 nM (62-80% glucose-lowering). The replacement with diethyl(pyridin-3-ylmethyl) phosphonate gives rise to compound **6c** resulting in enhanced potency as an EC₅₀ value of 98 nM

(66-78% glucose-lowering). In compound **6e**, diethyl((1H-pyrazol-1-yl) methyl) phosphonate substitution showed reduced potency as EC₅₀ value of 65 nM (79% glucose-lowering). Compound **6f** exhibits raised potency as EC₅₀ value 18 nM but lower % glucose-lowering as 44% when (thiazol-4-yl) methyl) methylphosphonate was incorporated. *In vitro* GK enzyme assay concluded that the compounds **6g-j** exhibited potent antidiabetic activity. The compound **6g** comprehends 2-(thiazol-4-ylmethyl)-1,4,2-dioxaphosphinane-2-oxide substitution results in an increase in potency with an EC₅₀ value of 9 nM. The substitution with diethyl((5-bromothiazol-4-yl) methyl) phosphonate gives rise to a compound **6h** with elevated potency as EC₅₀ value of 15 nM. Compound **6i** contain diethyl((5-chlorothiazol-4-yl) methyl) phosphonate exhibits potency with an EC₅₀ value of 22 nM. In comparison to compound **6e**, compound **6j** contain azetidin-1-yl(pyrazin-2-yl) methanone replacement instead of methyl-sulfonyl-phenoxy group and *i*-propoxy at place of (S)-((1-methoxypropan-2-yl)oxy) group leads to increase in potency as EC₅₀ value 38 nM [163].

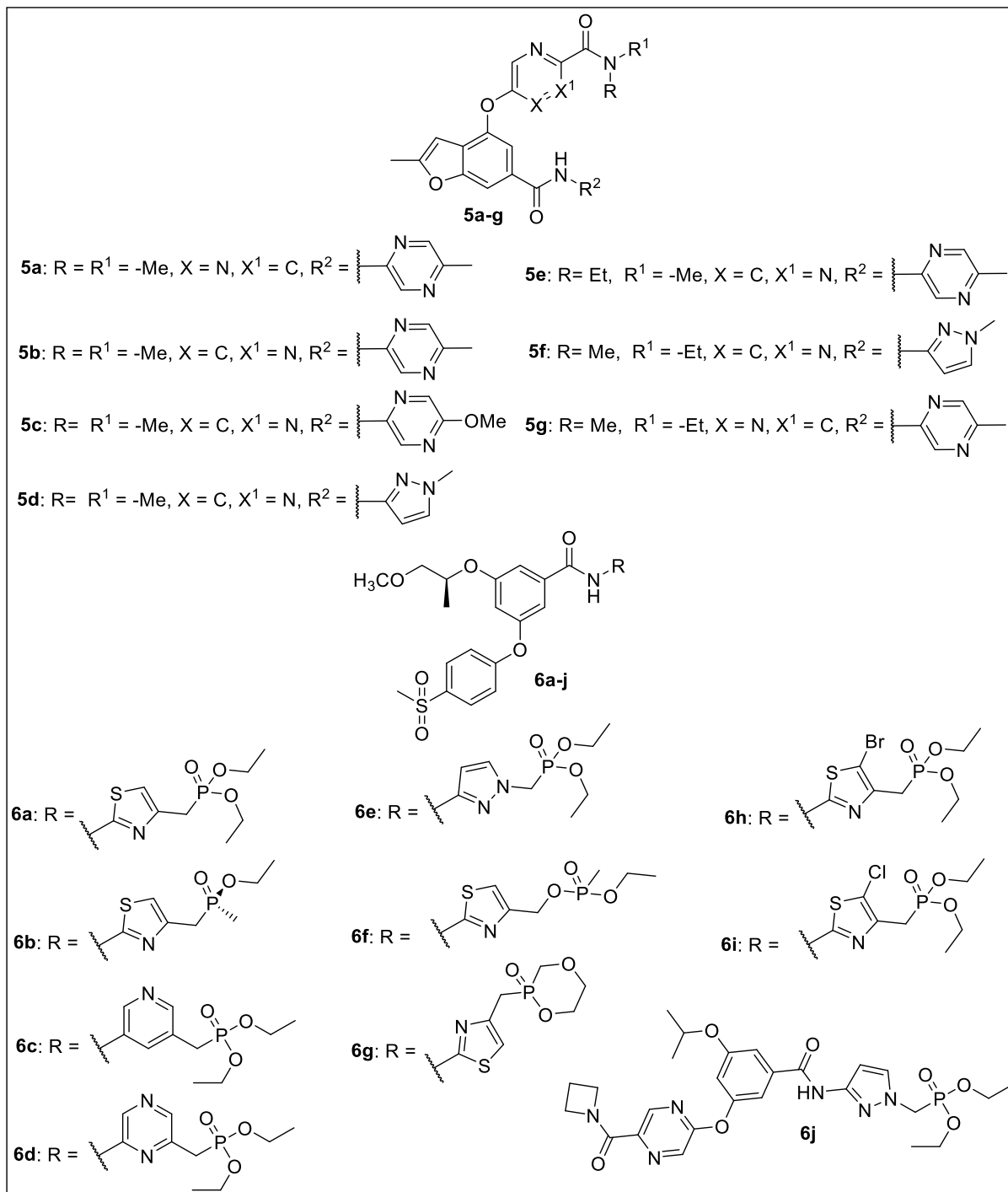


Figure 23. Benzofuranyl derivatives **5a-g** and phosphonate derivatives **6a-j**.

2.2.18.7 Foshan Saiweisi Pharmaceutical Technology Co. Ltd.

CN104892505A

Halogenated quinoline derivatives as GK activators for T2D treatment were patented by Foshan Saiweisi Pharmaceutical Technology Co. Ltd. Compounds **7a-e** (Figure 24A) have strong antidiabetic action, *in vitro* GK enzyme assay. These compounds are formed by substituting various halogens on the quinoline ring. The substitution with iodo and methyl leads to the existence of compound **7e** with an EC₅₀ value 10.3 nM and S_{0.5} (substrate concentration at which the rate of reaction is half of the limiting rate) as 3.5 mM. In compound **7d** bromo and methyl substitution exhibited raised potency as EC₅₀ value 8.8 nM and increased S_{0.5} as 3.0 mM. While the chloro substitution and methyl escalate compound **7b** with increased potency and improved S_{0.5} as EC₅₀ value of 4.5 nM, S_{0.5} as 1.6 mM. The replacement with fluoro gives rise to compound **7c** exhibiting reduced potency with an EC₅₀ value 6.7 nM, S_{0.5} as 2.1 mM. There is a vast drop in potency observed in compound **7a** when both R¹ and R² positions reserved with hydrogens as EC₅₀ value 24.1 nM and reduced S_{0.5} as 5.4 mM [164].

CN104628617A

Foshan Saiweisi Pharmaceutical Technology Co., Ltd. patented N-adamantane amide derivatives as GK activators for the treatment of T2D. *In vitro* GK assay, compounds **8a-e** (Figure 24A) exhibited a strong antidiabetic effect. The substitution with a phenyl ring gave rise to compound **8a**, which showed an EC₅₀ of 21.6 nM and S_{0.5} of 3.7 mM. The replacement with 4-methylphenyl gave rise to compound **8b** with enhanced potency, with an EC₅₀ value of 5.9 nM and S_{0.5} of 2.6 mM. The efficacy drop was observed in compound **8c** an EC₅₀ value of 10.4 nM and S_{0.5} of 3.2 mM when substituted with 4-ethylphenyl. Compound **8d** bears 3-methylphenyl, exhibited an EC₅₀ value of 8.2 nM and S_{0.5} of 2.6 mM. The replacement of 2-methylphenyl in compound **8e** resulted in reduced potency, with an EC₅₀ value of 9.3 nM and S_{0.5} of 3.7 mM [165].

CN104672218A

Glucosamide derivatives as GK activators for the treatment of T2D were patented by Foshan Saiweisi Pharmaceutical Technology Co., Ltd. *In vitro* glucokinase activity of the synthesized derivatives was determined, and compound **9a-h** (Figure 24A) exhibited promising antidiabetic activity. All these compounds are formed by distinct substitutions on the pyridine and pyrazine moieties of the general structure. In compound **9c**, both R¹ and R² as hydrogen, exhibited an

EC₅₀ value of 16.2 nM and S_{0.5} of 5.8 mM. Replacement of R² with methyl leads to the formation of compound **9a** with enhanced potency and improved S_{0.5}, with an EC₅₀ of 11 nM and S_{0.5} of 3.4 mM. Compound **9b**, which bears methyl groups at both R¹ and R², exhibited good GK activity with an EC₅₀ of 4.9 nM and S_{0.5} of 2.1 mM. The removal of the methyl group at the R² position of compound **9b** gave rise to compound **9h** showed reduced potency with an EC₅₀ value of 9.3 nM and S_{0.5} of 3.1 mM. Compound **9g** contains both R¹ and R² as ethyl groups, with an EC₅₀ of 8.9 nM and S_{0.5} of 3 mM. The removal of the ethyl group at R¹ of compound **9g** escalated compound **9e** with reduced potency, with an EC₅₀ value of 11.4 nM and S_{0.5} of 3.8 mM. In compound **9d** R¹ is ethyl and R² is methyl, which has an EC₅₀ of 7.1 nM and an S_{0.5} of 2.7 mM. While the vice versa of substitutions that is, R¹ as methyl and R² as ethyl of compound **9d**, forms compound **9f** that displays enhanced potency EC₅₀ value of 5.4 nM and S_{0.5} of 2.2 mM [166].

CN104557698A

In the realm of GKA research, Foshan Saiweisi Pharmaceutical Technology Co., Ltd. filed a patent application in 2015. A series of derivatives containing quinolyl and alkoxyphenyl moieties were identified using a GK enzyme-based assay, exemplified by compounds **10a-f** (Figure 24A). Compound **10a** holds methoxy and acetamide, exhibited an EC₅₀ of 9.4 nM and S_{0.5} of 2.1 mM. The substitution with hydroxy gave rise to compound **10b** which displayed reduced potency with an EC₅₀ value of 15.3 nM and S_{0.5} of 3.6 mM. In compound **10c** ethoxy and acetamide substitutions yielded an EC₅₀ value of 10.2 nM and S_{0.5} of 2.7 mM. The replacement with propionamide escalated compound **10f** with enhanced potency EC₅₀ values of 11.7 nM and S_{0.5} of 2.9 mM. Compound **10e** containing methoxy and propionamide showed an EC₅₀ value of 11 nM and S_{0.5} of 3.9 mM. The replacement of the R¹ position of compound **10e** with hydroxy led to the existence of compound **10d** which exhibited reduced potency with an EC₅₀ value of 16.6 nM and S_{0.5} of 3.2 mM [167].

2.2.18.8 Shanghai Inst. Materia Medica.

WO2016112863A1

Shanghai Inst Materia Medica has filed a patent including the development of N-substituted-3,5-disubstituted benzamide derivatives **11a-f** (Figure 24A) as GK activators for the treatment of T2D. Compound **11c** R¹ as N, N-dimethylformamide, R² as tert-butyl formate, and X as nitrogen,

exhibited an EC₅₀ value of 0.09 µmol/L. Replacing N, N-dimethylformamide at the R¹ position of compound **11c** with azetidine-1-carbonyl resulted in compound **11d** showed equipotent with an EC₅₀ value of 0.1 µmol/L. Compound **11e** comprehends R¹ as methylsulfonyl and R² as pyridyl. X as nitrogen offers an EC₅₀ value of 0.1 µmol/L. Compound **11a** comprehends R¹ as methylsulfonyl, R² as acetamide, and X as carbon exhibited the highest potency, with an EC₅₀ of 0.04 µmol/L. The substitution at the R¹ position of compound **11a** with azetidine-1-carbonyl and replacement of 1-methoxy-propanoxy with i-propoxy led to the formation of compound **11b** with almost equipotent activity, as indicated by an EC₅₀ value of 0.05 µmol/L. Compound **11f** had R¹ as ethylsulfonyl, R² as tert-butyl formate, and X as nitrogen, offering an EC₅₀ value of 0.14 µmol/L. The replacement of the R¹ position of compound **11f** with *c*-propyl sulfonyl group resulted in compound **11g** which exhibited reduced potency with an EC₅₀ value of 0.2 µmol/L.

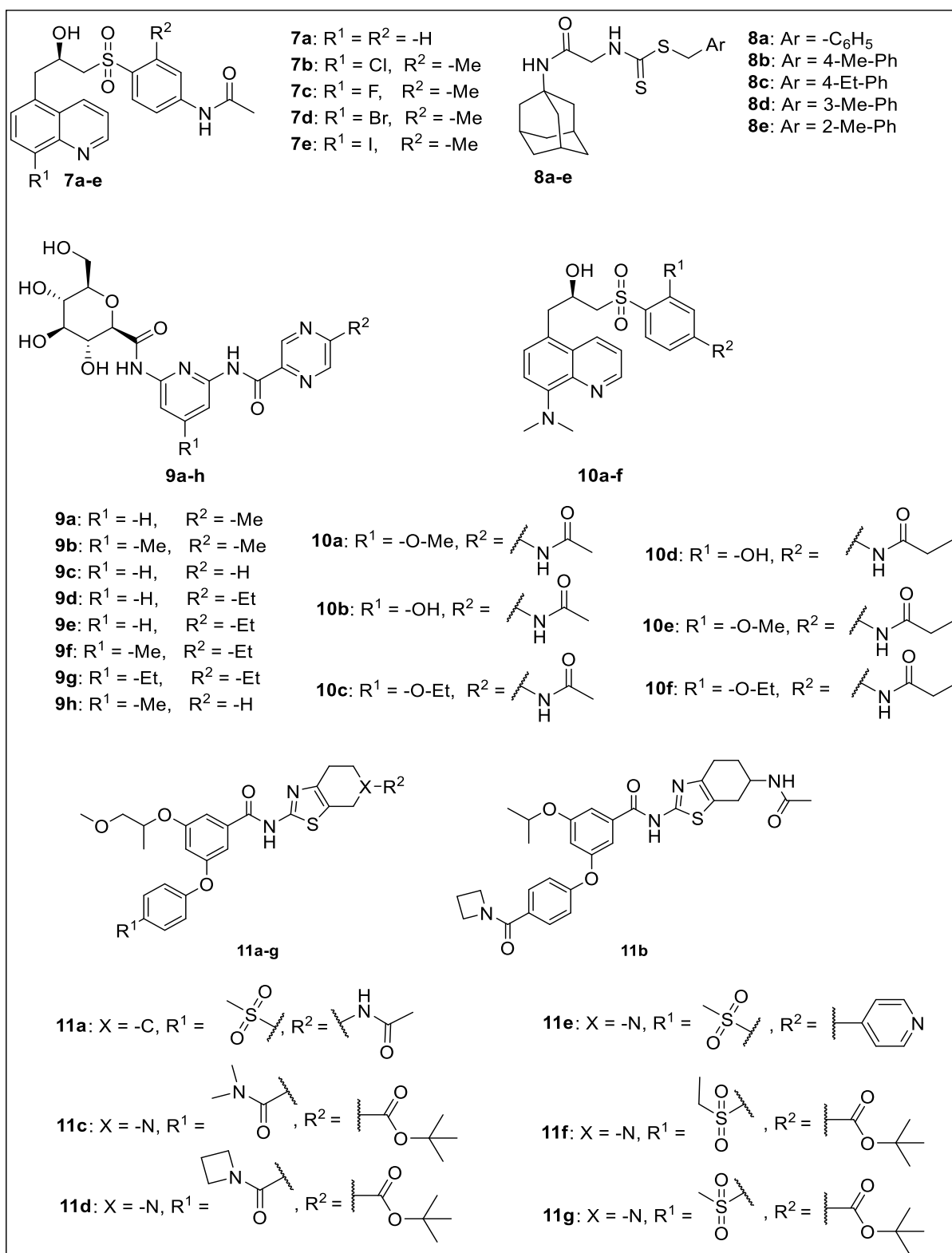


Figure 24A. Quinoline derivatives **7a-e**, adamantane amide derivatives **8a-e**, glucosamide derivatives **9a-h**, quinolyl derivatives **10a-f** and N-substituted-3,5-disubstituted benzamide derivatives **11a-g**.

The introduction of polar and bulky substituents at the R¹ position, including N, N-dimethylformamide (**11e**), azetidine-1-carbonyl (**11d**), ethyl sulfonyl (**11f**), and cyclopropyl sulfonyl (**11g**), while maintaining identical R² and X groups, led to a consistent decline in potency. This trend suggests that increased steric hindrance and/or polarity at R1 may negatively influence the target-binding affinity [168a].

WO2010150280

Cadila Healthcare has published several patent applications for potent glucokinase activators based on heterocyclic scaffolds. Aryl-centered compounds in which a 1,3,4-oxadiazole moiety was used as a bioisosteric substitution for the traditional sulfone or amide group were revealed in a 2010 filing, in which compound **11h** (Figure 24B) was demonstrated to be highly potent (EC₅₀ = 0.014 μM) [168b].

WO2011013141

This patent described structurally analogous containing pyrrolidinone substituents, in wherein compound **11i** (Figure 24B) exhibited high activity (EC₅₀ = 0.021 μM) and demonstrated moderate glucose-lowering effects (23.3%) in OGTT studies [168c].

IN2012MU01500

The scaffold was further diversified by a third application, which substituted the oxadiazole core with other heterocycles, including fused triazoles. Compound **11j** (Figure 24B) exhibited a more pronounced reduction (75.2%) in blood glucose levels. Altogether, these studies emphasize the importance of oxadiazole and its analogs in the context of increased glucokinase activation and glucose-lowering activity [168d].

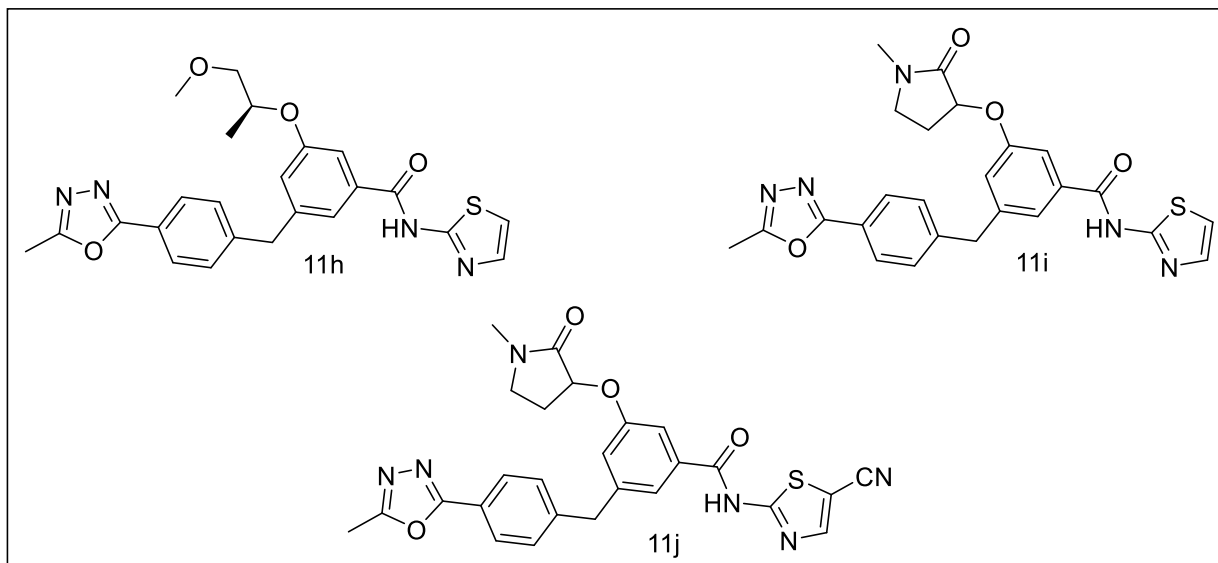


Figure 24B. Oxadiazole derivatives (11h-11i) as GKA.

2.2.19 Literature insights from prior research

3,5-disubstituted benzamide derivatives

A substitution was incorporated into the benzamide nucleus based on pharmacophoric requirements (N of benzamide nucleus) for ligand binding to the GK protein (where the Arg63 residue is essential for GK activity). According to docking studies, these derivatives exhibited significant binding interactions with the GK protein at Arg63. *In vitro* GK assay, compounds **12a-f** (Figure 25) showed promising GK activity of 1.74 ± 0.08 , 1.89 ± 0.06 , 1.68 ± 0.04 , and 2.11 ± 0.05 , respectively. 2.11 ± 0.09 and 1.99 ± 0.07 μM , respectively. A glucose-lowering effect of these derivatives was assessed in the rat model using OGTT assays. AUC and blood glucose levels at varied time intervals such as 0, 30, 60, 90 and 120 min following oral glucose administration, were used to measure antihyperglycemic activity. *In vivo* OGTT assay reveals that these compounds showed increased antihyperglycemic activity due to the substitution of electron-withdrawing groups such as chloro in compound **12d** and nitro in compound **12e** at the phenyl ring. Compounds **12d**, **12e** and **12f** exhibited glucose AUC reduction in the rat OGTT model as 250, 240, 260 mg/dL at 120 min respectively in a similar way to the standard anti-diabetic drug metformin (glucose AUC reduction in rat OGTT model as 230 mg/dL at 120 min) whereas compounds **12a-c** did not show a significant reduction in glucose AUC. Compounds **12d**, **12b** and **12c** showed improved

antihyperglycemic activity as the pyrimidine-2-yl ring was replaced with the thiazol-2-yl ring, as demonstrated by the OGTT assay results in comparison to compounds **12a**, **12b** and **12c**. *In vitro*, enzymatic test results were not in compliance with the *in vivo* OGTT assay results. The most potent compounds **12a-c** in terms of *in vitro* GK activity remained ineffective in glucose AUC reduction in the rat OGTT model (*in vivo*) [169].

Sulfonamido-benzamide hybrids

In addition to the carboxyl (C=O) group on the carboxyl side of benzamide, the amino (NH) group on the amino side of benzamide acts as an allosteric site (with Arg63) on the GK. The benzamide ring was modified by adding a substituted sulfone fragment at the 2nd position of the primary amino group to enhance oral bioavailability. To target the allosteric site in the 1V4S GK protein, heterocyclic moieties with hydrophobic interactions and strong hydrogen bonds with amino acid residues can be substituted for the amido nitrogen in the benzamide nucleus. The compounds **13a-e** (Figure 25) exhibited good *in vitro* GK activation. Compound **13a** contains ethyl and 5-chloro-thiazole substitutions showed an EC₅₀ of 495 nM. The replacement of 4-methyl phenyl with that of **13d** lowers GK activation (EC₅₀ = 657 nM). In compound **13b**, the 6-methylbenzothiazole replacement exhibited an EC₅₀ of 522 nM. The replacement with 4-methyl phenyl escalated compound **13e** with reduced potency, with an EC₅₀ of 836 nM. Compound **13c** displayed an EC₅₀ of 958 nM when 5-methyl-1,3,4-thiadiazole was introduced as a replacement. *In vivo* OGTT assay, compound **13a** showed an extreme decrease in blood glucose level to 135 mg/dL, followed by compound **13b** at 142 mg/dL at 120 min compared to the control. Compounds **13d** and **13e** showed a modest drop in blood glucose levels of 168 and 162 mg/dL, respectively. Compound **13c** showed a similar pattern of blood glucose drop as that of control 172 mg/dL (ineffective) [170].

Thiazol-2-yl benzamide derivatives

To enhance the activity of GK in the benzamide nucleus, thiazol-2-yl was substituted at the amido nitrogen, and aromatic moieties were substituted at the sulfonamide group. *In vitro* enzymatic testing, compounds **14a-d** (Figure 25) had a good profile of GK activation. Compound **14a-d** have R¹ as phenyl, 2-nitrophenyl, *c*-propyl, and 2-chlorophenyl, respectively, and R² as hydrogen in all except compound **14d** (R² is *p*-tolyl). These compounds exhibited GK activation by 1.65, 1.74, 1.48, and 1.82-fold, respectively. These compounds were subjected to *in vivo* OGTT in rats. *In*

in vivo OGTT assay results demonstrated that compound **14d** reduced blood glucose levels comparable to that of the standard drug (metformin blood glucose level reduction of 125 mg/dL) at 120 min. The substitution with electron-withdrawing groups, such as NO₂ and Cl, increased *in vivo* antidiabetic activity, as observed in compounds **14b** and **14d**. Compound **14a** exhibited reduced antidiabetic activity when the electron-withdrawing group was replaced with hydrogen. The results of the *in vivo* antidiabetic activity corresponded with the *in vitro* enzyme assay [171].

N-benzothiazol-2-yl benzamide derivatives

The N-benzothiazol-2-yl moiety was attached to the benzamide nucleus to form novel derivatives of GKA. Compound **15a-g** (Figure 25) exhibited hydrogen bond interactions with the Arg63 amino acid residue (which is essential for GK activity) and had dock scores of -9.9, -9.4, -8.4, -9.3, -8.6, -8.7, and -7.5, respectively. Compound **15a-d** comprehends R¹ as N-(2-methyl phenyl), N-4-bromophenyl, N-phenyl, and N-2-chloro-4-nitrophenyl, respectively, exhibited GK activation by 1.97, 1.84, 1.66, 1.69-folds respectively. According to the results obtained from the *in vitro* GK test, compounds **15a-d** exhibited improved GK activity when at R¹, substituted phenyl moieties were attached. In compound **15e** replacement with methyl reduced the potential by 1.34-fold. In contrast, the substitution with a higher alkyl homologue, such as N-propyl in compound **15f** and N-butyl in compound **15g**, remained ineffective in the GK assay. The docking results were consistent with those of the *in vitro* GK assay [172].

5-Alkyl-2-urea-substituted pyridines

The study includes designing and evaluating the GK activity of the prepared 5-alkyl-2-urea-substituted pyridines derivatives **16a-b**. By substituting the 5-position of methyl urea pyridine, different compounds were formed. The substitution with *c*-butanol at R¹ forms a compound **16a**, with higher S_{0.5}. This reduces the risk of hypoglycemia and maintains equivalent potency and V_{max} as that of AM-2394. AM-2394 was reported as potent GKA with an EC₅₀ of 65 nM, S_{0.5} of 0.64 and V_{max} of 1 in a study conducted by Dransfield, et.al [117]. While compound **16a** displayed a good potency, EC₅₀ as 110 nM, S_{0.5} of 0.66 and V_{max} of 0.97. During OGTT assay with doses of 3, 10 and 30 mg/kg, in a dose-dependent manner it reduces blood glucose levels, resulting in a 32% OGTT-AUC reduction at 30 mg/kg. Compound **16b** comprehends tetrahydro-2H-pyran exhibited EC₅₀ as 160 nM, S_{0.5} of 0.65 and V_{max} of 1.1. A dose-proportional diminution in blood glucose levels was observed when compound **16b** was administered at doses of 3, 10, 30, and 100 mg/kg

during the OGTT assay, displaying a 40% reduction in AUC at 100 mg/kg. Compounds **16a** and **16b** (Figure 25) demonstrated acceptable clearance, better oral bioavailability and successfully reduced plasma glucose levels in rodents [173].

Quinazolin-4-one derivatives

Design, synthesis, and evaluation of clubbed thiazole acetates and acetamide derivatives of quinazolin-4-one as GKA was studied by Khadse et. al. Using docking studies, best-fit conformations were evaluated for the allosteric site of human glucokinase 1V4S to evaluate the binding mode and interactions. The docking result concluded that compounds **17a** and **17b** (Figure 25) have the highest dock scores as -6.44 and -6.24, respectively, which is similar to standards RO-28-1675 (-6.39) and piragliatin (-7.89). Compound **17a** shows 2H-bond interactions, one between Arg63 residue and carbonyl oxygen (C=O) of the acetate group. Second H-bond interaction between N¹ of the quinazolinone ring and Thr65. Compound **17b** exhibits 3H-bond interactions; 1st between Tyr61 residue and NH of the amide group, the 2nd interaction with N of thiazole ring with Arg63 and the 3rd interaction with N1 of quinazolinone ring and Tyr214 (Figure 25). As compared with the standards RO-281675 and piragliatin (Figure 25), compound **17a** with an EC₅₀ of 632 nM, and compound **17b** with an EC₅₀ of 516 nM, demonstrated the highest *in vitro* GK activation. *In vivo* OGTT assays revealed that compounds **17a** and **17b** significantly reduced blood glucose levels to almost normal levels of 133 mg/dL and 135 mg/dL, respectively. *In vitro* enzymatic test, the OGTT assay results were in compliance with the docking scores of compounds **17a** and **17b** [174].

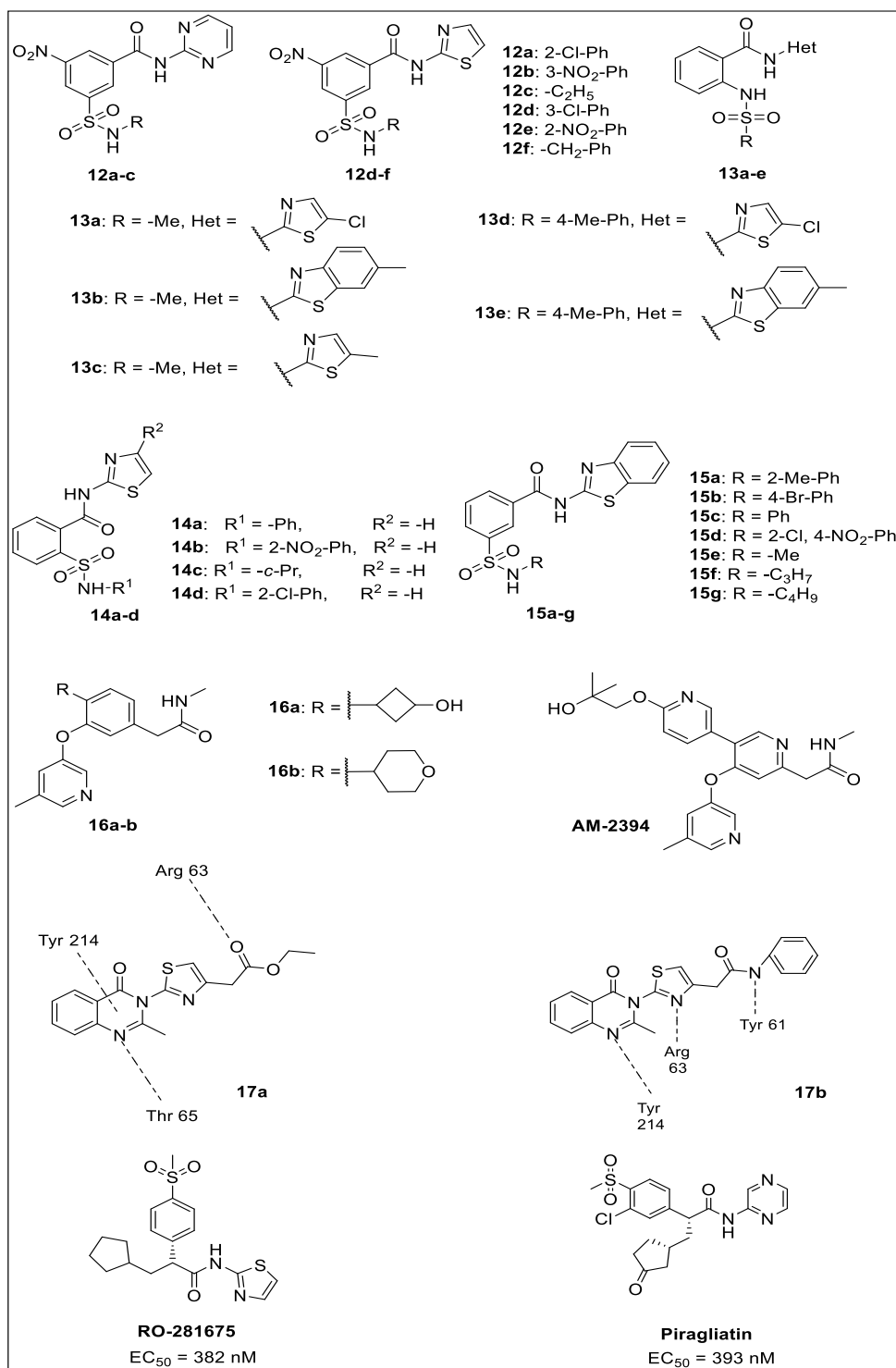


Figure 25. Substituted benzamide derivatives **12a-f**, **13a-e**, **14a-d** and **15a-g**, 5-alkyl-2-urea-substituted pyridine derivatives **16a-b** and quinazolin-4-one derivatives **17a-b**.

Amino phosphonate derivatives

To develop new potent leads, phosphate, amino, and aromatic moieties were combined in different orientations to specifically interact with allosteric residues. Hydrogen bonds can be formed between the amino group and the polar residues, while hydrogen bonds can be formed between the phosphate moiety and the polar residues. A complete π - π stacking interaction may form between the aromatic tyrosine residues within the allosteric site. Compound **18a** (Figure 26) is a potent compound in the series with the highest dock score of -7.689 Kcal/mol [175].

Sulfamoyl Benzamide Derivatives

GK was activated by addition of sulphonamide moieties at the aromatic ring and substitution at the amide linker. Compounds **19a** and **19b** showed favorable interactions in molecular docking studies (PDB. 3IMX) with the amino acid residues in the allosteric binding site of the GK protein. Both compounds **19a** and **19b** displayed the highest dock scores of -9.9 and -9.3 kcal/mol. The glucose-lowering effects of these derivatives were assessed in diabetic rats using the OGTT. Compounds **19a** and **19b** (Figure 26) exhibited a glucose-lowering effect equivalent to that of the standard drug metformin (190 mg/dl) at 190 and 200 mg/dl at a time interval of 2 h [176].

N-pyridin-2-yl benzamide derivatives

GK activators have been predominantly developed through the substitution of benzamide derivatives during drug discovery. These may be related to the allosteric site of the GK enzyme and its complementary orientation patterns. The development of new allosteric GK activators based on N-pyridin-2-yl substituted sulfamoyl benzamide derivatives was inspired by the importance of GK activators in T2D therapy. The *in silico* screening results showed that compounds **20a** and **20b** had the highest docking scores of -11.26 and -11.16 kcal/mol, respectively, in the molecular docking study (3IMX). Both compounds **20a** and **20b** (Figure 26) exhibited potent *in vitro* activity, with GK fold activation of 2.07 and 2.02 folds, respectively, at a concentration of 10 μ M. *In vivo* OGTT assay revealed that compounds **20a** and **20b** showed increased antihyperglycemic activity of 210 mg/dl and 200 mg/dl, respectively, compared to metformin (210 mg/dl) at an interval of 120 min. The *in silico*, *in vitro* and *in vivo* results were consistent with each other [177].

Pyridin-2-carboxamide derivatives

Novel Pyridin-2-carboxamide derivatives were designed by substituting the 3rd and 6th positions of the pyridine nucleus as GKA. In vitro assays revealed that compounds **21a** and **21b** (Figure 26) showed better GK activity with an IC₅₀ value of 0.001 μ M, 2.42 folds, and 0.012 μ M, 2.05 folds respectively. Compounds 21a and 21b demonstrated greater antihyperglycemic activity through substitution at the 3rd position of the pyridine ring with a hydrophobic group such as CH₃. After 120 min, compounds **21a** and **21b** in the OGGT assay lowered blood glucose levels by 122 mg/dL and 120 mg/dL, respectively, compared to metformin (110 mg/dL) [178].

Acetylenyl Benzamide Derivatives

The benzamide nucleus was combined with an acetylene group to design a novel GKA. Based on an in vivo OGTT study with favorable pharmacokinetic results, compound **22a** elicited a 47.4% reduction in AUC with an EC₅₀ of 27 nM. Additionally, compound **22a** (Figure 26) increased glucose uptake in rat primary hepatocytes by 2.16-fold and enhanced insulin release from rat pancreatic islets by 2.13-fold under glucose stimulation (glucose 0 mM to 5.6 mM). There was a dose-dependent improvement in the oral glucose tolerance of compound 22a in C57BL/6J mice, with favorable pharmacokinetics. At 30 mg/kg, this compound significantly reduced blood glucose AUC in C57BL/6J mice by 47.4%, whereas sitagliptin (standard drug) reduced it by 22.6% [179].

Diazole-benzamide Derivatives

Novel benzamide derivatives were designed by substituting the benzamide scaffold with diazole moieties. Docking analysis showed that the designed compounds fit the allosteric site on the GK protein in a complementary manner. Compounds **23a** and **23b** (Figure 26) showed favorable interactions in molecular docking studies (PDB. 5V4X) with the amino acid residues in the allosteric binding site of the GK protein. Considering hydrophobic interactions, PLP fitness, and hydrogen bonding, compounds **23a** and **23b** exhibited the best fitting with the active site, with dock scores of 77.95 and 70.23 kcal/mol. These compounds exhibited effective hydrophobic interactions with amino acid residues TYR 214 and TRP 99 by forming H-bonds [180].

Thiophene derivatives

Novel thiophene derivatives were designed by combining the thiophene ring with an amide and a heterocyclic ring as GKA. In vitro enzymatic testing of compounds **24a** showed a good profile of GK activation. In an in vitro GK assay, compound **24a** (Figure 26) showed an increase in GK activity 8.60 folds at a concentration of 10 μ M. The glucose-lowering effects of these derivatives were assessed in a rat model using OGTT assays. The AUC and blood glucose levels at various time intervals (0, 30, 60, 90, and 120 min) following oral glucose administration were used to measure antihyperglycemic activity. Compound **24a** in the OGTT assay resulted in lowering of blood glucose levels by 120 mg/dL as compared to piragliatin (120 mg/dL) [181].

N-benzimidazol-2-yl benzamide

Using a structure-based drug design approach, a series of novel N-benzimidazol-2-yl benzamide analogs were designed. The docking conformations of analogs **25a** and **25b** (Figure 26) revealed strong hydrogen bond interactions between the amide NH group and the N group of the benzimidazol-2-yl group in the Arg63 residue, with the backbone carbonyl and amide NH group also interacting with the Arg63 residue. Among these newly identified derivatives, there was a two-fold increase in the GK catalytic activity of derivatives **25a** and **25b** (>2.0-fold increase). An in vitro docking study of the GK enzyme showed results similar to those of the in silico docking study. Compared with compounds with alkyl groups, compounds **25a** and **25b** had improved GK activities when the substituted phenyl moiety was replaced with SO₂NH [182a].

Kaur et al. performed molecular docking on 91 phytoconstituents of *Allium hirtifolium* to determine their use as glucokinase activators. Out of the screened phytoconstituents, quercetin and kaempferol were found to be the most binding (-7.6 and 7.5 kcal/mol), which suggests a high level of association with the allosteric site of the glucokinase enzyme. The binding affinity of **26a** (Figure 26) (quercetin) and **26b** (kaempferol) (Figure 26) was high as they created 2 hydrogen bonds with the residue of glucokinase Arg63 (3.08 Å, 4.49 Å and 4.9 Å, 4.7 Å), which is an indicator of the availability to stabilize the enzyme-ligand complex and predicts their potential in the use as allosteric activators [182b].

To determine the potential of the selected phytoconstituents of *Allium hirtifolium* as glucokinase activators, in silico molecular docking studies of the selected phytoconstituents were conducted. Of

the compounds under investigation, kaempferol and quercetin had high binding affinities (-8.1 and -8.4 kcal/mol, respectively) and reacted mainly in the allosteric site of the enzyme. Arg63 and Ser69 were important residues involved in the major interactions of hydrogen bonding, anchoring the ligands in the binding cavity. **26a** (quercetin) (Figure 26) and **26b** (kaempferol) (Figure 26) exhibited a high level of interaction with Arg63, indicating that it is a significant ligand-binding residue. These data indicate that hydrogen bonding with Arg63/Ser69 and hydrophobic interactions within the pocket are critical for effective glucokinase activation [182c].

Coumarin hydrazide and oxadiazole-based derivatives have been reported as promising bioactive scaffolds, exhibiting notable antitubercular, anthelmintic, and antimicrobial activities [182d-182g].

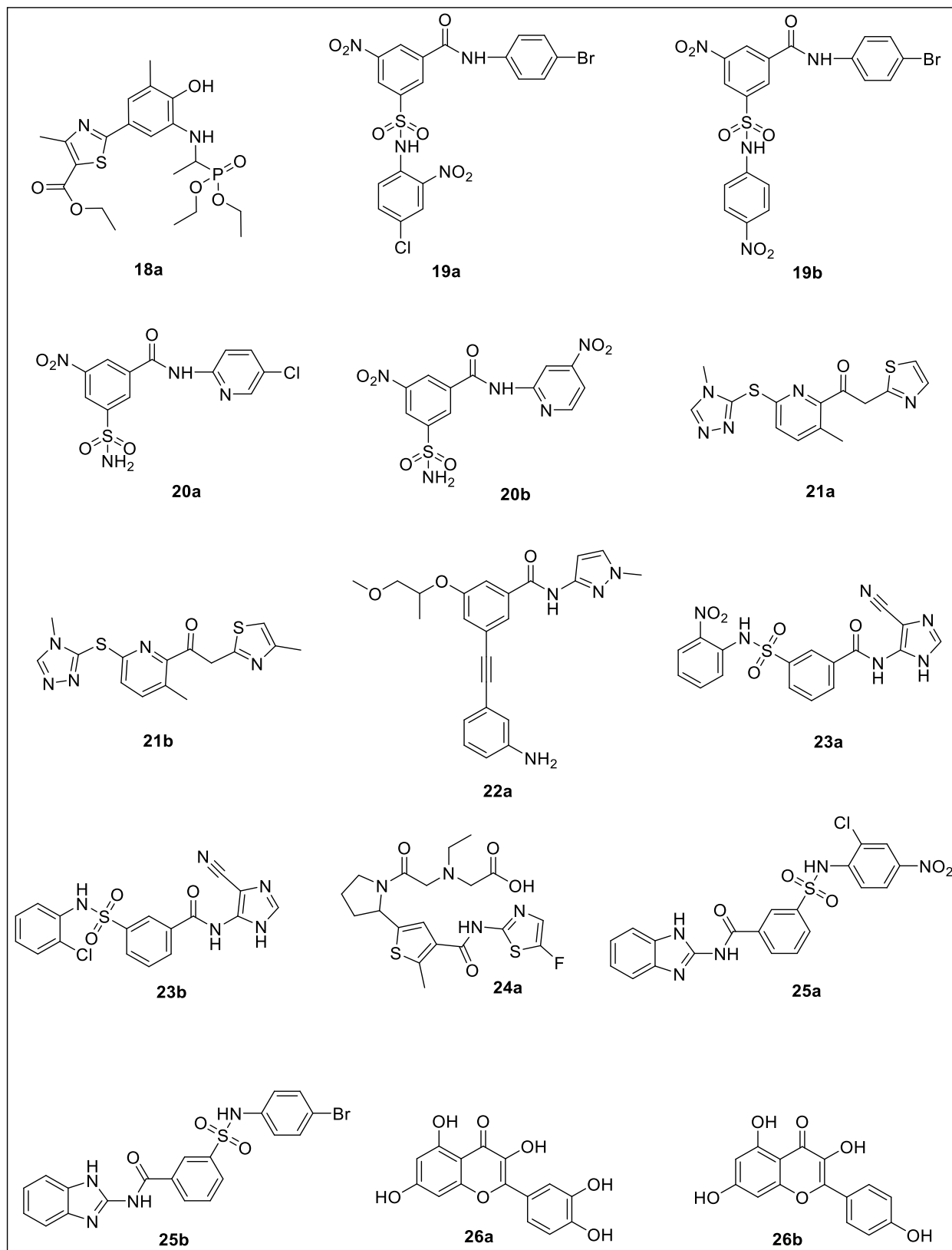
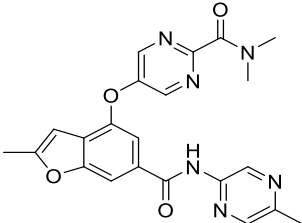
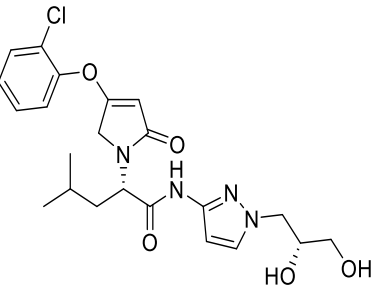
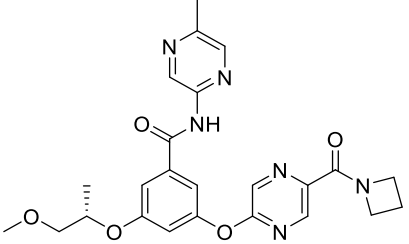
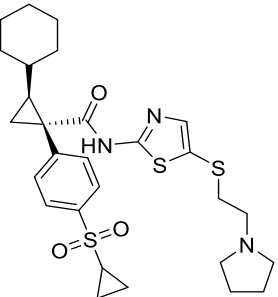


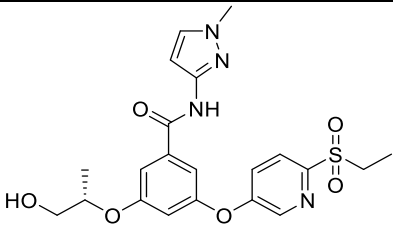
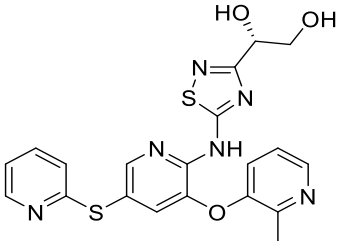
Figure 26. Substituted derivatives as GKAs

2.2.20 Clinical trial status of GKAs

Targeted dual action on the liver and pancreas, GKA is a novel class of drugs for diabetes treatment that provides the body with greater sensitivity to glucose. Some small-molecule GKAs have entered the clinical stage of research. Novel GKAs such as PB-201, Dorzagliatin (phase 4), AZD-1656, TTP-399 (phase 2), Globalagliatin, MK-0941, ARRY-403 (phase 1) (Table 4) are attracting considerable attention in the pharmaceutical industry [161, 183, 184, 185, 186, 187, 188].

Table 4. Glucokinase activators under clinical trials.

Sr. No.	Name/Code	Structure	Status	Reference
1.	PB-201		Phase 3	[161]
2.	Dorzagliatin		Phase 4	[183]
3.	AZD-1656		Phase 2	[184]
4.	TTP399	ND	Phase 3	[185]
5.	Globalagliatin		Phase 1	[186]

6.	MK-0941		Phase 2	[187]
7.	ARRY-403		Phase 1	[188]

2.2.21 Research Gap

Out of the existing measures currently on antidiabetic treatment, chronic use of metformin has been associated with the emergence of resistance to the treatment. Moreover, some oral hypoglycaemics, like pyrazolidines, sulfonylureas, and acarbose, are linked to serious adverse reactions as well as a number of restrictions, including inappropriate clinical efficacy [189]. As a result, there is an urgent requirement of discovery of new antidiabetic agents. The glucokinase-activators (GKAs) have become the top candidate for the management of T2D by increasing the cellular uptake of glucose, and by successfully lowering the levels of glucose in the bloodstream. However, the GKA development should be aimed achieving high efficacy while minimizing unwanted effects [190]. In a perfect scenario, GKAs ought to have a selective action on the liver and pancreatic tissues and not perform harmful effects on the non-target organs such as the brain and the cardiovascular system. In addition, these agents are not supposed to affect other critical physiological processes like insulin responsiveness or lipid homeostasis. Long-term studies are needed to establish the safety profile of GKAs. Overcoming the resistance to available oral antidiabetic drugs is another highly important goal of GKA-based therapies. The molecular pathways that cause drug resistance cannot be fully understood without a detailed insight into the pathways that lead to this phenomenon and more importantly, how to devise a strategy to prevent or reverse the occurrence of this phenomenon. Also, the use of GKAs in combination with the existing antidiabetic medications, including metformin, can result in improved glycemic control in patients with T2D [191]. Several previously reported GKAs, such as piragliatin and MK-0941, showed promising antihyperglycemic activity but did not succeed in clinical development due to

hypoglycemia, loss of sustained efficacy, and negative metabolic effects. Therefore, there is still a strong need to find new scaffolds with better safety and drug absorption profiles. The coumarin scaffold has gained significant interest because of its structural flexibility, beneficial pharmacological properties, and known antidiabetic potential. This makes it a promising foundation for creating safer and more effective glucokinase activators.



CHAPTER 3

RATIONALE, AIM, OBJECTIVES AND WORK PLAN

“ *Transforming Research Gaps into Scientific Opportunities.* ”



Research Gap Identification



Hypothesis Formulation



Aim



Objectives



Work Plan



RESEARCH GAP IDENTIFICATION



HYPOTHESIS FORMULATION



AIM



OBJECTIVES



WORK PLAN

3. RATIONALE, AIM, OBJECTIVES AND WORK PLAN

3.1. Rationale

Diabetes mellitus (DM) is a metabolic syndrome characterized by hyperglycemia due to impaired glucose homeostasis [192a]. This disease is associated with multiple pathologies, including decreased pancreatic β -cell function; insulin resistance in the liver, skeletal muscle, and adipose tissue; increased lipolysis in fat cells; resistance in the gastrointestinal tract; and impaired kidney function [192b]. If diabetes remains untreated, it leads to severe damage to the body's systems, such as blood vessels and nerves, and oxidative stress. DM leads to extensive disruption of cellular redox homeostasis [192c-192f]. A continued increase in glucose enhances oxidative phosphorylation in the mitochondria, leading to the overproduction of reactive oxygen species (ROS), overpowering the ability of the antioxidant defenses inherent to the body. Oxidative stress results from an imbalanced production of ROS and the effectiveness of the endogenous antioxidant defense system [192i-192k]. This imbalance plays a leading role in the development and advancement of many pathological diseases, such as DM, heart anomalies, and neurodegenerative illnesses [192l]. Exogenous antioxidant supplementation to counteract oxidative stress is a common practice in the therapeutic management of diabetes [192m-192o]. However, adjunctive therapy increases the burden of antioxidants, resulting in low compliance among patients. A molecular scaffold was rationally designed by incorporating a chromene nucleus as the head moiety, due to its well-established antioxidant properties [192p]. In this design approach, antioxidant activity is included in the pharmacophore, which could reduce the multi-drug regimen and increase compliance [192q-192r]. Extensive literature shows that the linker region mainly contains an amide bond with a variable tail moiety, which is mostly an aromatic ring that can be substituted or unsubstituted [192s-192t]. The head group was attached to the tail group (lipophilic) through an amide or oxadiazole linker. It is worth mentioning that earlier research has established that an increase in lipophilicity is positively correlated with molecular potency. In this respect, the current design uses substituted/unsubstituted aromatic rings as the tail group to enhance lipophilicity, which correlates positively with molecular potency.

Pharmacophoric studies revealed the involvement of the amide group in anchoring the ligand to the allosteric site, and an interaction between these groups and Arg63 [192u]. The H-bond interactions with Arg63 may be due to the linker fragment, which is the H-bond donor-acceptor

moiety [192v]. GK activators can stabilize the GK enzyme in its active conformation by creating essential H-bond interactions with Arg63 [192w]. This study aimed to identify new lead compounds with higher efficacy and superior pharmacokinetic attributes. We recently developed a privileged-fragment-merging (PFM) strategy (Figure 27) and synthesized a series of coumarin hydrazide and coumarin oxadiazole analogs. Hydrazide or oxadiazole linkers are important components that contribute to high GK potency, with the former substituent in the tail being the most potent.

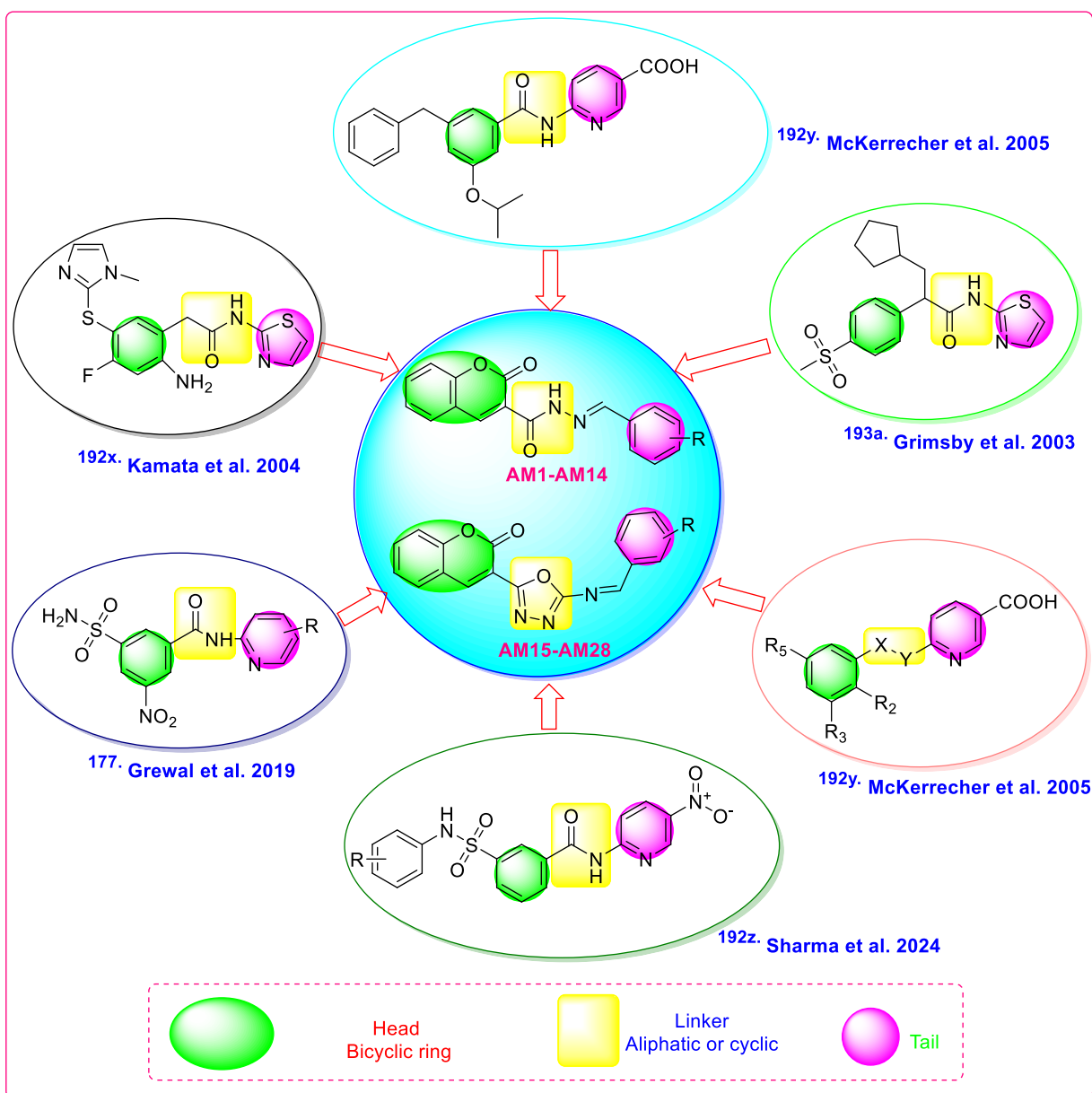


Figure 27. Rationale designing of hydrazide or oxadiazole-based derivatives

3.2. Aim

Design, Synthesis and Evaluation of Heterocyclic Compounds as Potential Antidiabetic Agents

3.3. Objectives

1. Design of glucokinase activators using a drug design approach
2. To synthesize and characterize the designed molecules.
3. To perform the *in vitro* glucokinase activation assay
4. Structure-activity relationship of glucokinase activators via *in vitro-in silico* correlation
5. To evaluate the efficacy of selected molecules using a suitable *in vivo* animal model

3.4. Plan of work

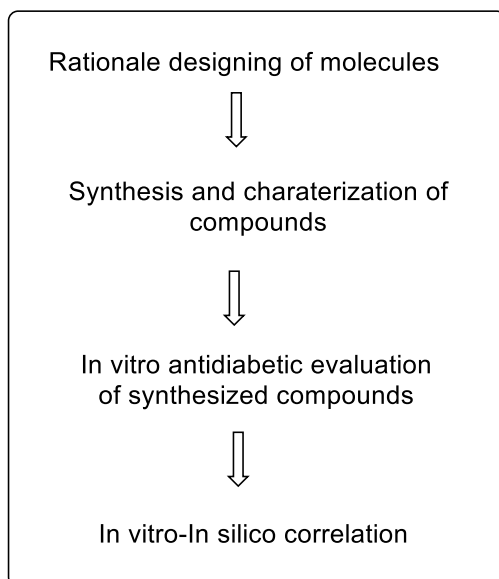


Figure 28. Work Plan



CHAPTER 4

MATERIALS AND METHODS

“ Systematic Methodology
Ensures Reliable
Scientific Discovery. ”



Chemicals
and Reagents



Synthesis
of Compounds



Characterization
(FT-IR, NMR, HRMS)



Biological
Studies



Computational
Studies



4. MATERIALS AND METHODS

The current exploration entails several methodological parts, which comprise a pharmacophore-based design plan that will help create new heterocyclic scaffolds. This study also entails the production of coumarin analogs and their subsequent testing to determine their activation potential in relation to glucokinase, the principal metabolic enzyme essential for glucose homeostasis. Further molecular docking and density functional theory (DFT) analyses of the target receptors were conducted to develop correlations between *in silico* predictions and the experimental results of the *in vitro* experiments to provide a more mechanistic understanding of the observed biological activities.

4.1. Materials

The chemicals, solvents, and reagents used in this study were of analytical grade and purchased from reputable suppliers. Diethyl malonate, hydrazine hydrate, different aldehydes, CNBr, solvents, and reagents were acquired from Sigma-Aldrich, BLD Pharma, Merck India, and Loba Chemie. All aqueous solutions were prepared using deionized water. The progress of the reaction was monitored using pre-coated thin-layer chromatographic (TLC) plates. Using *n*-hexane: ethyl acetate as the eluent with fluorescent indicator F254 (Merck, India) and TLC spot visualization was performed using a UV cabinet (Lab line, LSC-130) and a self-prepared iodine chamber (in Lab, School of Pharmaceutical Sciences, LPU).

The characteristics of the synthesized compounds were determined using melting point, FT-IR, MS, ¹H-NMR, and ¹³C-NMR spectroscopy. FT-IR spectra were recorded at the Central Instrumentation Facility (CIF) of Lovely Professional University (LPU). A FT NMR Spectrometer (model AV NEO, 400 MHz) was used to obtain the ¹H (400 MHz) and ¹³C NMR (100 MHz) spectra at BITS Pilani, Pilani Campus. Chemical shifts were measured in ppm, and CDCl₃ was employed as a solvent, with tetramethyl silane (TMS) as an internal reference in the calibration of chemical shifts. A GC-MS/MS, namely Shimadzu TQ8040 (CIF, LPU) in the mass range of 0 to 500 *m/z* detected the mass spectra (ESI-MS). The melting points were measured using a droplet RSW 138 B (Central Instrumentation Lab, School of Pharmaceutical Sciences, LPU) melting-point device.

The binding affinity and stability of the synthesized compounds with the target enzyme were predicted using *in silico* validation methods, such as DFT and molecular docking. Using molecular docking, the binding poses and affinity of each synthesized compound (ligand) to the active site of the target protein (1V4S) were predicted using AutoDock 4.2.6. ChemBioDraw Ultra 4.0 software was used to draw and design all structures (2D and 3D). The docking and DFT target proteins were glucokinase, which was downloaded from the RCSB (www.rcsb.org) protein data bank (PDB) with the PDB ID: 1V4S. The molecular structures, sequences, and correlations with the binding pocket with different amino acids were analyzed using the Discovery Studio Visualizer software (<https://discover.3ds.com/disclosure-studio-visualizer-download>). Finally, the study included *in silico* ADME (Absorption, Distribution, Metabolism, Excretion) was performed using the SwissADME web tool (<http://swissadme.ch>) and toxicity studies using pKCSM (<https://biosig.lab.uq.edu.au/pkcsml/>).

4.2. Methods

The experimental methodology was classified into the following:

4.2.1. Part-I: Synthesis and characterization of designed compounds

4.2.2. Part-II: *In vitro* antidiabetic studies

4.2.3. Part-III: *In vivo* assay

4.2.4. Part-IV: *In silico* study

4.2.1. Part-I: Synthesis and characterization of designed compounds

4.2.1.1. Synthesis

Two different groups of heterocyclic derivatives, hydrazide-based (Series I) and oxadiazole-based (Series II) coumarins, were designed and synthesized to initiate the study. The synthetic procedures for the target compounds are described in detail.

4.2.1.2. Characterization

Following the successful production of the targeted compounds, purification was performed using the relevant solvent systems via recrystallization and column chromatography. The melting point range of each purified compound was then determined. All the synthesized compounds were structurally characterized by FT-IR, mass spectrometry (MS), ¹H NMR, and ¹³C NMR spectroscopy was used to establish the chemical structures of the compounds in the synthetic strategy that was planned to occur in line with the structure of the designed synthetic strategy.

4.2.2. Part-II: *In vitro* antidiabetic studies

4.2.2.1 *In vitro* Glucokinase activation assay

Glucokinase (GK) activity was spectrophotometrically determined using a coupled enzyme assay with glucose-6-phosphate dehydrogenase (G6PDH). In this test, GK catalyzes the phosphorylation of glucose to form glucose-6-phosphate (G-6-P), which is then oxidized by G6PDH, and this reduction is accompanied by the reduction of NADP⁺ to NADPH. NADPH development was determined by measuring the absorbance at 340 nm, which was then calculated as the change in absorbance at 340 nm using a microplate reader/UV-Vis spectrophotometer. Each of the test compounds was dissolved in DMSO and assayed in 96-well plates with a final reaction volume of 100 μ L of 50 mM HEPES buffer (pH 7.4), 5 mM glucose, 25 mM KCl, 2 mM MgCl₂, 1 mM DTT, 1 mM ATP, 1 mM NADP⁺, 2.5 U/mL G6PDH, and 0.5 μ g GK. To determine the EC₅₀ of the different compounds, varying concentrations of each compound were assayed, and the increase in activity relative to the control was plotted in a four-parameter logistic model in GraphPad Prism (version 4) to estimate the EC₅₀ values of the compounds.

$$\text{Enzyme Activity (\%)} = (\text{Abs Treated}) / (\text{Abs Control}) \times 100$$

Abs T = Absorbance of Treated Sample

Abs C = Absorbance of control

4.2.2.2. Statistical analysis

GraphPad Prism version 5.0 was applied to conduct statistical analyses and the results were expressed in terms of mean standard deviation (SD). Testing of statistical significance was performed with one-way analysis of variance (ANOVA) and Dunnetts post hoc t-test, in which p value of below 0.05 is regarded as statistically significant.

4.2.3. Part-III: *In vivo* antidiabetic assay

The rats used in this study were male Sprague Dawley 7-8 weeks old, with a weight of approximately 160 ± 20 g, and were bred in the institutional animal facility. Housing of the animals was done in five animals per polypropylene cage under controlled conditions of temperature $23 \pm 2^{\circ}\text{C}$; humidity 50-60%; light 300 lx at floor level with regular 12 h light/dark cycle. The animals were provided standard pellet diet and water ad libitum. The Institutional Animal Ethics Committee (IAEC) of the CSIR-Central Drug Research Institute, Lucknow, approved all experimental procedures (Ref. No. IAEC/2022/6/ Renew-3/ Sr.). This study was conducted according to the guidelines of the Committee on the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, No-12 dated 30.06.2025). Rats were induced with streptozotocin (55 mg/kg in 0.1M citrate buffer pH 4.5) by intraperitoneal injection after fasting for 72 h. The blood glucose level was measured 72 h after the injection, and rats with blood glucose levels above 280 mg/dl were considered diabetic and included in the study [193b, 193c]. Diabetic rats were further separated into groups of five. In the experiment, the animals were dry-fed for 6h before oral administration of the test sample suspension (100 mg/kg), standard Dorzagliatin (100 mg/kg), or vehicle (0.5% CMC). Blood glucose levels were measured at 0, 30, 60, 90, and 120 min following treatment. In the experiment, food was withheld, but free access to water was provided to the animals.

4.2.4. Part-IV: *In Silico* Studies

4.2.4.1. Materials and Methods

AutoDock 4.2.6 was used to assess the activation of glucokinase (GK) by estimating ligand-protein binding energies in kcal/mol and compared them with experimental optimal docked positions of the synthesized molecules. The two dimensional and three dimensional molecular structures of the compounds were built in ChemBioDraw Ultra and ChemBioDraw 3D respectively. The energy optimization of every structure was performed with the MM2 force field to give the most stationary molecular conformations before subjecting it to docking analysis [193d]. The derivatives of the molecules were then converted by AutoDock Vina in a readable file format that is compatible in docking simulations. The protein structure of glucokinase (PDB ID: 1V4S) was obtained at the Protein Data Bank to research on the role of enzyme activation.

The complexes of the docked ligand and protein were visualized and analyzed using BIOVIA Discovery Studio Visualizer and PyMOL software to calculate the orientations of binding and main interactions.

4.2.4.2. Default Directory Configuration

The default directory configuration is established by default. Default Directory Configuration
The default directory configuration is defined by default.

The AutoDock default working directory was set as follows: go to File, Preferences, Set, and type C: workplace as the starting directory, press Set, and make sure by clicking Make default. This arrangement ensures that all files pertaining to docking are automatically stored in the specific directory when docking is performed.

4.2.4.3. Selection and Preparation of Proteins

The crystal structure of glucokinase (PDB ID: 1V4S), obtained from the RCSB Protein Data Bank (<https://www.rcsb.org/>), was used in the present study for molecular docking. The downloaded protein structure was imported into AutoDock Vina to dissociate the macromolecule and ligand components, and docking was performed. The main aim of this process was to determine the possible lead compounds with a high binding affinity for the target protein. AutoDock Tools was accessed through a desktop shortcut. First, the workspace directory was opened with File > Read Molecule and the macromolecular file (1V4S). pdb) was opened. The water was used to remove all crystallographic water molecules. Hydrogen atoms were inserted by clicking on Edit Hydrogens Add All Hydrogens, and this was confirmed. Edit Hydrogens: Merge Non-Polar was used to merge non-polar hydrogens. Gasteiger charges were then allocated using the menu Edit > Charges > Compute Gasteiger. The altered protein structure was stored using File > Save > Write PDB, and the workspace folder was updated with the processed file. Finally, the ready macromolecule was chosen under Grid > Macromolecules > Choose and stored in PDBQT format as protein.pdbqt in the same folder to be used later in docking experiments.

4.2.4.4. Protein Selection

1V4S was resolved to 2.30 Å by X-ray diffraction, to 1V4S, providing a high-resolution view of the enzyme's active site, allosteric sites, and conformational states. The high-resolution structure

is an accurate template for studying ligand binding and performing computational methodologies for drug discovery, such as molecular docking. 1V4S is exemplary for revealing the binding of small molecules (such as activators) to the allosteric site, separate from the glucose-binding catalytic site. It is a key GKAs target for the enzyme GK that increases its activity without competing with glucose, which improves drug selectivity and decreases off-target effects. 1V4S is one of the first to highlight the allosteric regulation mechanism of the enzyme. Therefore, wild-type human glucokinase without mutations (1V4S) is more physiologically relevant for designing GKAs aimed at normal human enzyme interactions. Studies on glucokinase activators and structure-based drug design have been frequently reported 1V4S [193e, 194]. The widespread use of this model serves to validate its use as a standard model for glucokinase activator research. It has proven useful in elucidating structure-activity relationships (SAR) for developing GKAs, especially in understanding the interaction of small molecules at the allosteric site. 1V4S is suitable for other PDBs as it strikes a good balance between resolution, physiological relevance (wild-type structure), and critical insights into the allosteric activation mechanism. Because of its detailed representation of the allosteric site, it is ideally suited for the development of glucokinase activators, which are promising therapeutic agents for T2D.

4.2.4.5. Protein Active Site Determination

The protein structure was imported into Discovery Studio for active site determination. Crystallographic water molecules and HETATM entries were removed prior to analysis. Hydrogen atoms were subsequently added to improve the accuracy of binding site prediction. The “Define and Edit Binding Site” tool was used to define the binding site of the target protein by using the receptor–ligand interaction protocol. Pockets were generated using the receptor cavity detection method, and also the PDB site record option was taken into account if the protein data file contained information on the receptor sites. The software recognized up to 4 possible binding sites which were termed Site 1, Site 2, Site 3 and Site 4 respectively. From these, Site 1 was chosen to be docked with other molecules in order to determine the best predicted binding cavity. The X, Y, Z and radius of the region representing the active site were found to be 39.919040 Å, 15.954920 Å, 62.682120 Å and 7.149165 Å respectively and the region was visualized as a spherical region in the protein structure (39.919040, 15.954920, 62.682120, 7.149165) (Figure 29).

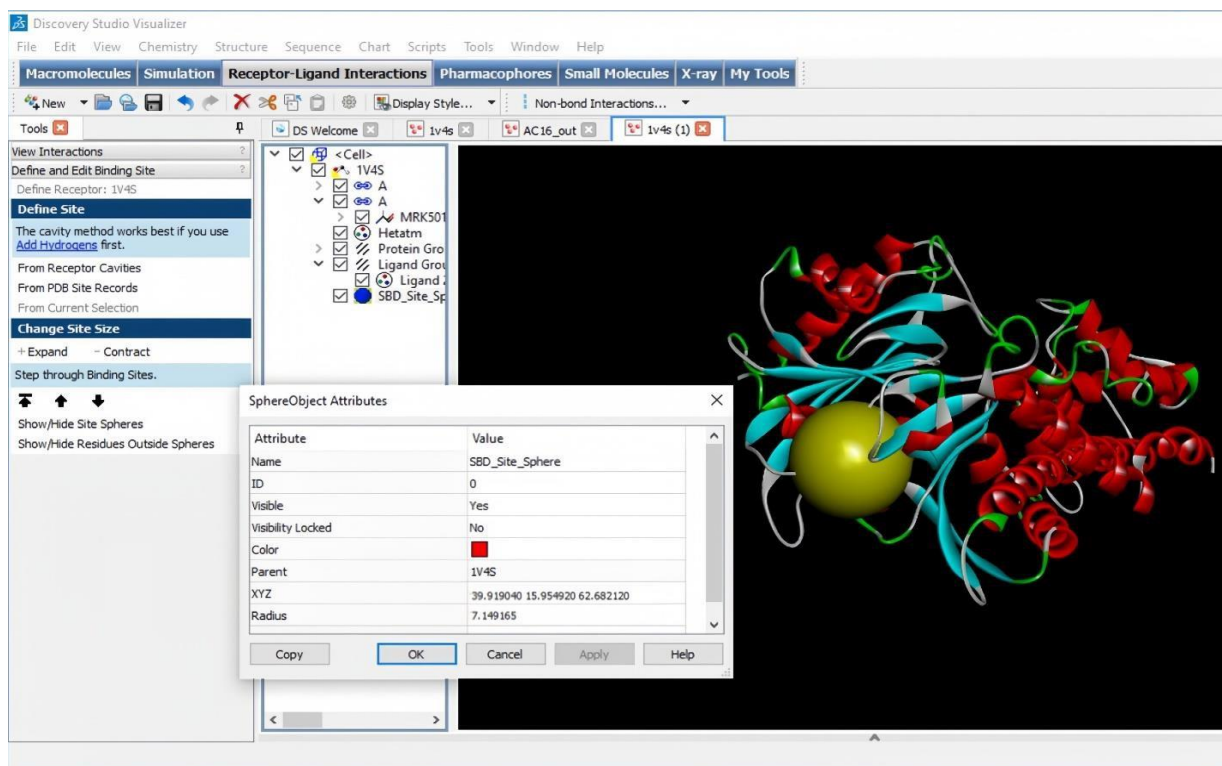


Figure 29. Active site coordinates of co-crystallized ligand

The point count in the cavity detection showed the possibility of a high binding affinity at the selected coordinate region of the protein (Figure 30).

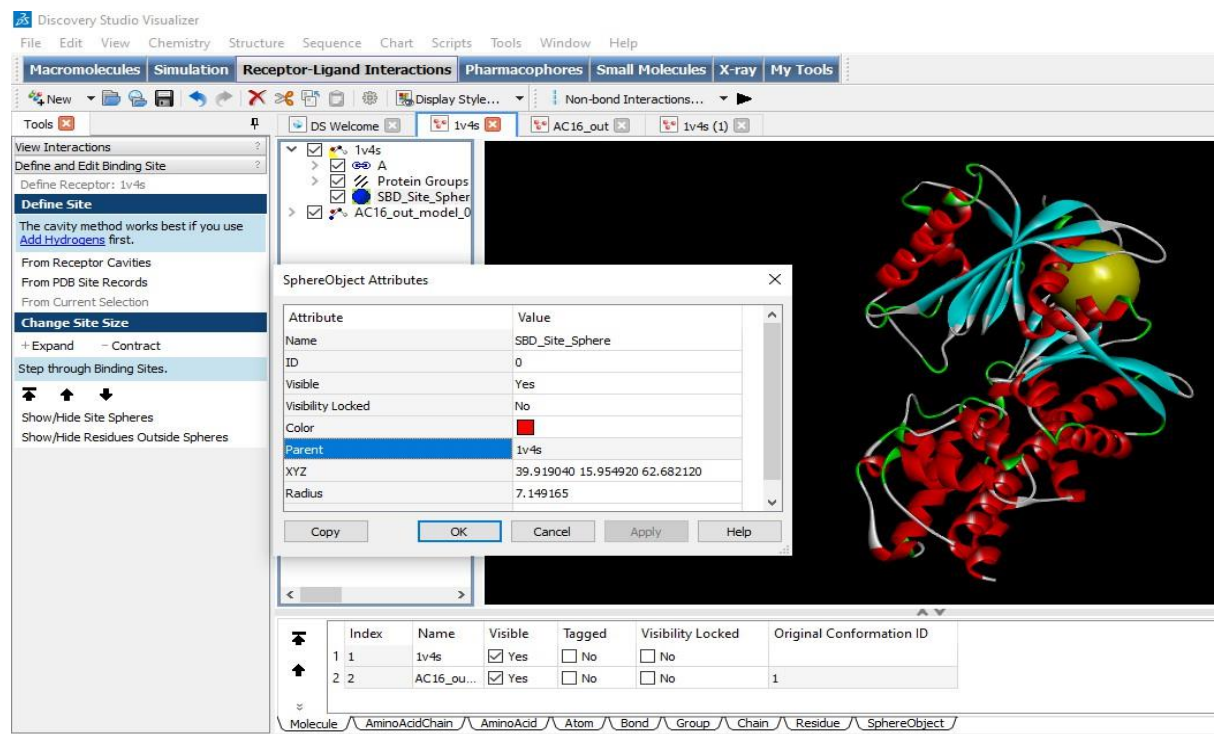


Figure 30. Active site coordinates of AM16 ligand

4.2.4.6. Validation Studies

The analysis was done by the ligand-protein interaction with the glucokinase crystal structure (PDB ID: 1V4S) that was repeatedly docked in the specified active site area. Root Mean Square Deviation (RMSD) computations were conducted to determine the consistency and repeatability of the docking protocol by looking at the position of the redocked ligand and comparing it to its experimentally calculated crystallographic orientation. Redocking of the native co-crystallized ligand into its original binding pocket (Figure 16B) without changing its conformation or chemical state was used to validate the docking protocol. The resulting value of RMSD in the MRK was shown to be equivalent to 1 Å with the docked pose overlaid on the crystallographic structure, which revealed that the positional accuracy was high. One application of RMSD analysis in molecular docking studies is to determine the degree to which the predicted binding pose is reproducible to the experimental binding pose. In general, the RMSD of less than 1 Å can be classified as very accurate, whereas the range of 1 to 3 can be classified as acceptable to use in pose prediction. The virtual screening results were also tested in terms of reproducibility through the re-run of the docking simulations with the same datasets and docking parameters. The binding conformations so generated, the pose with the highest spatial overlap with the co-crystallized ligand (Figure 31) was chosen to perform further evaluation of binding energy and amino acid interactions. Figure 32 shows the superimposed binding orientations of the native ligand and the new designed compounds.

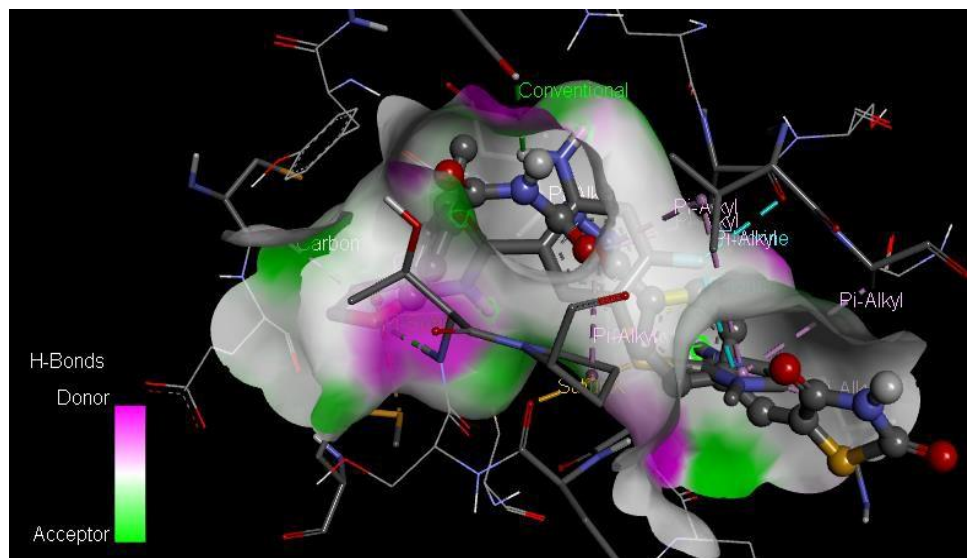


Figure 31. Overlapping of the co-crystallised ligand and designed ligands to ensure validation.

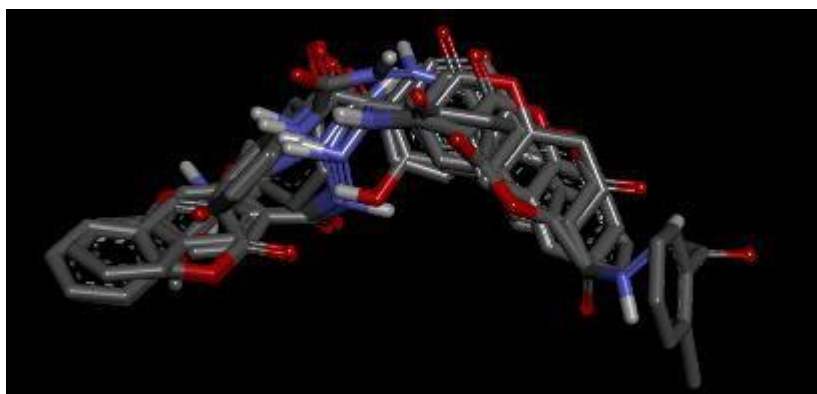


Figure 32. Overlapping pose of co-crystallised ligand and designed ligands.

4.2.4.7. Ligand Preparation

Heterocyclic compound library A broad library of heterocyclic products was tested to identify candidates with the best binding affinity for the target enzyme. Two-dimensional chemical structures were first drawn using ChemBioDraw 2D software. These were then translated into their respective three-dimensional conformations using ChemBioDraw 3D. The ligand structures that had been prepared were then imported into AutoDock Vina 1.5.6 to dock these ligands and saved as Protein Data Bank (.pdb).

In AutoDock, the ligand file (i.e., AM1.pdb) was opened by selecting Ligand >Input > Open. The software automatically resorted to processing the ligand by assigning partial charges and finding rotatable bonds. In this process, the aromatic carbon atoms and the overall number of torsional degrees of freedom were observed. The ligand root was defined by selecting Ligand > Torsion Tree > Detect Root, which was graphically displayed as a green marker in the displayed window. The processed ligand was then exported in the PDBQT format (Ligand > Output > Save as PDBQT). This preparation scheme was used for each ligand in the series, AM2–AM28.

4.2.4.8. Grid Box Configuration

Parameters in the grid box were used to identify the potential binding regions of the target macromolecule. The structure of the glucokinase protein was chosen using the Grid > Macromolecule > Choose option and saved as a PDBQT file in the working directory. The reference ligand (MRK) was chosen through Ligand > Select Ligand, and the map types were set through Grid > Set Map types. This was followed by the definition of the grid box, where the selection was made as Grid > Grid Box. When the grid size was finalized, the grid was saved by

simply closing the grid window and exporting the grid parameters by clicking Grid > Output > Save. The resultant configuration file was written to the workspace directory under the name conf.

4.2.4.9. Molecular Docking Study

The designed ligand library was simulated using molecular docking to determine the possible glucokinase activators in the library. Glucokinase (PDB ID: 1V4S) was used as the target protein, and the ligand that co-crystallized with it was cleared to confirm the docking protocols. The protein structure was made as a result of removing the water molecules, fixing missing atoms and introducing polar hydrogen atoms. Kollman charges were generated, and the protein was transformed into the PDBQT format for docking [195,196].

The grid box was focused on the ligand-binding region of the enzyme to obtain the correct sampling of the active site. The grid output file was used to state the grid box dimensions on the x, y, and z axes and was integrated within the conf.txt configuration file. These ready-made inputs were then used to conduct docking simulations and test the interactions between ligands and proteins.

Center_x = 39.91040, Center_y = 15.954920, Center_z = 62.682120

Size_x = 30, Size_y = 44, Size_z = 24

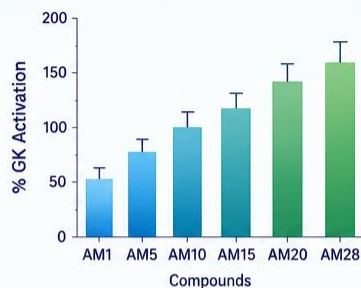
A command prompt was opened and the command "program files/the Scripps research institute/vina/vina.exe - config conf.txt - log log.txt" was typed to perform molecular docking with Vina using the AutoDock Tool.

CHAPTER 5

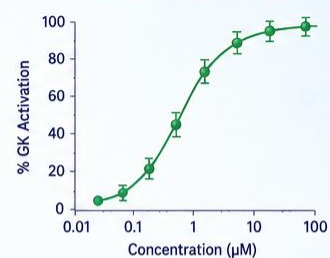
RESULTS AND DISCUSSION

“Interpreting Experimental Evidence to Generate Meaningful Insights.”

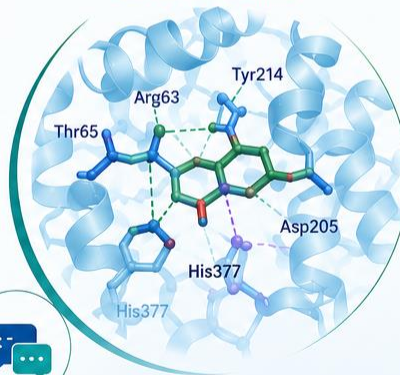
BIOLOGICAL ACTIVITY RESULTS



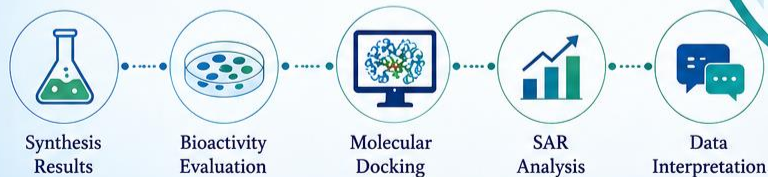
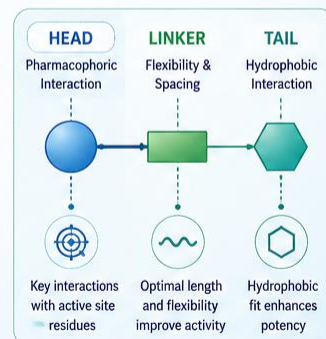
DOSE-RESPONSE CURVE



MOLECULAR DOCKING



STRUCTURE-ACTIVITY RELATIONSHIP (SAR)



5. RESULTS AND DISCUSSION

5.1. Synthesis

Coumarin-hydrazide derivatives AM1-AM14 compounds were prepared by refluxing equal amounts of 2-oxo-2H-chromene-3-carbohydrazide and benzaldehydes in methanol for 5-6 hours. The resulting solids were separated and purified using column chromatography. Coumarin-oxadiazole derivatives (AM15-AM28) were obtained after 6-8 hours of reflux reaction of 3-(5-amino-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one and benzaldehydes in a methanolic medium in an equimolar ratio. The reaction mixture was concentrated under reduced pressure, and the crude product was purified by treatment with ice-cold water, followed by recrystallization and column chromatography (CC). Progress throughout the reaction in either of the synthetic routes was observed using thin-layer chromatography (TLC) (*n*-hexane: ethyl acetate) solvent system.

5.1.1. Synthesis of 3, 4 and 5

Salicylaldehyde (1) (0.02 mol) was condensed with diethyl malonate (2) (0.02 mol) in the presence of piperidine and glacial acetic acid (Figure 33). The reaction mixture was refluxed for 5 hours. The reaction progress was monitored by using TLC (*n*-hexane: ethyl acetate (7:3). After completion of the reaction, the reaction mixture was cooled to room temperature until the precipitates of 3-carboxycoumarin (3) were obtained. The compound was recrystallized from ethanol (80% yield). Compound 4 was synthesized by refluxing a mixture of ethyl 2-oxo-2H-chromene-3-carboxylate (3) (0.001 mol) (0.2 g) with hydrazine hydrate (0.003 mol) (12 ml) for 4 hours at 65-70°C. The reaction progress was monitored by using TLC (*n*-hexane: ethyl acetate (6:4). The mixture was poured on distilled water and extracted with ethyl acetate. The ethyl acetate was distilled off by rota evaporator, and the dried compound was collected (60% yield). Compound 5 was synthesized by refluxing a solution of 2-oxo-2H-chromene-3-carbohydrazide (0.01 mol) and cyanogen bromide (0.01 mol) in methanol at 55–60 °C for 5 hours. After completion of the reaction, the mixture was allowed to cool to room temperature and then neutralized with a sodium bicarbonate solution. The resulting solid was filtered, washed, and dried to afford the product (70% yield). The reaction progress was monitored by TLC using ethyl acetate (30:70) as the mobile phase.

Synthesis of AM1-AM14

An equimolar mixture of 2-oxo-2H-chromene-3-carbohydrazide (0.01 mol) and benzaldehyde (0.01 mol) was dissolved in methanol (30 mL) (Figure 33) and subjected to reflux for 6-8 hours. Upon completion of the reaction, the solvent was removed under reduced pressure. The crude product was precipitated by adding the reaction mixture to ice-cold water. The obtained solid was purified by column chromatography using an appropriate eluent system (*n*-hexane acetate) and further recrystallized from methanol. The other derivatives in the AM1-AM14 series were prepared following the same methods. The reaction progress was monitored using TLC (*n*-hexane: ethyl acetate).

5.1.2. Synthesis of AM15-AM28

A solution of 3-(5-amino-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (0.01 mol) and benzaldehyde (0.01 mol) in methanol (30 mL) was refluxed for 5-6 hours. After the reaction was complete, the solvent was evaporated under reduced pressure. The solid product was obtained by pouring the mixture into ice-cold water. The obtained solid was purified by column chromatography using an appropriate eluent system (*n*-hexane acetate) and further recrystallized from methanol. The remaining compounds in the AM15-AM28 series were synthesized using an identical procedure. The reaction progress was monitored using TLC (*n*-hexane: ethyl acetate).

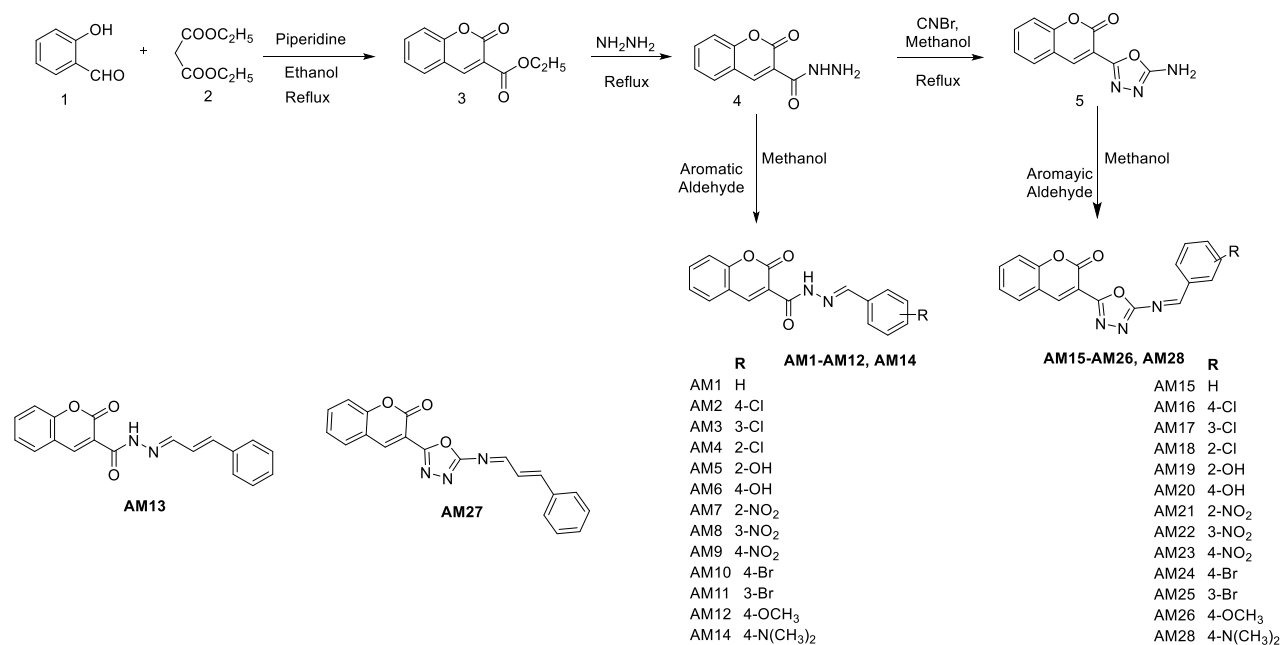


Figure 33. Synthetic scheme of heterocyclic derivatives

5.2. *In vitro* Assay

5.2.1. *In vitro* Glucokinase activation

We evaluated the efficacy of the synthesized coumarin analogs (AM1-AM28) for their efficacy as glucokinase activators to determine their antidiabetic potential. Glucokinase (GK) is a regulatory enzyme of glucose homeostasis that catalyzes the phosphorylation of glucose to glucose-6-phosphate in the liver and pancreas. GK activation leads to increased glucose uptake and metabolism, insulin secretion, and decreased hyperglycemia. Therefore, GK activation is a promising approach for the treatment of diabetes and regulation of postprandial glucose levels. The potency and glucokinase (GK) activation profiles of all the synthesized compounds are presented in Table 5 and depicted in Figures 34 and 35, respectively.

Table 5. Compounds AM1-AM28 with EC₅₀ and GK activation potential (n = 3).

Compound Code	EC ₅₀ (μM) (Mean ± SEM)	Enzyme activity (%)
AM1	6.12 ± 0.14	136.23
AM2	13.92 ± 0.20	124.43
AM3	11.36 ± 0.18	129.97
AM4	4.64 ± 0.12	132.68
AM5	5.48 ± 0.15	139.28
AM6	13.60 ± 0.21	132.60
AM7	5.12 ± 0.15	134.20
AM8	12.84 ± 0.20	125.93
AM9	5.93 ± 0.13	134.33
AM10	4.08 ± 0.11	137.46
AM11	10.92 ± 0.17	128.20
AM12	12.41 ± 0.19	120.82
AM13	9.84 ± 0.16	131.34
AM14	5.09 ± 0.13	136.30
AM15	6.72 ± 0.11	135.28
AM16	4.56 ± 0.12	144.93
AM17	6.02 ± 0.11	140.38
AM18	3.51 ± 0.10	156.18

AM19	5.84 ± 0.11	151.60
AM20	5.89 ± 0.12	154.79
AM21	3.63 ± 0.10	148.72
AM22	3.77 ± 0.11	151.74
AM23	4.82 ± 0.12	139.26
AM24	4.77 ± 0.12	145.30
AM25	5.66 ± 0.10	147.56
AM26	5.22 ± 0.13	143.89
AM27	6.32 ± 0.12	144.32
AM28	7.14 ± 0.14	142.61
Dorzagliatin	19.82 ± 0.25	120.11

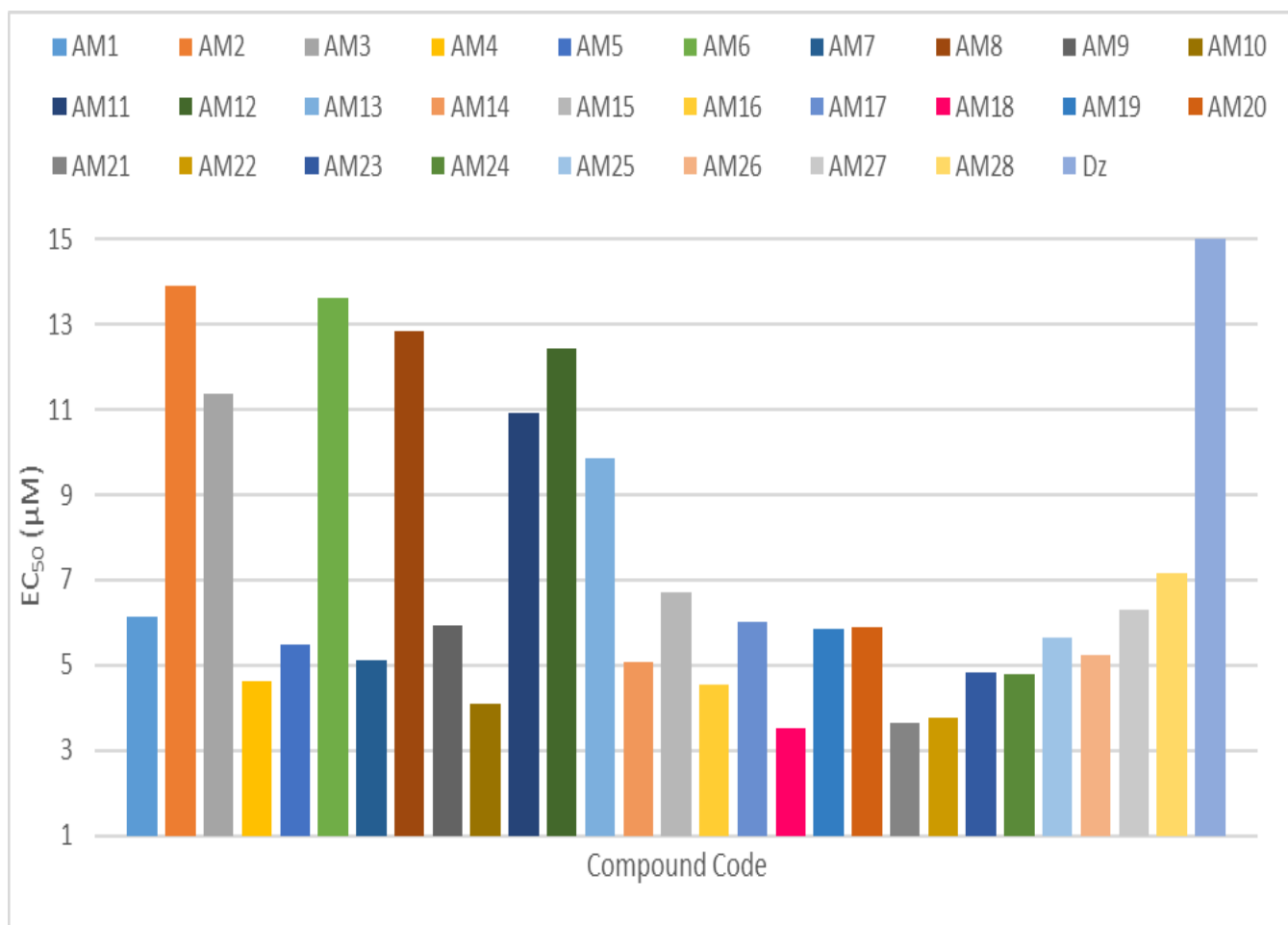


Figure 34. EC_{50} of compounds AM1-AM28

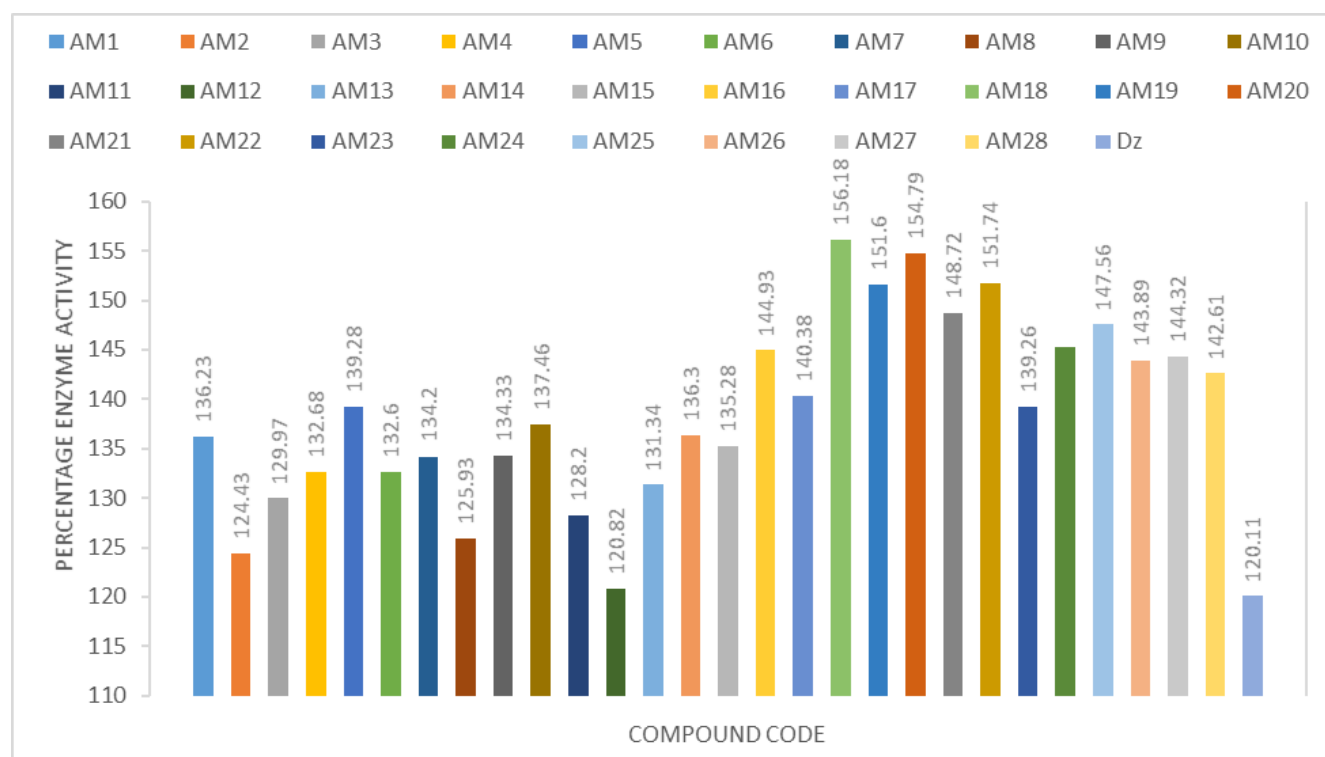


Figure 35. GK activation potential of compounds (AM1-AM28)

A significant increase in enzymatic activity was observed for AM18 and AM20 compared with Dorzagliatin ($p < 0.001$). This amplified enzyme activity indicates that these substances achieve the active shape of glucokinase, thus supporting the process of glucose phosphorylation and enhancing glucose sensing in pancreatic β -cells and hepatocytes. The increase in AM18 and AM20 activity could be explained by the preferred interaction of these ligands with the protein, including hydrogen bonding and hydrophobic stabilization, resulting in the increased catalytic performance of the enzyme (Figure 36).

Although this is statistically significant, the difference between AM18 and AM20 alone is not significant ($p > 0.05$), indicating that both compounds are likely to adopt a similar binding mode and interaction pattern in the allosteric site of glucokinase. Moreover, the fact that AM18 and AM20 perform better than Dorzagliatin also demonstrates the possibility of the designed scaffold achieving a higher biological performance, potentially by promoting a better binding orientation or stabilizing the active enzyme conformation. Altogether, this discussion provides a more in-depth insight into the mechanism of glucokinase activation and underlines the importance of the fact that both compounds are much more effective than the standard drug.

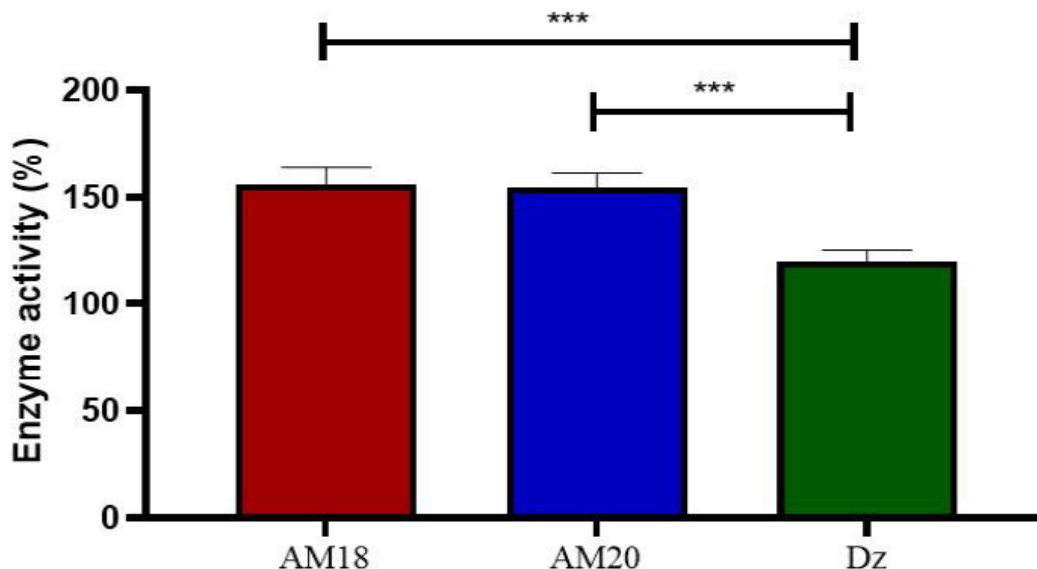


Figure 36. GK activation potential comparison of AM18, AM20 and Dorzagliatin

5.2.2. MTT Assay

The cytotoxic capacity of the selected glucokinase-activating analogs was systematically assessed using the HepG2 cell line, an *in vitro* model that is well-established and well-regulated and is extensively used in the initial safety examination of antidiabetic agents. The results indicate the dependence of cell viability on the concentration of the reduction of cell viability, which proves that the cytotoxic action of the synthesized compounds is dose-dependent and appears to be noticeable at higher concentrations (50-100 $\mu\text{g/mL}$).

None of the compounds affected cellular viability (>70-90%), suggesting that the analogs studied are non-toxic in the pharmacological concentration spectrum. This fact is especially critical since glucokinase activators perform their activities in liver tissues; therefore, it is essential to keep hepatocytes alive to use them in therapy. This decrease in viability with increasing concentrations indicates that at high doses, cellular tolerance limits are surpassed, possibly because of the cumulative stress within the cell. A comparative analysis of the IC_{50} values indicated different cytotoxicity patterns for the analogs. AM18 and AM20 had the largest IC_{50} (97.92 and 104.48 $\mu\text{g/mL}$, respectively), indicating that they were the least cytotoxic and safest. Even at high concentrations, these derivatives were relatively stable; thus, their structural scaffolds might restrict excessive accumulation into the cell or limit interactions with important

cellular structures, such as mitochondrial enzymes. In contrast, other compounds, such as AM16, AM21, and AM25, had much lower IC_{50} (34.41-42.29 $\mu\text{g/mL}$), indicating higher cytotoxicity. This could be explained by the increased toxicity of these derivatives owing to the structural features that facilitate an increase in membrane permeability or localization in the mitochondria, resulting in the disruption of cellular respiration. As the MTT assay is based on the presence of mitochondrial succinate dehydrogenase, which reduces tetrazolium salt to formazan, any dysfunction of the compound in the mitochondria would lead to a direct decrease in absorbance and a seemingly loss of viability. Thus, these compounds can cause off-target toxicity in mitochondria, which may restrict their future use in development unless optimized at the molecular level. AM16 had the lowest IC_{50} value (34.41 $\mu\text{g/mL}$), suggesting relatively high cytotoxicity against HepG2 cells, may be due to structural features that have greater interaction with cells and mitochondria. AM18 and AM20, on the other hand, exhibited significantly higher IC_{50} values (97.92 and 104.48 $\mu\text{g/mL}$, respectively), indicating increased hepatoprotection and hepatotolerance. The results suggest that the structural changes have a remarkable effect on the cytotoxic activity profile of the synthetic analogues.

Other analogs (AM22, AM24, AM26, and AM27) had intermediate IC_{50} (73.78-94.43 $\mu\text{g/mL}$), indicating a good compromise between biological and cytotoxic risks. These compounds are especially useful for lead optimization because they allow for a compromise in which efficacy can be preserved and toxicity can be controlled. These results underscore the fact that minor structural changes have a major effect on cytotoxicity, highlighting the need to fine-tune substituent effects during drug development.

Dorzagliatin, the reference drug, had the highest IC_{50} value (114.23 $\mu\text{g/mL}$) (Table 6), indicating its high safety and clinical relevance. Notably, some of the synthesized products, especially AM18 and AM20, demonstrated similar cytotoxicity profiles, indicating that these molecules could be effective leads with safety profiles similar to that of the drug of interest. Mechanistically, increased cytotoxicity was observed due to several interrelated pathways. The overload of compounds into the cell can result in the depolarization of the mitochondrial membrane, production of reactive oxygen species (ROS), and initiation of apoptotic cascades. In addition, because glucokinase is a major modulator of hepatic glucose metabolism, hyperactivation or malregulation could cause metabolic stress in hepatocytes, which may reduce their viability. Alternatively, it is reasonable that some substituents would increase redox cycling

or electrophilic reactions, which would further increase oxidative damage.

Moreover, a comparison of cytotoxicity data with glucokinase activation (EC_{50}) indicated a significant relationship between efficacy and toxicity. Compounds with a large activation but low IC_{50} value can have a narrow therapeutic index, whereas compounds with medium activation but high IC_{50} values (e.g., AM18 and AM20) are more drug-like candidates with higher safety margins. This balance is of utmost importance in the development of antidiabetic drugs, as chronic toxicity requires low hepatotoxicity. In general, the results of the MTT test showed that although all compounds are cytotoxic in a dose-dependent manner, some of the analogs remain safe at tolerable levels. Specifically, compounds with higher IC_{50} values, which can be maintained at intermediate concentrations and have positive glucokinase activation, are good candidates for further preclinical studies. The proposed mechanisms of cytotoxicity should be confirmed by future research, which should include apoptosis studies, ROS measurements, and studies on the mitochondrial membrane potential, which will help improve the safety profile of the compounds discussed.

Table 6. Percentage cell viability and IC_{50} of compounds.

S. No.	Test Compound	% Viability (Concentration $\mu\text{g/ml}$)					IC_{50}
		6.25	12.5	25	50	100	
1.	AM16	81.09 $\pm$ 0.34	71.33 $\pm$ 0.20	59.45 $\pm$ 0.27	39.15 $\pm$ 0.43	32.79 $\pm$ 0.32	34.41
2.	AM18	91.22 $\pm$ 1.34	79.40 $\pm$ 1.60	73.22 $\pm$ 0.36	61.65 $\pm$ 1.24	49.66 $\pm$ 1.56	97.92
3.	AM19	73.64 $\pm$ 0.22	61.89 $\pm$ 0.64	57.22 $\pm$ 0.48	45.04 $\pm$ 0.35	32.19 $\pm$ 0.83	43.63
4.	AM20	91.56 $\pm$ 1.37	81.55 $\pm$ 1.67	71.45 $\pm$ 1.15	61.38 $\pm$ 1.08	51.84 $\pm$ 1.57	104.48
5.	AM21	74.32 $\pm$ 0.38	68.16 $\pm$ 0.78	61.72 $\pm$ 0.62	48.76 $\pm$ 0.37	41.93 $\pm$ 0.73	36.41
6.	AM22	81.74 $\pm$ 0.46	64.28 $\pm$ 0.40	63.17 $\pm$ 0.59	55.78 $\pm$ 0.23	46.78 $\pm$ 0.37	93.34
7.	AM24	71.55 $\pm$ 0.23	69.52 $\pm$ 0.35	67.81 $\pm$ 0.63	56.95 $\pm$ 0.46	45.44 $\pm$ 0.89	76.09
8.	AM25	83.62 $\pm$ 0.58	73.84 $\pm$ 0.21	56.43 $\pm$ 0.76	44.85 $\pm$ 0.61	32.98 $\pm$ 0.67	42.29
9.	AM26	83.16 $\pm$ 0.45	69.22 $\pm$ 0.47	65.87 $\pm$ 0.91	64.54 $\pm$ 0.86	44.61 $\pm$ 0.22	73.78
10.	AM27	81.94 $\pm$ 0.53	73.68 $\pm$ 0.37	63.97 $\pm$ 0.59	52.73 $\pm$ 0.16	48.96 $\pm$ 0.41	94.43
11.	Dorzagliatin	79.63 $\pm$ 0.24	79.68 $\pm$ 0.52	67.93 $\pm$ 0.17	63.42 $\pm$ 0.74	59.87 $\pm$ 0.28	114.23

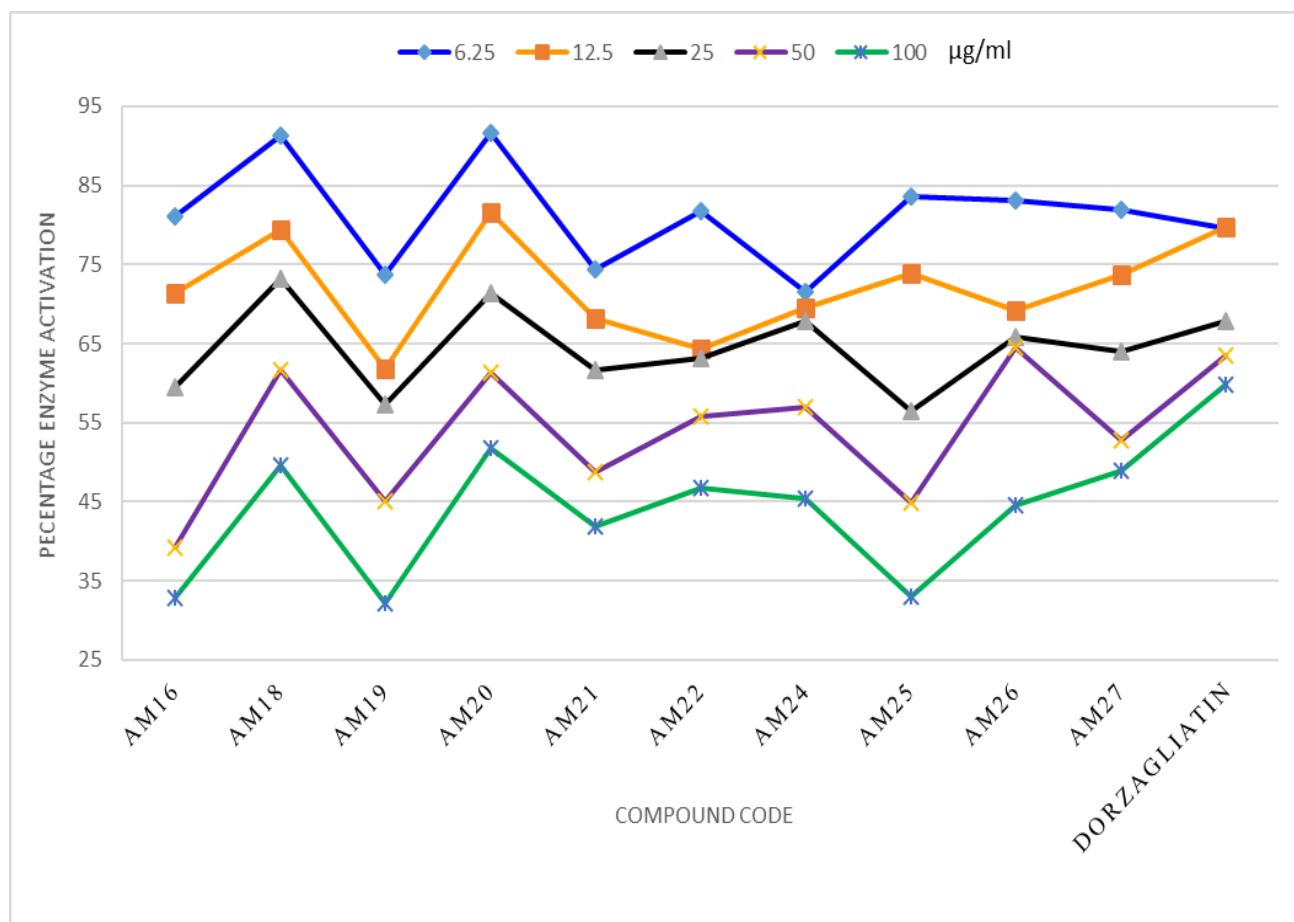


Figure 37. Effect of test compounds and standard (Dorzagliatin) at five different concentrations on percentage cell viability.

5.3. *In vivo* antihyperglycemic activity

Based on the positive cytotoxicity data from the MTT assay, AM18 and AM20 were selected for *in vivo* use in the STZ-induced diabetic rat model, a well-established experimental system that replicates several important aspects of diabetes, including insulin-secretion impairment, liver-increased glucose secretion, and peripheral glucose-utilization distortion. These compounds were tested against the oral glucose tolerance test using a dose of 100 mg/kg, and their antihyperglycemic action was observed by measuring the levels of glucose in the blood at a particular time (0, 30, 60, 90, and 120 min); thus, the time curve of glucose regulation was obtained. These findings clearly showed that both AM18 and AM20 resulted in a significant and time-dependent decrease in blood glucose levels, suggesting strong and sustained

antihyperglycemic action. The slow reduction in glucose concentration, specifically between 60 and 120 min, indicates that these compounds have long-term pharmacodynamic effects that are necessary to provide stable glycemic regulation in diabetic diseases.

A comparative evaluation with the reference drug Dorzagliatin showed that AM18 and AM20 had better glucose-lowering effects during the study period. This increased activity can be attributed to better pharmacokinetic and pharmacodynamic characteristics, including increased absorption, higher bioavailability, or increased interaction with the target enzyme. The main pathway of the effect mechanism is probably linked to the activation of glucokinase, which is one of the main forces of glucose homeostasis, as it catalyzes the phosphorylation of glucose to glucose-6-phosphate in hepatocytes. This response acts as a gateway in glycolysis and glycogen synthesis in metabolism, thus enhancing the uptake of glucose in the liver and subsequently lowering the glucose concentration in the blood. In addition to hepatic effects, glucokinase activation increases glucose sensing in pancreatic β -cells, resulting in improved glucose-stimulated insulin secretion. The fact that AM18 and AM20 can significantly decrease the blood glucose level even in STZ-induced diabetic conditions, when the functions of the β -cell are partly impaired, implies that the compounds could be used to enhance the remaining insulin secretion and, at the same time, increase hepatic glucose clearance.

The time-dependent decrease in glucose levels is also useful for understanding the pharmacological actions of these compounds. This is due to the fact that there was no sudden drop in glucose levels at early time points, with a gradual and persistent drop which is a controlled mode of action that reduces the chances of hypoglycemia. This feature is especially desirable in antidiabetic treatment, which allows the normalization of glucose levels without causing acute metabolic disorders. Moreover, the observed long-term effect (up to 120 min) suggests that the compounds can be sufficiently metabolically stable and have a good half-life, which can ensure therapeutic levels over a prolonged period.

The higher in vivo performance of AM18 and AM20 is correlated with the reduced cytotoxicity profile of these agents in the MTT assay, indicating a close relationship between cell safety and systemic efficacy. Compounds with lower cytotoxicity have a low chance of disrupting cellular integrity and metabolic activity, thus ensuring physiological compatibility and improved therapeutic effects. The physical attributes of AM18 and AM20 can contribute to favorable

physicochemical properties, such as moderate lipophilicity, appropriate polarity, and reduced electrophilic reactivity, all of which are conducive to enhancing membrane permeability, target engagement, and metabolic resilience. Also, they can be found to have better binding interactions in the allosteric activation site of glucokinase, which leads to more effective enzyme activation than the conventional drug.

In addition to direct glucokinase activation, it is reasonable to assume that AM18 and AM20 have complementary antihyperglycemic effects through other metabolic mechanisms. These effects may involve the inhibition of hepatic gluconeogenesis, elevation of peripheral glucose uptake in muscle and adipose tissues, and insulin sensitivity, all of which lead to glucose homeostasis. Combined modulation of these pathways might be the reason why the glucose-lowering activity of these pathways is better than that of Dorzagliatin. Furthermore, the fact that these compounds can work in a compromised diabetic model highlights their robustness and adaptability under pathological conditions.

Collectively, the *in vivo* results lead to a strong belief that AM18 and AM20 are good lead candidates with strong and sustained antihyperglycemic effects that are better than the standard drug in this experimental model. Both *in vitro* and *in vivo* studies have shown that they have a good balance between efficacy and safety, making them potential candidates for further development as antidiabetic agents. However, further studies on chronic dosing phases, insulin concentration, liver glycogen, oxidative stress markers, and thorough pharmacokinetic characterization are required to fully determine their therapeutic use. These studies will provide more information on their mechanism of action, long-term safety, and clinical translatability.

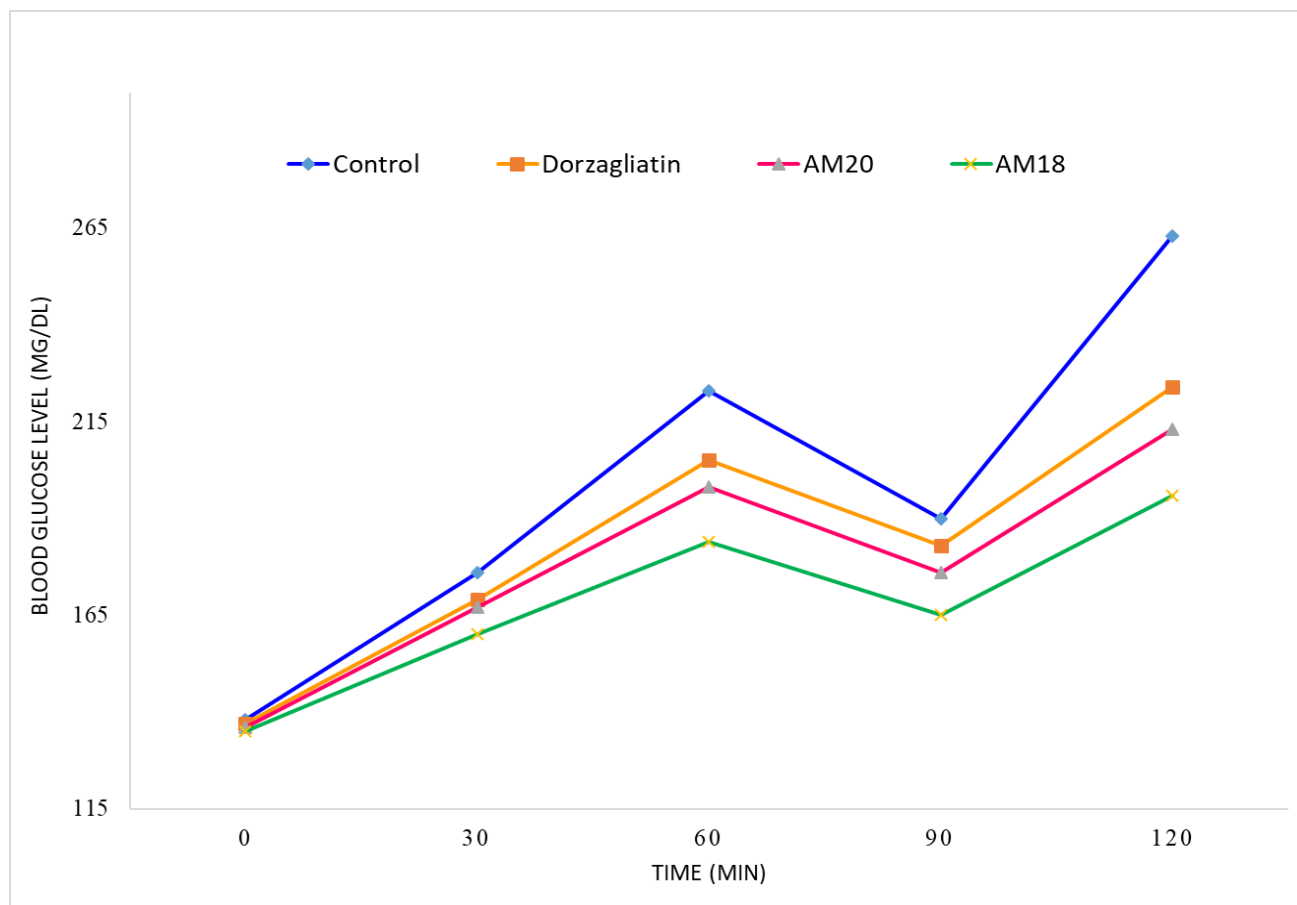


Figure 38. Effects of AM18 and AM20 on the level of blood glucose at different time intervals in diabetic rats.

5.4. *In silico* study

5.4.1. Docking Analysis

Once docking was complete, the output file (dock.output) was opened via the Analyze menu in AutoDock Tools. The ligand file (AM1.pdbqt) was selected using the Analyze > Macromolecule > Choose. The obtained results were then compared using conformational analysis, where the Analyze menu was used to select the Conformations menu, which was then used to organize the results based on the energy rankings. All predicted binding positions were individually analyzed and tested.

This analytical procedure was methodically reiterated for all the compounds in the ligand sequence so that all compounds were evaluated equally. The output files contained docking scores as binding-free energy values (kcal/mol). For the most promising compounds that demonstrated good interactions in the glucokinase active site, in silico analysis of hydrogen bonding patterns and bond distances was carried out using BIOVIA Discovery Studio Visualizer software. The most important interaction parameters for the optimized ligands are summarized in Table 11.

5.4.2. Molecular Docking Interactions

Docking-based screening was conducted based on the predicted binding free energy values (kcal/mol) to screen the interaction with GK, a major glucose-sensing protein involved in the regulation of insulin release by pancreatic 3D cells and as a metabolic gatekeeper in hepatocytes by facilitating glucose uptake and glycogen synthesis. Most of the developed compounds showed a stronger binding affinity for GK than the reference ligands.

In the active site of GK, the reference ligand MRK showed a binding free energy of -7.2 kcal/mol. Similarly, the estimated binding energy of Dorzagliatin, a glucokinase activator authorized by the Japan FDA in 2022, was the same, at -7.2 kcal/mol. In contrast, several newly constructed compounds exhibited significantly higher binding affinities. The binding energy of compounds AM18, AM21, AM22, AM19, AM20, AM25, AM15, AM27, AM10 and AM28 was found to be -11.0, -10.7, -10.7, -10.6, -10.6, -10.6, -10.5, -10.2, -10, and -10 kcal/mol, respectively, and thus, much stronger binding was found than that between MRK (-7.2 kcal/mol) and Dorzagliatin (-7.2 kcal/mol). Table 7 provides a detailed overview of all docked ligands and their binding scores, along with the interactions of the ligand with the major amino acids in the GK binding pocket. In general, the binding energy of the most active compounds was predicted to be between -11 and -10 kcal/mol, which demonstrates their likely activity as effective glucokinase-activators.

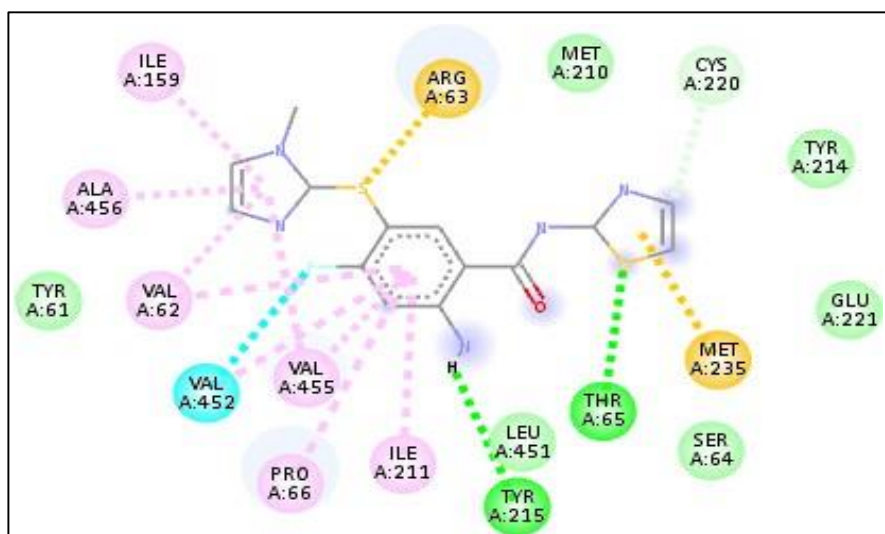


Figure 39. MRK showing interactions in the GK.

As shown in Figure 39, MRK formed hydrogen bond interactions with TYR215 and THR65. The π -alkyl interactions were found with ILE211, PRO66, VAL455, VAL62, ALA456, and ILE159. π -Sulfur interactions with MET235 and ARG63. The Van der Waals interactions with LEU451, SER64, GLU221, TYR214, MET210, and TYR61.

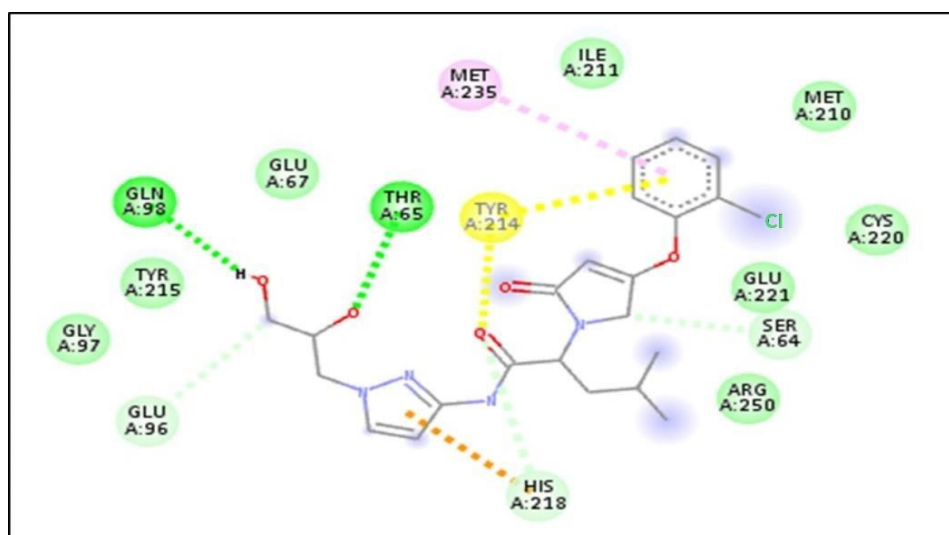


Figure 40. Dorzagliatin showing interactions in the GK.

As shown in Figure 40, Dorzagliatin had hydrogen bond interactions with GLN98 and THR65. π -Alkyl interactions were observed with MET235. π -cation interactions with TYR214. The Van der Waals interactions with GLU67, TYR215, GLY97, GLU96, HIS218, ARG250, SER64, GLU221, CYS220, MET210, and ILE211.

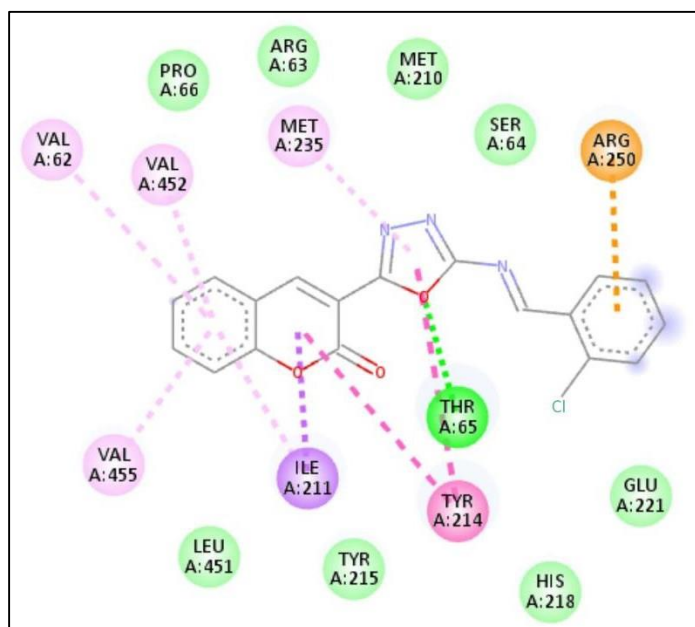


Figure 41. AM18 showing interactions in the GK.

As shown in Figure 41, AM18 formed hydrogen bond interactions with THR65. The π -alkyl interactions were found with VAL62, VAL455, VAL452, and MET235 and π -sigma interaction with ILE211. Van der Waals interactions were observed with PRO66, ARG63, MET210, SER64, LEU451, TYR215, HIS21, and GLU221.

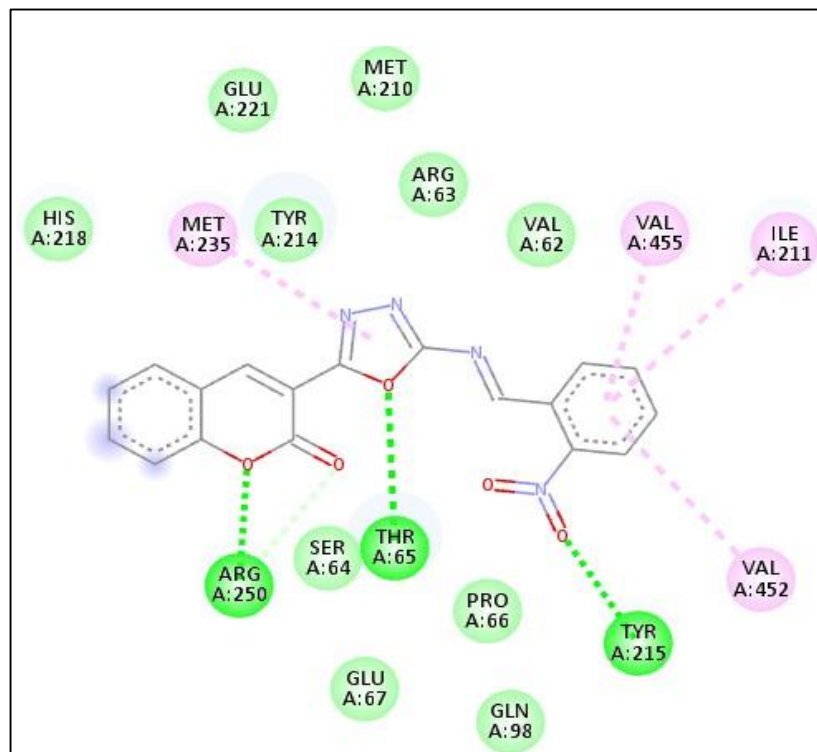


Figure 42. AM21 showing interactions with GK.

As shown in Figure 42, AM21 had hydrogen-bond interactions with THR65, TYR215, and ARG250. π -Alkyl interactions were observed with VAL455, VAL452, MET235, and ILE211. Van der Waals interactions with GLU221 and MET210.

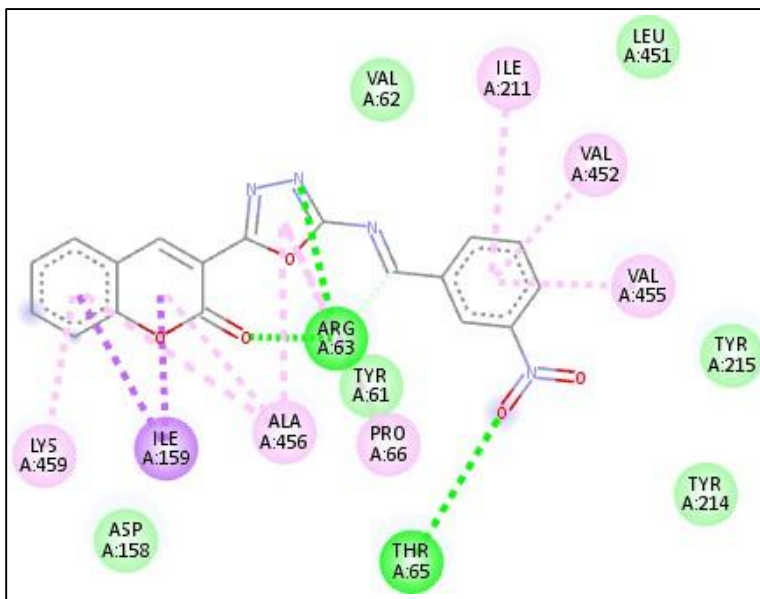


Figure 43. AM22 showing interactions with GK.

As shown in Figure 43, AM22 formed hydrogen bond interactions with THR65 and ARG63. π -Alkyl interactions were observed with LYS459, ALA456, PRO66, ILE211, VAL452, and VAL455. The π -sigma interaction was observed with ILE159. The Van der Waals interactions with VAL62, LEU451, TYR215, TYR214, TYR61, and ASP158.

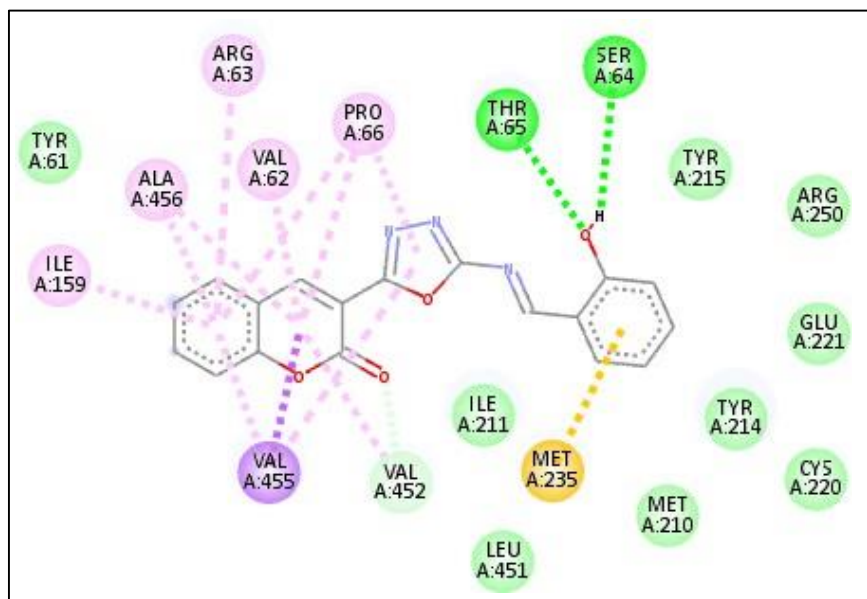


Figure 44. AM19 showing interactions with GK.

As shown in Figure 44, AM19 had a hydrogen bond interaction with THR65 and SER64. π -alkyl interactions with ILE159, ALA456, ARG63, VAL62, and PRO66. π -sigma interactions were observed with VAL455. The Van der Waals interactions with TYR61, ILE211, VAL452, LEU451, MET210, CYS220, TYR214, GLU221, ARG250, and TYR215 were also observed.

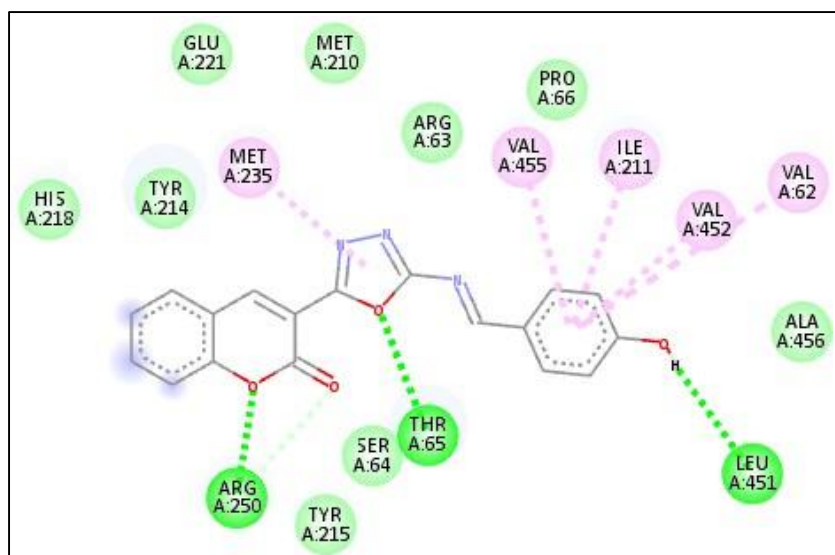


Figure 45. AM20 showing interactions with GK.

As shown in Figure 45, AM20 had hydrogen bond interactions with ARG250, THR65, and LEU451. π -Alkyl interactions were found with MET235, VAL455, ILE211, VAL452, and

VAL62. Van der Waals interactions were observed with HIS218, TYR214, GLU221, MET210, ARG63, PRO66, ALA456, TYR215, and SER64.

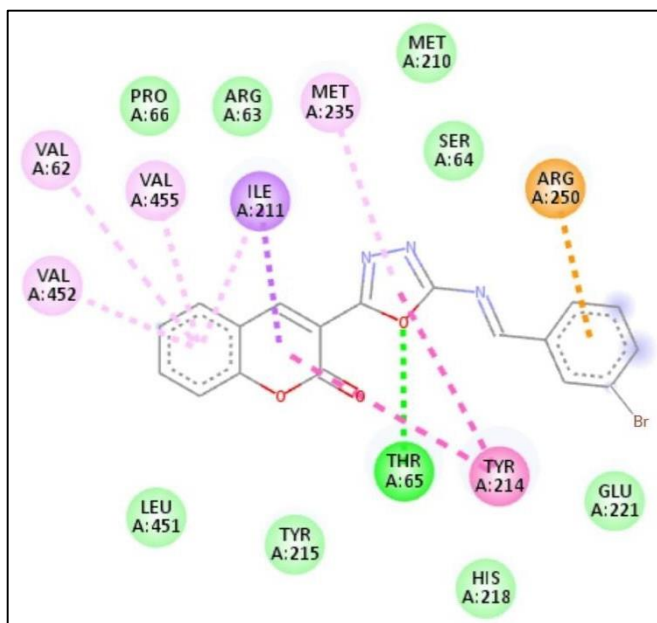


Figure 46. AM25 showing interactions with GK

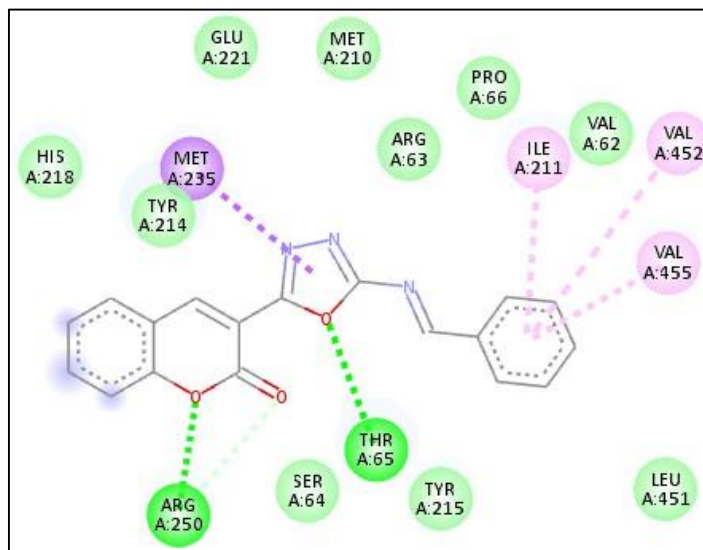


Figure 47. AM15 showing interactions with GK.

As shown in Figure 46, AM25 exhibited hydrogen-bond interactions with THR65. π -alkyl interactions with VAL62, VAL455, VAL452, MET235, and TYR214. The π sigma interactions

were found with ILE211. Van der Waals interactions were observed with PRO66, ARG63, MET210, SER64, GLU221, LEU451, TYR215, and HIS218.

As shown in Figure 47, AM15 exhibited hydrogen bonding interactions with ARG250 and THR65. π -alkyl interactions with ILE211, VAL452, and VAL455. π -sigma interactions with MET235. Van der Waals interactions were observed with HIS218, TYR214, GLU221, MET210, ARG63, PRO66, VAL62, LEU451, TYR215, and SER64.

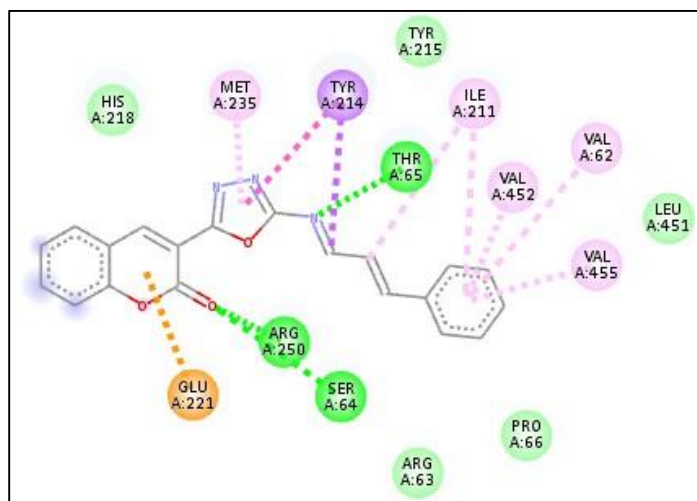


Figure 48. AM27 showing interactions with GK.

As shown in Figure 48, AM27 had hydrogen-bond interactions with ARG250, SER64, and THR65. π -alkyl interactions with MET235, ILE211, VAL452, VAL62, and VAL455. π -Sigma interactions were observed with TYR214. Van der Waals interactions were observed with HIS218, LEU451, PRO66, and ARG63.

Interactions	
	van der Waals
	Conventional Hydrogen Bond
	Carbon Hydrogen Bond
	Pi-Donor Hydrogen Bond
	Pi-Sigma
	Pi-Sulfur
	Pi-Pi Stacked
	Pi-Pi T-shaped
	Pi-Alkyl
	Halogen (Fluorine)

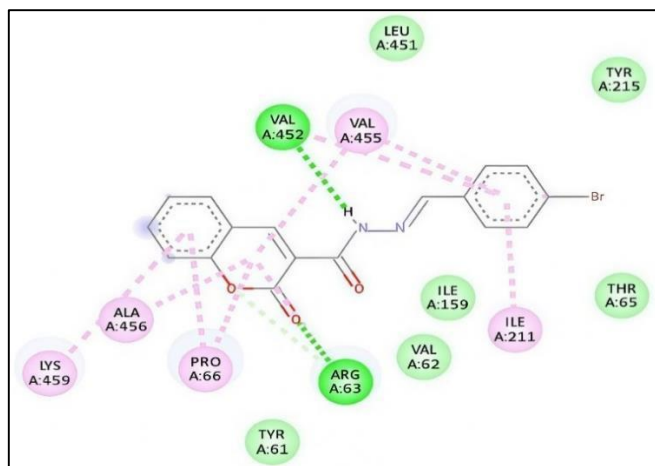


Figure 49. AM10 showing interactions with GK.

As shown in Figure 49, AM10 had hydrogen-bond interactions with VAL452 and ARG63. π -alkyl interactions with LYS459, ALA456, PRO66, VAL455, and ILE211. Van der Waals interactions were observed with TYR61, VAL62, ILE159, THR65, TYR215, and LEU451.

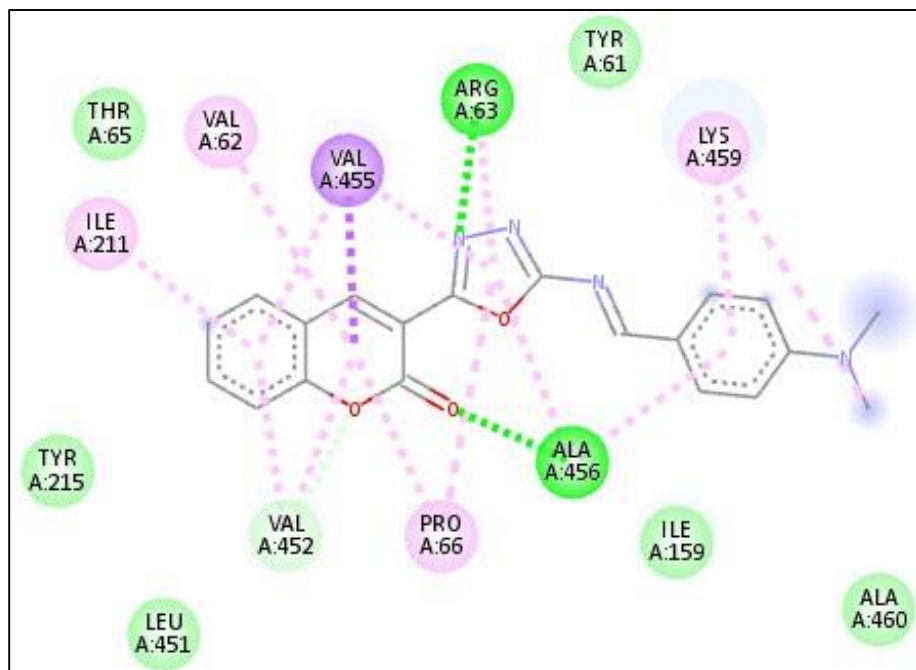


Figure 50. AM28 showing interactions with GK.

As shown in Figure 50, AM28 exhibited hydrogen bonding interactions with ARG63 and ALA456. π -alkyl interactions with ILE211, VAL62, and LYS459. π -sigma interactions with VAL455. Van der Waals interactions were observed with TYR215, LEU451, VAL452, PRO66, ILE159, and ALA460.

Table 7. The interaction strength of the identified hit compounds, highlighting their hydrogen-bonding patterns and additional contacts with surrounding amino acid residues within the GK active site.

Sr. No.	Compound	Binding affinity (Kcal/mol)	H-bond	π -alkyl	π -sigma	Van der Waals interaction
1.	AM1	-9.5	TYR215	MET235 VAL452	VAL62 VAL455	GLU221 SER64 PRO66 THR65 ARG63 TYR61 ILE159 ALA456 ILE211 LEU451
2.	AM2	-8.3	ARG63	LYS459 VAL62 VAL452 ILE211	VAL455	TYR61 TYR215 PRO66 THR65 ALA456 ILE159 LEU451
3.	AM3	-8.9	TYR215 GLN98	MET210 MET235 ILE211 PRO66 TYR214	VAL62 VAL455	GLU221 CYS220 THR65 ARG63 ILE159 SER64 LEU451 VAL452
4.	AM4	-9.8	TYR215	VAL452 MET235 TYR214	VAL455 VAL452	GLU221 SER64 THR65 PRO66 ARG63 TYR61 ALA456 CYS220 ILE211 ILE159 LEU451

5.	AM5	-9.5	PRO66 GLU67	ALA456 ARG63 VAL452	VAL62 VAL455	ILE159 TYR61 THR65 ILE211 GLY68 LYS458
6.	AM6	-8.3	THR65	MET210 VAL455 PRO66 TYR214	VAL62	CYS220 GLU221 TYR61 ILE211 ARG63 VAL452 SER64 TYR215 GLN98 ALA456 ILE159 TYR61
7.	AM7	-9.6	ARG63	LYS458 PRO66 VAL452 VAL62 ALA456	VAL455	ILE159 GLU67 GLY68 ILE211 TYR61
8.	AM8	-8.5	TYR215	TYR214 MET235 VAL452	VAL62 VAL455	GLU221 CYS220 ARG250 SER64 THR65 ILE211 MET210 PRO66 ARG63 ALA456 TYR61 ILE159 LEU451
9.	AM9	-9.7	VAL452 ARG63	LYS459 ALA456 PRO66 VAL455 ILE211	-	TYR61 VAL62 ILE159 THR65 TYR215 LEU451
10.	AM10	-10.0	ARG63	VAL455 ILE211 PRO66	TYR214	TYR215 LEU451 VAL452

				ALA456 LYS459		VAL62 THR65 ILE159 TYR61
11.	AM11	-8.9	ARG63	PRO66 VAL455 ILE211 ALA456 LYS459	VAL62	GLU67 GLY68 THR65 ILE159 TYR61
12.	AM12	-8.6	ARG63 TYR61	VAL452 ILE211 VAL455 PRO66	-	LEU451 ILE159 ALA456 TYR215 VAL62 THR65
13.	AM13	-9.1	TYR215	MET235 TYR214 VAL62 VAL452	VAL455	ARG250 ILE211 SER64 GLU221 MET210 CYS220 MET210 THR65 PRO66 TYR61 ARG63 ALA456 ILE159
14.	AM14	-9.6	ARG63	LYS458 PRO66 ALA456 VAL452	VAL455 VAL62	GLY68 GLU67 LYS459 ILE159 TYR61 ILE211
15.	AM15	-10.5	ARG250 THR65	ILE211 VAL452 VAL455	MET235	HIS218 TYR214 GLU221 MET210 ARG63 PRO66 VAL62 LEU451 TYR215 SER64
16.	AM16	-9.9	ARG63	ALA456	ILE159	TYR215

				LYS459 VAL452 ILE211 VAL455 PRO66 ALA456 LYS459		LEU451 THR65 VAL62 TYR61 ASP158
17.	AM17	-10.4	ARG63 ALA456	ILE211 VAL62 LYS459 PRO66	VAL455	THR65 TYR215 LEU451 VAL452 ILE159 TYR61
18.	AM18	-11.0	THR65	VAL62 VAL455 VAL452 MET235	ILE211	PRO66 ARG63 MET210 SER64 LEU451 TYR215 HIS21 GLU221
19.	AM19	-10.6	THR65 SER64	ILE159 ALA456 ARG63 VAL62 PRO66	VAL455	TYR61 ILE211 VAL452 LEU451 MET210 CYS220 TYR214 GLU221 ARG250 TYR215
20.	AM20	-10.6	ARG250 THR65 LEU451	MET235 VAL455 ILE211 VAL452 VAL62	-	HIS218 TYR214 GLU221 MET210 ARG63 PRO66 ALA456 TYR215 SER64
21.	AM21	-10.7	THR65 TYR215 ARG250	VAL455 VAL452 MET235 ILE211	-	GLU221 MET210
22.	AM22	-10.6	THR65	LYS459	ILE159	VAL62

			ARG63	ALA456 PRO66 ILE211 VAL452 VAL455		LEU451 TYR215 TYR214 TYR61 ASP158
23.	AM23	-9.9	THR65 ARG250	TYR214 MET235 VAL62 ALA456 PRO66	ILE211 VAL455	GLN219 HIS218 GLU221 SER64 ARG63 ILE159 VAL452 LEU451 TYR215
24.	AM24	-9.9	ARG63 LEU451	VAL62 PRO66 TYR214	ILE211	CYS220 TYR214 GLU221 ARG250 TYR215 HIS218 ARG250 SER64
25.	AM25	-10.6	THR65	VAL62 VAL455 VAL452 MET235 TYR214	ILE211	PRO66 ARG63 MET210 SER64 GLU221 LEU451 TYR215 HIS218
26.	AM26	-9.6	ARG63	ALA456 VAL62 PRO66 TYR214 LYS459	VAL455 ILE159	THR65 TYR215 LEU451 VAL452 TYR61 ASP158 ASP160
27.	AM27	-10.2	ARG250 SER64 THR65	MET235 ILE211 VAL452 VAL62 VAL455	TYR214	HIS218 LEU451 PRO66 ARG63
28.	AM28	-10.0	ARG63 ALA456	ILE211 VAL62 LYS459	VAL455	TYR215 LEU451 VAL452

						PRO66 ILE159 ALA460
29.	Dorzagliatin	-7.2	GLN98 THR65	MET235	-	GLU67 TYR215 GLY97 GLU96 HIS218 ARG250 SER64 GLU221 CYS220 MET210 ILE211
30.	MRK	-7.2	TYR215 THR65	ILE211 PRO66 VAL455 VAL62 ALA456 ILE159	-	LEU451 SER64 GLU221 TYR214 MET210 TYR61

5.4.3 ADME Studies

The top ten lead compounds were assessed based on their pharmacokinetic properties using the SwissADME web-based platform (<http://www.swissadme.ch>). The chemical structures were imported or created manually using the built-in molecular sketching tool using ChemDraw, which can be used to design two-dimensional molecular structures. Moreover, the compounds can be entered, edited, or pasted directly into SMILES notations, where SMILES identifiers are supported to input a molecule in SwissADME. Key physicochemical and pharmacokinetic parameters, such as P-glycoprotein substrate likelihood, adherence to the Lipinski rule of five, blood-brain barrier permeability, and gastrointestinal absorption, were evaluated using the BOILED-Egg model. This in silico screening method helped in the discovery of lead molecules with desirable drug-like characteristics for the targeted activation of glucokinase.

5.4.4 ADME Analysis

The physicochemical properties of an active compound are important for defining its pharmacokinetic characteristics. A summary of the key parameters of all compounds considered is presented in Table 8, including parameters such as molecular weight, partition coefficient (Log P), and number of hydrogen bond acceptors and donors. The bioavailability scores of the chosen hit compounds were predicted to be equal to the bioavailability score of the reference compound MRK, and the scores were 0.55 each. The Lipinski rule of five drug-like calculator showed that all hits covered the criteria. Of these, AM18 (molecular weight = 351.74 Log P = 3.31) and AM21 (molecular weight = 362.30, Log P = 2.50) proved to be the most promising results, which were confirmed by the positive physicochemical features and a high docking rate.

Table 8. The drug-likeness property of hit heterocyclic derivatives using SwissADME

Sr. No	Compound	Mol. Wt.	H-bond acceptor	H-bond donor	LOG P	GI absorption	Bioavailability Score
1.	AM1	292.29	4	1	2.28	High	0.55
2.	AM2	326.73	4	1	2.71	High	0.55
3.	AM3	326.73	4	1	2.71	High	0.55
4.	AM4	326.73	4	1	2.71	High	0.55
5.	AM5	308.29	5	2	2.15	High	0.55
6.	AM6	308.29	5	2	2.15	High	0.55
7.	AM7	337.29	6	1	2.04	High	0.55
8.	AM8	337.29	6	1	2.04	High	0.55
9.	AM9	337.29	6	1	2.04	High	0.55
10.	AM10	371.18	4	1	2.76	High	0.55
11.	AM11	371.18	4	1	2.76	High	0.55
12.	AM12	322.31	5	1	2.47	High	0.55
13.	AM13	318.33	4	1	2.70	High	0.55
14.	AM14	335.36	4	1	2.72	High	0.55
15.	AM15	317.30	6	0	2.83	High	0.55
16.	AM16	351.74	6	0	3.24	High	0.55
17.	AM17	351.74	6	0	3.24	High	0.55
18.	AM18	351.74	6	0	3.24	High	0.55
19.	AM19	333.30	7	1	2.57	High	0.55
20.	AM20	333.30	7	1	2.57	High	0.55
21.	AM21	362.30	8	0	2.50	High	0.55
22.	AM22	362.30	8	0	2.50	High	0.55
23.	AM23	362.30	8	0	2.50	High	0.55

24.	AM24	396.19	6	0	3.20	High	0.55
25.	AM25	396.19	6	0	3.20	High	0.55
26.	AM26	347.32	7	0	3.37	High	0.55
27.	AM27	343.34	6	0	3.34	High	0.55
28.	AM28	360.37	6	0	3.35	High	0.55
29.	Dorzagliatin	462.93	6	3	3.08	High	0.55
30.	MRK	349.41	4	2	2	Low	0.55

AM18's moderate molecular weight and optimal Log P (3.31) suggest excellent membrane permeability and absorption potential, whereas its lack of H-bond donors (0) may enhance passive diffusion. AM21, despite its slightly higher MW (362.30), exhibits a favorable Log P (2.50) and a higher number of H-bond acceptors (8), which could improve aqueous solubility without severely compromising permeability. Both compounds maintain high GI absorption, aligning well with oral drug development. Their inability to cross the BBB may be advantageous for peripheral targets by minimizing CNS-related off-effects. Among the other leads, compounds with lower molecular weight (AM1 = 292.29, AM16 = 317.30) demonstrated better solubility and membrane permeability, enhancing absorption potential. Higher molecular weight compounds, Dorzagliatin (462.93) may face challenges in oral absorption but could offer prolonged activity. Both AM21 and AM22 contain 8 H-bond acceptors, showing increased solubility but potentially reduced permeability, while those with balanced donors and acceptors such as AM19 and AM20 with 7 acceptors and 1 donor achieved optimal solubility-permeability balance. Log P values between 2 and 3.5 (AM18 with 3.31 and AM28 with 3.35) indicated favorable lipophilicity for absorption, whereas MRK (Log P = 2) represented a more hydrophilic profile. All compounds except MRK exhibited high GI absorption, supporting their suitability for oral delivery, while MRK's low absorption may require alternative formulations. None of the compounds could cross the blood-brain barrier, eliminating CNS side effects but restricting neurological applications. The consistent bioavailability (0.55) across compounds suggested predictable systemic exposure, though further optimization of Log P and hydrogen bonding could enhance performance. Compound AM9 (MW = 371.18, Log P = 2.76, four acceptors, and one donor) and AM27 (MW = 343.34, Log P = 3.34) also emerged as strong candidates because of their balanced properties, making them ideal for oral drug development. AM18 and AM21 stand out not only for their docking performance but also for their ADME-compliant profiles, positioning them as top-tier candidates for further preclinical evaluation.

5.4.5 Toxicity Prediction pkCSM software was used for the comprehensive toxicity assessment of the top 10 compounds. The toxicity profiles of all compounds were analyzed based on their maximum tolerated dose (MTD) in humans, oral acute toxicity (ORAT LD₅₀), skin sensitization potential, *Tetrahymena pyriformis* toxicity, and minnow toxicity (Table 9). None of the compounds caused skin sensitization, indicating good dermal safety. Compounds AM18 and AM1 exhibited the lowest human MTD values (-0.028 log mg/kg/day), suggesting potential dose limitations, whereas AM27 (0.449 log mg/kg/day) and Dorzagliatin (0.428 log mg/kg/day) demonstrated the highest tolerability, allowing for wider therapeutic windows. In acute oral toxicity, Dorzagliatin showed the highest LD₅₀ value (3.328 mol/kg), indicating superior safety for oral administration, followed by AM21 (2.928 mol/kg) and AM22 (2.953 mol/kg) administration. The co-crystallized ligand MRK had the lowest LD₅₀ (0.969 mol/kg), necessitating careful dose consideration. For environmental toxicity, AM18 and AM1 displayed the highest *T. pyriformis* toxicity (0.923 log µg/mL), whereas MRK showed the lowest (0.285 log µg/mL). In the aquatic toxicity assessment, Dorzagliatin (2.394 mM) and MRK (1.657 mM) were the least toxic to minnows, whereas AM27 (-3.07 mM) and AM22 (-2.675 mM) showed higher toxicity values. Overall, Dorzagliatin combines favorable properties, including high human MTD, excellent oral safety (high LD₅₀), and low environmental toxicity, making it particularly suitable for further development. Compounds AM21 and AM22 also demonstrated promising safety profiles with high LD₅₀ values and moderate environmental toxicity.

Table 9. Toxicity Profile of Potent compounds

Sr. No	Compound	MTD (human) log mg/kg/day	ORAT (LD ₅₀) mol/kg	Skin Sensiti zation	<i>T.Pyriformis</i> toxicity log µg/mL	Minnow toxicity (mM)
1.	AM1	-0.028	1.336	No	0.923	0.913
2.	AM2	0.075	2.099	No	0.934	0.664
3.	AM3	0.017	2.115	No	0.941	0.3
4.	AM4	-0.023	2.058	No	0.923	0.598
5.	AM5	-0.185	2.298	No	0.655	1.355
6.	AM6	0.207	2.07	No	0.654	1.356
7.	AM7	-0.15	2.855	No	0.542	0.52
8.	AM8	-0.197	2.87	No	0.583	0.424
9.	AM9	-0.21	2.882	No	0.588	0.318
10.	AM10	0.073	2.11	No	0.933	0.518
11.	AM11	0.015	2.126	No	0.941	0.154

12.	AM12	0.06	2.092	No	0.747	0.811
13.	AM13	-0.018	1.921	No	0.93	-0.244
14.	AM14	0.035	2.104	No	0.878	0.64
15.	AM15	0.379	2.206	No	0.306	-1.36
16.	AM16	0.242	2.247	No	0.306	-1.58
17.	AM17	0.266	2.271	No	0.306	-2.058
18.	AM18	0.341	2.282	No	0.309	-1.727
19.	AM19	0.074	2.684	No	0.305	-0.445
20.	AM20	0.428	2.711	No	0.302	-0.625
21.	AM21	0.406	2.928	No	0.291	-2.196
22.	AM22	0.262	2.953	No	0.291	-2.675
23.	AM23	0.195	2.973	No	0.291	-2.364
24.	AM24	0.246	2.255	No	0.305	-1.726
25.	AM25	0.269	2.279	No	0.306	-2.204
26.	AM26	0.316	2.339	No	0.299	-1.609
27.	AM27	0.449	2.183	No	0.327	-3.07
28.	AM28	0.247	2.292	No	0.305	-1.4
29.	Dorzagliatin	0.545	0.969	No	0.285	1.657
30.	MRK	0.428	3.328	No	0.308	2.394

*MTD = maximum tolerated dose, ORAT = oral rat acute toxicity, LD₅₀ = lethal dose

Although AM18 shows concerning human MTD and environmental toxicity values, its structural analogs with improved properties (AM21 and AM22) may offer safer alternatives. The consistent absence of skin sensitization across all compounds is advantageous for patient acceptance and regulatory approval. These findings enable the prioritization of lead compounds based on their comprehensive toxicity profiles for subsequent development stages. AM21's exceptional docking scores are complemented by its favorable toxicity profile, including high oral safety, tolerable MTD, and no sensitization, making it a prime candidate for lead optimization.

5.4.6 DFT analysis

DFT analysis represents further insight into the electronic properties and reactivity of the coumarin-hydrazide (AM1-AM14) and coumarin-oxadiazole (AM15-AM28) derivatives in comparison with the reference glucokinase activators, Dorzagliatin and MRK (co-crystallized ligand). The obtained frontier molecular orbital (FMO) parameters of the energies of HOMO and LUMO, energy gap (del), chemical hardness, and electronegativity showed specific patterns that are correlated with the biological behavior of the compounds (Table 10). HOMO, LUMO energy gap of molecules is a valid measure of molecular reactivity, stability, and transfer ability of

electrons. The AM14 had the smallest energy gap (2.3851 eV) when compared to all the candidates, implying that it was more chemically soft and capable of forming more interactions with the glucokinase active site residues in charge transfer reaction. Dorzagliatin and the co-crystallized ligand surprisingly exhibited relatively bigger gaps (4.9667 eV and 4.5658 eV, respectively) suggesting a higher chemical hardness and less reactivity, which is characteristic of stable reference molecules already optimized to bind.

In both series, most derivatives had moderate energy gaps of 3.03.5 eV, and thus a good balance between stability and reactivity that would allow for interaction with enzymes. Other compounds with greater biological activity, such as AM18, AM21, and AM25, also exhibited relatively low-to-moderate ΔE values, supporting the claim that optimal electronic softness promotes charge delocalization and orbital overlap with the active site, making the binding and activation of glucokinase a facile process. The tendency in HOMO energies was based on the ability of the compounds to give off electrons; the higher the HOMO energy (closer to zero), the better the electron-donating ability, and the lower the LUMO energy, the higher the ability to accept electrons. High glucokinase activators tended to have low LUMO values, in which the electron-accepting power was better in the ligand–protein interaction (Figure 51).

Table 10. Calculated Frontier Molecular Orbital Energies, Electronegativity, and Chemical Hardness of molecules

Compound	HOMO	LUMO	E	Hardness	Electro negativity
AM1	-5.9596	-2.7168	3.2428	1.6214	4.3382
AM2	-6.1452	-2.8341	3.3111	1.6555	4.4896
AM3	-6.1571	-2.7312	3.3560	1.6787	4.4877
AM4	-6.1838	-2.7930	3.3908	1.6954	4.4884
AM5	-5.8736	-2.8014	3.0722	1.5361	4.3375
AM6	-5.6553	-2.6743	2.9810	1.4905	4.1648
AM7	-6.4701	-3.0556	3.4145	1.7072	4.7628
AM8	-6.4924	-2.9505	3.5418	1.7709	4.7215
AM9	-6.6186	-3.1211	3.4975	1.7487	4.8699
AM10	-6.0698	-2.8158	3.2539	1.6270	4.4428
AM11	-6.1892	-2.8254	3.3639	1.6819	4.5073
AM12	-5.5655	-2.6485	2.9171	1.4585	4.1070
AM13	-5.6279	-2.7078	2.9201	1.4600	4.1678
AM14	-4.8970	-2.5119	2.3851	1.1925	3.7044
AM15	-6.3324	-2.8518	3.4806	1.7403	4.5921

AM16	-6.4673	-2.9938	3.4735	1.7368	4.7306
AM17	-6.4576	-2.9356	3.4535	1.7315	4.7189
AM18	-6.4456	-2.9568	3.4888	1.7444	4.7012
AM19	-6.2837	-2.9328	3.3508	1.6754	4.6083
AM20	-6.1041	-2.7791	3.3250	1.6625	4.4416
AM21	-6.5911	-3.1220	3.4692	1.7346	4.8566
AM22	-6.6867	-3.2365	3.4501	1.7251	4.9616
AM23	-6.7558	-3.5609	3.1949	1.5974	5.1583
AM24	-6.4145	-2.9677	3.4469	1.7234	4.6911
AM25	-6.4850	-2.9859	3.4991	1.7496	4.7355
AM26	-6.0023	-2.7394	3.2629	1.6315	4.3708
AM27	-6.0042	-2.8700	3.1342	1.5671	4.4371
AM28	-5.3862	-2.5503	2.8360	1.4180	3.9682
Co-cryst Ligand (MRK)	-6.0959	-1.5301	4.5658	2.2829	3.8130
Dorzagliatin	-6.1188	-1.1521	4.9666	2.4833	3.6354

This trend was further supported by the calculated chemical hardness (η) and electronegativity (χ) parameters. Lower hardness, moderate electronegativity compounds (AM14, AM18, and AM25) were shown to have good electronic adjustability to dynamic interactions in the enzyme pocket and thus showed stronger stabilization of the enzyme ligand pocket (Figure 52).

. On the other hand, the harder the better (e.g., AM7, AM8, and AM22), the more polarization resistant they are, and the less they can be redistributed favorably by binding. Among the candidates, Dorzagliatin and the co-crystal ligand exhibited the largest energy gaps (4.9667 and 4.5658 eV, respectively) and were chemically hard. This small gap suggests a higher reactivity (chemically soft) and a greater propensity for chemical reactions, such as binding with a target protein. In general, the DFT calculations support the hypothesis (Figure 53) that any structural changes that decrease the HOMO energy gap and increase orbital delocalization lead to an increase in the binding affinity and activation potential. The results support the fact that coumarin-based analogs, especially those with electron-withdrawing substituents to maximize electronic softness, have an attractive electronic structure for glucokinase activation, which is in agreement with the experimental EC₅₀ and percent activation data.

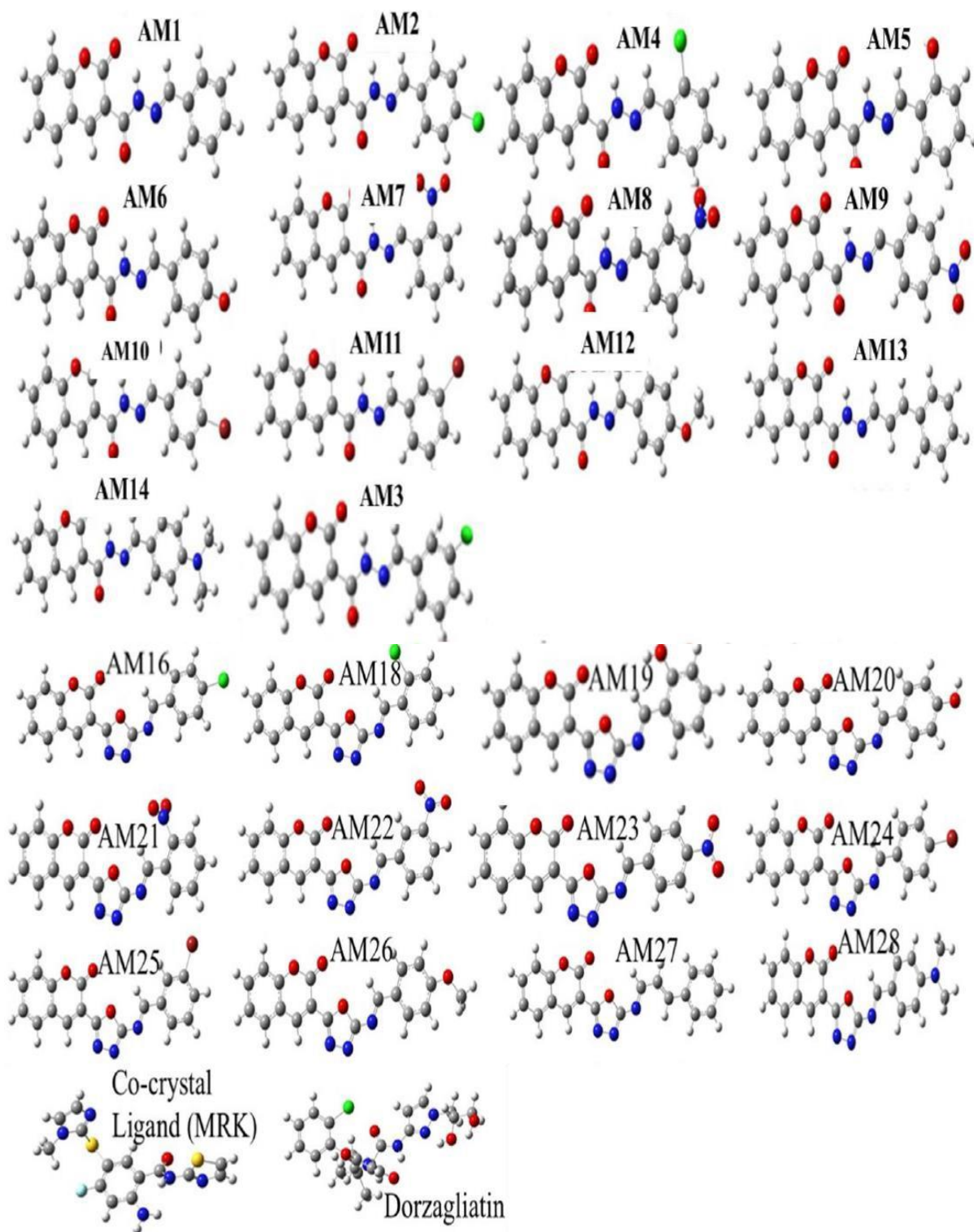


Figure 51. The optimized geometries of the drug molecules are displayed. The structures were optimized using Gaussian 16 at the B3LYP/6-31G level of theory.

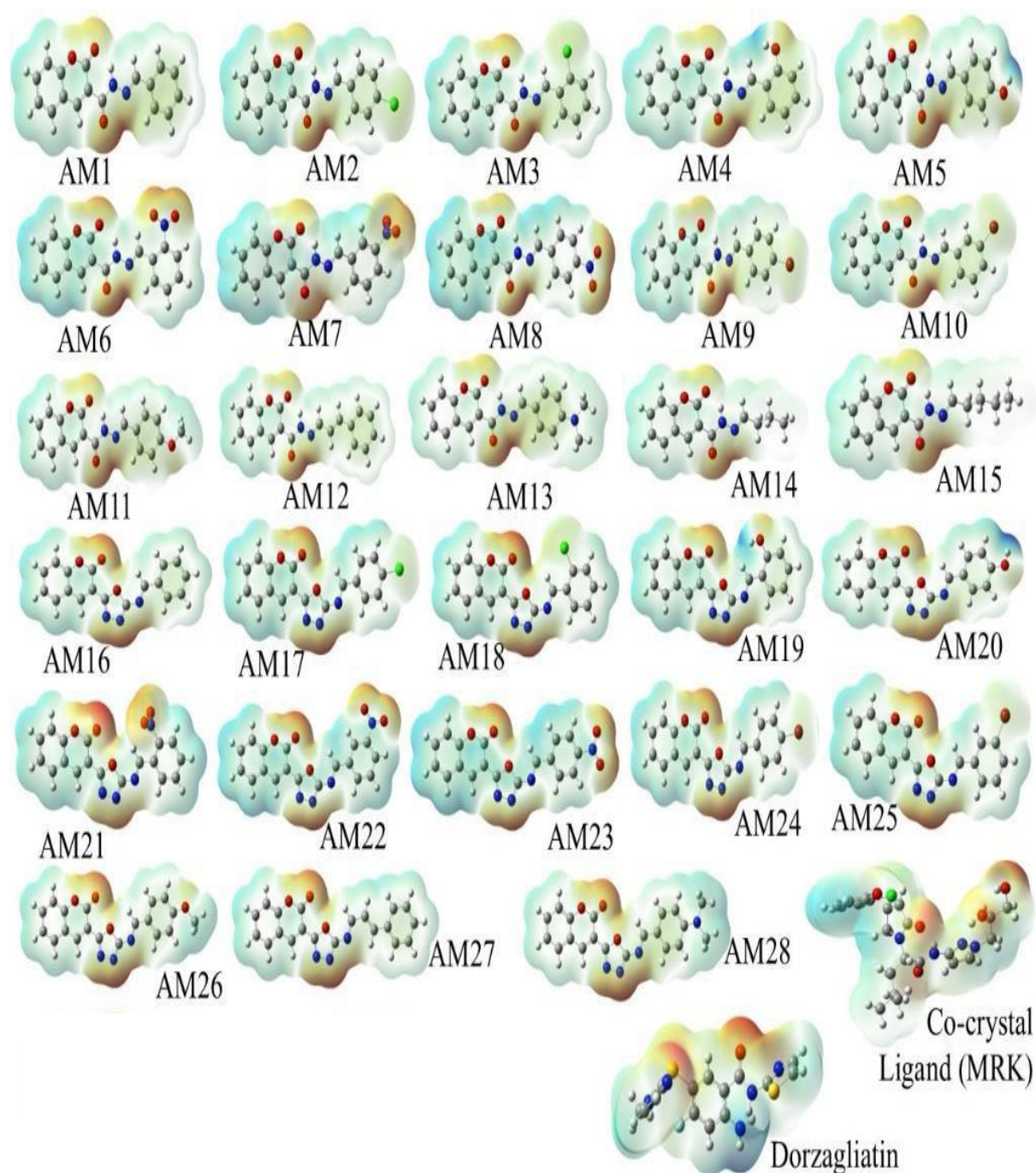


Figure 52. The molecular electrostatic potential plots of the drug molecules. The red and blue colour indicates the electron-dense and electron-deficient regions, respectively. The Intermediate Colours (Orange, Yellow, Green) represent a gradient of potential values between the negative (red) and positive (blue) regions.

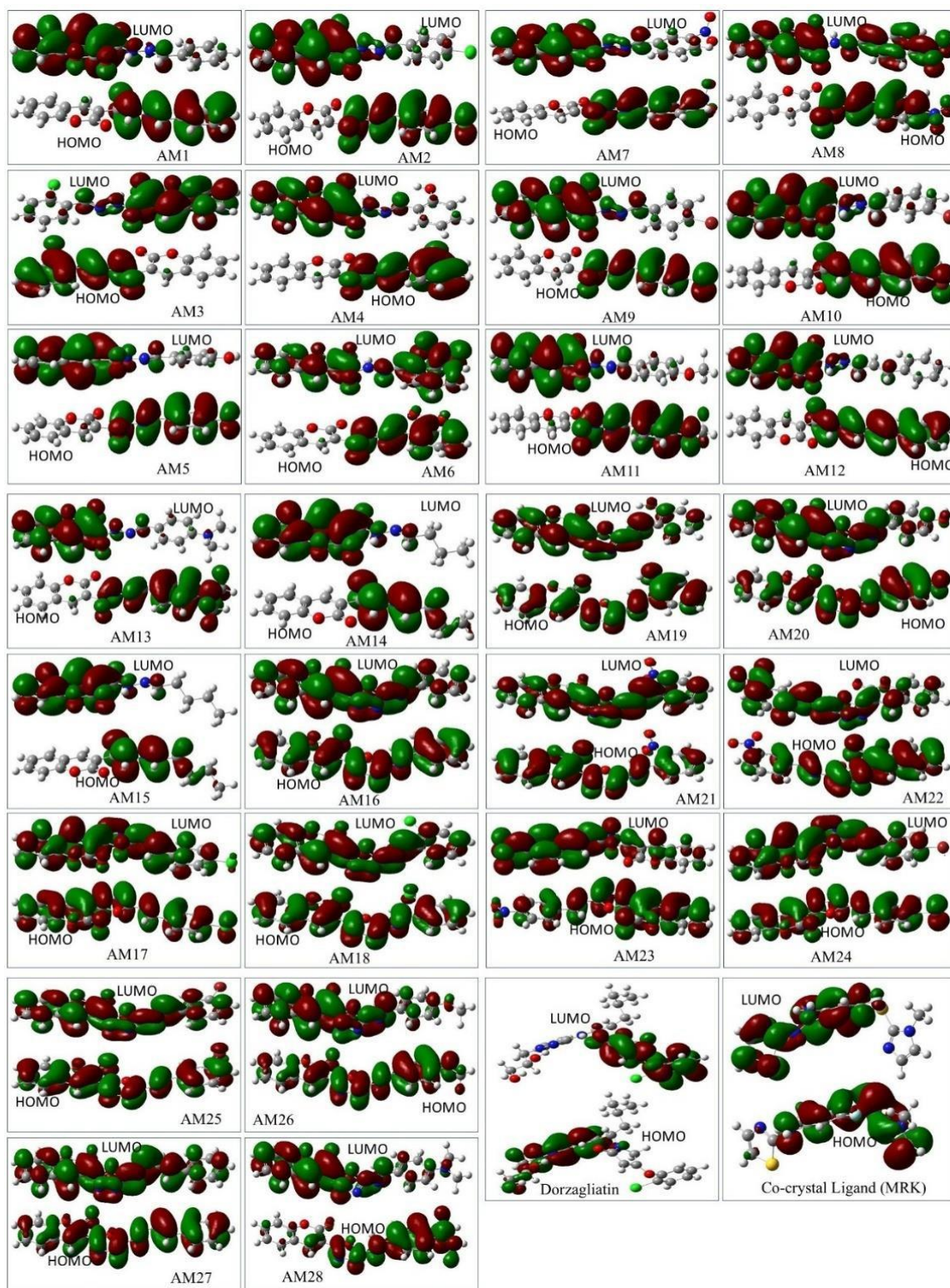


Figure 53. The frontier molecular orbitals (HOMO and LUMO) of drug molecules. The positive and negative phases of the molecular orbitals are presented by red and green colours, respectively.

5.5 Characterization

Ethyl 2-oxo-2H-chromene-3-carboxylate (3)

Yield: 70%, yellow solid crystals, M.P.: 113-116°C, R_f: 0.17 (*n*-Hexane: ethyl acetate (7:3), FT-IR (ν, cm⁻¹): 3052.98 (CH-Ar), 2752.59 (CH-aliphatic), 2639.34 (CH-aliphatic), 1685.97 (C=O), 1575.16 (C=C aromatic), 1477.82 (aromatic stretch), 1426.65 (C=C), 1380.19 (C-N), 1259.44 (C-O), 1232.61 (C-O), 1209.73 (C-O), 1188.06 (C-C), 975.10 (Ar-H oop), 942.18 (C-H bending), 891.22 (C-H oop), 855.94 (Ar-H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 1.22 – 1.25 (t, J = 7.103 Hz, 3H), 4.19 – 4.23 (q, J = 6.958 Hz, 2H), 7.35 – 7.38 (m, 1H), 7.44 (dd, J = 1.283, 8.518 Hz, 1H), 7.61 – 7.64 (m, 1H), 7.67 – 7.69 (m, 1H), 8.49 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 165.49 (C=O, coumarin lactone C-2), 158.11 (C-O, C-7), 156.65 (C-O, C-9), 149.90 (C-4a/C=N-type aromatic carbon), 134.23 (C-3, ester carbonyl adjacent), 131.13 (C-5), 126.82 (C-6), 126.09 (C-8), 117.90 (C-4), 116.46 (C-3), 62.91 (O-CH₂ of ethyl ester), 15.49 (CH₃ of ethyl ester), LCMS (ESI) m/z: 241.0496 [M + H]⁺

2-oxo-2H-chromene-3-carbohydrazide (4)

Yield: 65%, off-white crystals, M.P.: 134-137°C, R_f: 0.67 (*n*-Hexane: ethyl acetate (6:4), FT-IR (ν, cm⁻¹): 3243.17 (N-H, ν/NH stretch), 1668.13 (C=O), 1594.18 (C=C aromatic), 1341.09 (C-N), 1333.74 (C-N), 1295.57 (C-O/C-N), 1272.32 (C-O), 1217.90 (C-O), 1121.34 (C-O/C-N), 1015.83 (C-N/C-O), 963.62 (Ar-H oop), 890.50 (C-H bending), 747.40 (Ar-H oop) and 684.93 (Ar-H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 4.49 (d, J = 4.346 Hz, 2H), 7.50 – 7.54 (m, 1H), 7.56 – 7.62 (m, 2H), 7.77 – 7.80 (td, J = 1.454, 7.628 Hz, 1H), 8.55 (s, 1H), 10.01 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 166.88 (C=O, coumarin lactone C-2), 161.76 (C=O, carbohydrazide C=O), 155.05 (C-O, C-7), 148.22 (C-4), 134.40 (C-5), 130.70 (C-6), 126.98 (C-8), 120.47 (C-3), 118.91 (C-4), 118.05 (C-9), LCMS (ESI) m/z: 227.0913 [M + H]⁺

3-(5-amino-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (5)

Yield: 68%, light beige crystals, M.P.: 204-206°C, R_f: 0.41 (*n*-Hexane: ethyl acetate (7:3), FT-IR (ν, cm⁻¹): 3275.19 (N-H stretch), 2938.01 (C-H aliphatic stretch), 2750.95 (C-H), 1665.90 (C=O stretch), 1579.24 (C=C aromatic), 1487.62 (C=C aromatic), 1193.16 (C-O), 1380.87 (C-N), 1442.82 (C=C aromatic), 903.74 (Ar-H oop), 749.58 (C-H bending), 729.13 (Ar-H oop), ¹H

NMR (400 MHz, CDCl₃, ppm): δ 6.76 (s, 2H), 7.67 – 7.75 (m, 3H), 8.04 – 8.06 (m, 1H), 8.59 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 167.03 (C=O, coumarin lactone C-2), 161.91 (C=N–O of oxadiazole), 155.20 (C–O, C-7), 148.37 (C-4 / heteroaromatic quaternary C), 134.55 (C-5), 130.85 (C-6), 127.13 (C-8), 120.62 (C-3a), 119.06 (C-4), 118.20 (C-9), LCMS (ESI) m/z: 252.0539 [M + H]⁺

(E)-N'-(benzylidene-2-oxo-2H-chromene-3-carbohydrazide (AM1).

Yield: 75%, cream-white crystals, M.P.: 202–205°C, R_f: 0.82 (*n*-Hexane: ethyl acetate (7:3), FT-IR (ν , cm⁻¹): 3043.24 (CH–Ar), 2638.27 (CH-aliphatic), 1617.87 (C=O/C=N), 1571.34 (C=C aromatic), 1485.31 (aromatic stretch), 1316.32 (C–N), 1031.56 (C–N), 974.74 (Ar–H oop), and 747.26 (Ar–H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 7.24–7.30 (m, 2H), 7.33–7.36 (m, 4H), 7.53 (d, *J* = 7.4 Hz, 1H), 7.59 (d, *J* = 7.1 Hz, 2H), 8.02 (s, 1H), 8.33 (s, 1H), 10.63 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 162.98 (C=O), 161.46 (C=O), 154.87 (C=N), 148.96 (C–Ar), 142.62 (C–Ar), 134.22, 133.67, 131.92, 130.52, 130.21, 129.03, 126.80, 120.37, 117.87, 117.81 (CH–Ar), HRMS (ESI) m/z: 293.0815 [M + H]⁺

(E)-N'-(4-chlorobenzylidene)-2-oxo-2H-chromene-3-carbohydrazide (AM2).

Yield: 76%, pale brownish crystals, M.P.: 211–215°C, R_f: 0.78 (*n*-Hexane: ethyl acetate (8:2), FT-IR (ν , cm⁻¹): 3043.78 (CH–Ar), 2752.59 (CH-aliphatic), 2639.34 (CH-aliphatic), 1616.36 (C=O/C=N), 1572.00 (C=C aromatic), 1485.86 (aromatic stretch), 157.53 (C=C), 1316.47 (C–N), 1046.65 (C–N), 727.93 (Ar–H oop) and 747.29 (Ar–H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 7.36–7.39 (m, 1H), 7.45 (d, *J* = 8.1 Hz, 2H), 7.57 (d, *J* = 8.1 Hz, 2H), 7.60–7.66 (m, 3H), 8.14 (s, 1H), 8.44 (s, 1H), 10.73 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 163.15 (C=O), 161.64 (C=O), 155.05 (C=N), 149.39 (C–Ar), 142.79 (C–Ar), 138.37, 134.39, 132.40, 131.92, 130.69, 130.63, 126.97, 120.54, 118.04, 117.98 (CH–Ar), HRMS (ESI) m/z: 327.0501 [M + H]⁺

(E)-N'-(3-chlorobenzylidene)-2-oxo-2H-chromene-3-carbohydrazide (AM3).

Yield: 77%, ivory solid crystals, M.P.: 214–217°C, R_f: 0.75 (*n*-Hexane: ethyl acetate (7:3), FT-IR (ν , cm⁻¹): 3344.03 (CH–Ar), 2645.73 (CH-aliphatic), 1643.69 (C=O), 1617.27 (C=N), 1569.18 (C=C aromatic), 1472.54 (aromatic stretch), 1454.23 (C=C), 1322.61 (C–N), and 981.14 (Ar–H oop) and 752.41 (Ar–H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 7.18–7.23 (m, 1H), 7.32–7.39

(m, 4H), 7.46-7.48 (m, 1H), 7.52 (d, $J = 7.3$ Hz, 1H), 7.57-7.58 (t, $J = 2.1$ Hz, 1H), 8.03 (s, 1H), 8.32 (s, 1H), 10.61 (s, 1H), ^{13}C NMR (100 MHz, CDCl_3 , ppm): δ 162.85 (C=O), 161.33 (C=O), 154.74 (C=N), 145.45 (C-Ar), 142.49 (C-Ar), 136.34, 135.75, 134.09, 131.74, 130.39, 130.34, 129.49, 126.67, 126.11, 120.24, 117.74, 117.68 (CH-Ar), HRMS (ESI) m/z : 327.0512 $[\text{M} + \text{H}]^+$

(E)-N'-(2-chlorobenzylidene)-2-oxo-2H-chromene-3-carbohydrazide (AM4).

Yield: 68%, off-cream solid crystals, M.P.: 215–216°C, R_f : 0.58 (*n*-Hexane: ethyl acetate (7:3), FT-IR (ν , cm^{-1}): 3043.98 (CH-Ar), 2639.57 (CH-aliphatic), 1617.27 (C=O/C=N), 1572.06 (C=C aromatic), 1486.00 (aromatic stretch), 1446.72 (C=C), 1386.08 (C-N), 1031.99 (C-N), 728.43 (Ar-H oop) and 748.03 (Ar-H oop), ^1H NMR (400 MHz, CDCl_3 , ppm): δ 7.15-7.18 (m, 1H), 7.21-7.25 (m, 2H), 7.29-7.34 (m, 3H), 7.48-7.51 (m, 2H), 8.29 (s, 1H), 8.32 (s, 1H), 10.36 (s, 1H), ^{13}C NMR (100 MHz, CDCl_3 , ppm): δ 163.33 (C=O), 161.83 (C=O), 155.24 (C=N), 145.45 (C-Ar), 142.99 (C-Ar), 135.86, 134.59, 133.87, 133.55, 130.89, 130.62, 130.32, 130.16, 127.17, 120.74, 118.24, 118.18 (CH-Ar), HRMS (ESI) m/z : 327.0511 $[\text{M} + \text{H}]^+$

(E)-N'-(2-hydroxybenzylidene)-2-oxo-2H-chromene-3-carbohydrazide (AM5).

Yield: 69%, faint beige solid crystals, M.P.: 183–186°C, R_f : 0.70 (*n*-Hexane: Ethyl acetate (8:2), FT-IR (ν , cm^{-1}): 3053.72 (CH-Ar), 2752.9 (CH-aliphatic), 2645.11 (CH-aliphatic), 1621.34 (C=O/(C=N), 1565.71 (C=C aromatic), 1489.65 (aromatic stretch), 1451.70 (C=C), 1382.56 (C-N), 1023.54 (C-N), 967.46 (Ar-H oop) and 756.13 (Ar-H oop), ^1H NMR (400 MHz, CDCl_3 , ppm): δ 6.78-6.82 (m, 2H), 7.15-7.18 (m, 1H), 7.21-7.25 (m, 1H), 7.33-7.39 (m, 3H), 7.53 (d, $J = 7.1$ Hz, 1H), 8.16 (s, 1H), 8.33 (s, 1H), 10.33 (s, 1H), 11.72 (s, 1H), ^{13}C NMR (100 MHz, CDCl_3 , ppm): δ 162.83 (C=O), 161.33 (C=O), 159.19 (C-Ar), 154.74 (C=N), 148.85 (C-Ar), 142.49 (C-Ar), 134.09, 134.02, 131.76, 130.39, 126.67, 122.42, 120.24, 118.49, 117.74, 117.71, 116.98 (CH-Ar), HRMS (ESI) m/z : 309.0826 $[\text{M} + \text{H}]^+$

(E)-N'-(4-hydroxybenzylidene)-2-oxo-2H-chromene-3-carbohydrazide (AM6).

Yield: 71%, milky-white solid crystals, M.P.: 184–188°C, R_f : 0.52 (*n*-Hexane: ethyl acetate (8:2), FT-IR (ν , cm^{-1}): 3044.03 (CH-Ar), 2752.69 (CH-aliphatic), 2640.01 (CH-aliphatic), 1618.62 (C=O/C=N), 1572.23 (C=C aromatic), 1485.90 (aromatic stretch), 1446.80 (C=C), 1385.79 (C-N), 1031.93 (C-N), 893.28 (Ar-H oop) and 748.51 (Ar-H oop), ^1H NMR (400

MHz, CDCl₃, ppm): δ 6.76-6.78 (m, 2H), 7.31-7.36 (m, 2H), 7.35-7.40 (m, 4H), 7.55 (d, J = 7.2 Hz, 1H), 8.05 (s, 1H), 8.35 (s, 1H), 10.64 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 162.75 (C=O), 161.24 (C=O), 156.48 (C–Ar), 154.65 (C=N), 148.98 (C–Ar), 142.39 (C–Ar), 133.99, 133.53, 130.29, 126.81, 126.57, 120.14, 117.64, 117.58, 116.11 (CH–Ar), HRMS (ESI) m/z : 309.0836 [M + H]⁺

(E)-N'-(2-nitrobenzylidene)-2-oxo-2H-chromene-3-carbohydrazide (AM7).

Yield: 70%, light brown solid crystals, M.P.: 268–270°C, R_f: 0.52 (*n*-Hexane: ethyl acetate (8:2), FT-IR (ν , cm⁻¹): 2640.84 (CH–Ar), 1620.51 (C=O), 1519.31 (C=N), 1570.92 (C=C aromatic), 1486.56 (aromatic stretch), 1386.29 (C=C / NO₂ sym), 1305.62 (C–N), 1032.32 (C–N), 784.33 (Ar–H oop) and 729.89 (Ar–H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 7.28-7.32 (m, 1H), 7.36-7.40 (t, J = 8.1 Hz, 2H), 7.52-7.60 (m, 3H), 7.87 (d, J = 8.4 Hz, 1H), 7.93 (d, J = 6.5 Hz, 1H), 8.26 (s, 1H), 8.33 (s, 1H), 10.43 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 163.04 (C=O), 161.55 (C=O), 154.96 (C=N), 147.57 (C–Ar), 144.54, 142.70, 134.30, 133.75, 132.25, 130.60, 130.41, 130.10, 126.88, 125.20, 120.45, 117.95, 117.92 (CH–Ar), HRMS (ESI) m/z : 338.0731 [M + H]⁺

(E)-N'-(3-nitrobenzylidene)-2-oxo-2H-chromene-3-carbohydrazide (AM8).

Yield: 74%, tan-colored solid crystals, M.P.: 270–273°C, R_f: 0.47 (*n*-Hexane: ethyl acetate (7:3), FT-IR (ν , cm⁻¹): 2734.90 (CH–Ar), 1681.14 (C=O), 1572.15 (C=N), 1519.31 (C=C aromatic), 1487.65 (aromatic stretch), 1386.29 (NO₂ sym), 1364.35 (C–N), 985.63 (C–N), 729.89 (Ar–H oop) and 703.48 (Ar–H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 7.29-7.34 (m, 1H), 7.39 (d, J = 8.4 Hz, 2H), 7.53-7.60 (m, 2H), 7.91 (d, J = 7.3 Hz, 1H), 7.91 (s, 1H), 8.13-8.15 (t, J = 8.408 Hz, 1H), 8.34-8.36 (m, 2H), 10.76 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 162.78 (C=O), 161.27 (C=O), 154.68 (C=N), 148.54 (C–Ar), 146.45 (C–Ar), 142.42, 135.99, 134.02, 133.16, 130.68, 130.32, 126.60, 125.35, 122.91, 120.18, 117.67, 117.62 (CH–Ar), HRMS (ESI) m/z : 338.0711 [M + H]⁺

(E)-N'-(4-nitrobenzylidene)-2-oxo-2H-chromene-3-carbohydrazide (AM9)

Yield: 72%, pale orange solid crystals, M.P.: 271–275°C, Rf: 0.50 (*n*-Hexane: ethyl acetate (7:3), FT-IR (ν , cm^{-1}): 3114.92 (CH–Ar), 1595.04 (C=O/C=N), 1518.83 (C=C aromatic), 1380.90 (aromatic stretch), 1344.50 (NO₂ sym), 1330.65 (C–N), 1011.81 (C–N), and 782.72 (Ar–H oop) 745.37 (Ar–H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 7.31–7.35 (m, 1H), 7.37–7.42 (m, 2H), 7.55 (d, *J* = 7.3 Hz, 1H), 7.82–7.85 (m, 2H), 8.06 (s, 1H), 8.14–8.16 (d, 2H), 8.35 (s, 1H), 10.64 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 162.86 (C=O), 161.35 (C=O), 154.76 (C=N), 149.57 (C–Ar), 149.00 (C–Ar), 142.50, 137.63, 134.10, 130.40, 127.45, 126.68, 125.71, 120.26, 117.75, 117.70 (CH–Ar), HRMS (ESI) *m/z*: 338.0721 [*M* + *H*]⁺

(E)-N'-(4-bromobenzylidene)-2-oxo-2H-chromene-3-carbohydrazide (AM10).

Yield: 76%, brownish solid crystals, M.P.: 223–227°C, Rf: 0.85 (*n*-Hexane: ethyl acetate (6:4), FT-IR (ν , cm^{-1}): 3044.23 (CH–Ar), 2941.76 (CH-aliphatic), 2639.81 (CH-aliphatic), 1686.44 (C=O), 1619.05 (C=N), 1572.15 (C=C aromatic), 1482.85 (aromatic stretch), 1446.73 (C=C), 1398.87 (C–N), 1175.11 (C–N), 748.68 (Ar–H oop) and 728.90 (Ar–H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 7.30 (d, *J* = 8.5 Hz, 1H), 7.34–7.40 (m, 4H), 7.49–7.51 (m, 2H), 7.55–7.58 (m, 1H), 8.07 (s, 1H), 8.36 (s, 1H), 10.65 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 163.26 (C=O), 161.75 (C=O), 155.16 (C=N), 149.41 (C–Ar), 142.90 (C–Ar), 134.50, 133.13, 132.61, 130.80, 130.28, 127.08, 125.94, 120.66, 118.15, 118.10 (CH–Ar), HRMS (ESI) *m/z*: 371.0124 [*M* + *H*]⁺

(E)-N'-(3-bromobenzylidene)-2-oxo-2H-chromene-3-carbohydrazide (AM11).

Yield: 72%, sand color solid crystals, M.P.: 224–227°C, Rf: 0.78 (*n*-Hexane: ethyl acetate (7:3), FT-IR (ν , cm^{-1}): 3056.17 (CH–Ar), 2938.43 (CH-aliphatic), 2642.75 (CH-aliphatic), 1692.17 (C=O), 1617.86 (C=N), 1584.27 (C=C aromatic), 1477.32 (aromatic stretch), 1453.67 (C=C), 1327.45 (C–N), 1031.63 (C–N), 725.71 (Ar–H oop) and 757.13 (Ar–H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 7.30–7.34 (m, 2H), 7.35–7.39 (m, 2H), 7.51–7.57 (m, 3H), 7.89 (t, *J* = 2.0 Hz, 1H), 8.01 (s, 1H), 8.35 (s, 1H), 10.69 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 162.99 (C=O), 161.47 (C=O), 154.88 (C=N), 145.78 (C–Ar), 142.63 (C–Ar), 136.51, 134.22, 132.81, 131.71, 131.19, 130.52, 126.81, 126.37, 124.22, 120.38, 117.88, 117.82 (CH–Ar), HRMS (ESI) *m/z*: 371.0014 [*M* + *H*]⁺

(E)-N'-(4-methoxybenzylidene)-2-oxo-2H-chromene-3-carbohydrazide (AM12).

Yield: 75%, light cream solid crystals, M.P.: 176–179°C, R_f: 0.74 (*n*-Hexane: ethyl acetate (7:3), FT-IR (ν, cm⁻¹): 3043.91 (CH–Ar), 2752.90 (CH-aliphatic), 2640.01 (CH-aliphatic), 1618.16 (C=O/C=N), 1571.91 (C=C aromatic), 1486.00 (aromatic stretch), 1446.94 (C=C), 1331.11 (C–N), 1031.60 (C–N), 783.44 (Ar–H oop) and 748.59 (Ar–H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 3.68 (s, 3H), 6.94–6.97 (m, 2H), 7.21–7.25 (m, 1H), 7.34–7.38 (m, 2H), 7.44–7.46 (m, 2H), 7.53–7.56 (t, J = 7.2 Hz, 1H), 8.04 (s, 1H), 8.34 (s, 1H), 10.63 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 163.17 (C=O), 162.99 (C=O), 161.65 (C=N), 155.06 (C–Ar), 149.35 (C–Ar), 142.81 (C–Ar), 134.41, 130.71, 130.32, 128.10, 126.99 (CH–Ar), 120.56, 118.06, 118.00, 115.93 (CH–Ar), 56.87 (OCH₃), HRMS (ESI) m/z: 323.1062 [M + H]⁺

2-oxo-N'-((1E,2E)-3-phenylallylidene)-2H-chromene-3-carbohydrazide (AM13).

Yield: 79%, soft beige solid crystals, M.P.: 154–156°C, R_f: 0.80 (*n*-Hexane: ethyl acetate (6:4), FT-IR (ν, cm⁻¹): 3043.92 (CH–Ar), 2639.80 (CH-aliphatic), 1618.82 (C=O/C=N), 1572.05 (C=C aromatic), 1486.04 (aromatic stretch), 1447.09 (C=C), 1386.37 (C–N), 1031.97 (C–N), 893.46 (Ar–H oop) and 748.16 (Ar–H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 6.76–6.81 (m, 1H), 6.92–6.95 (m, 1H), 7.09–7.16 (m, 3H), 7.31 (d, J = 8.3 Hz, 1H), 7.34–7.38 (t, J = 8.5 Hz, 2H), 7.53–7.56 (t, J = 7.1 Hz, 1H), 7.64–7.67 (m, 3H), 8.34 (s, 1H), 11.39 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 162.72 (C=O), 161.35 (C=O), 154.77 (C=N), 147.34 (C–Ar), 142.51 (C–Ar), 137.45, 136.64, 134.11, 130.96, 130.41, 130.15, 129.53, 126.69, 125.79, 120.26, 117.76, 117.58 (CH–Ar), HRMS (ESI) m/z: 319.1024 [M + H]⁺

(E)-N'-(4-(dimethylamino)benzylidene)-2-oxo-2H-chromene-3-carbohydrazide (AM14).

Yield: 77%, pale peach solid crystals, M.P.: 205–206°C, R_f: 0.60 (*n*-Hexane: ethyl acetate (8:2), FT-IR (ν, cm⁻¹): 2908.44 (CH–Ar), 2638.16 (CH-aliphatic), 1613.61 (C=O/C=N), 1582.43 (C=C aromatic), 1524.43 (aromatic stretch), 1446.47 (C=C), 1364.35 (C–N), 984.12 (C–N), 785.41 (Ar–H oop) and 751.69 (Ar–H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 2.78 (s, 6H), 6.57–6.59 (m, 2H), 7.21–7.25 (m, 1H), 7.32–7.36 (t, J = 8.0 Hz, 2H), 7.41–7.43 (m, 2H), 7.52 (d, J = 7.6 Hz, 1H), 8.02 (s, 1H), 8.32 (s, 1H), 10.61 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 163.08 (C=O), 161.57 (C=O), 154.98 (C=N), 153.52 (C–Ar), 149.33 (C–Ar), 142.72 (C–Ar), 134.32, 130.62,

128.64, 126.90, 125.88, 120.47, 117.97, 117.91, 113.37 (CH–Ar), 41.74 (N(CH₃)₂), HRMS (ESI) m/z: 336.1314 [M + H]⁺

3-(5-(benzylideneamino)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (AM15).

Yield: 78%, almond-colored solid crystals, M.P.: 208–212°C, R_f: 0.85 (*n*-Hexane: ethyl acetate 7:3), FT-IR (ν, cm⁻¹): 3043.93 (CH–Ar), 2638.91 (CH-aliphatic), 1618.29 (C=O/C=N), 1572.14 (C=C aromatic), 1485.77 (aromatic stretch), 1446.67 (C=C), 1385.61 (C–N), 1031.90 (C–N), 893.25 (Ar–H oop) and 747.72 (Ar–H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 7.29–7.34 (m, 6H), 7.61–7.63 (m, 1H), 7.73–7.76 (m, 2H), 8.16 (s, 1H), 8.40 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 168.07 (C=O), 166.87 (C–O), 165.87 (C–N), 162.09 (C–Ar), 161.21 (C–Ar), 155.13 (C=N), 136.01, 133.87, 132.07, 131.56, 130.94, 130.10, 126.60, 120.33, 117.61, 110.13 (CH–Ar), HRMS (ESI) m/z: 318.0857 [M + H]⁺

3-(5-((4-chlorobenzylidene)amino)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (AM16).

Yield: 80%, beige-white solid crystals, M.P.: 194–197°C, R_f: 0.86 (*n*-Hexane: ethyl acetate (7:3)), FT-IR (ν, cm⁻¹): 3049.83 (CH–Ar), 2846.51 (CH-aliphatic), 1619.34 (C=O/C=N), 1582.13 (C=C aromatic), 1493.52 (aromatic stretch), 1465.72 (C=C), 1372.24 (C–N), 782.89 (Ar–H oop), and 743.92 (Ar–H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 7.32–7.36 (m, 3H), 7.51–7.53 (m, 2H), 7.59–7.61 (m, 1H), 7.72–7.74 (m, 2H), 8.14 (s, 1H), 8.37 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 168.27 (C=O), 166.97 (C–O), 166.07 (C–N), 162.29 (C–Ar), 161.41 (C–Ar), 155.33 (C=N), 138.19 (C–Ar), 134.51, 134.07, 132.73, 131.14, 130.75, 126.80, 120.53, 117.81, 110.33 (CH–Ar), HRMS (ESI) m/z: 352.0401 [M + H]⁺

3-(5-((3-chlorobenzylidene)amino)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (AM17).

Yield: 81%, grey solid crystals, M.P.: 197–201°C, R_f: 0.82 (*n*-Hexane: ethyl acetate (6:4)), FT-IR (ν, cm⁻¹): 3053.21 (CH–Ar), 2916.78 (CH-aliphatic), 1621.75 (C=O/C=N), 1566.28 (C=C aromatic), 1489.42 (aromatic stretch), 1453.14 (C=C), 1369.52 (C–N), 1067.59 (C–N), 782.89 (Ar–H oop) and 728.76 (Ar–H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 7.30–7.34 (m, 4H), 7.36–7.38 (t, J = 7.4 Hz, 1H), 7.53–7.54 (t, J = 2.1 Hz, 1H), 7.58 (d, J = 6.1 Hz, 1H), 7.66–7.68

(m 1H), 8.13 (s, 1H), 8.33 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 167.35 (C=O), 167.31 (C–N), 165.13 (C–N), 161.35 (C–Ar), 160.48 (C–Ar), 154.39 (C=N), 135.68, 135.43, 133.14, 130.93, 130.53, 130.20, 129.79, 129.10, 125.86, 119.60, 116.87, 109.39 (CH–Ar), HRMS (ESI) m/z: 352.0411 [M + H]⁺

3-(5-((2-chlorobenzylidene)amino)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (AM18).

Yield: 84%, beige-white solid crystals, M.P.: 198–201°C, R_f: 0.60 (*n*-Hexane: ethyl acetate (7:3), FT-IR (ν, cm⁻¹): 3043.02 (CH–Ar), 2639.43 (CH-aliphatic), 1617.89 (C=O/C=N), 1571.21 (C=C aromatic), 1485.34 (aromatic stretch), 1446.56 (C=C), 1385.51 (C–N), 1031.63 (C–N), 747.86 (Ar–H oop) and 728.63 (Ar–H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 7.18–7.25 (m, 5H), 7.53 (d, J = 7.5 Hz, 1H), 7.59–7.61 (m, 1H), 7.75–7.77 (m, 1H), 8.15 (s, 1H), 8.48 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 168.25 (C=O), 166.27 (C–O), 166.02 (C–N), 162.24 (C–Ar), 161.37 (C–Ar), 155.28 (C=N), 137.96, 134.03, 133.91, 133.41, 132.12, 131.09, 130.86, 130.06, 126.75, 120.49, 117.76, 110.28 (CH–Ar), HRMS (ESI) m/z: 352.0321 [M + H]⁺

3-(5-((2-hydroxybenzylidene)amino)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (AM19).

Yield: 83%, cream-tan solid crystals, M.P.: 243–245°C, R_f: 0.50 (*n*-Hexane: Ethyl acetate (8:2), FT-IR (ν, cm⁻¹): 3043.83 (CH–Ar), 2752.74 (CH-aliphatic), 2639.76 (CH-aliphatic), 1691.08 (C=O), 1617.41 (C=N), 1571.67 (C=C aromatic), 1485.49 (aromatic stretch), 1446.60 (C=C), 1385.43 (C–N), 1031.78 (C–N), 893.06 (Ar–H oop) and 747.21 (Ar–H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 6.77–6.82 (m, 2H), 7.14–7.17 (m, 1H), 7.21–7.25 (m, 3H), 7.47 (d, J = 7.1 Hz, 1H), 7.59 (d, J = 6.9 Hz, 1H), 8.14 (s, 1H), 8.45 (s, 1H), 10.58 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 168.99 (C=O), 168.13 (C=O), 166.11 (C–O), 162.73 (C–N), 162.33 (C–Ar), 161.45 (C–Ar), 155.37 (C=N), 134.79, 134.32, 134.11, 131.18, 126.84, 120.79, 120.57, 119.20, 118.71, 117.85, 110.37 (CH–Ar), HRMS (ESI) m/z: 334.0716 [M + H]⁺

3-(5-((4-hydroxybenzylidene)amino)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (AM20).

Yield: 84%, light orange solid crystals, M.P.: 245–247°C, R_f: 0.55 (*n*-Hexane: ethyl acetate (8:2), FT-IR (ν, cm⁻¹): 3043.62 (CH–Ar), 2751.08 (CH-aliphatic), 2639.42 (CH-aliphatic), 1617.74 (C=O/C=N), 1571.62 (C=C aromatic), 1485.58 (aromatic stretch), 1446.69 (C=C), 1385.69 (C–

N), 975.00 (C–N), 747.77 (Ar–H oop) and 728.41 (Ar–H oop), ^1H NMR (400 MHz, CDCl_3 , ppm): δ 6.77–6.79 (m, 2H), 7.19 (d, J = 2.6 Hz, 1H), 7.22–7.25 (m, 3H), 7.50–7.52 (m, 2H), 7.59 (d, J = 6.3 Hz, 1H), 8.14 (s, 1H), 8.39 (s, 1H), ^{13}C NMR (100 MHz, CDCl_3 , ppm): δ 168.24 (C=O), 167.13 (C–O), 166.04 (C–N), 162.26 (C–Ar), 161.39 (C–Ar), 156.64 (C–Ar), 155.30 (C=N), 134.05, 133.93, 131.11, 129.28, 126.77, 120.51, 117.78, 117.64, 110.30 (CH–Ar), HRMS (ESI) m/z : 334.0725 $[\text{M} + \text{H}]^+$

3-(5-((2-nitrobenzylidene)amino)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (AM21).

Yield: 85%, light amber solid crystals, M.P.: 290–292°C, R_f: 0.52 (*n*-Hexane: Ethyl acetate (8:2), FT-IR (ν , cm^{-1}): 3049.13 (CH–Ar), 2763.24 (CH-aliphatic), 2642.57 (CH-aliphatic), 1615.23 (C=O), 1574.67 (C=N), 1482.11 (aromatic stretch), 1382.19 (NO_2 sym), 1337.25 (C–N), 1042.65 (C–N), 893.08 (Ar–H oop) and 745.13 (Ar–H oop), ^1H NMR (400 MHz, CDCl_3 , ppm): δ 7.20–7.25 (m, 3H), 7.48–7.52 (m, 1H), 7.59 (d, J = 8.0 Hz, 2H), 7.79 (d, J = 6.5 Hz, 1H), 7.89 (d, J = 8.2 Hz, 1H), 8.13 (s, 1H), 8.92 (s, 1H), ^{13}C NMR (100 MHz, CDCl_3 , ppm): δ 168.39 (C=O), 166.19 (C–O), 166.12 (C–N), 162.41 (C–Ar), 161.48 (C–Ar), 155.45 (C=N), 150.83 (C–Ar), 136.24, 134.11, 131.05, 130.90, 129.81, 126.71, 120.71, 117.89, 110.46 (CH–Ar), HRMS (ESI) m/z : 363.0746 $[\text{M} + \text{H}]^+$

3-(5-((3-nitrobenzylidene)amino)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (AM22).

Yield: 81%, dark brown solid crystals, M.P.: 292–296°C, R_f: 0.60 (*n*-Hexane: ethyl acetate (7:3), FT-IR (ν , cm^{-1}): 3051.72 (CH–Ar), 2754.35 (CH-aliphatic), 2639.15 (CH-aliphatic), 1619.34 (C=O), 1570.75 (C=N), 1486.12 (aromatic stretch), 1385.46 (NO_2 sym), 1321.44 (C–N), 1045.51 (C–N), 895.77 (Ar–H oop) and 747.90 (Ar–H oop), ^1H NMR (400 MHz, CDCl_3 , ppm): δ 7.20–7.25 (m, 3H), 7.51 (d, J = 8.4 Hz, 1H), 7.56–7.58 (m, 1H), 7.91–7.94 (m, 1H), 8.09–8.12 (m, 2H), 8.39–8.40 (t, J = 2.1 Hz, 1H), 8.53 (s, 1H), ^{13}C NMR (100 MHz, CDCl_3 , ppm): δ 168.21 (C=O), 166.92 (C–O), 165.93 (C–N), 162.15 (C–Ar), 161.23 (C–Ar), 155.14 (C=N), 148.90 (C–Ar), 143.23, 136.10, 134.07, 131.04, 129.97, 126.63, 120.28, 117.66, 110.17 (CH–Ar), HRMS (ESI) m/z : 363.0723 $[\text{M} + \text{H}]^+$

3-(5-((4-nitrobenzylidene)amino)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (AM23).

Yield: 82%, dusky green solid crystals, M.P.: 294–297°C, R_f: 0.67 (*n*-Hexane: ethyl acetate (7:3), FT-IR (ν , cm^{-1}): 3043.72 (CH–Ar), 2751.92 (CH-aliphatic), 2639.85 (CH-aliphatic), 1617.74

(C=N), 1571.55 (NO₂ asym), 1485.66 (aromatic stretch), 1446.74 (C=C), 1385.72 (C–N), 1031.83 (C–N), 893.08 (Ar–H oop) and 747.66 (Ar–H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 7.17-7.20 (m, 2H), 7.21-7.24 (m, 1H), 7.56 (d, J = 6.3 Hz, 1H), 7.89-7.92 (m, 2H), 8.07-8.11 (m, 3H), 8.35 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 168.35 (C=O), 166.14 (C–O), 166.06 (C–N), 162.39 (C–Ar), 161.47 (C–Ar), 155.45 (C=N), 150.83 (C–Ar), 142.52, 136.14, 134.04, 131.06, 127.99, 126.78, 120.69, 117.93, 110.45 (CH–Ar), HRMS (ESI) m/z: 363.0745 [M + H]⁺

3-(5-((4-bromobenzylidene)amino)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (AM24).

Yield: 83%, pale orange solid crystals, M.P.: 266–268°C, R_f: 0.80 (*n*-Hexane: ethyl acetate (7:3), FT-IR (ν, cm⁻¹): 2638.47 (CH-aliphatic), 1618.46 (C=N), 1571.43 (C=C aromatic), 1485.67 (aromatic stretch), 1446.64 (C=C), 1385.77 (C–N), 749.25 (Ar–H oop), and 748.16 (Ar–H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 7.24-7.31 (m, 3H), 7.41-7.44 (m, 2H), 7.61-7.66 (m, 3H), 8.16 (s, 1H), 8.39 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 168.10 (C=O), 166.89 (C–O), 165.90 (C–N), 162.12 (C–Ar), 161.24 (C–Ar), 155.16 (C=N), 134.78, 133.93, 133.02, 131.06, 130.14, 129.23, 126.64, 120.36, 117.64, 110.16 (CH–Ar), HRMS (ESI) m/z: 396.0917 [M + H]⁺

3-(5-((3-bromobenzylidene)amino)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (AM25).

Yield: 84%, light amber solid crystals, M.P.: 268–272°C, R_f: 0.78 (*n*-Hexane: ethyl acetate (6:4), FT-IR (ν, cm⁻¹): 3043.64 (CH–Ar), 2752.15 (CH-aliphatic), 2639.60 (CH-aliphatic), 1617.98 (C=N), 1571.81 (C=C aromatic), 1485.83 (aromatic stretch), 1446.70 (C=C), 1385.79 (C–N), 1031.86 (C–N), 783.34 (Ar–H oop) and 747.95 (Ar–H oop), ¹H NMR (400 MHz, CDCl₃, ppm): δ 7.17-7.24 (m, 4H), 7.47-7.49 (m, 1H), 7.56-7.58 (m, 1H), 7.71 (d, J = 7.1 Hz, 2H), 8.12 (s, 1H), 8.35 (s, 1H), ¹³C NMR (100 MHz, CDCl₃, ppm): δ 168.42 (C=O), 166.24 (C–O), 166.08 (C–N), 162.45 (C–Ar), 161.52 (C–Ar), 155.47 (C=N), 150.82 (C–Ar), 143.01, 136.18, 134.08, 131.08, 130.02, 126.71, 120.77, 117.96, 110.47 (CH–Ar), HRMS (ESI) m/z: 396.0528 [M + H]⁺

3-(5-((4-methoxybenzylidene)amino)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (AM26).

Yield: 85%, grey solid crystals, M.P.: 182–185°C, R_f: 0.72 (*n*-Hexane: ethyl acetate (7:3), FT-IR (ν, cm⁻¹): 2943.46 (CH-aliphatic), 1680.74 (C=O), 1619.37 (C=N), 1571.03 (C=C aromatic), 1485.67 (aromatic stretch), 1447.14 (C=C), 1386.63 (C–N), 824.15 (Ar–H oop) and 749.59 (Ar–

H oop), ^1H NMR (400 MHz, CDCl_3 , ppm): δ 3.68 (s, 3H), 6.84-6.86 (m, 2H), 7.18-7.24 (m, 3H), 7.60-7.62 (m, 1H), 7.68-7.70 (m, 2H), 8.16 (s, 1H), 8.40 (s, 1H), ^{13}C NMR (100 MHz, CDCl_3 , ppm): δ 168.13 (C=O), 166.86 (C–O), 165.93 (C–N), 162.67 (C–Ar), 162.14 (C–Ar), 161.27 (C–Ar), 155.18 (C=N), 133.93, 133.33, 131.00, 128.45, 126.65, 120.39, 117.67, 115.52, 110.19 (CH–Ar), 56.53 (OCH_3), HRMS (ESI) m/z : 348.0910 $[\text{M} + \text{H}]^+$

3-(5-(((E)-3-phenylallylidene)amino)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (AM27).

Yield: 80%, cream-buff solid crystals, M.P.: 173–175°C, R_f: 0.82 (*n*-Hexane: ethyl acetate (6:4), FT-IR (ν , cm^{-1}): 3049.74 (CH–Ar), 2639.80 (CH-aliphatic), 1689.74 (C=O), 1615.63 (C=N), 1578.19 (C=C aromatic), 1482.45 (aromatic stretch), 1451.33 (C=C), 1337.21 (C–N), 727.18 (Ar–H oop) and 743.15 (Ar–H oop), ^1H NMR (400 MHz, CDCl_3 , ppm): δ 6.79-6.87 (m, 2H), 6.96-7.04 (m, 3H), 7.10-7.18 (m, 3H), 7.48 (d, J = 6.4 Hz, 1H), 7.50-7.53 (m, 2H), 8.02 (s, 1H), 8.18 (d, J = 8.1 Hz, 1H), ^{13}C NMR (100 MHz, CDCl_3 , ppm): δ 170.19 (C=O), 166.18 (C–O), 163.10 (C–N), 162.40 (C–Ar), 161.48 (C–Ar), 155.44 (C=N), 145.96 (C–Ar), 137.91, 134.19, 131.25, 131.17, 130.36, 129.74, 128.21, 126.91, 120.65, 117.93, 110.44 (CH–Ar), HRMS (ESI) m/z : 344.1024 $[\text{M} + \text{H}]^+$

3-(5-(((4-(dimethylamino)benzylidene)amino)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (AM28).

Yield: 79%, faint tan-cream solid crystals, M.P.: 185–189°C, R_f: 0.58 (*n*-Hexane: ethyl acetate (8:2), FT-IR (ν , cm^{-1}): 3043.93 (CH–Ar), 2752.33 (CH-aliphatic), 2640.10 (CH-aliphatic), 1680.74 (C=O), 1618.73 (C=N), 1572.22 (C=C aromatic), 1446.98 (C=C), 1386.01 (C–N), 1032.01 (C–N), 853.37 (Ar–H oop) and 747.51 (Ar–H oop), ^1H NMR (400 MHz, CDCl_3 , ppm): δ 2.80 (s, 6H), 6.57-6.59 (m, 2H), 7.19-7.24 (m, 3H), 7.47-7.50 (m, 2H), 7.59-7.61 (m, 1H), 8.15 (s, 1H), 8.38 (s, 1H), ^{13}C NMR (100 MHz, CDCl_3 , ppm): δ 168.22 (C=O), 167.15 (C–O), 166.01 (C–N), 162.23 (C–Ar), 161.36 (C–Ar), 155.27 (C=N), 153.34 (C–Ar), 134.02, 132.47, 131.08, 128.33, 126.74, 120.48, 117.76, 113.05, 110.27 (CH–Ar), 41.58 ($\text{N}(\text{CH}_3)_2$), HRMS (ESI) m/z : 361.1208 $[\text{M} + \text{H}]^+$

5.6 Structure activity relationship (SAR)

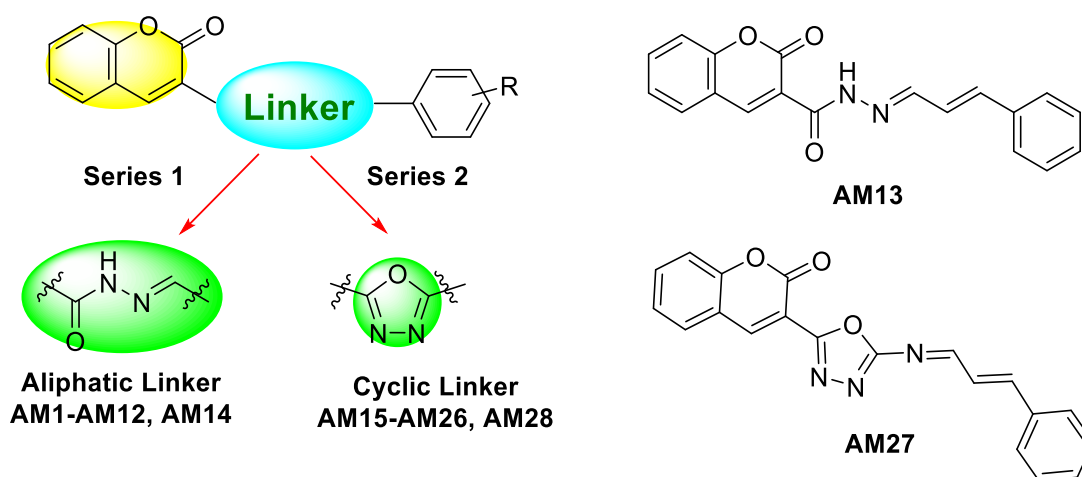


Figure 54. General formula for series 1 and series 2

Table 11. Comparison of binding affinity, EC₅₀ and percent GK activation of compounds

Sr. No	Compound	R	Binding affinity (Kcal/mol)	EC ₅₀ (μM) (Mean ± SEM)	Enzyme activity (%)
1.	AM1	H	-9.5	6.12 ± 0.14	136.23
2.	AM2	4-Cl	-8.3	13.92 ± 0.20	124.43
3.	AM3	3-Cl	-8.9	11.36 ± 0.18	129.97
4.	AM4	2-Cl	-9.8	4.64 ± 0.12	132.68
5.	AM5	2-OH	-9.5	5.48 ± 0.15	139.28
6.	AM6	4-OH	-8.3	13.60 ± 0.21	132.60
7.	AM7	2-NO ₂	-9.6	5.12 ± 0.15	134.20
8.	AM8	3-NO ₂	-8.5	12.84 ± 0.20	125.93
9.	AM9	4-NO ₂	-9.7	5.93 ± 0.13	134.33
10.	AM10	4-Br	-10.0	4.08 ± 0.11	137.46
11.	AM11	3-Br	-8.9	10.92 ± 0.17	128.20
12.	AM12	4-OCH ₃	-8.6	12.41 ± 0.19	120.82
13.	AM13	-	-9.1	9.84 ± 0.16	131.34
14.	AM14	4-N(CH ₃) ₂	-9.6	5.09 ± 0.13	136.30
15.	AM15	H	-10.5	6.72 ± 0.11	135.28
16.	AM16	4-Cl	-9.9	4.56 ± 0.12	144.93
17.	AM17	3-Cl	-10.4	6.02 ± 0.11	140.38
18.	AM18	2-Cl	-11.0	3.51 ± 0.10	156.18
19.	AM19	2-OH	-10.6	5.84 ± 0.11	151.60
20.	AM20	4-OH	-10.6	5.89 ± 0.12	154.79
21.	AM21	2-NO ₂	-10.7	3.63 ± 0.10	148.72
22.	AM22	3-NO ₂	-10.6	3.77 ± 0.11	151.74
23.	AM23	4-NO ₂	-9.9	4.82 ± 0.12	139.26
24.	AM24	4-Br	-9.9	4.77 ± 0.12	145.30
25.	AM25	3-Br	-10.6	5.66 ± 0.10	147.56

26.	AM26	4-OCH ₃	-9.6	5.22 ± 0.13	143.89
27.	AM27	-	-10.2	6.32 ± 0.12	144.32
28.	AM28	4-N(CH ₃) ₂	-10.0	7.14 ± 0.14	142.61
29.	Dorzagliatin	-	-7.2	19.82 ± 0.25	120.11

Structure-activity relationship (SAR) analysis of the coumarin-based glucokinase activator library (AM1-AM28) demonstrated an exceptionally consistent yet chemically complex pattern of correlations between minor alterations in the electronic nature, steric distribution, linker structure, and substituent orientation in determining the binding affinities, EC₅₀ (potency), and % GK activation. The SAR shows two pronounced Series, Series I (aliphatic hydrazide linker) and Series II (oxadiazole linker), which have characteristic substituent-dependent effects but still obey various general principles governing GK activation. A detailed analysis below provides a comprehensive, position-specific, substituent-specific, and series-specific interpretation of the data and encompasses the exceptions in which the docking scores deviate with respect to the actual biological activity. This general SAR develops a unified chemical rationale for why certain compounds are more potent in terms of EC₅₀ or % enzyme activation (Table 11) and the behavior of electron-donating and electron-withdrawing groups across the linker scaffolds.

The hydrazide linker Series I, includes AM1-AM14; AM1, in which the coumarin aryl linkage is subjected to less torsional constraint, establishing a system in which the substituents act on the system predominantly by torsional control, modulation of electron density, and hydrophobic surface complementarity, and to a lesser extent by rigid geometrical constraints. The parent compound AM1 provides the baseline (-9.5 kcal/mol; 6.12 μM; 136.23%, respectively), which proves that even in the absence of substitution, the coumarin core and hydrazide linker form constructive interactions within the allosteric site. Nevertheless, the general trend in Series I is that the greatest benefit is provided by *ortho* substitution because of the capacity of the *ortho* groups to create a pseudo-locked structure caused by the formation of a mild steric repulsion that curtails the rotational freedom of the phenyl ring, thereby increasing the binding specificity and augmenting the electron dipole reactions. This was apparent for AM4 (2-Cl), AM5 (2-OH), and AM7 (2-NO₂). The moderately sized chloro atom of AM4 increases the lipophilicity and enhances the shape complementarity, which leads to a higher potency (-9.8 kcal/mol; 4.64 μM; 132.68%). The polar substituent, AM5 (2-OH), exhibited increased activity (-9.5 kcal/mol; 5.48 μM; and 139.28%) owing to the hydrogen-bonding nature of the OH polar substituent, which temporarily

interacted with the polar site of the binding pocket. This *ortho* advantage is again supported by AM7 (2-NO₂), which exhibits strong electron-withdrawing activity (-9.6 kcal/mol; 5.12 μM; 134.20%) owing to the strong electron-withdrawing activity of the nitro group, which enhances the π -acceptor character, stabilizes the coumarin-aryl conjugation, and promotes polar residue interactions. In contrast, AM6 (2-Me) is a far worse performer (-8.3 kcal/mol; 13.60 μM; 132.60%), indicating that not all *ortho* substituents are productive. Methyl is a small, hydrophobic, and electronically neutral molecule that cannot adequately dominate the phenyl torsion. It also failed to improve electronic distribution, resulting in a less positive orientation. Despite, the compounds AM2 and AM6 with moderate binding affinities of -8.3 kcal/mol showed comparatively lower biological activity with their respective EC₅₀ values of 13.92 μM and 13.60 μM. It is suggested that these reduced activities are a result of less optimal hydrogen bonding and hydrophobic interactions with amino acid residues in the active site. In addition, the nature and position of their substituents could have resulted in less than ideal steric or electronic effects, which could decrease the effective interactions between the ligand and the receptor. This shows a very important point about Series I: *ortho* advantage can only be realized when the substituent can cause a conformational or electronic advantage, and not only through occupation of the site. With *meta* substitution, the activity of compounds such as AM3 (3-Cl), AM11 (3-Br), and AM8 (3-NO₂) consistently displayed weaker activity. This position effect is rational because *meta* groups do not significantly affect the resonance or significantly limit the torsional motion. Consequently, they do not increase the binding affinity via geometrical effects, resulting in EC₅₀ values of 10-13 μM. This apparent trend lays down the fact that *meta* substitution gives the flexible series a little SAR value, irrespective of electronic merit. However, a rich and varied SAR landscape was provided by *para* substitutions. *Para* halogen (4-Cl) in AM2 and (4-Br) in AM10 disrupted the distribution of electrons around the phenyl ring, which could increase conjugation and allow extensive hydrophobic interactions in the binding tunnel. AM10 (4-Br) represents one of the most potent Series I molecules (-10.0 kcal/mol; 4.08 μM; 137.46%), owing to the size and polarizability of bromine, which promotes π -halogen interactions and hydrophobic anchoring. The potency of AM9 (4-NO₂) is also strong (-9.7 kcal/mol; 5.93 μM; 134.33%), which confirms that *para*-EWGs facilitate electron withdrawal, which stabilizes the aromatic system and enhances binding.

Nevertheless, the exception in Series I is AM2 (4-Cl): although its docking score was recorded as

good docking score of -8.3 kcal/mol, low EC₅₀ of 13.92 μ M and 124.43% enzyme activation. Such divergence proves that docking is significantly more aggressive in predicting the value of *para*-chloro substitution in the flexible system, probably due to the fact that the conformational entropy penalty in actual binding conditions is not well represented in docking simulations. *Para* electron-donating groups, such as AM12 (4-OMe) and AM14 (4-NMe₂), provide the most instructive insights into electronic contributions. AM14 (4-NMe₂) performed moderately well (-9.6 kcal/mol; 5.09 μ M; 136.30%) because its bulkier dimethylamino group created steric hindrance that partially stabilized the orientation despite being an electron-donating group. However, AM12 (4-OMe), although with a fairly good docking score, also shows poor activity (-8.6 kcal/mol; 12.41 μ M; 120.82%), which confirms that the methoxy group brings about polarity and the aromatic ring has also been ejected out of the optimal position. This confirms that *para*-EDGs destabilize productive binding in the flexible aliphatic series. With an extended conjugated system, AM13 slightly modulates hydrophobic and π - π interactions (-9.1 kcal/mol; 9.8 μ M, and 132.34%). Together, Series I illustrates an SAR, which is dominated by the interaction of position-dependent steric steering and electronic modulation, with major docking-activity exceptions involving AM6 and AM8.

Series II (AM15-AM28) exhibited better SAR behavior because the cyclic oxadiazole linker fits into the allosteric binding pocket with more favorable interactions. The effect of the cyclic linker, where the parent compound AM15 already demonstrates better docking and increased enzyme activation (-10.5 kcal/mol; 6.72 μ M; 135.28%) than AM1 itself, can be managed by scaffold rigidity. The most significant positive effect on the entire dataset is in this rigidified system, that is, *ortho* substitution. AM18 (2-Cl) is the strongest glucokinase activator among both series, with an excellent activity (-11.0 kcal/mol; 3.51 μ M; 156.18%). The combination of steric volume, high level of hydrophobicity, and electron modulation predetermines this good docking scores. The cyclic linker in the scaffold ensures that *ortho*-Cl can precisely fix the aryl ring into the hydrophobic cleft without torsional strain. In a similar manner, AM21 (2-NO₂) provides an excellent potency (-10.7 kcal/mol; 3.63 μ M; 148.72%), which proves the fact that *ortho*-EWGs facilitate the orientation of the nitro group involved in π -stacking and dipolar stabilization. AM19 with 2-OH was highly potent (-10.6 kcal/mol; 5.84 μ M; 151.60%), implying that the *ortho*-hydroxyl group can stabilize the ligand via hydrogen bonding and enhance the overall binding affinity. In comparison, AM20 (-10.6 kcal/mol; 5.89 μ M; 154.79%) is also very active, but the

para-hydroxyl group in the AM20 molecule is engaged by hydrogen bonding and consequently, it is slightly stronger than the *ortho* analog. These trends, when considered together, demonstrated that the hydroxyl substituents can optimize glucokinase activation using different positional effects, with *ortho* substitution supporting conformational preorganization and *para* substitution supporting direct polar interactions in the allosteric cavity.

The moderately positive effect of *meta* substitution in the rigid series is preferred to Series I, with compounds such as AM17 (3-Cl) and AM25 (3-Br) retaining spectacular activity (≈ 5 -6 μ M) owing to the reduced reliance on torsion control provided by the rigid linker and hence, the high-energy acceptability of the *meta* position and the side effects of the substituents. This is in contrast to Series I, where rigidification can rescue otherwise weak *meta* substitutions, allowing EWGs and halogens to effectively modulate the electron density. Again, *para* substituents showed high dependence on the electronic character of the group. *Para* halogen (AM16 with 4-Cl, AM24 with 4-Br) strongly acted with EC₅₀ values of less than 5 μ M and maintained high enzyme activation. *Para*-NO₂ (AM23) exhibits strong docking and slightly lower activation (-9.9 kcal/mol; 4.82 μ M; 139.26%). *Para*-EDG, AM26 (4-OMe), and AM28 (4-NMe₂) showed inferior performance compared to EWGs, which confirms that electron-donating groups destabilize the aromatic system even in rigid scaffolds; however, the effect is not as bad as in Series I because of the stabilizing effect of rigidity. However, their activity (5-7 μ M) is always lower than that of the halogenated or nitro analogs. The most intriguing SAR insight is that AM22 (3-NO₂) has obtained good potency even with combined steric bulk (-10.6 kcal/mol; 3.77 μ M; 151.74%). Another notable feature is AM27 (extended conjugation), which has a strong docking value (but moderate activity, (-10.2 kcal/mol; 6.32 μ M; 144.32%). AM27 proves that strong conjugation increases the aromatic flexibility.

The Combined SAR Interpretation (Series I vs. Series II) clearly revealed that scaffold rigidity plays an important role in the conversion of docking affinity into actual biological potency. *Ortho* substitution was found to be positive, but its effect was phenomenally increased in the oxadiazole series. *Meta* substitution showed lower activity in Series I but was more favorable in Series II and demonstrated the significance of linker-controlled conformational alignment. In both series, *para* EWGs performed better than *para* EDGs. The oxadiazole linker ensures higher potency and better docking activity correlations. AM18 was the best activator in both series.



CHAPTER 6

CONCLUSION

“Summarizing Discoveries
and Scientific
Contributions.”



Findings



Validation



Lead Molecules



Impact



Contributions

CONCLUSION

The current study involved rational drug design, synthesis, and biological testing of a glucokinase activator (GKA) library composed of coumarin hydrazide/oxadiazole-based heterocycles (AM1-AM28). This study incorporates medicinal chemistry, experimental pharmacology, and computational modelling to establish the essential molecular attributes that promote strong GK activation.

The design strategy was based on an understanding of the glucokinase targets, particularly the structural constraints and vital interactions with Arg63, Tyr61, Thr65, and Tyr214. Two series of compounds were designed, synthesized, and biologically evaluated: hydrazide-linked Series I (AM1-AM14) and oxadiazole-linked Series II (AM15-AM28). The SAR of the two series allowed a systematic assessment of linker variations, altering the chain length, electronic distribution, steric factors, H-bonding, lipophilicity, and substituent orientation affecting the affinity of glucokinase binding and activation of the enzyme. The structures of all synthesized compounds were confirmed using spectral techniques. The synthesized compounds displayed distinct SAR trends in the glucokinase activation assay, with several derivatives which were found to be more potent than the reference (Dorzagliatin).

One of them, AM18, was the lead compound, with the lowest EC₅₀ value, highest enzyme activation, and good correlation with docking energies. Docking studies revealed that oxadiazole derivatives, especially AM18, effectively occupied the allosteric cavity and established stable hydrogen-bond networks and hydrophobic contacts that are important for activation of the target protein. The results of AM20 showed excellent *in vitro* assays, reliable *in vivo* anti-hyperglycemic effects, and desirable docking interactions, further suggesting that it is one of the best results in Series II. In addition to the lead compounds AM18 and AM20, other analogs, such as AM16, AM19, AM21, AM22, AM24, AM25, AM26, and AM27, also exhibited significant glucokinase activation, collectively strengthening the overall SAR consistency across the synthesized library. DFT studies also supported the role of perfect electronic softness and charge distribution in allowing effective ligand-enzyme interactions.

DISCUSSION SUMMARY

This study on coumarin-based heterocyclic derivatives (AM1–AM28) designed as glucokinase (GK) activators for antidiabetic potential. Two series were synthesized: coumarin-hydrazide derivatives and coumarin-oxadiazole derivatives, both prepared via reflux condensation with substituted benzaldehydes. All 28 compounds were evaluated for *in vitro* GK activation, with most surpassing the reference drug Dorzagliatin. Compounds AM18 and AM20 exhibited the strongest activation (EC_{50} of 3.51 and 5.89 μ M, respectively) and the least cytotoxicity in the MTT assay on HepG2 cells. *In vivo* studies using STZ-induced diabetic rats confirmed that AM18 and AM20 produced a significant, time-dependent reduction in blood glucose levels, outperforming Dorzagliatin. Molecular docking revealed AM18 had the highest binding affinity (–11.0 kcal/mol). ADME analysis confirmed all compounds met Lipinski's drug-likeness criteria with high GI absorption. DFT calculations supported favorable electronic properties for GK activation. Structure-activity relationship (SAR) analysis identified that *ortho* substitution and the rigid oxadiazole linker greatly enhanced potency, with AM18 (2-Cl, oxadiazole series) emerging as the most promising lead candidate for further antidiabetic drug development.



CHAPTER 7

FUTURE OUTLOOK

“Advancing Discoveries for
Safe, Effective, and Clinically
Relevant Antidiabetic Agents.”



Pharmacokinetic
& Metabolite
Studies



In Vivo Studies
& GK Activation



Medicinal Chemistry
Optimization



In Silico Approaches
for Next-Generation
Analogues



Safe, Efficacious
& Clinically Relevant
Antidiabetic Agents



FUTURE OUTLOOK

This study provides several prospective approaches for developing GKA. In the future, efforts will be made to perform pharmacokinetic and metabolite studies to determine the absorption, distribution, biotransformation, and clearance profiles necessary for diabetes treatment. *In vivo* research using chronic dosing models will play a critical role in assessing the anti-hyperglycemic effects and GK activation. Medicinal chemistry optimization will also be performed to enhance the efficacy of the synthesized molecules, which will enable the refinement of their effectiveness and metabolic stability. Application of various *in silico* methods will accelerate the discovery of next-generation analogues. These results provide a roadmap for the transformation of these scaffolds into safe, efficacious, and clinically relevant antidiabetic agents.



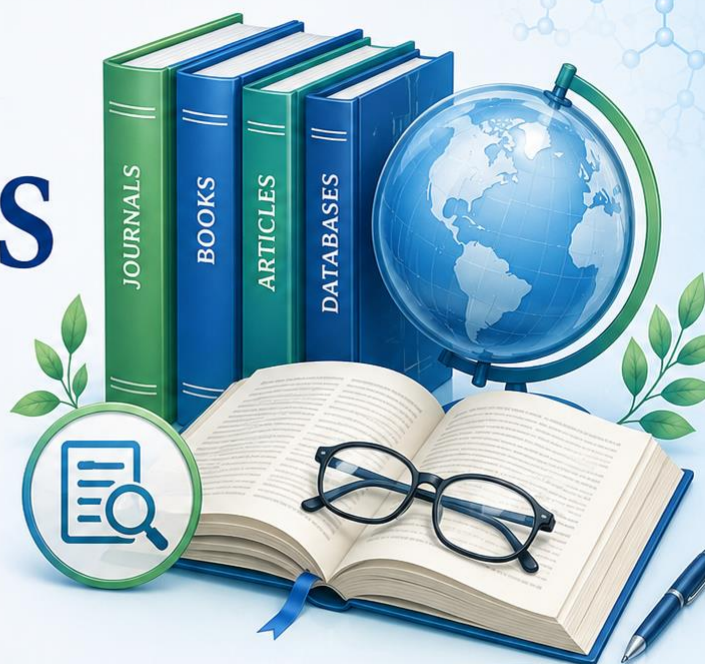
CHAPTER 8

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*Acknowledging the Foundations
that Support Discovery
and Innovation.*

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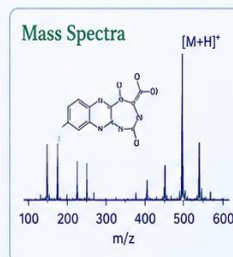
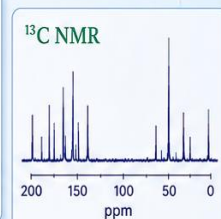
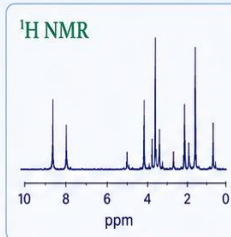
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CHAPTER 9

ANNEXURES

“Supporting Information
that Strengthens
Scientific Integrity.”



¹H & ¹³C NMR and Mass Spectra

Confirms the structural
elucidation and
purity of synthesized
compounds.



Publication Details

Highlights the research
output and contribution
to the scientific
community.



Animal Ethical Committee Approval

Ensures ethical conduct
in animal studies and
compliance with
regulatory guidelines.



Conference Attended Details

Demonstrates academic
engagement and
dissemination of
research findings.



ANNEXURES

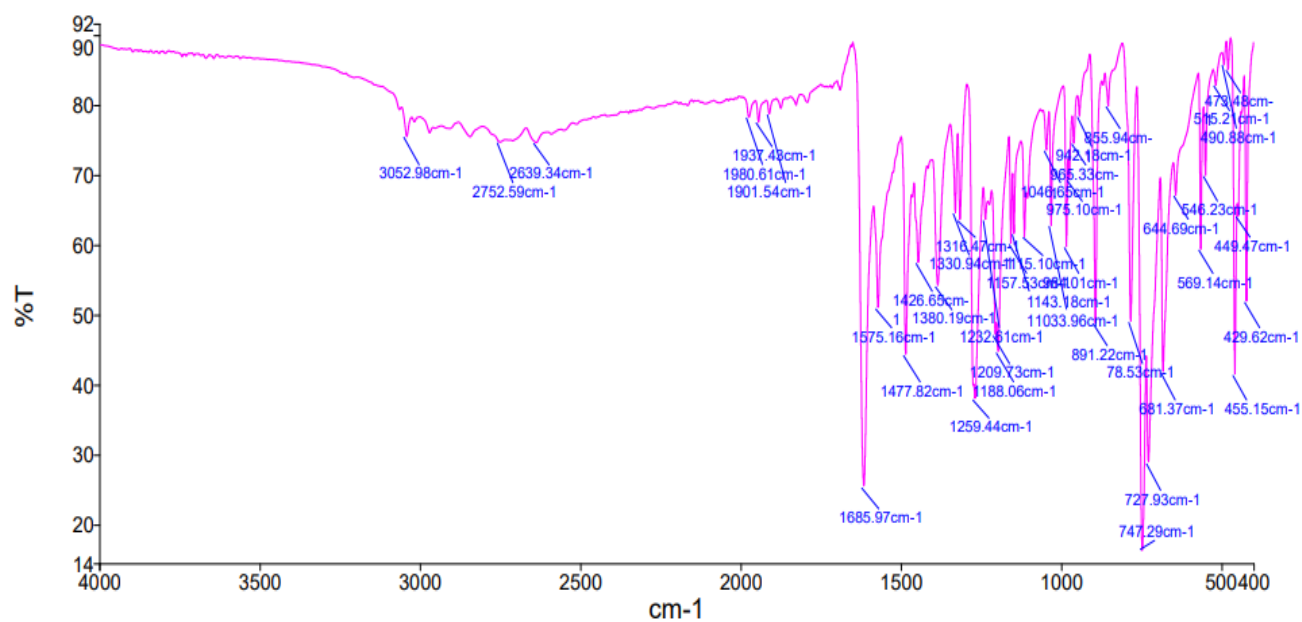


Figure S1. IR spectrum of compound 3

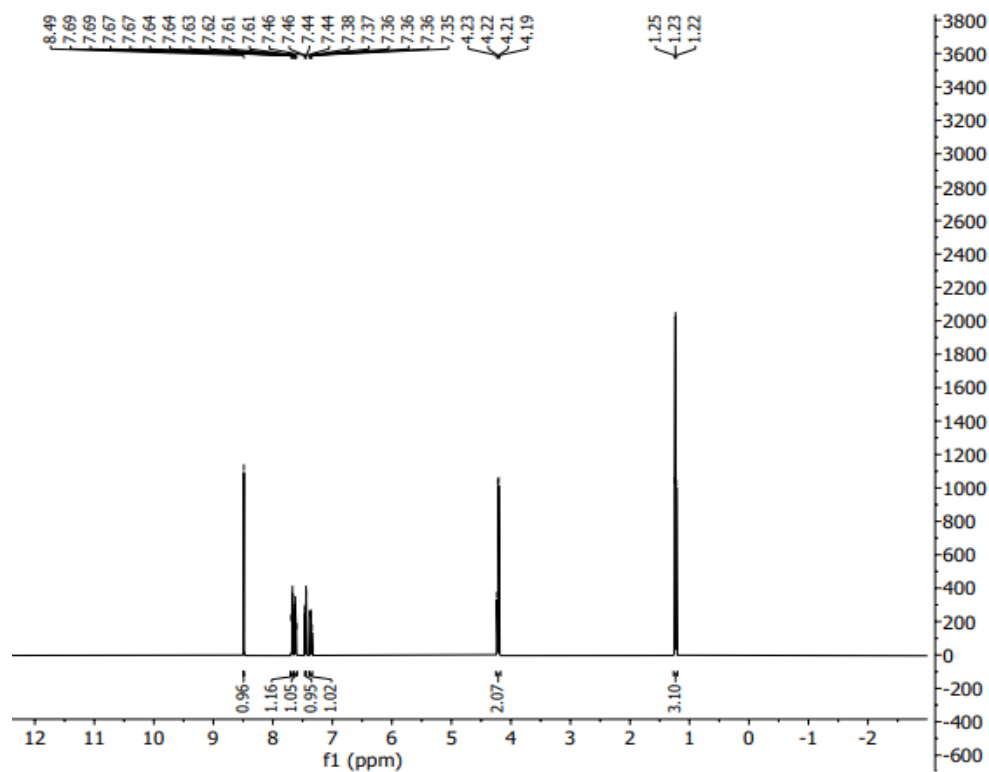


Figure S2. ¹H-NMR spectrum of compound 3

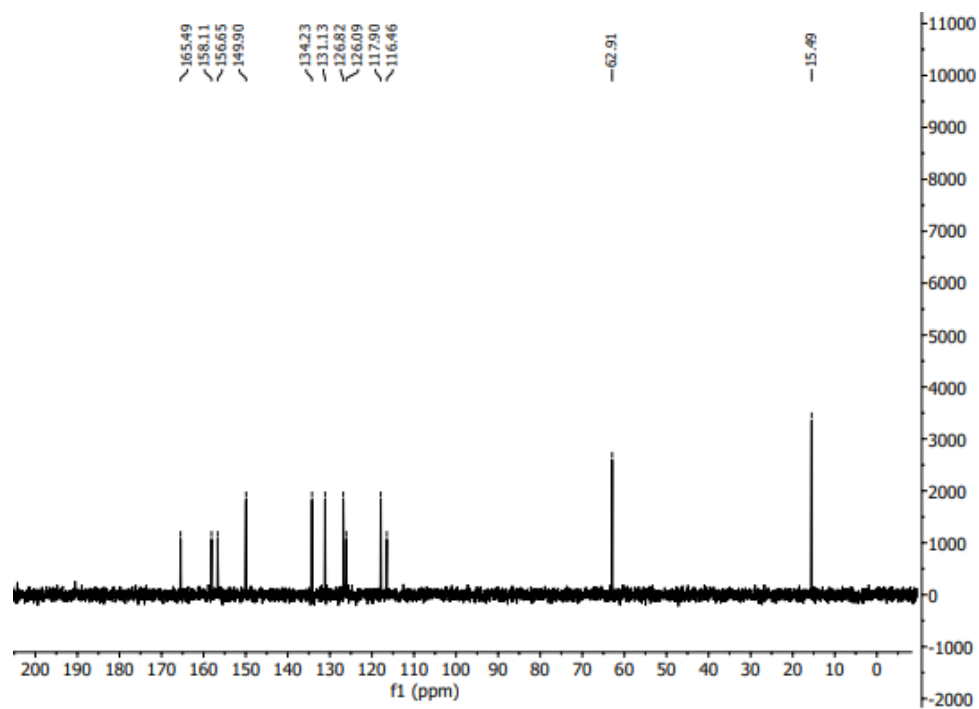


Figure S3. ¹³C-NMR spectrum of compound 3

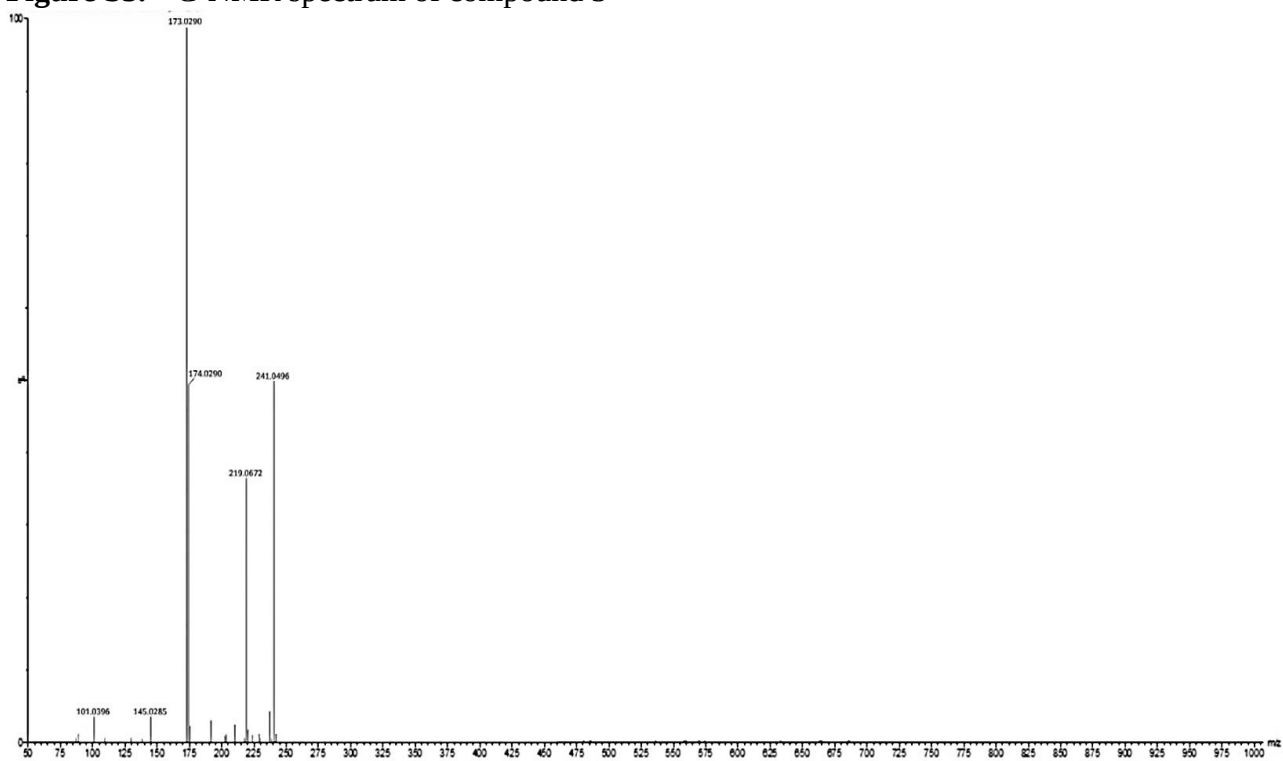


Figure S4. LCMS spectrum of compound 3

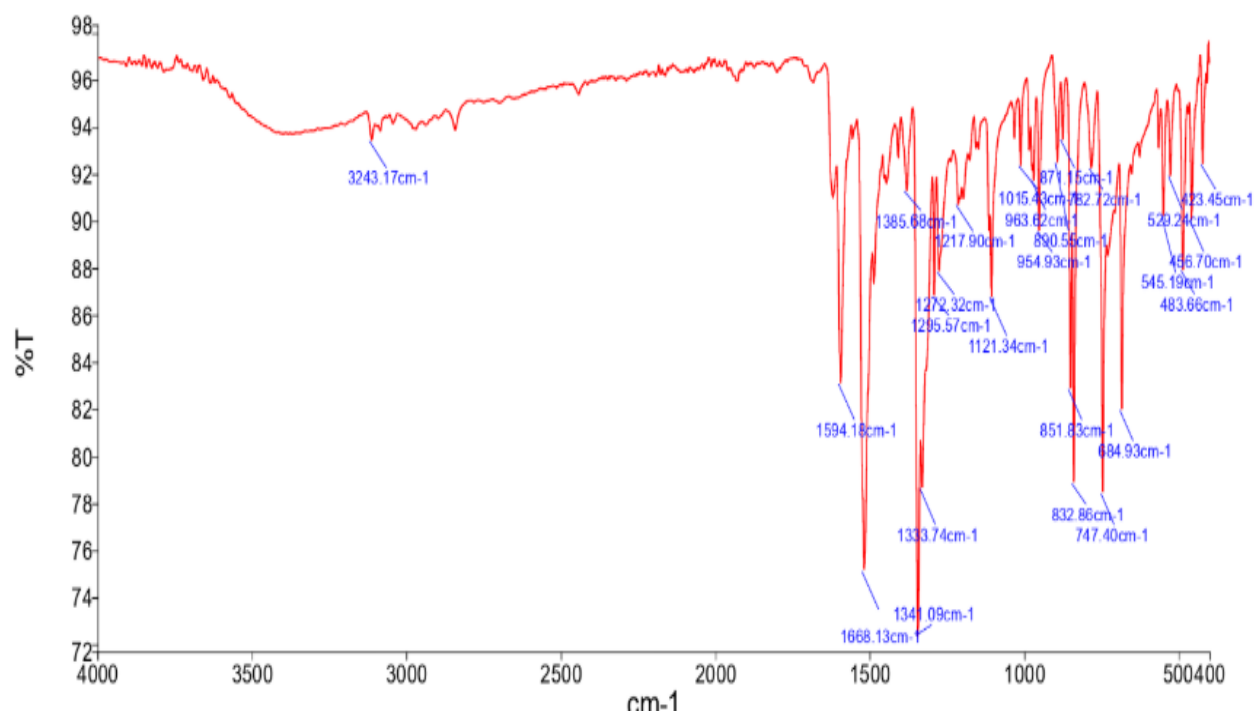


Figure S5. IR spectrum of compound 4

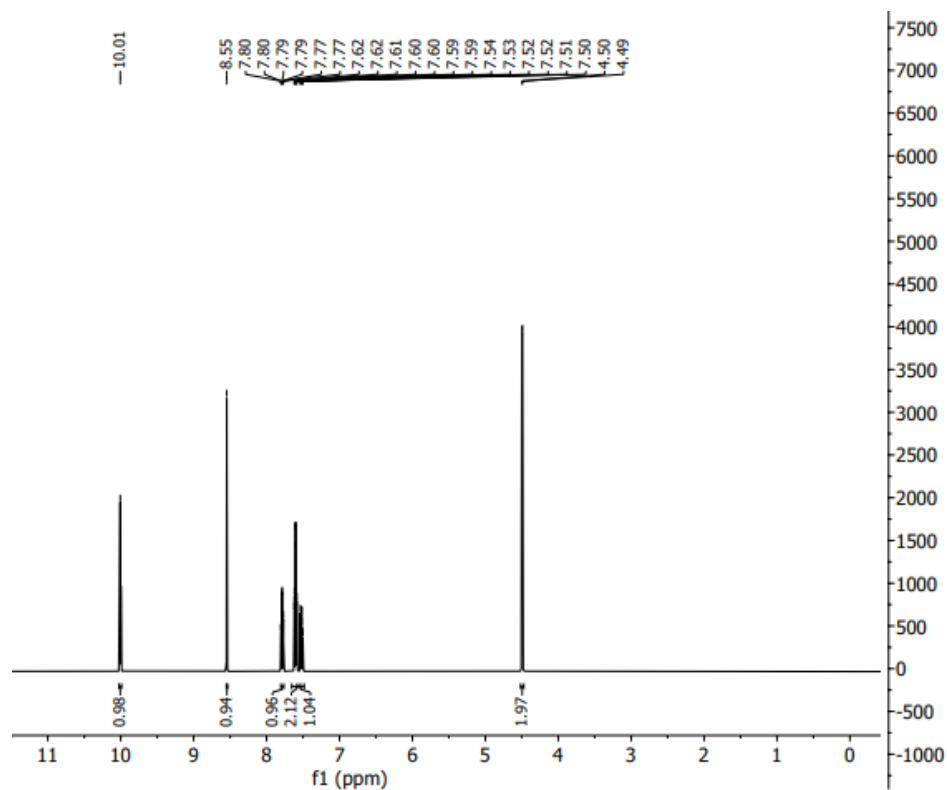


Figure S6. ^1H -NMR spectrum of compound 4

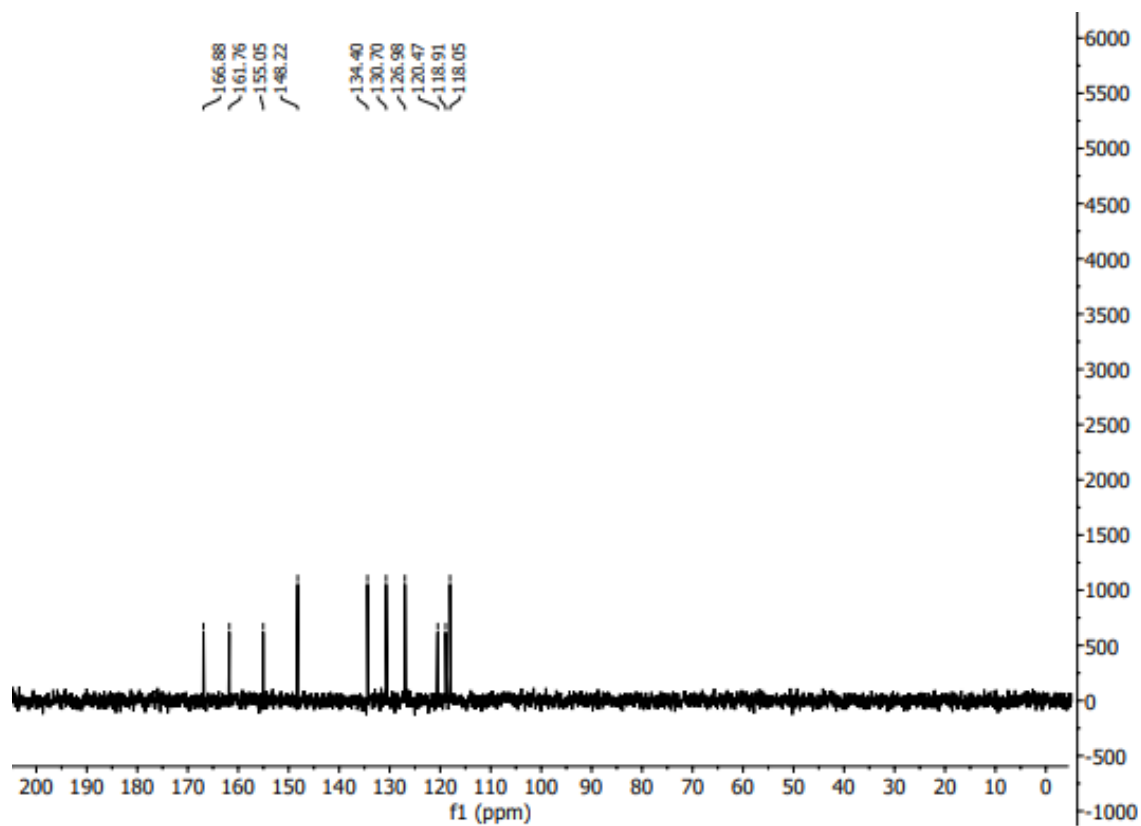


Figure S7. ¹³C-NMR spectrum of compound 4

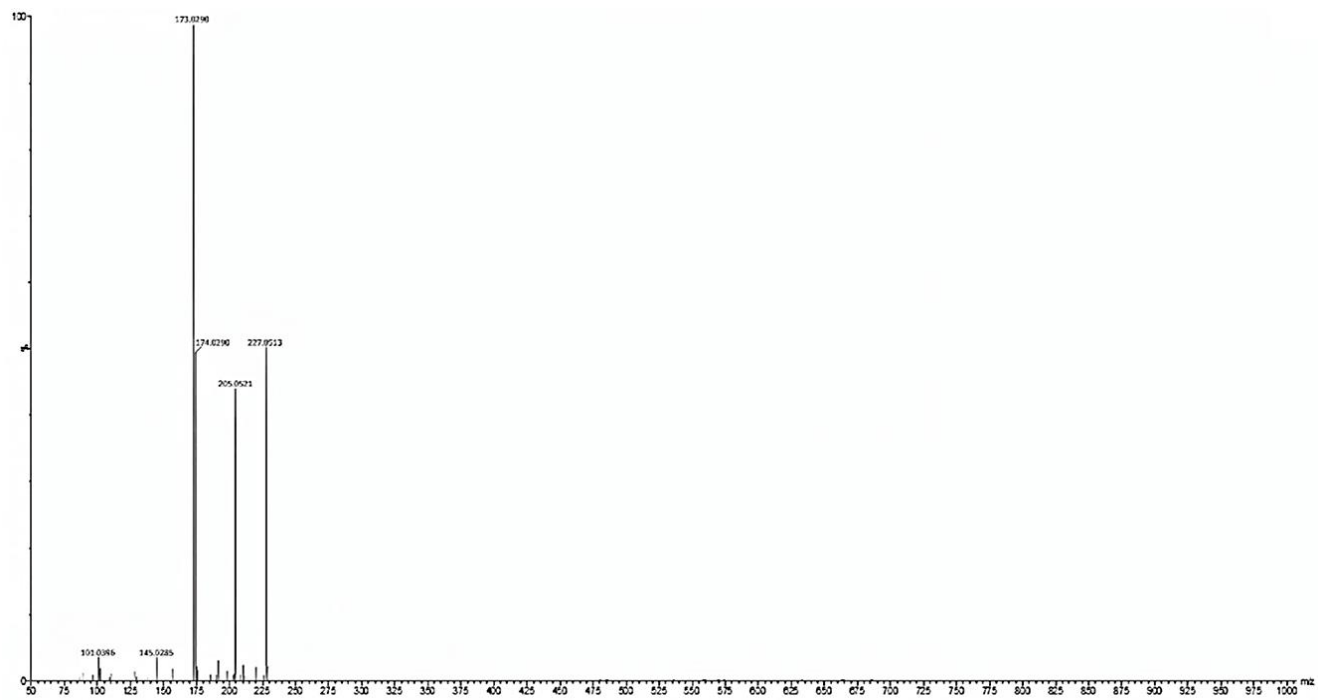


Figure S8. LCMS spectrum of compound 4

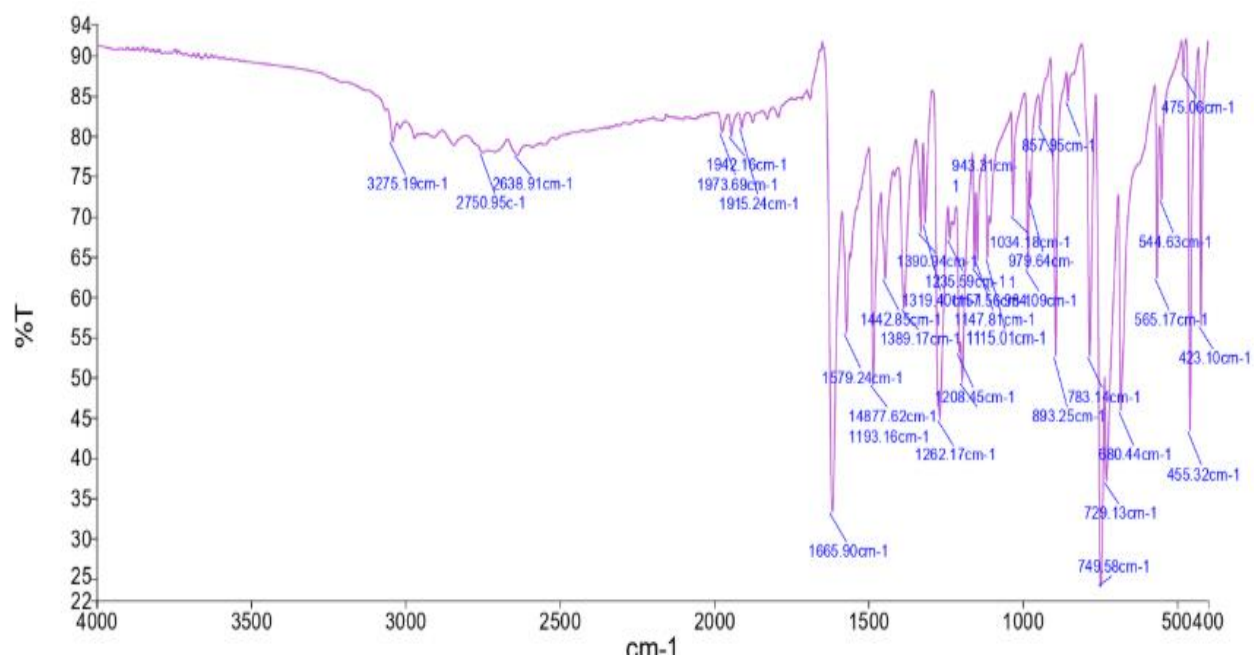


Figure S9. IR spectrum of compound 5

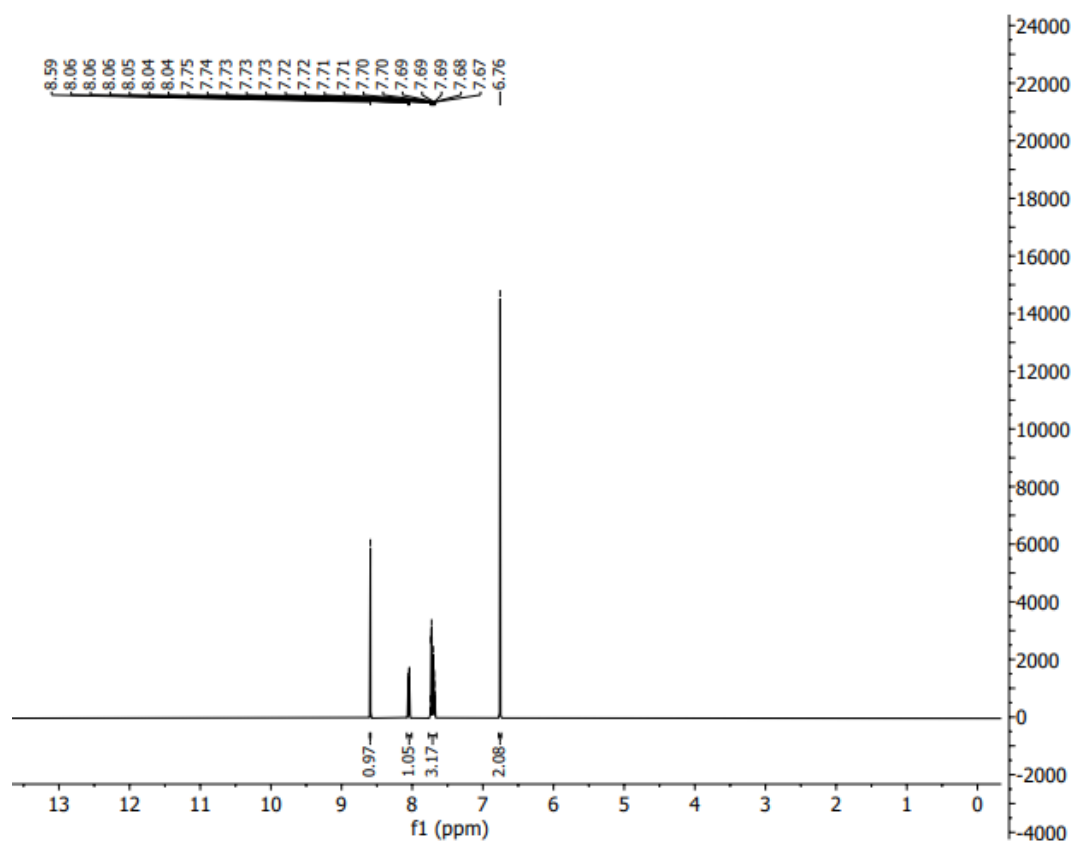


Figure S10. ¹H-NMR spectrum of compound 5

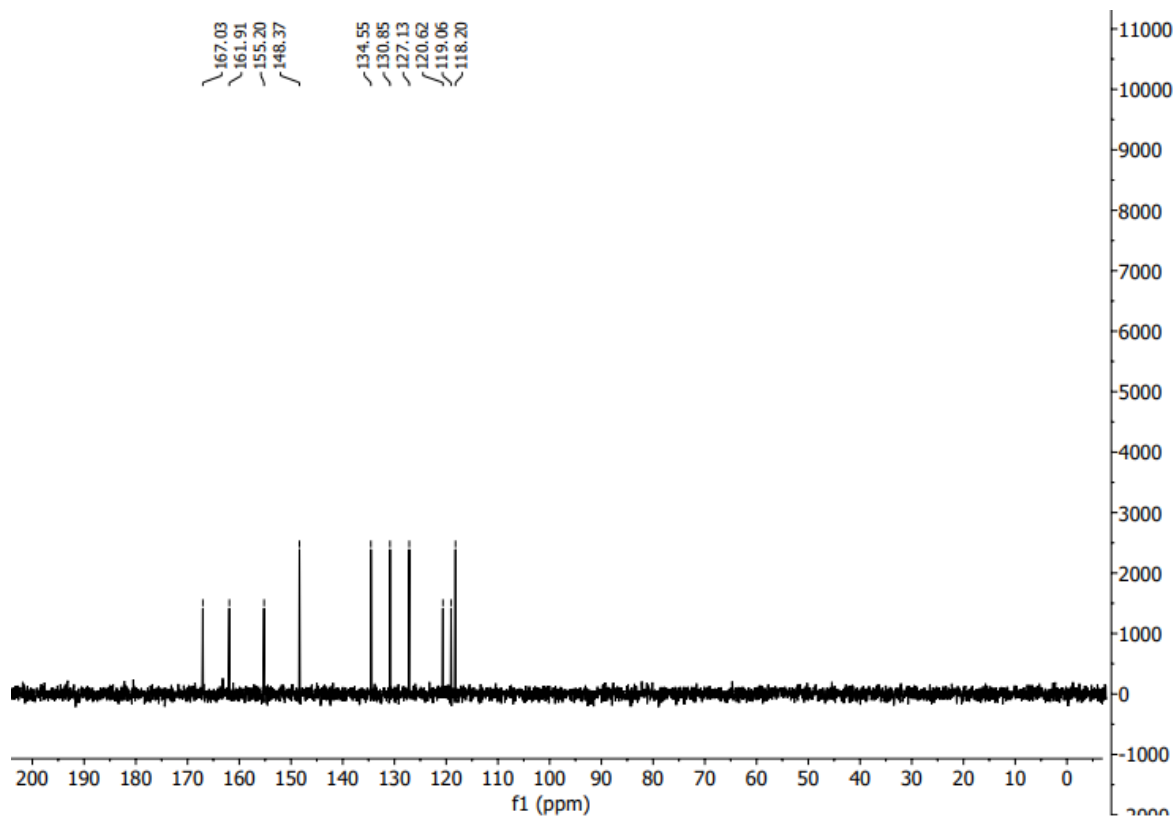


Figure S11. ¹³C-NMR spectrum of compound 5

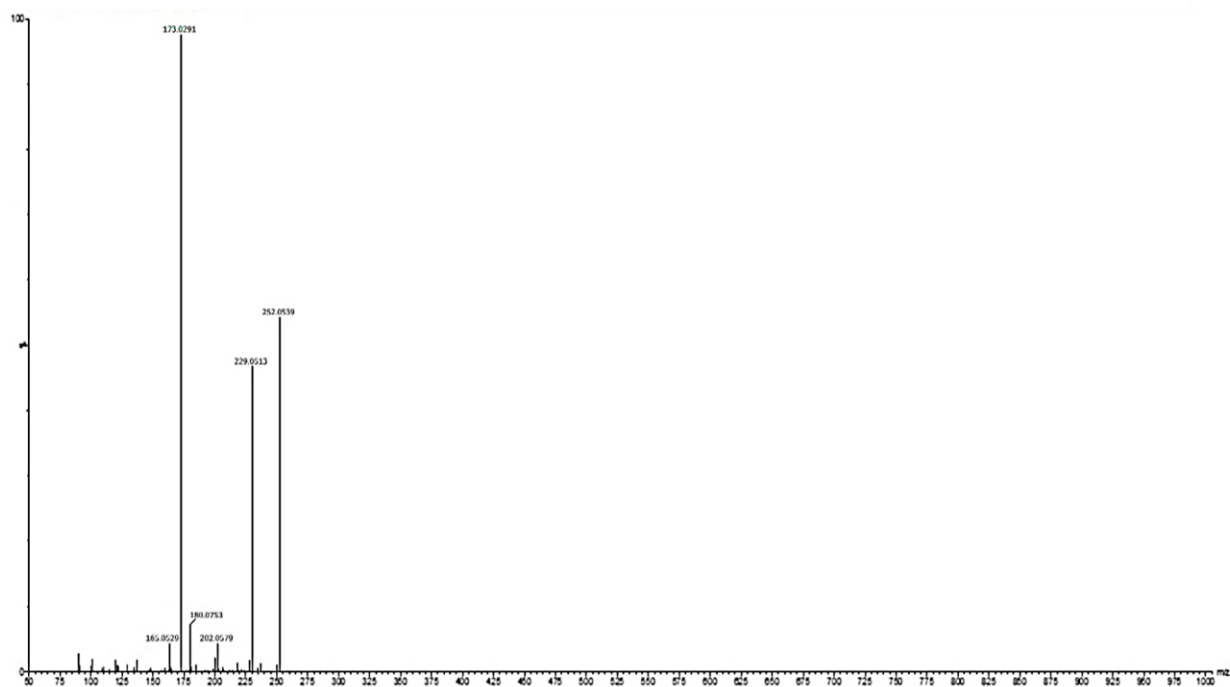


Figure S12. LCMS spectrum of compound 5

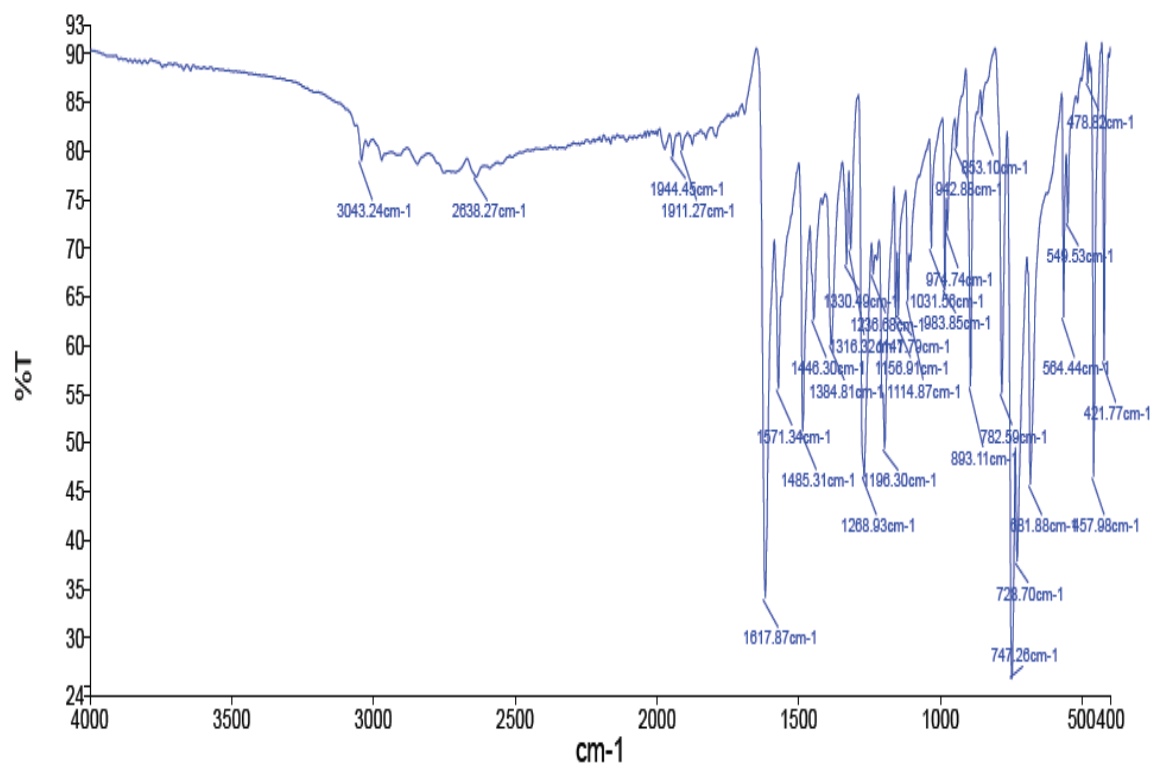


Figure S13. IR spectrum of compound AM1

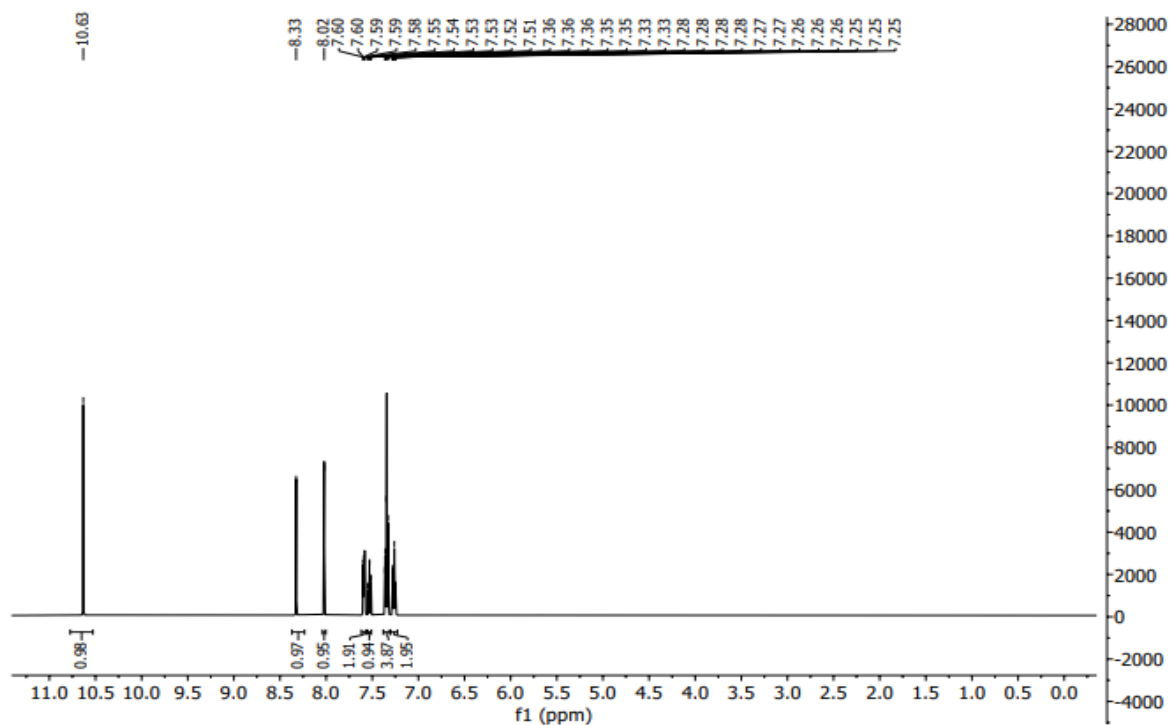


Figure S14. ^1H -NMR spectrum of compound AM1

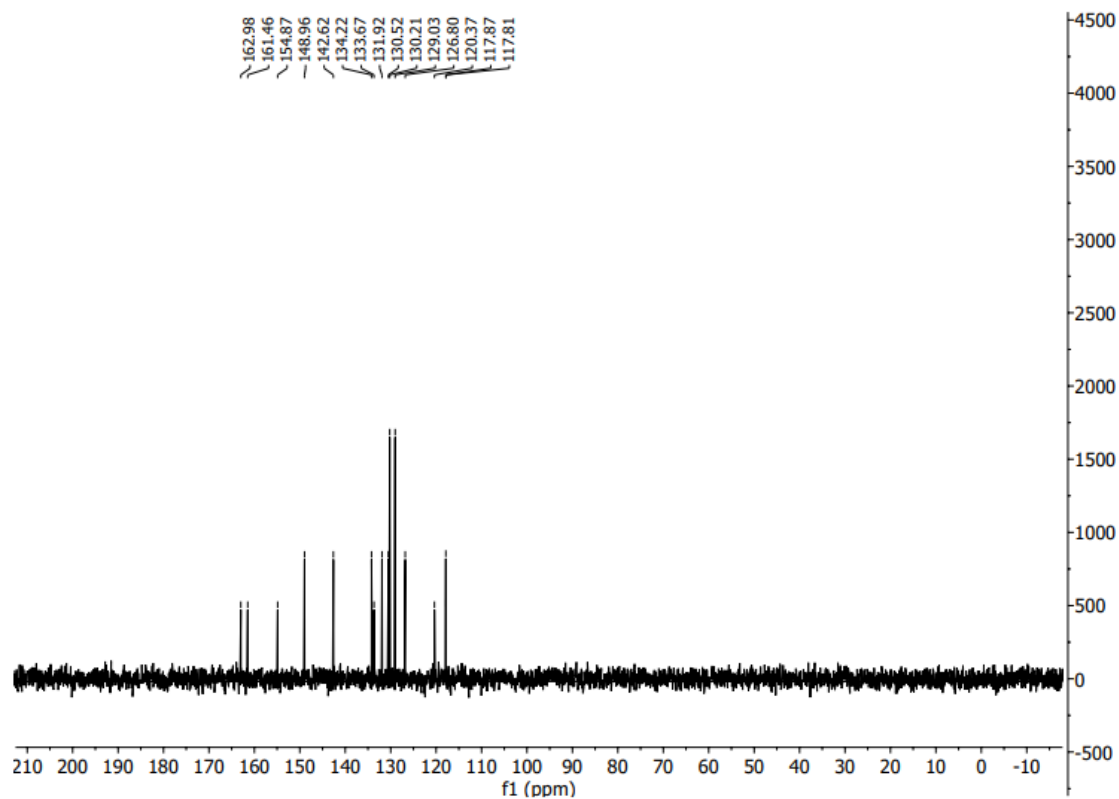


Figure S15. ^{13}C -NMR spectrum of compound AM1

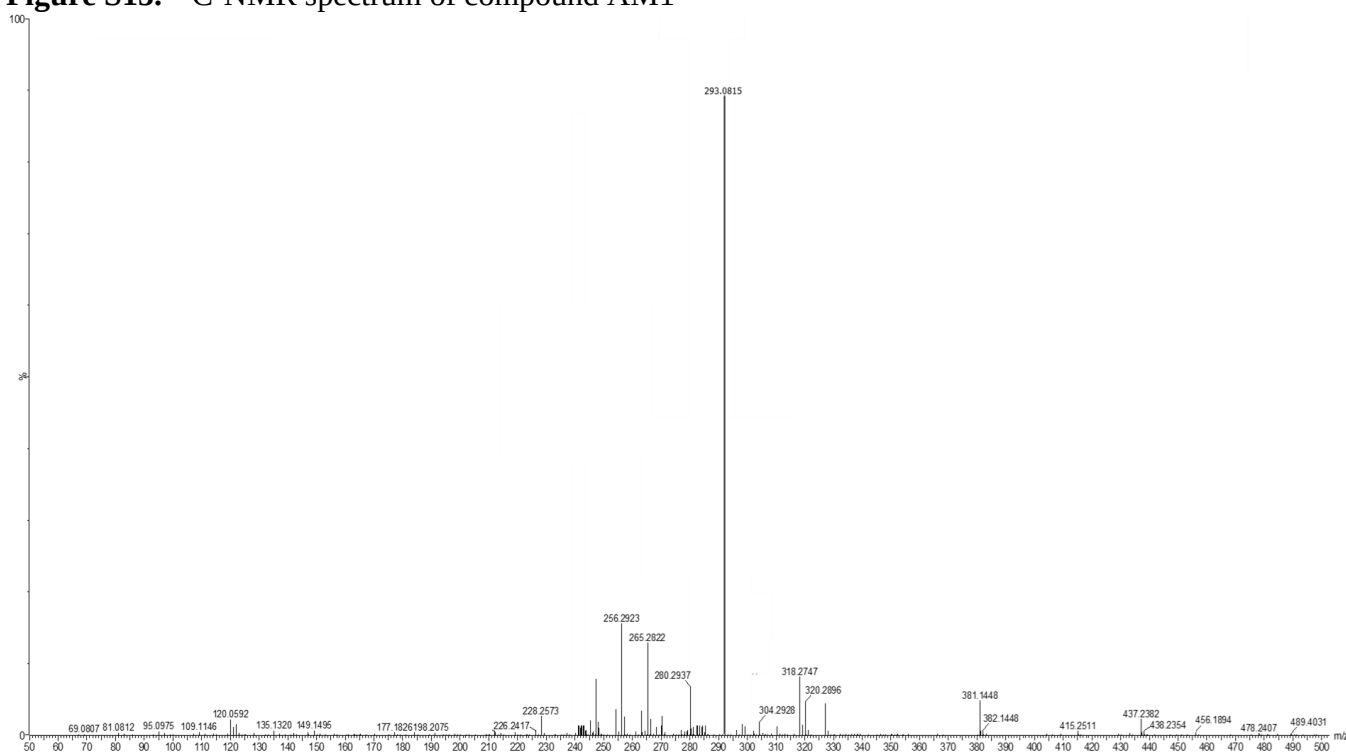


Figure S16. HRMS spectrum of compound AM1

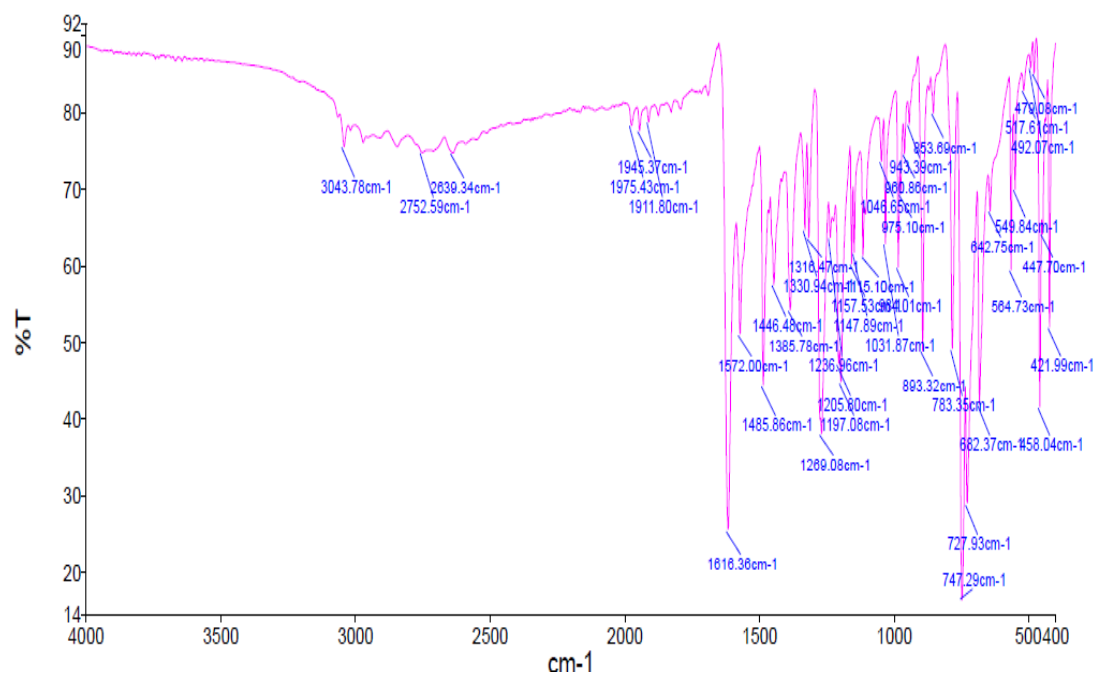


Figure S17. IR spectrum of compound AM2

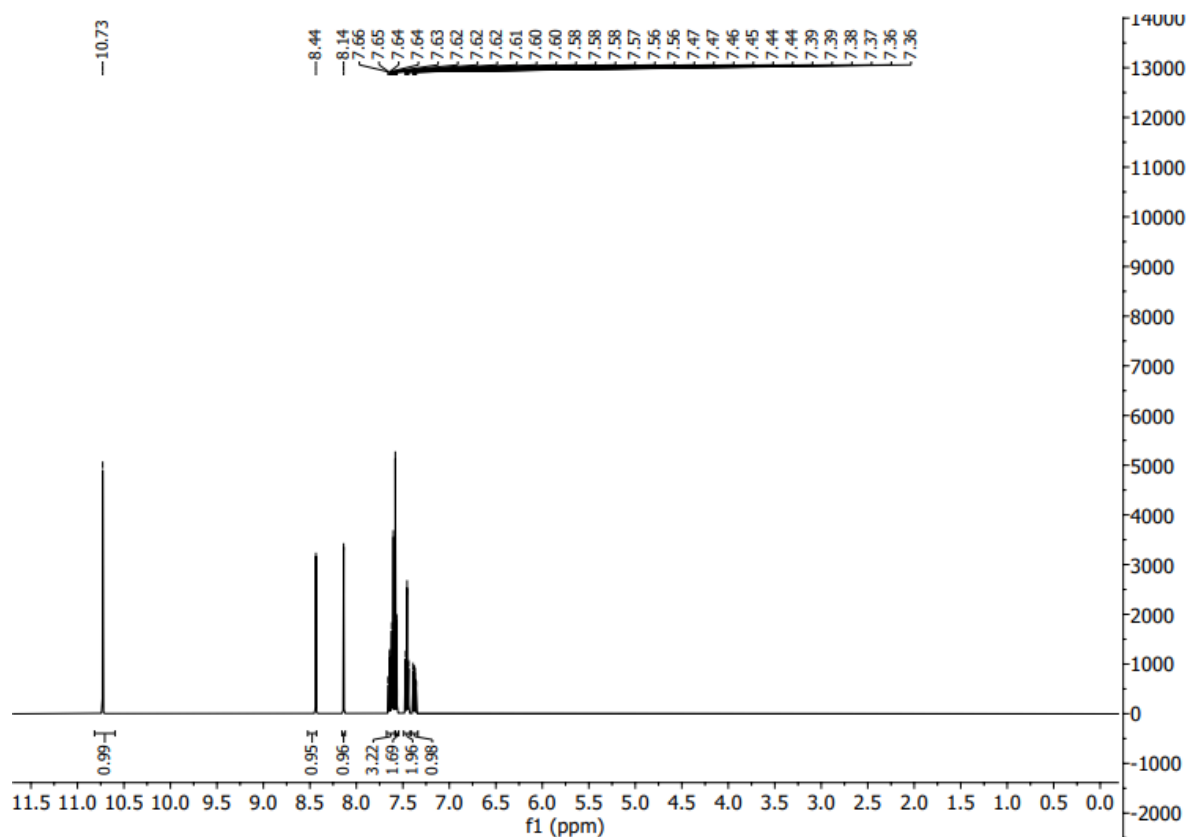


Figure S18. ¹H-NMR spectrum of compound AM2

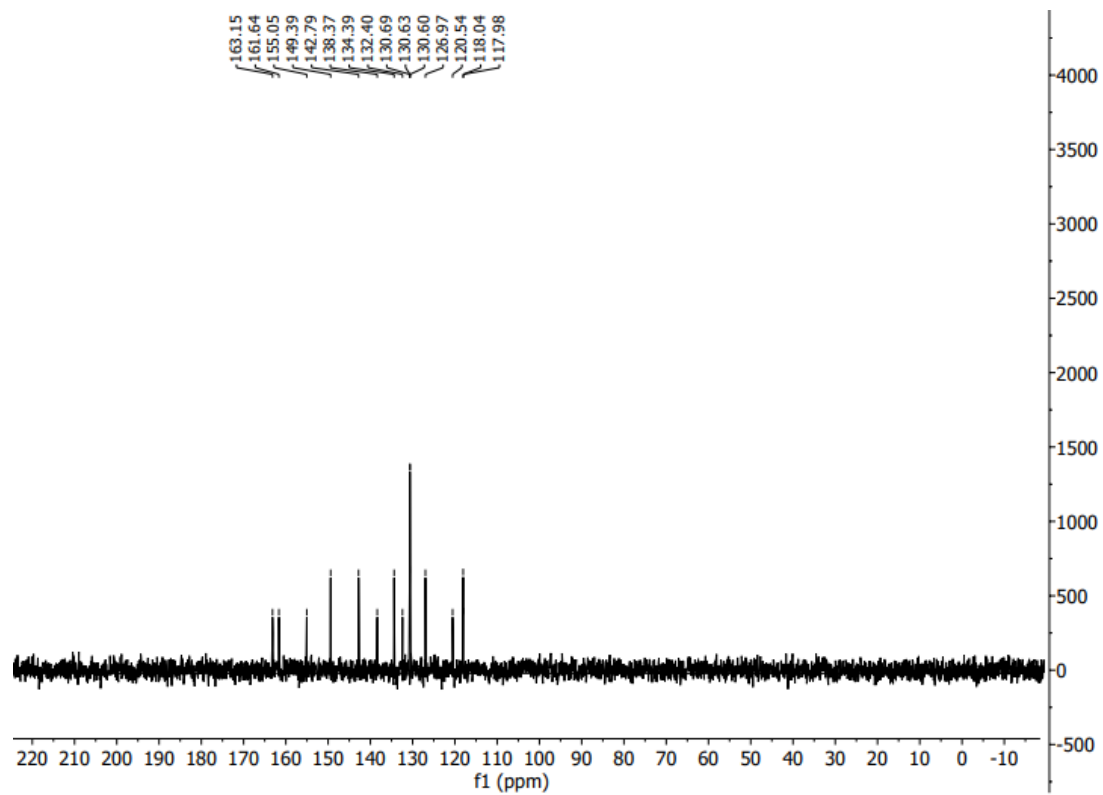


Figure S19. ^{13}C -NMR spectrum of compound AM2

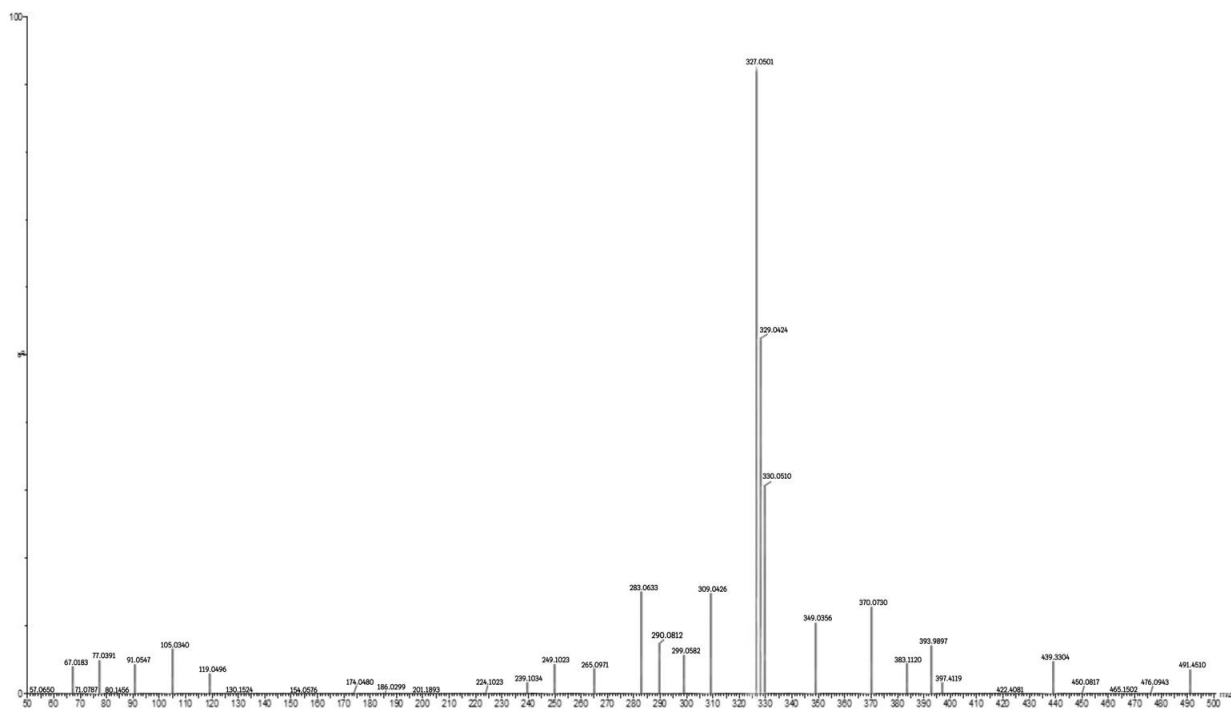
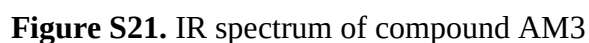


Figure S20. HRMS spectrum of compound AM2



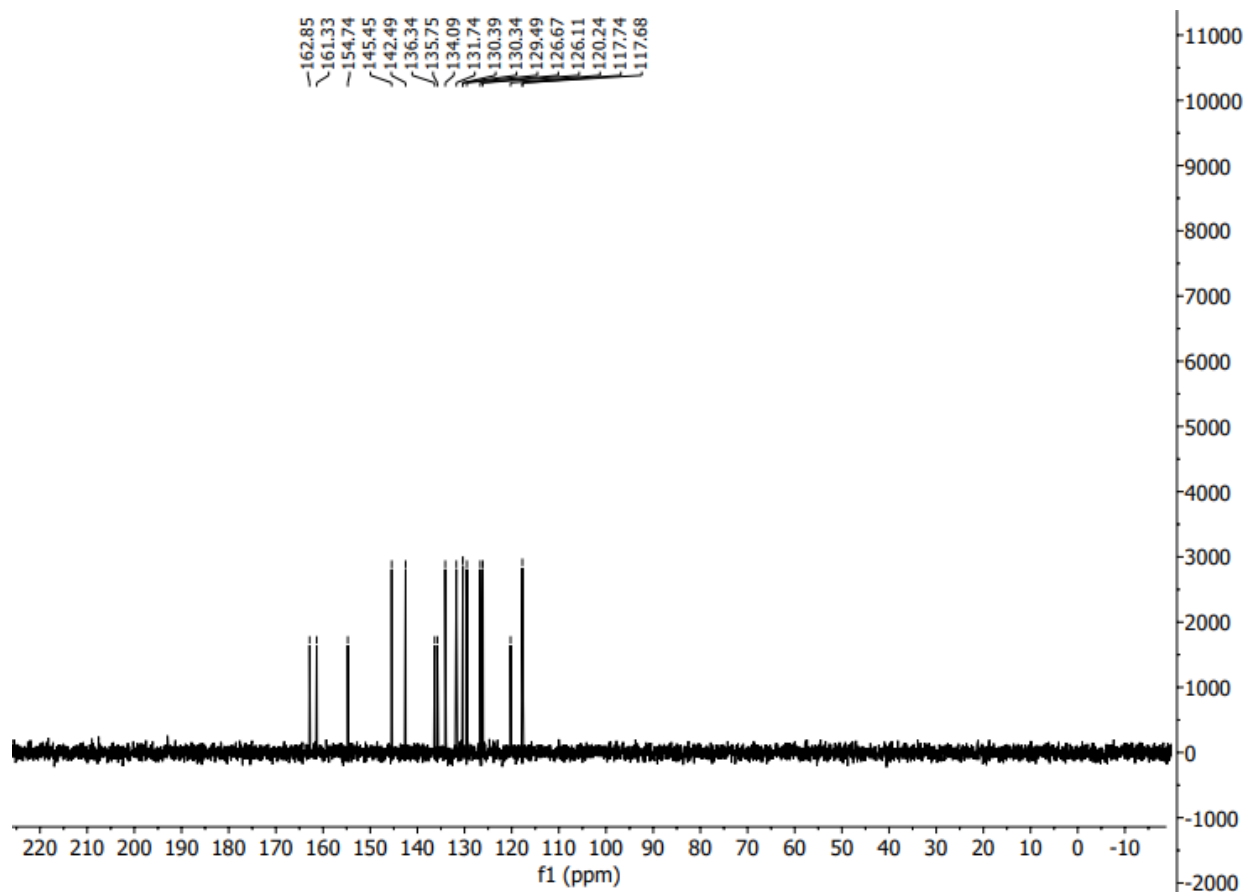


Figure S23. ^{13}C -NMR spectrum of compound AM3

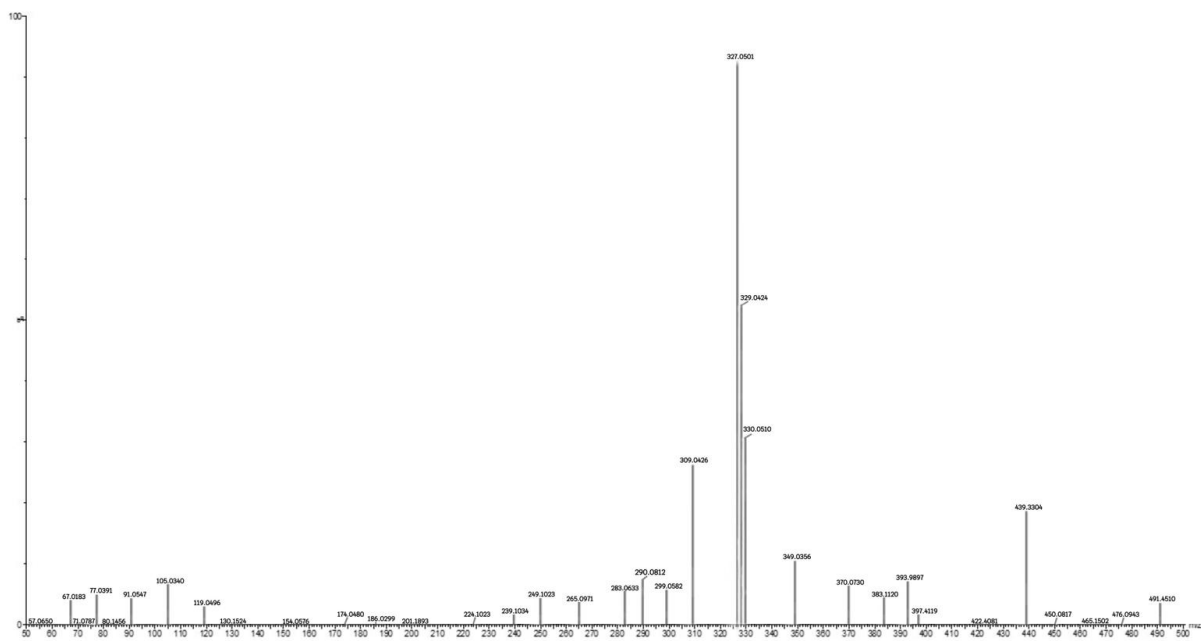
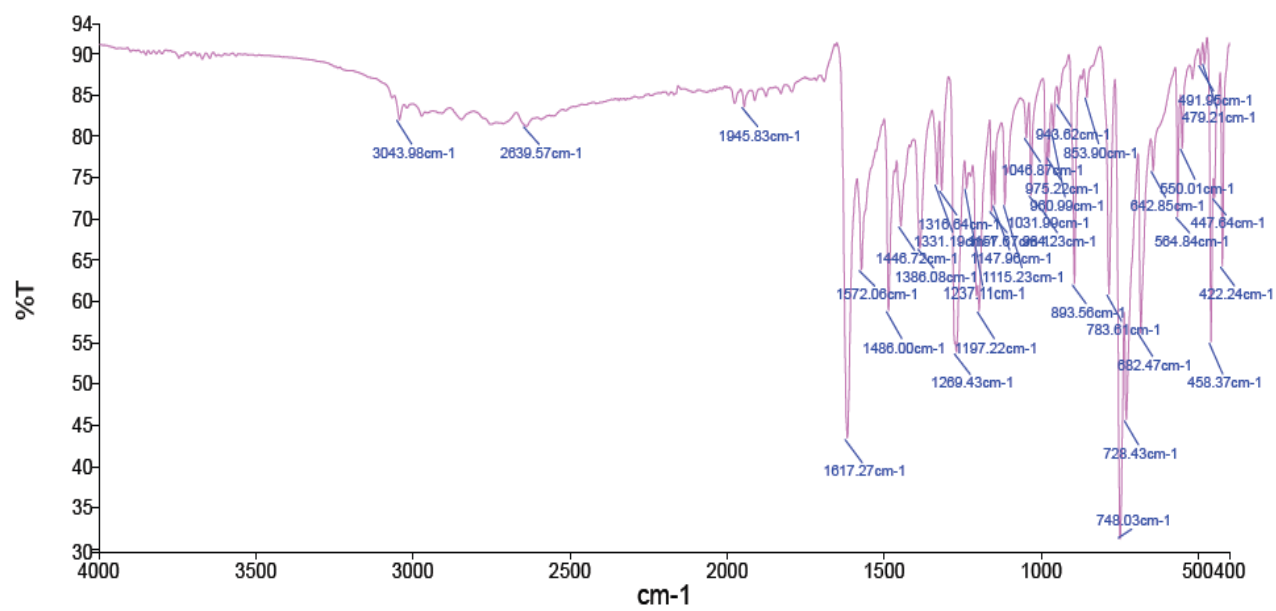


Figure S24. HRMS spectrum of compound AM3



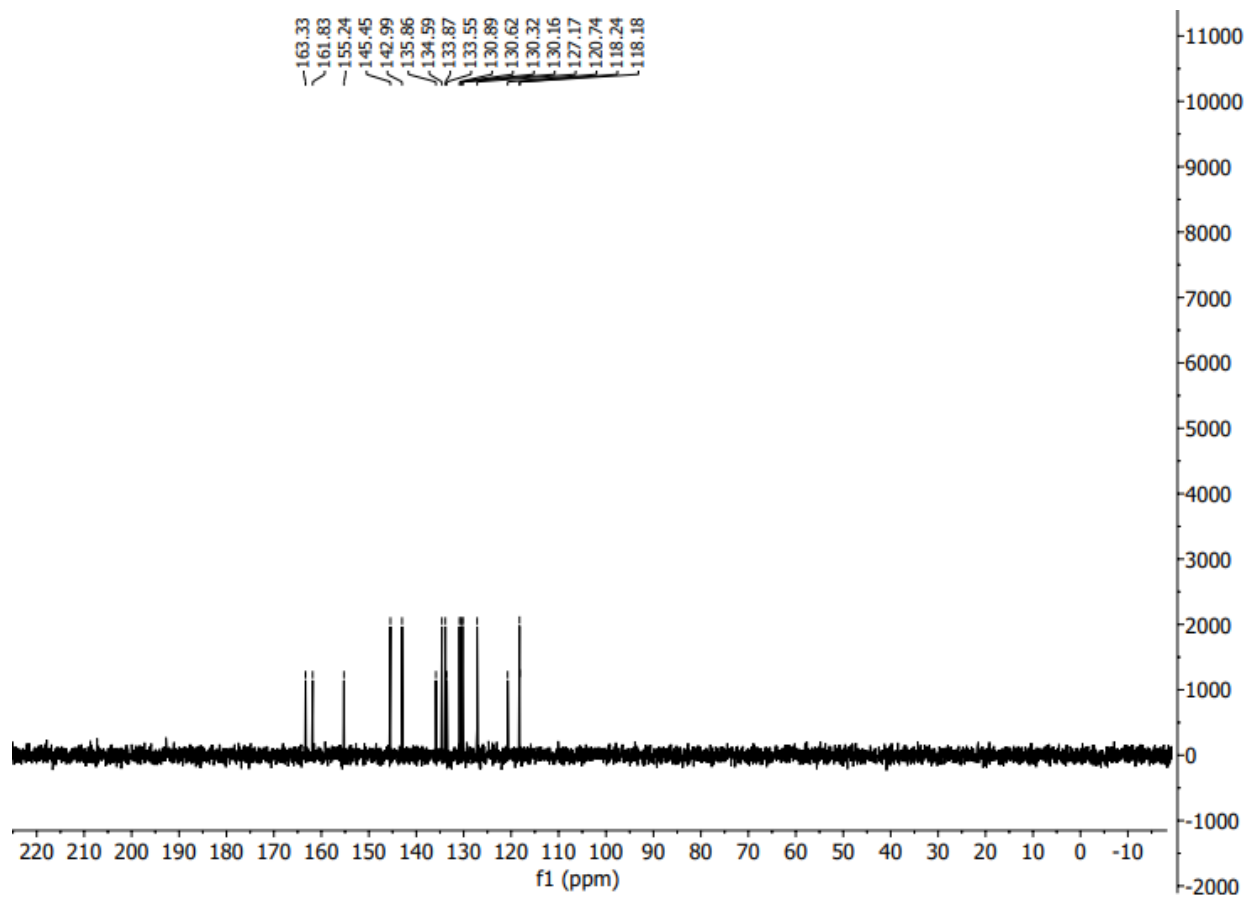


Figure S27. ^{13}C -NMR spectrum of compound AM4

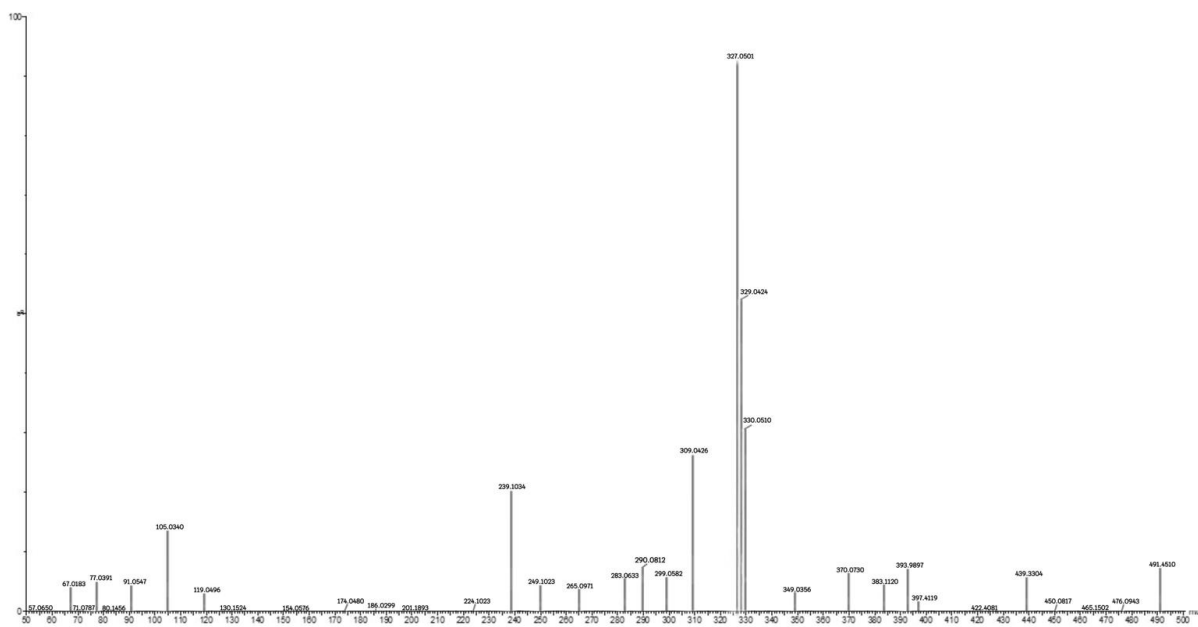


Figure S28. HRMS spectrum of compound AM4

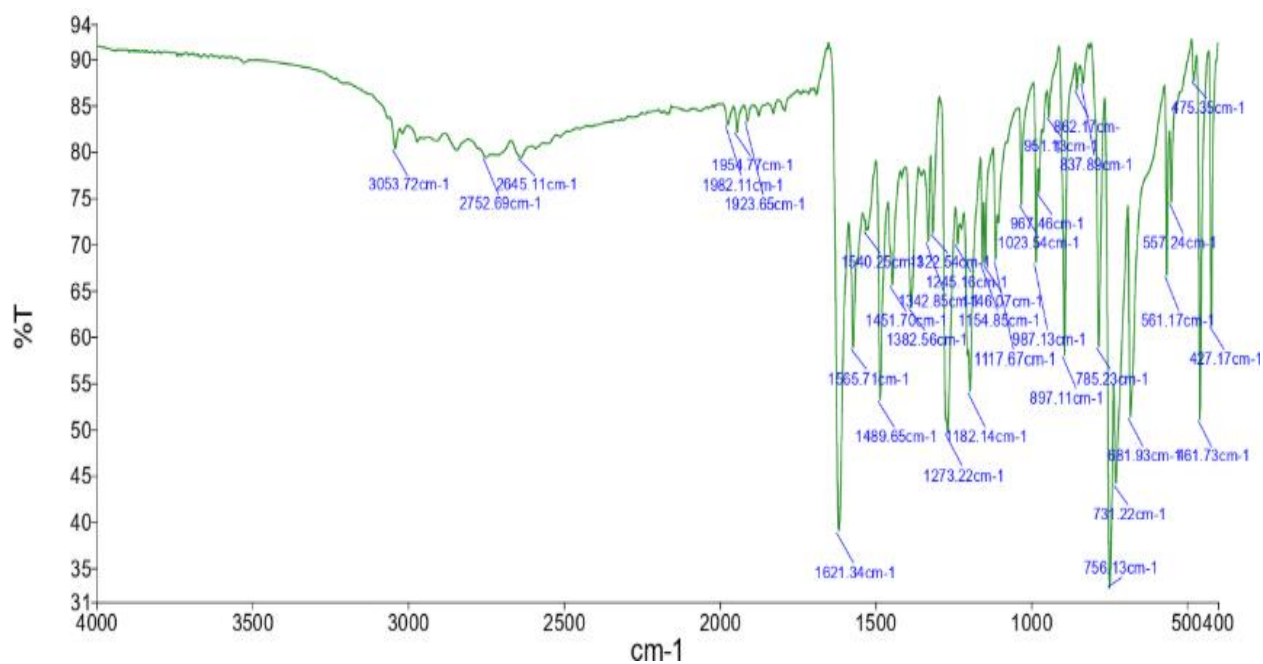
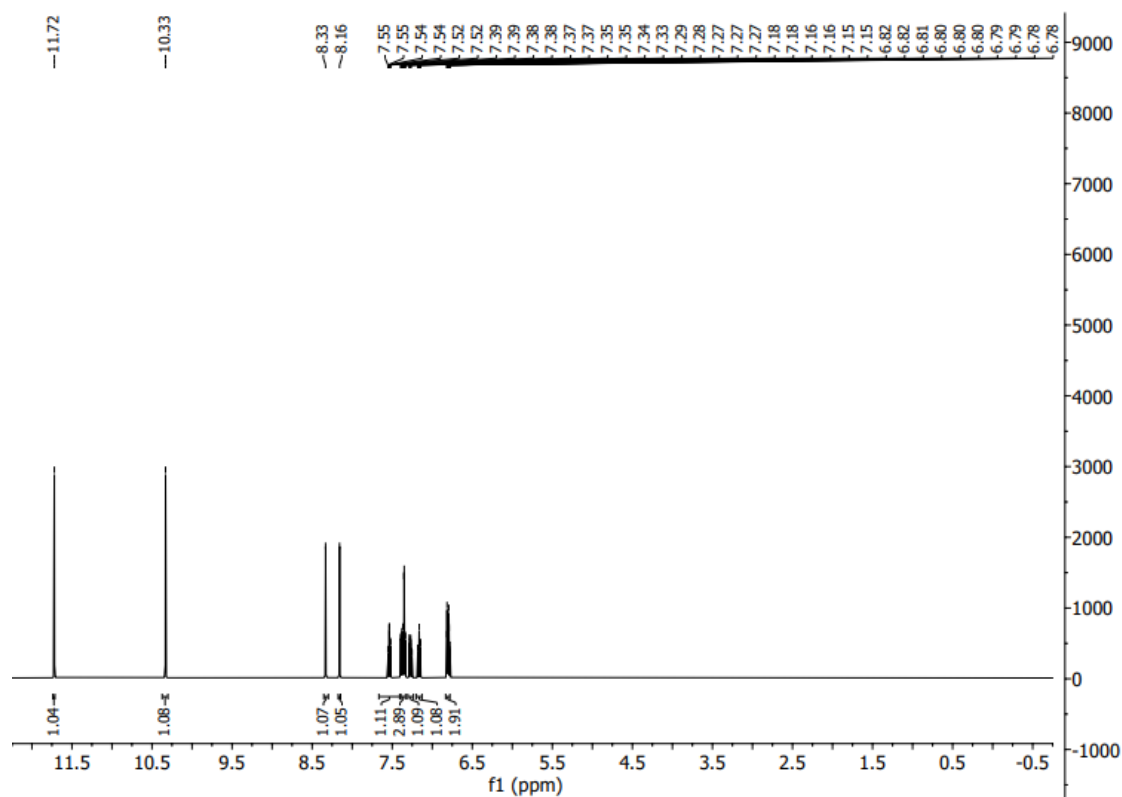


Figure S29. IR spectrum of compound AM5



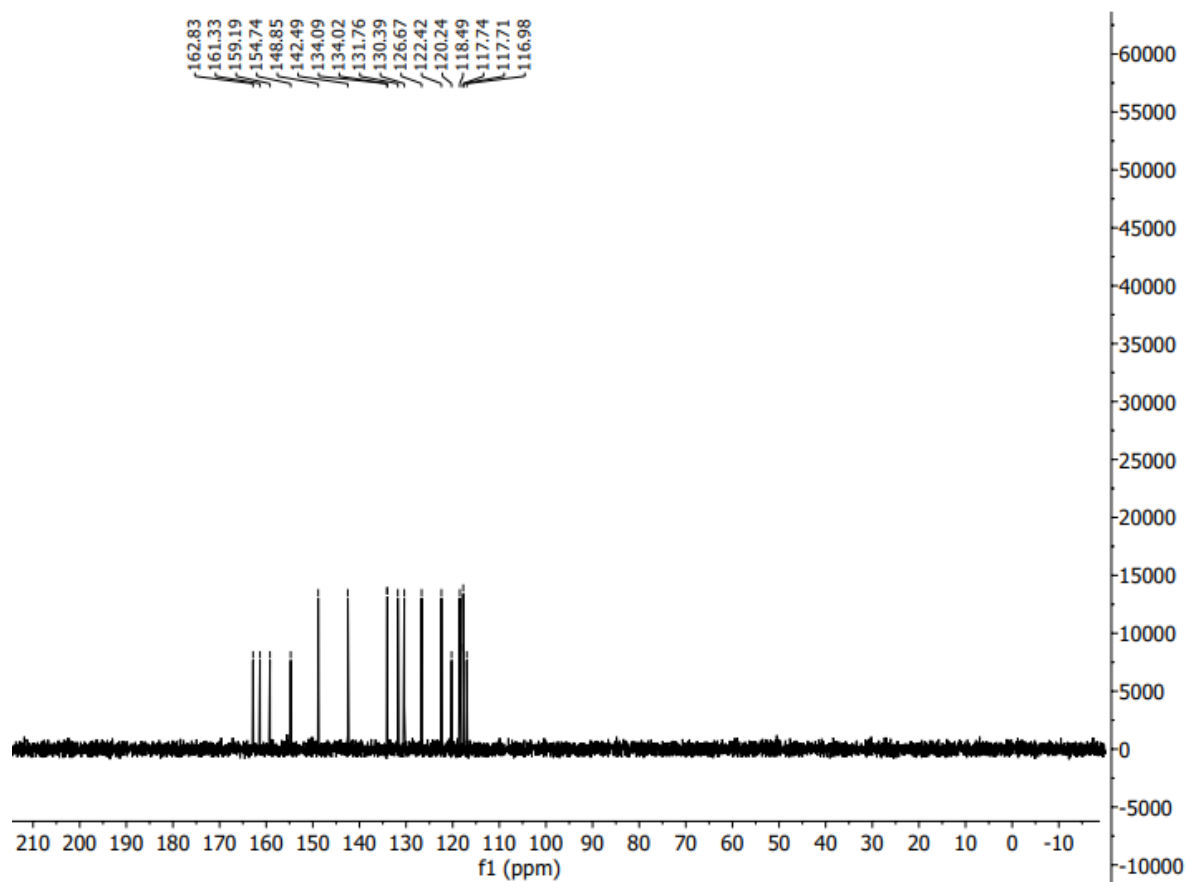


Figure S31. ¹³C-NMR spectrum of compound AM5

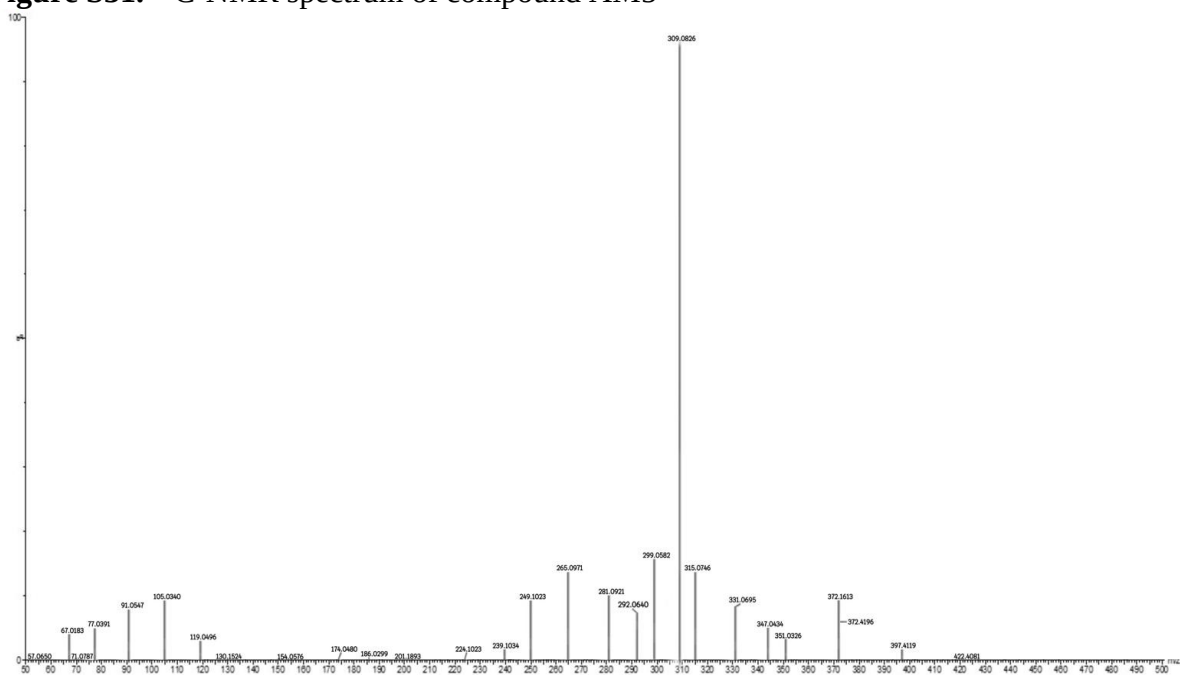


Figure S32. HRMS spectrum of compound AM5

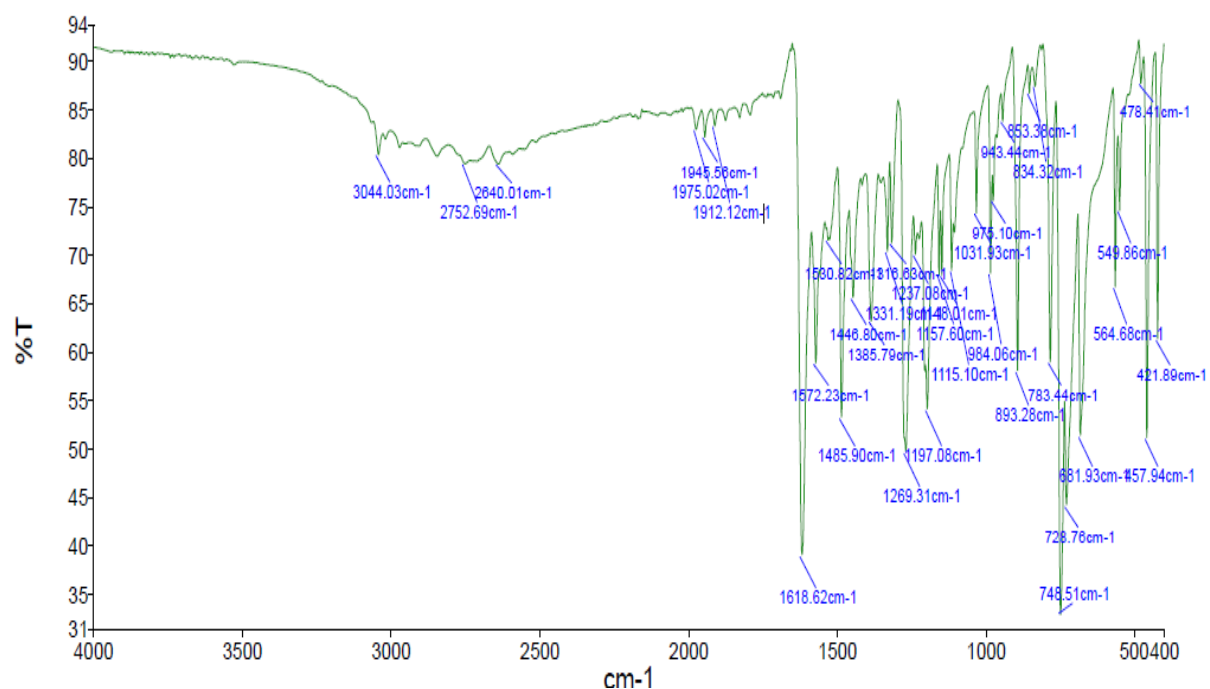


Figure S33. IR spectrum of compound AM6

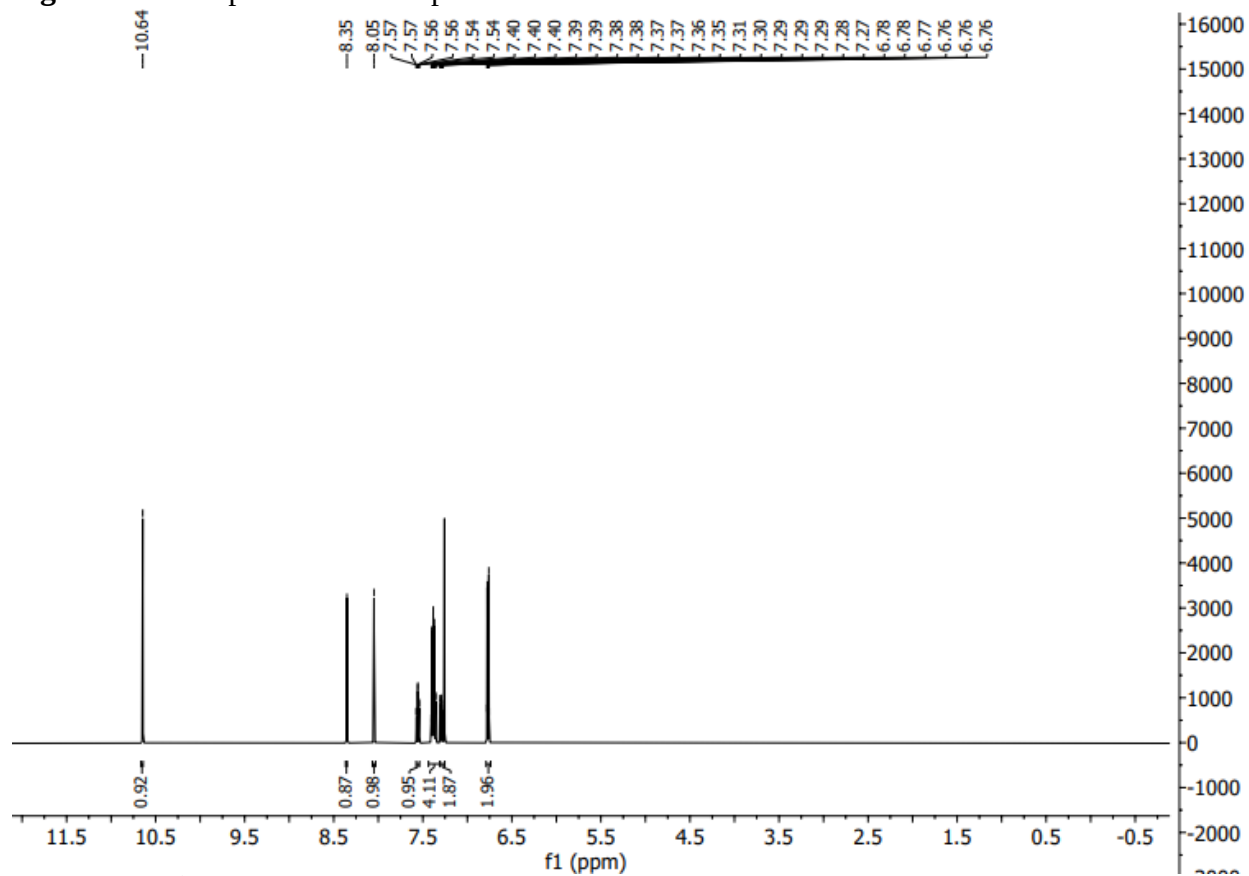


Figure S34. ^1H -NMR spectrum of compound AM6

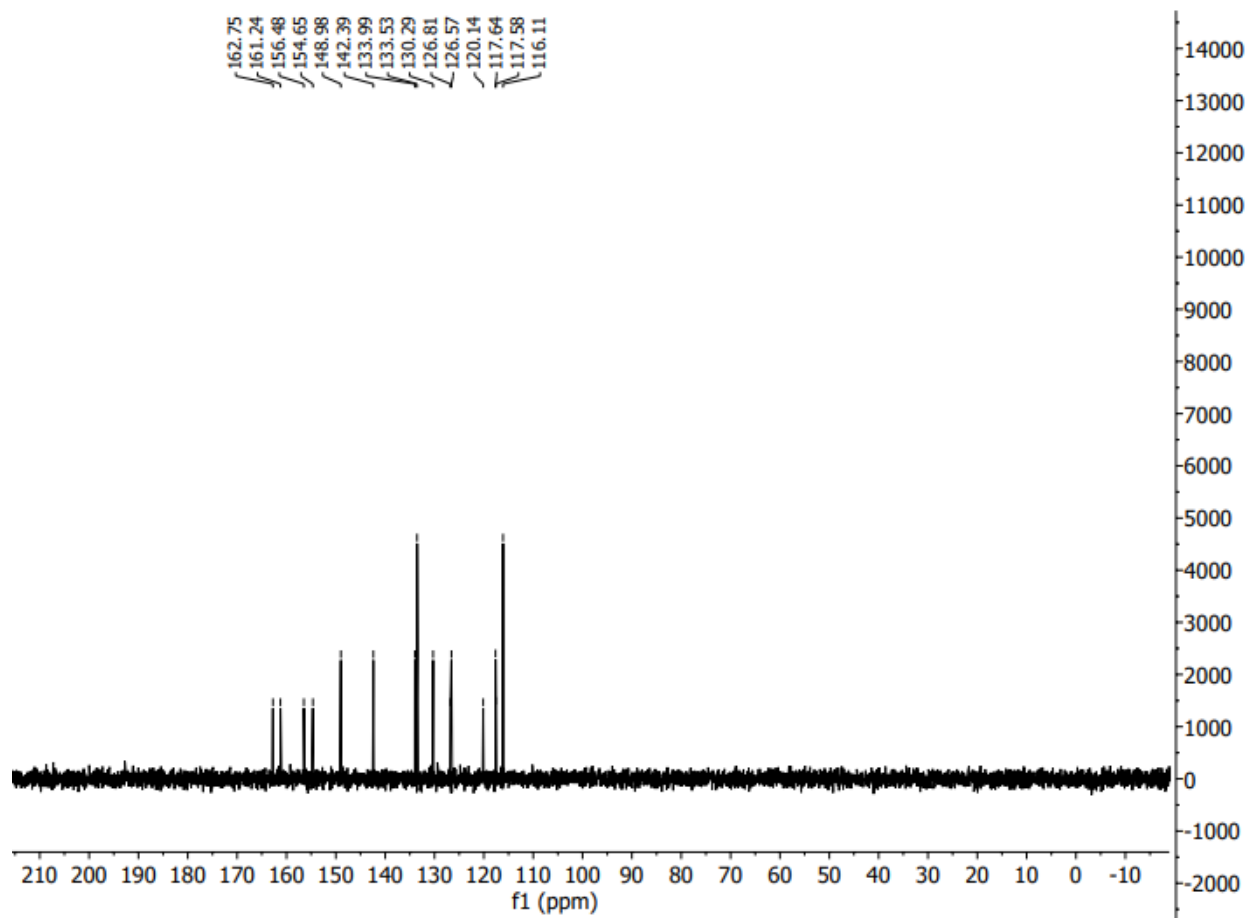


Figure S35. ^{13}C -NMR spectrum of compound AM6

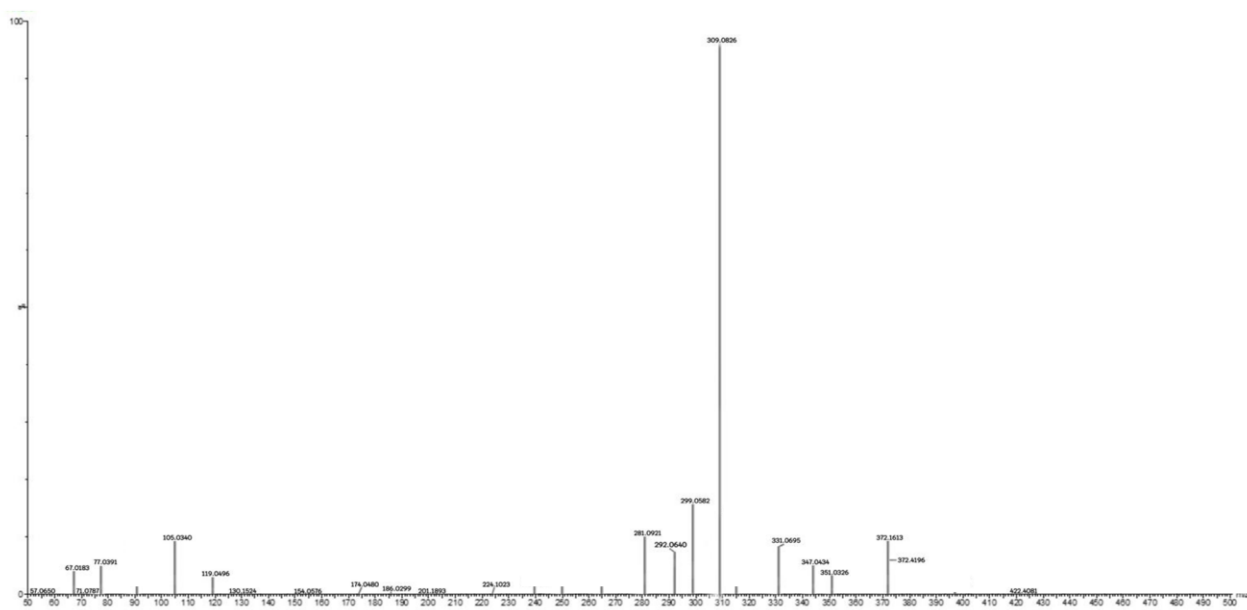


Figure S36. HRMS spectrum of compound AM6

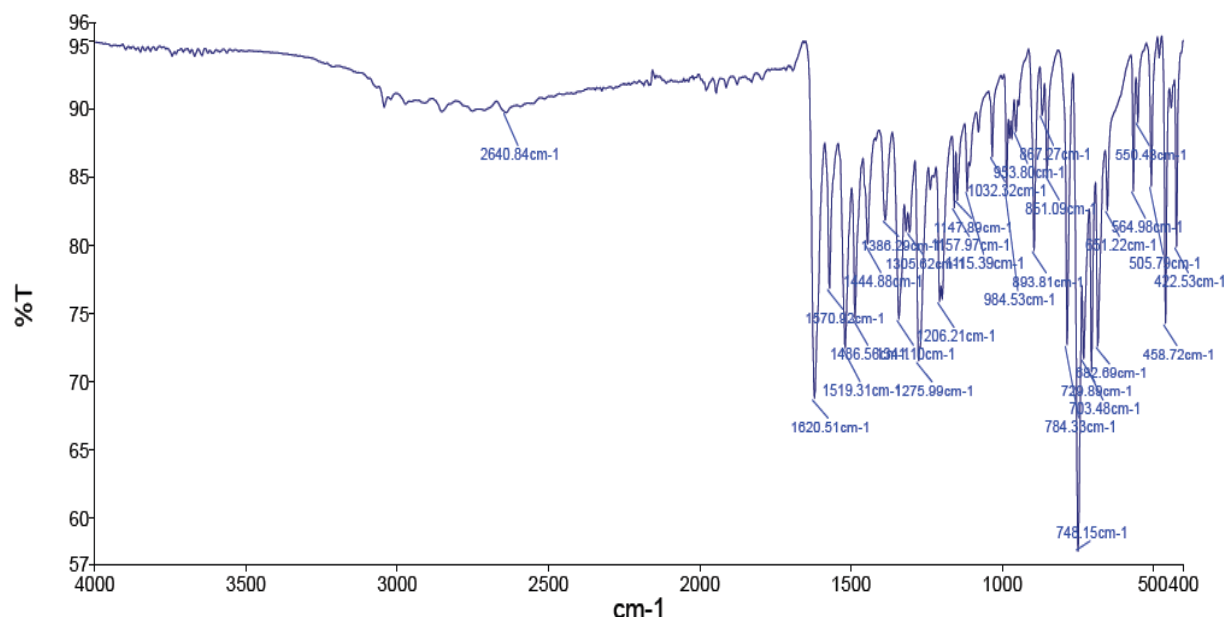


Figure S37. IR spectrum of compound AM7

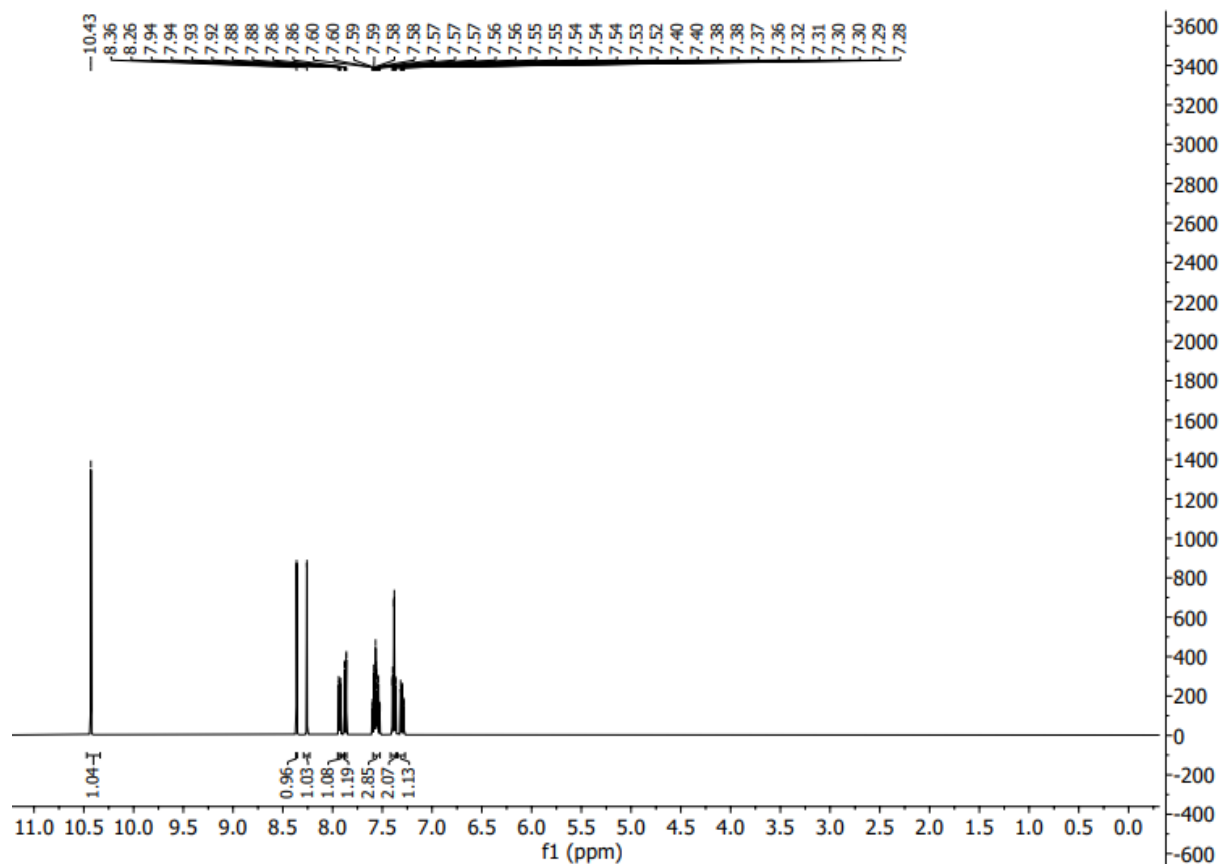


Figure S38. ^1H -NMR spectrum of compound AM7

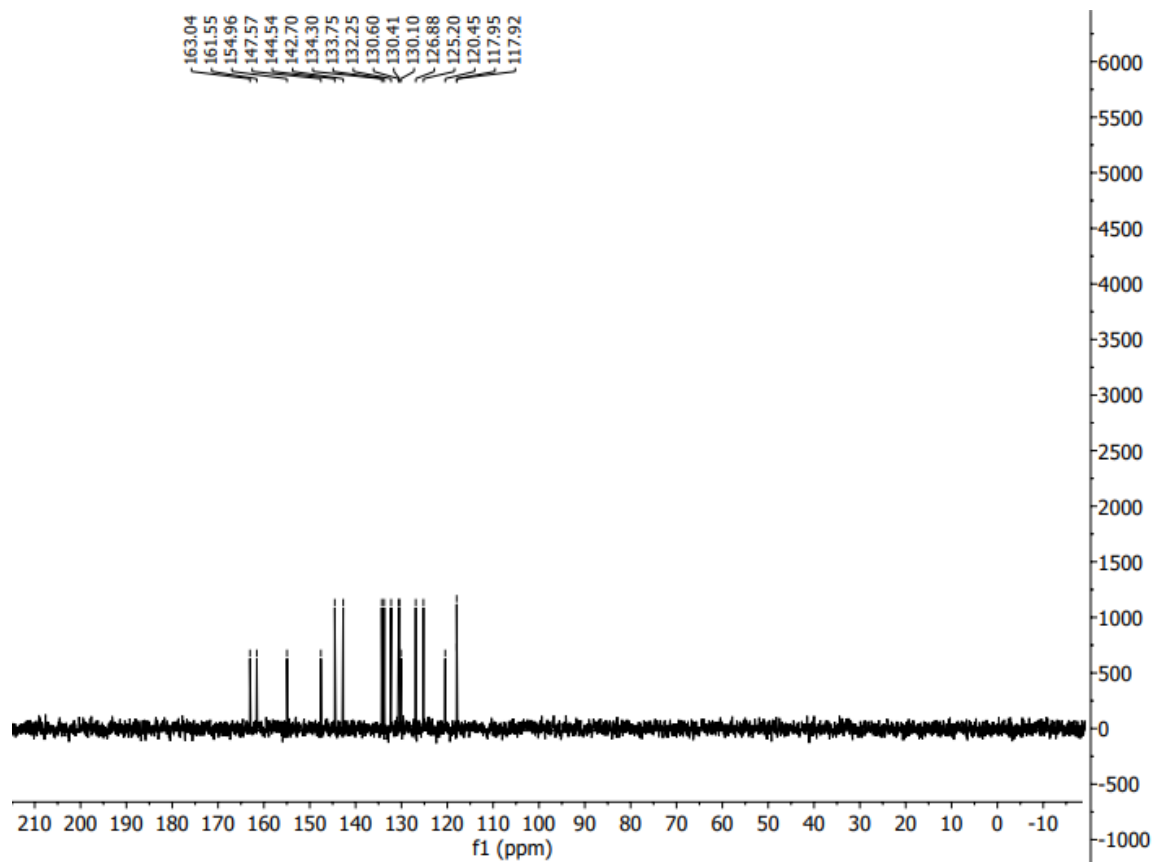


Figure S39. ^{13}C -NMR spectrum of compound AM7

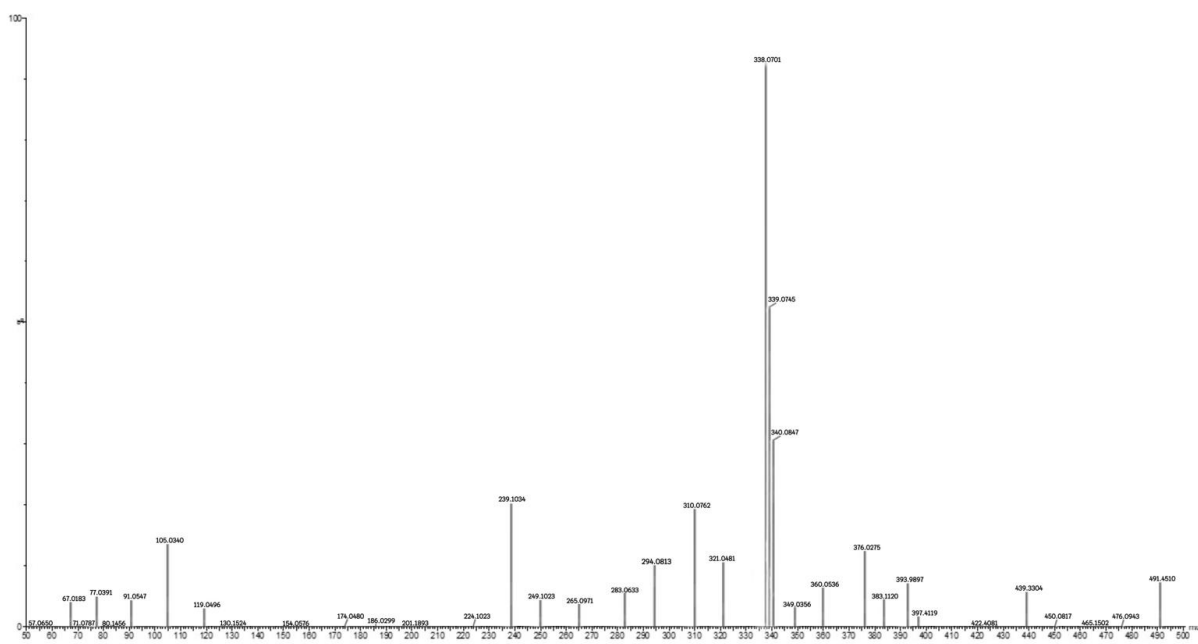


Figure S40. HRMS spectrum of compound AM7

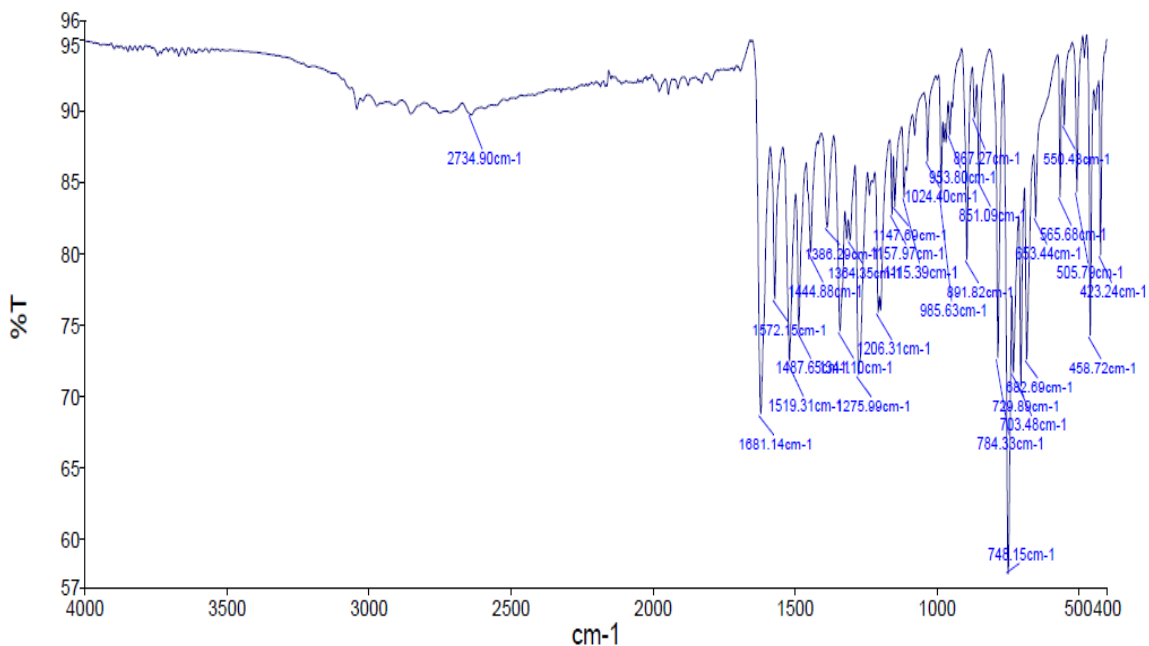


Figure S41. IR spectrum of compound AM8

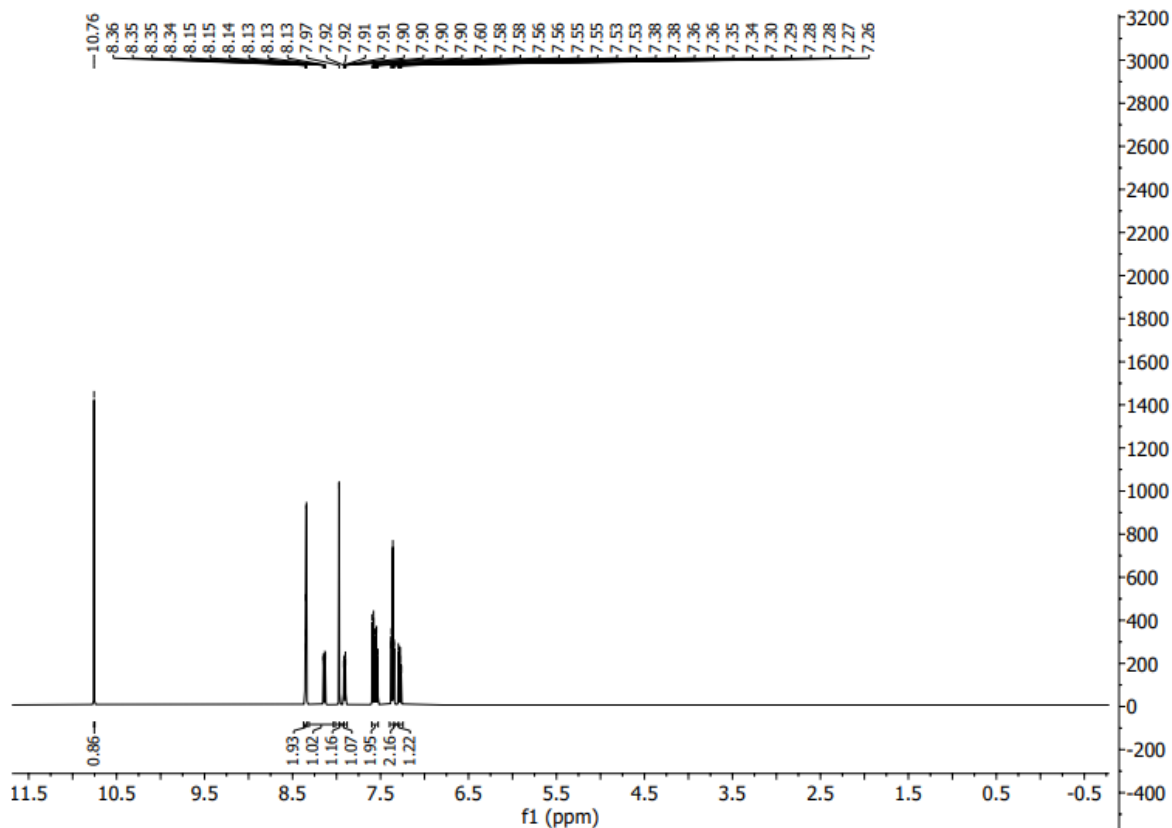


Figure S42. ¹H-NMR spectrum of compound AM8

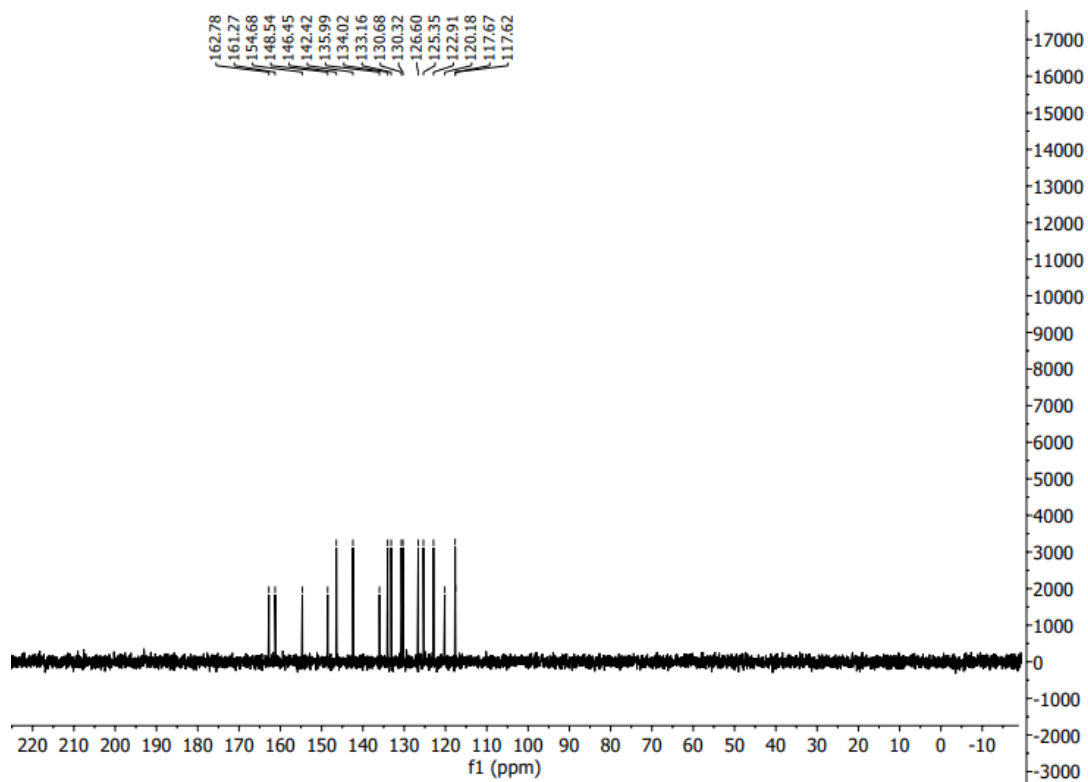


Figure S43. ^{13}C -NMR spectrum of compound AM8

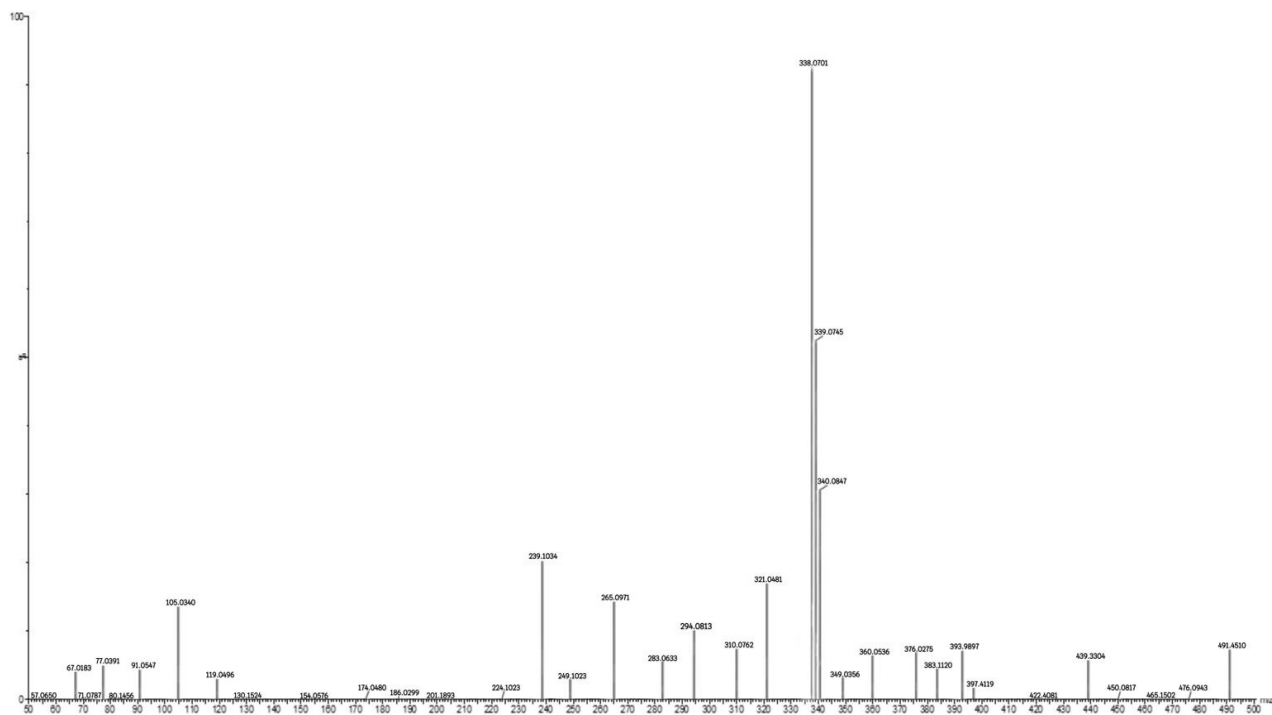


Figure S44. HRMS spectrum of compound AM8

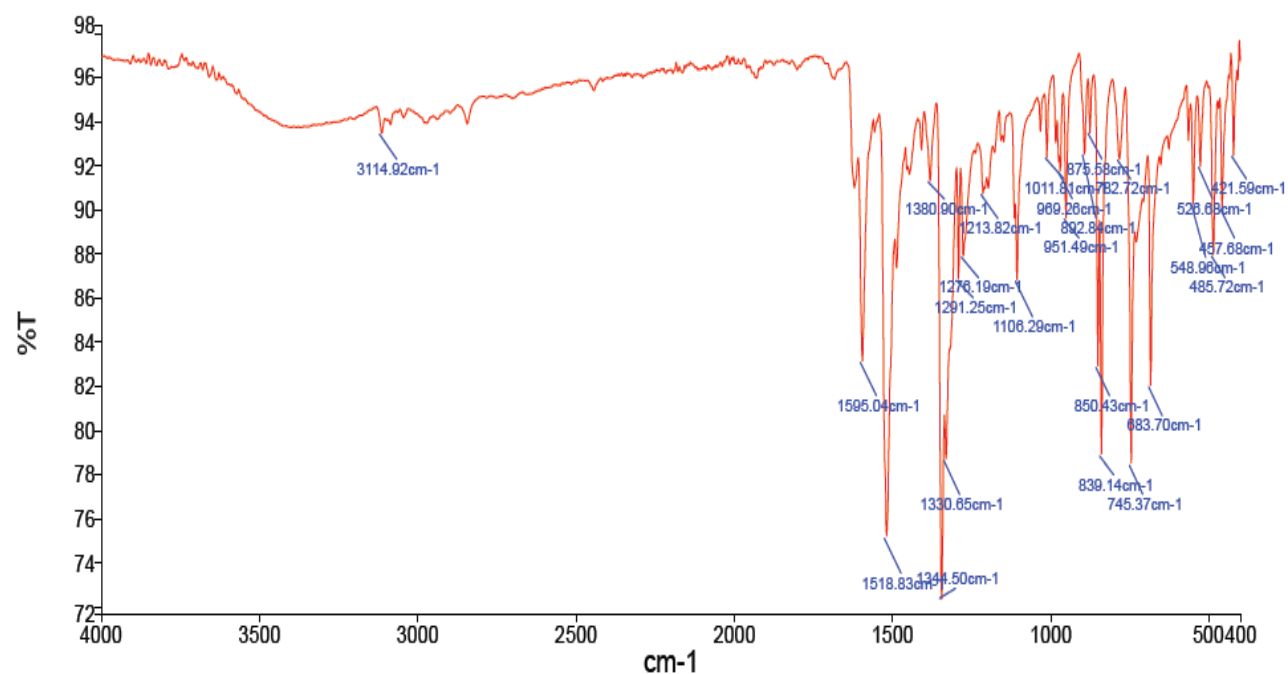


Figure S45. IR spectrum of compound AM9

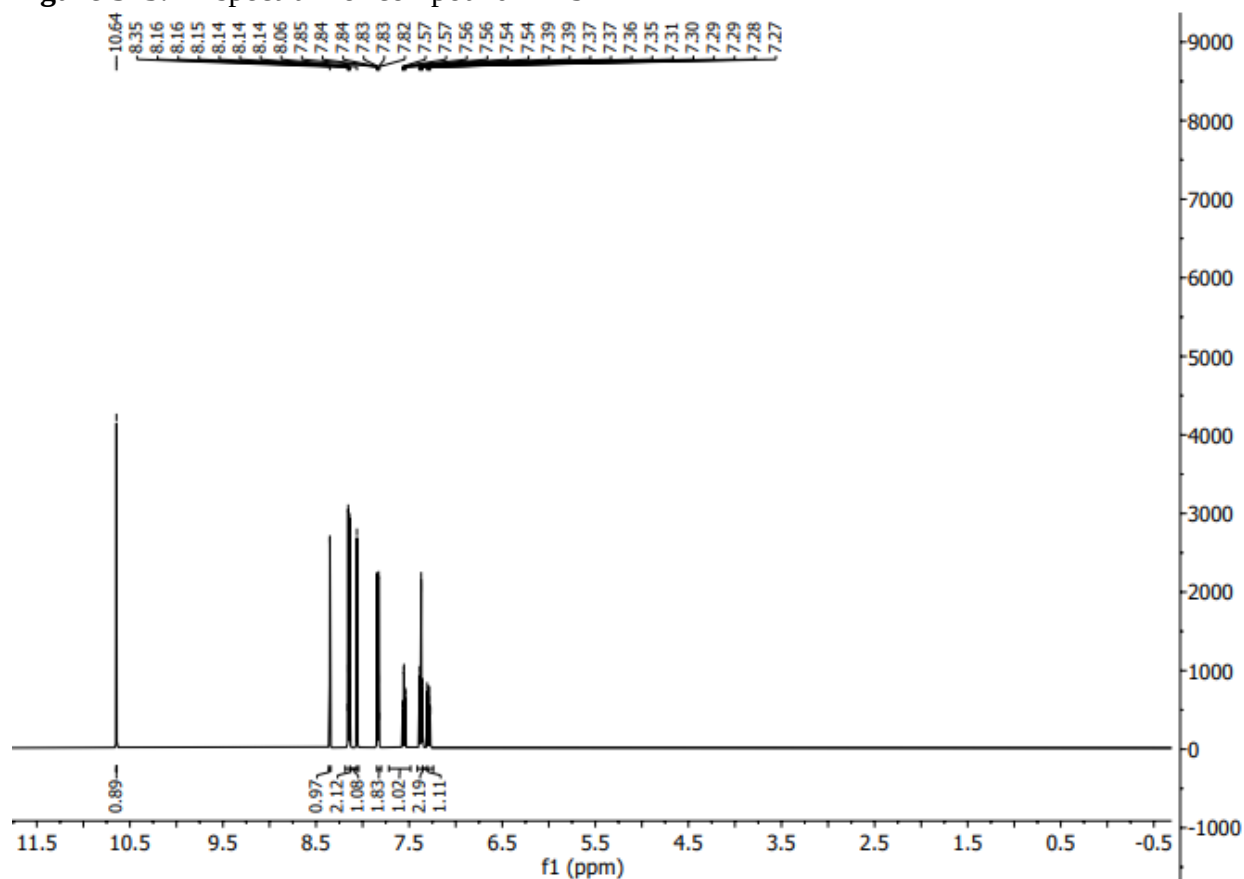


Figure S46. ¹H-NMR spectrum of compound AM9

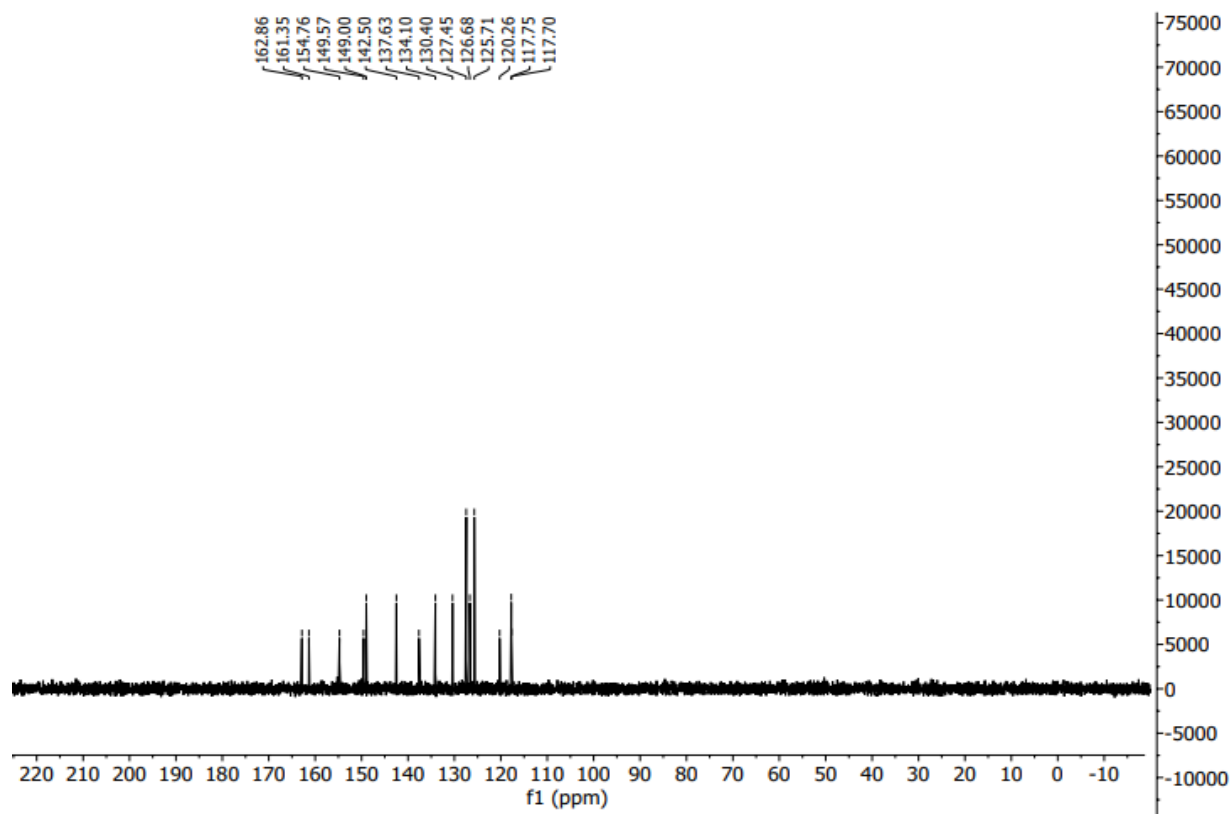


Figure S47. ¹³C-NMR spectrum of compound AM9

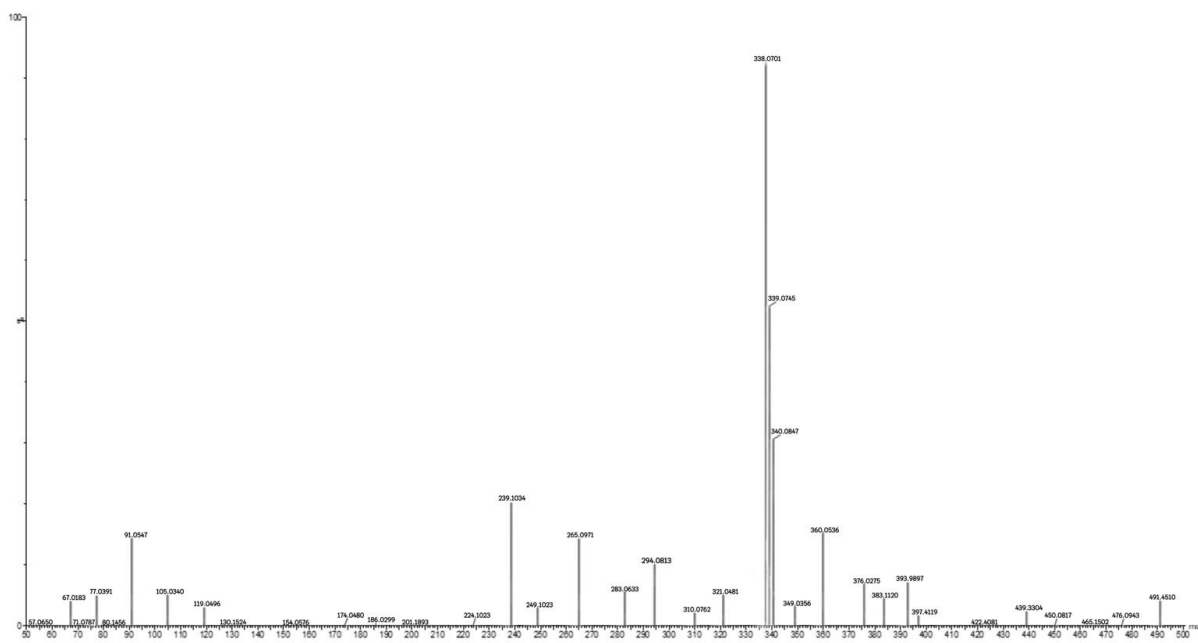


Figure S48. HRMS spectrum of compound AM9

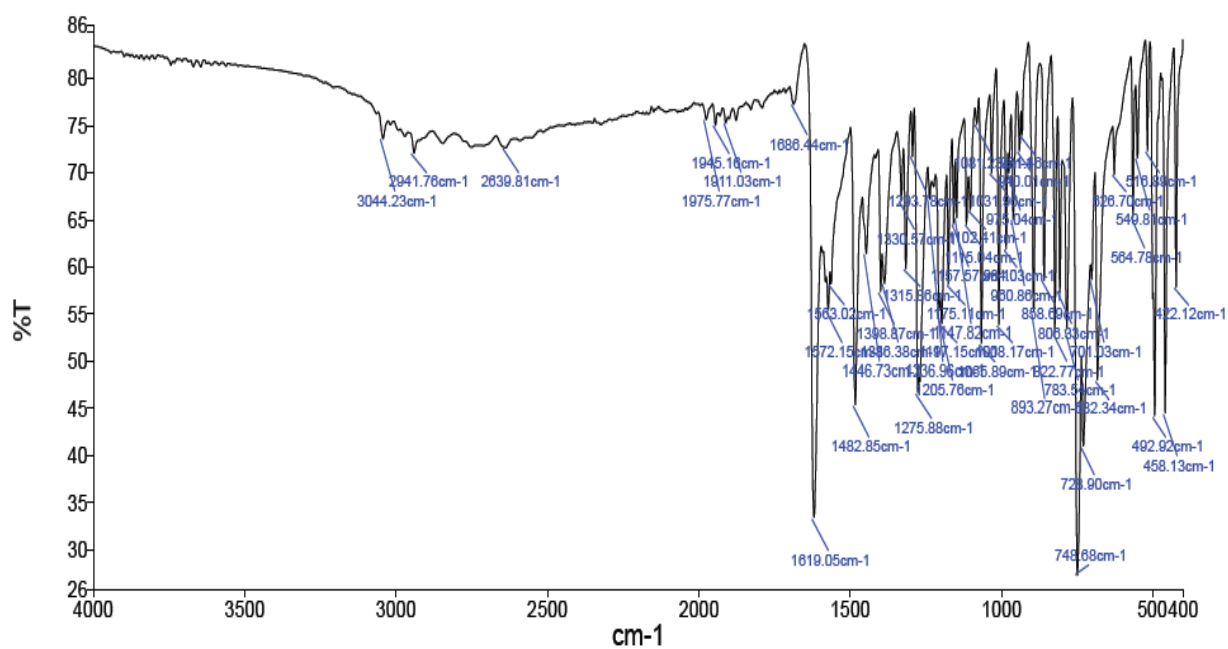


Figure S49. IR spectrum of compound AM10

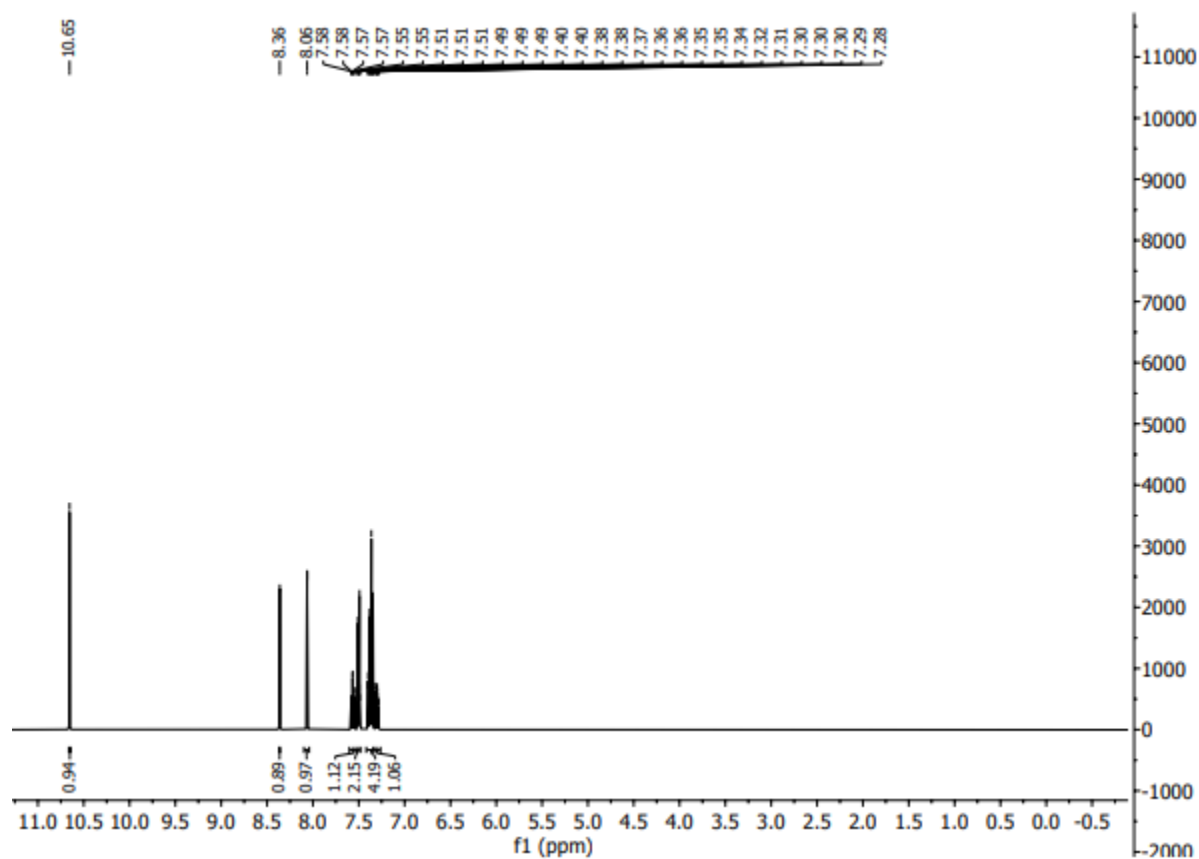


Figure S50. ¹H-NMR spectrum of compound AM10

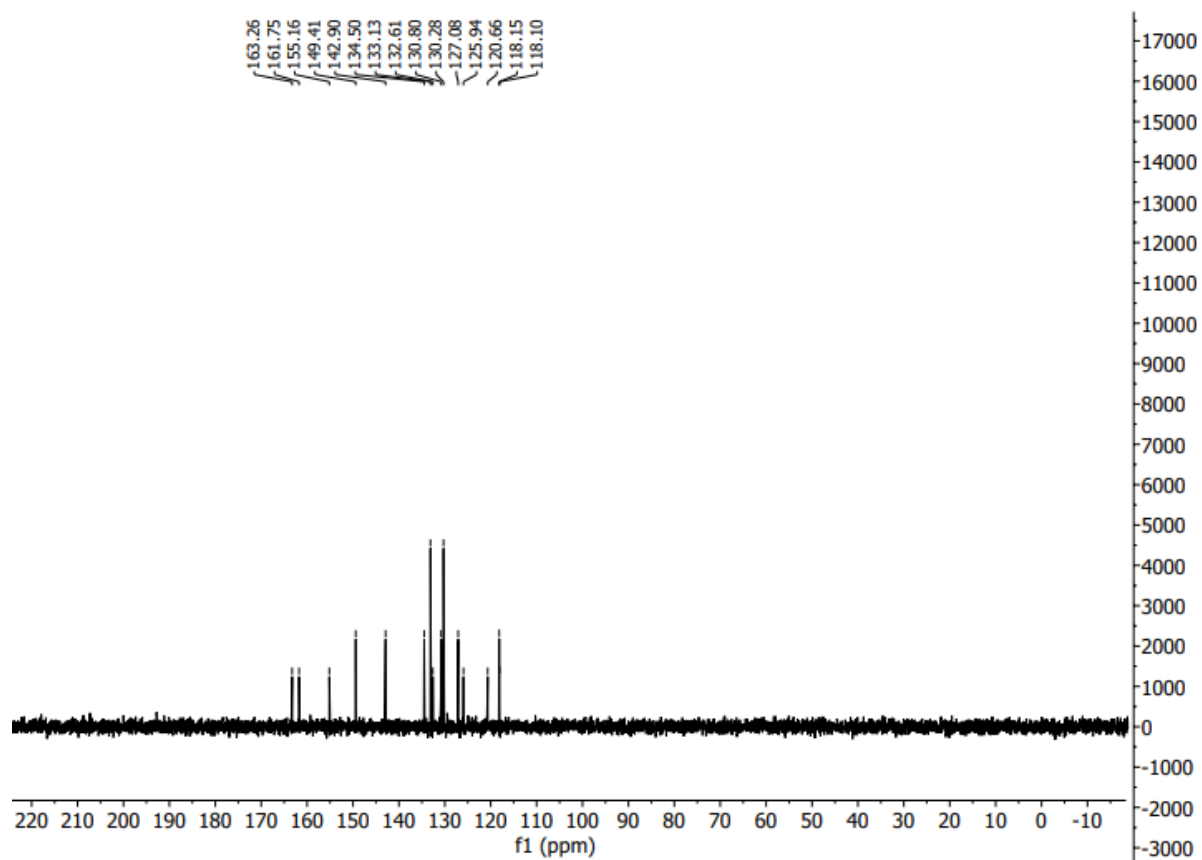


Figure S51. ^{13}C -NMR spectrum of compound AM10

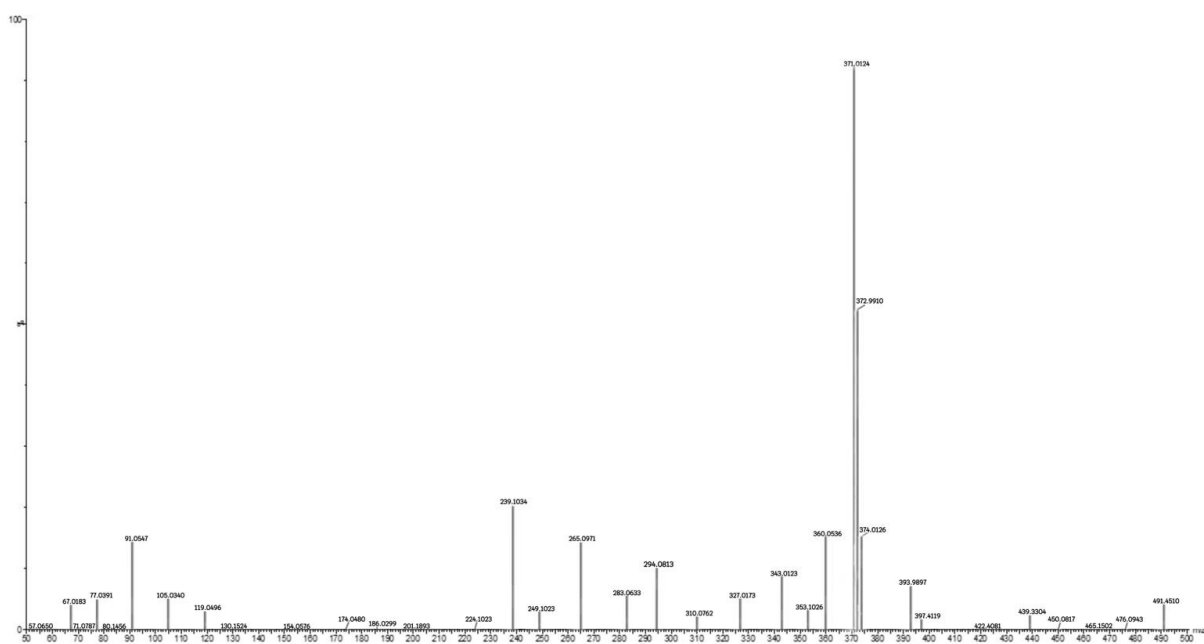


Figure S52. HRMS spectrum of compound AM10

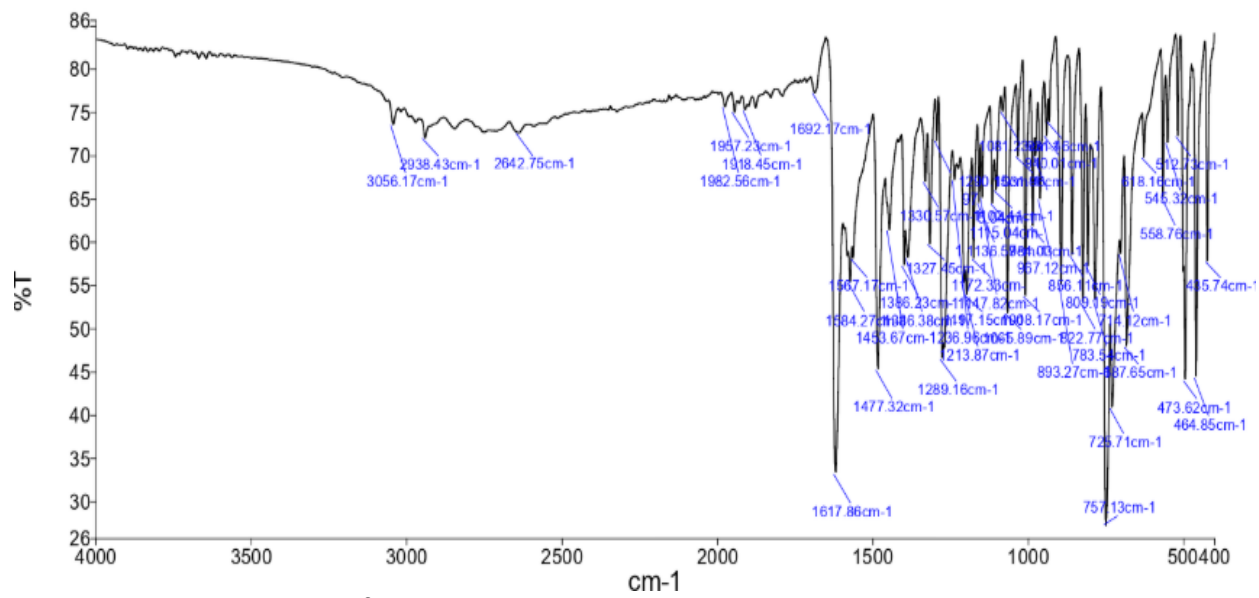


Figure S53. IR spectrum of compound AM11

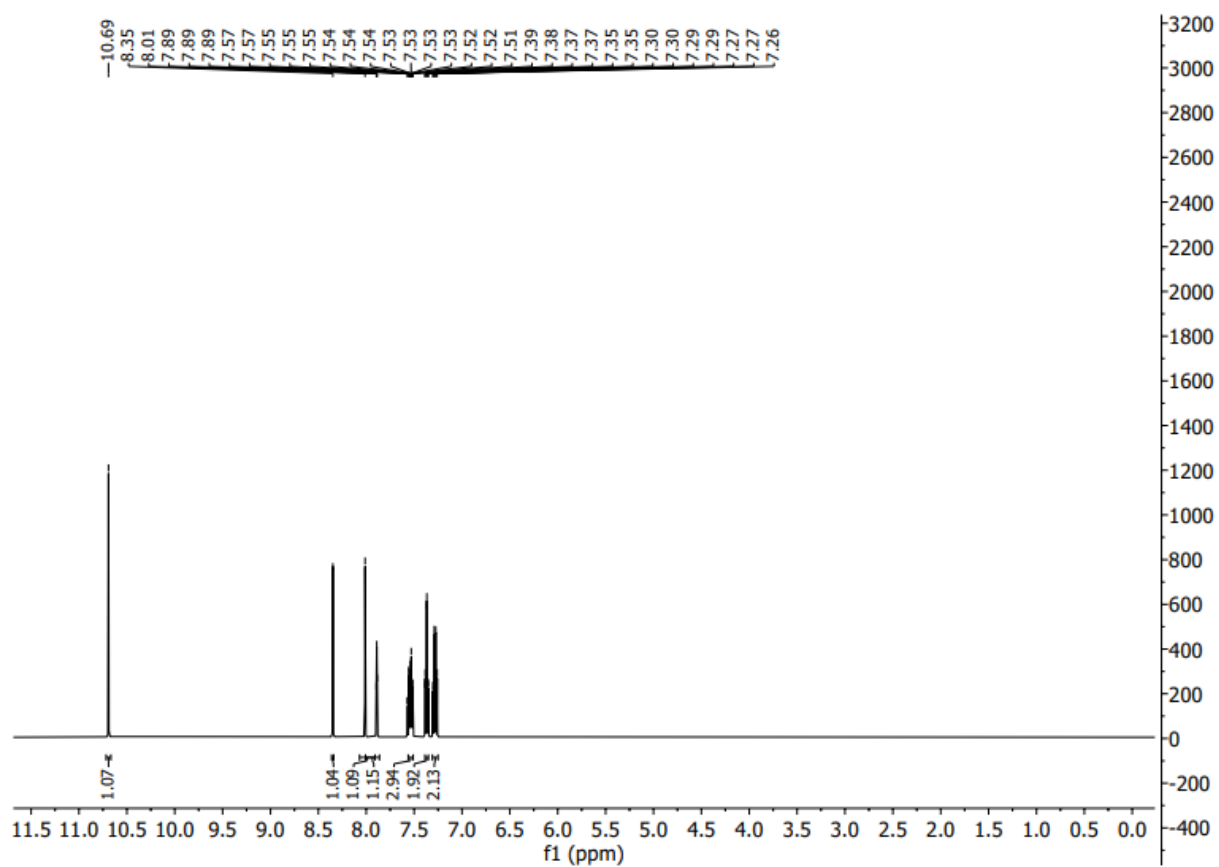


Figure S54. ¹H-NMR spectrum of compound AM11

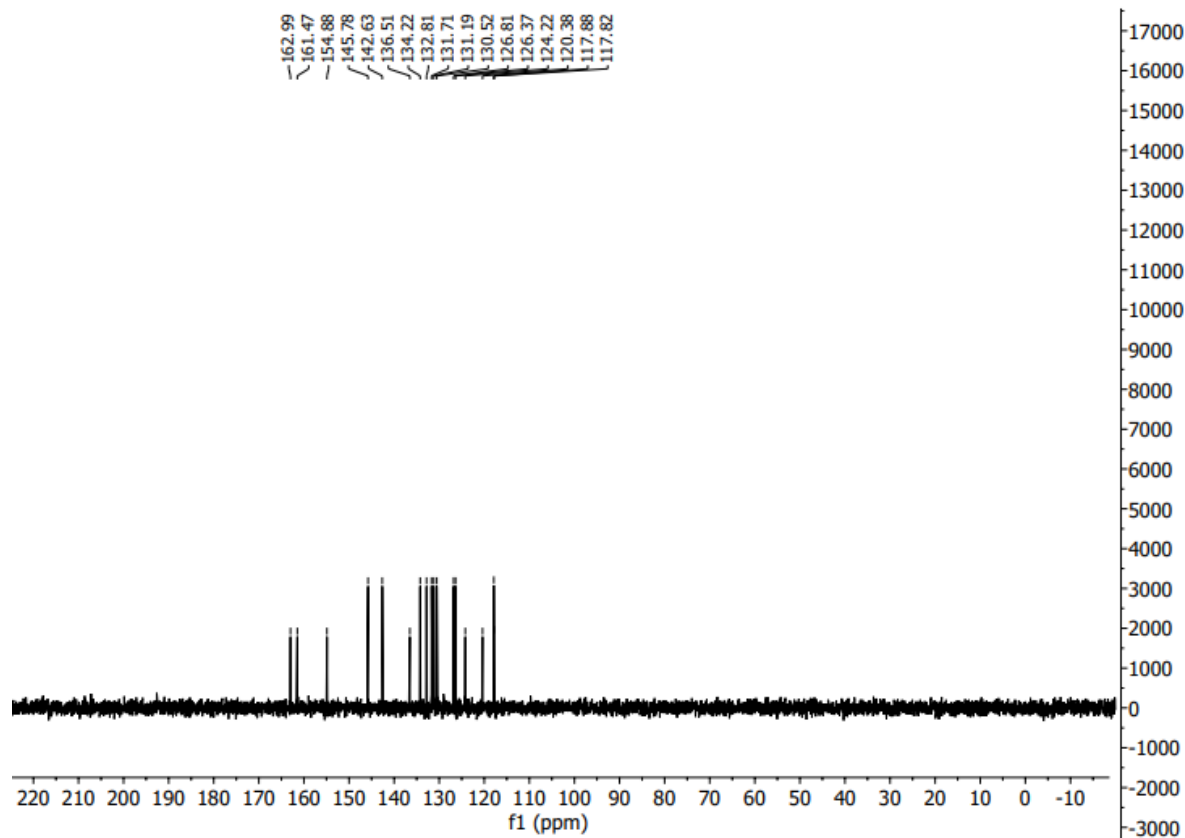


Figure S55. ^{13}C -NMR spectrum of compound AM11

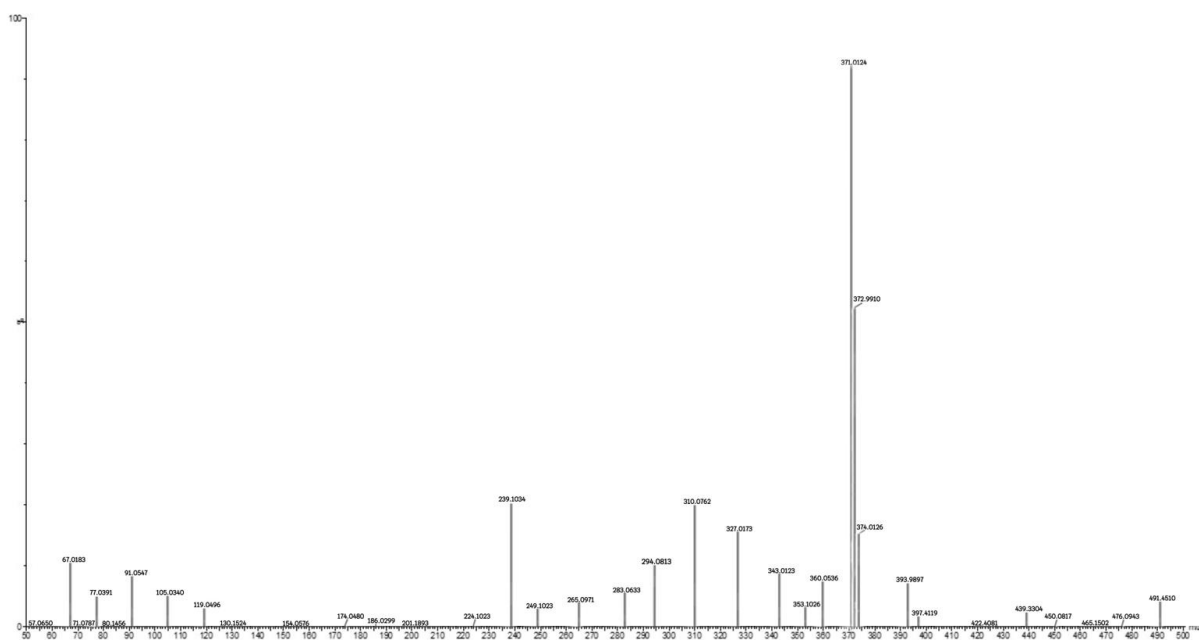


Figure S56. HRMS spectrum of compound AM11



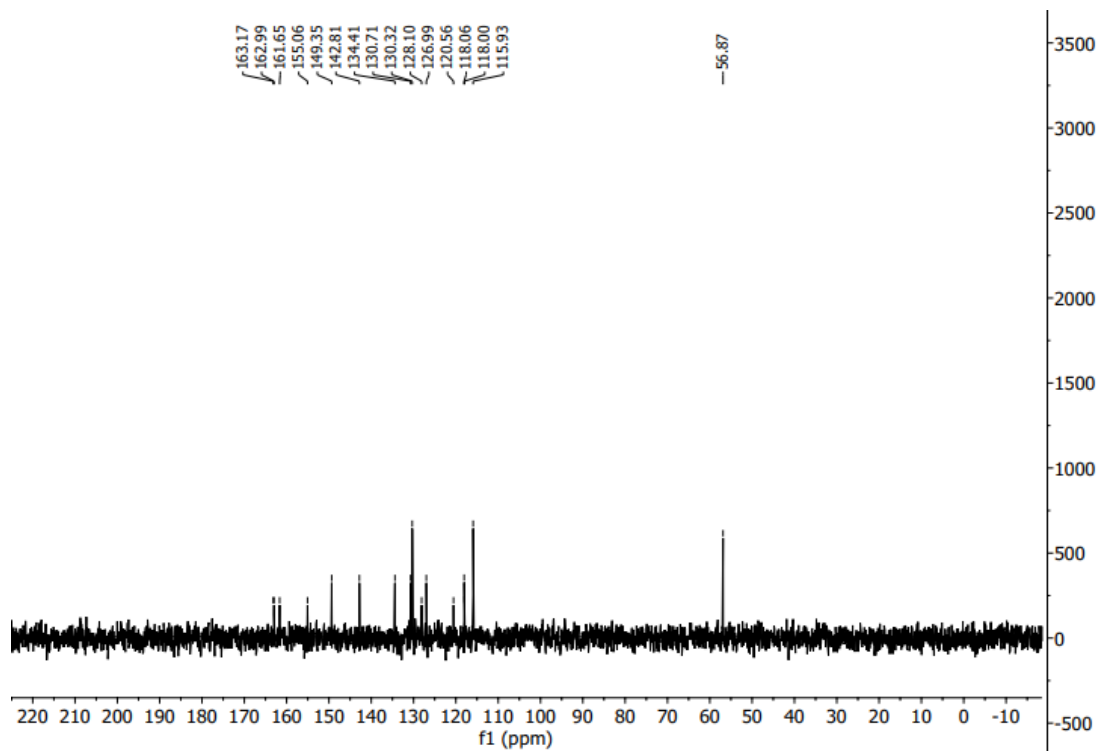


Figure S59. ^{13}C -NMR spectrum of compound AM12

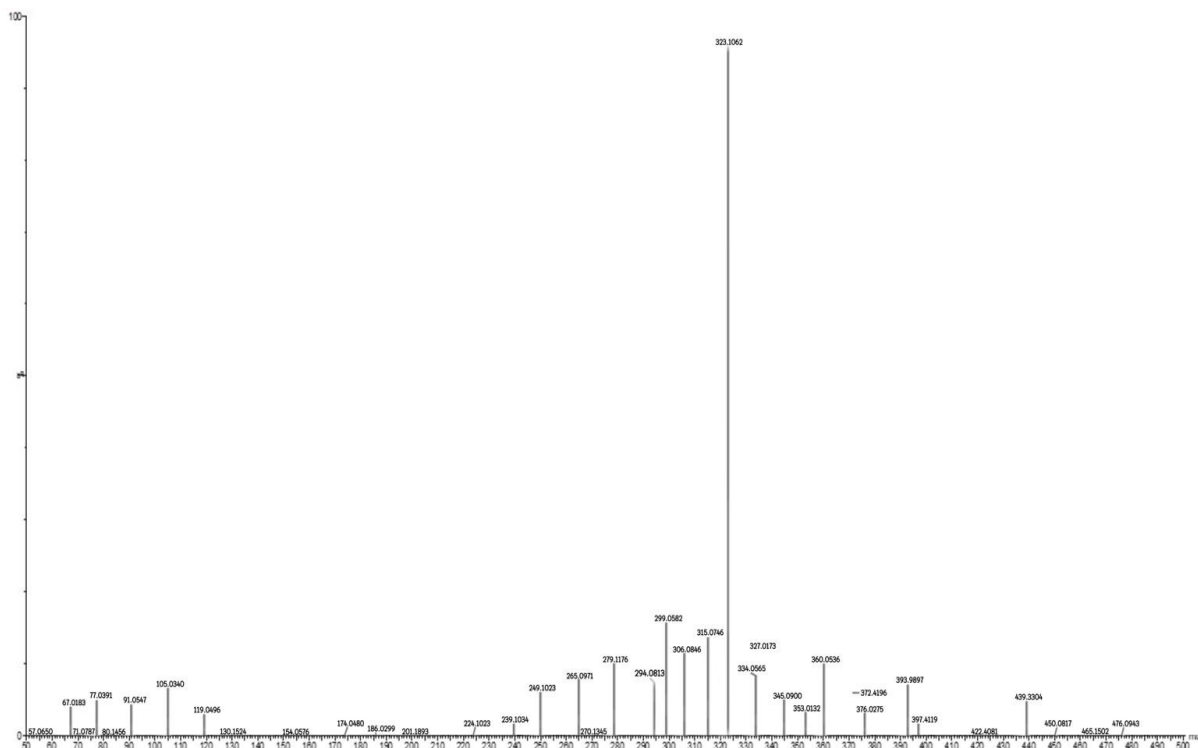


Figure S60. HRMS spectrum of compound AM12

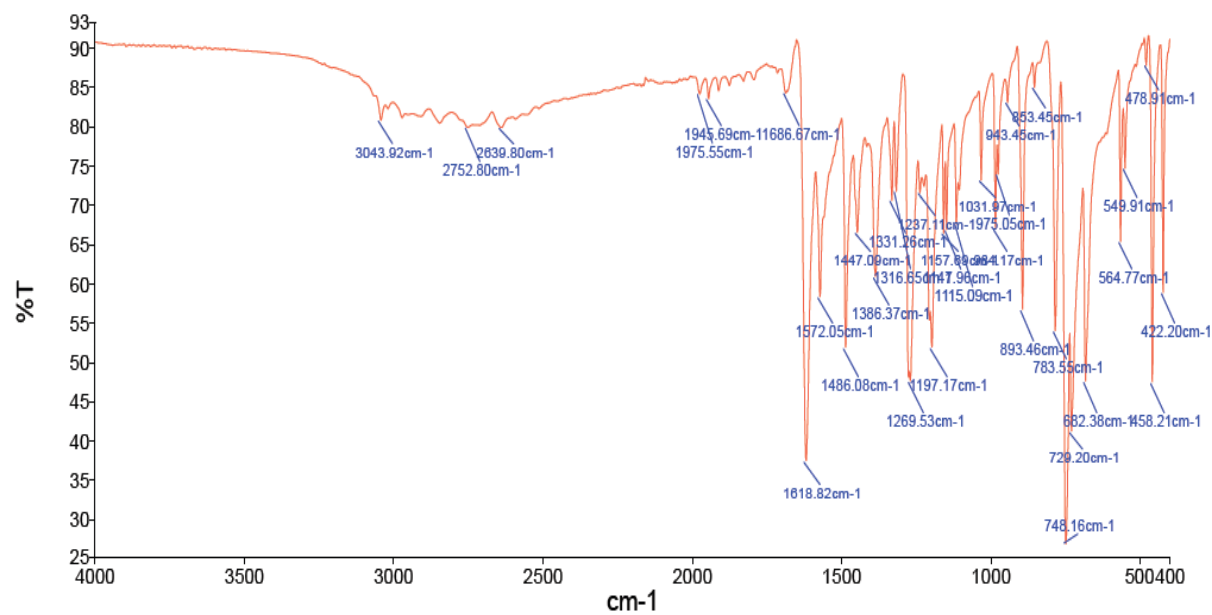


Figure S61. IR spectrum of compound AM13

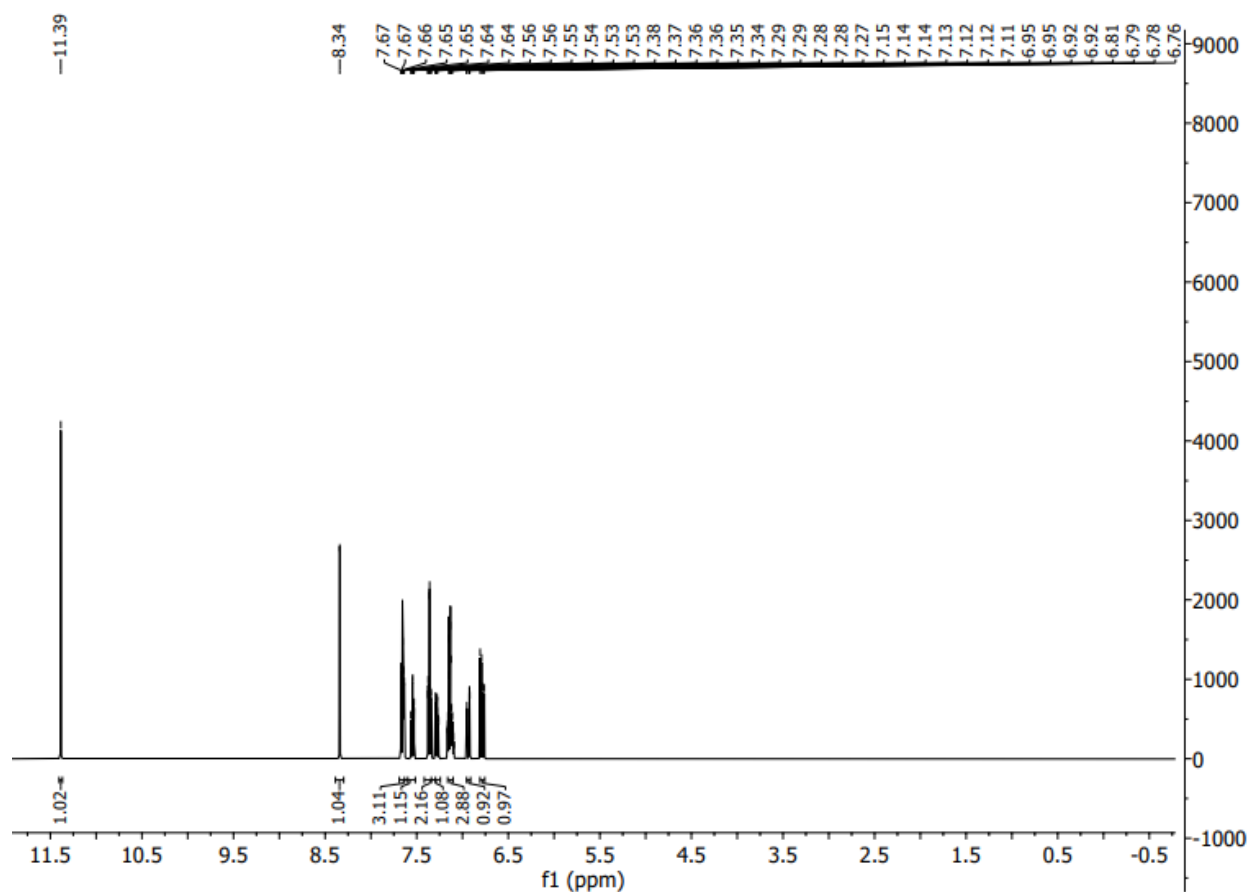


Figure S62. ¹H-NMR spectrum of compound AM13

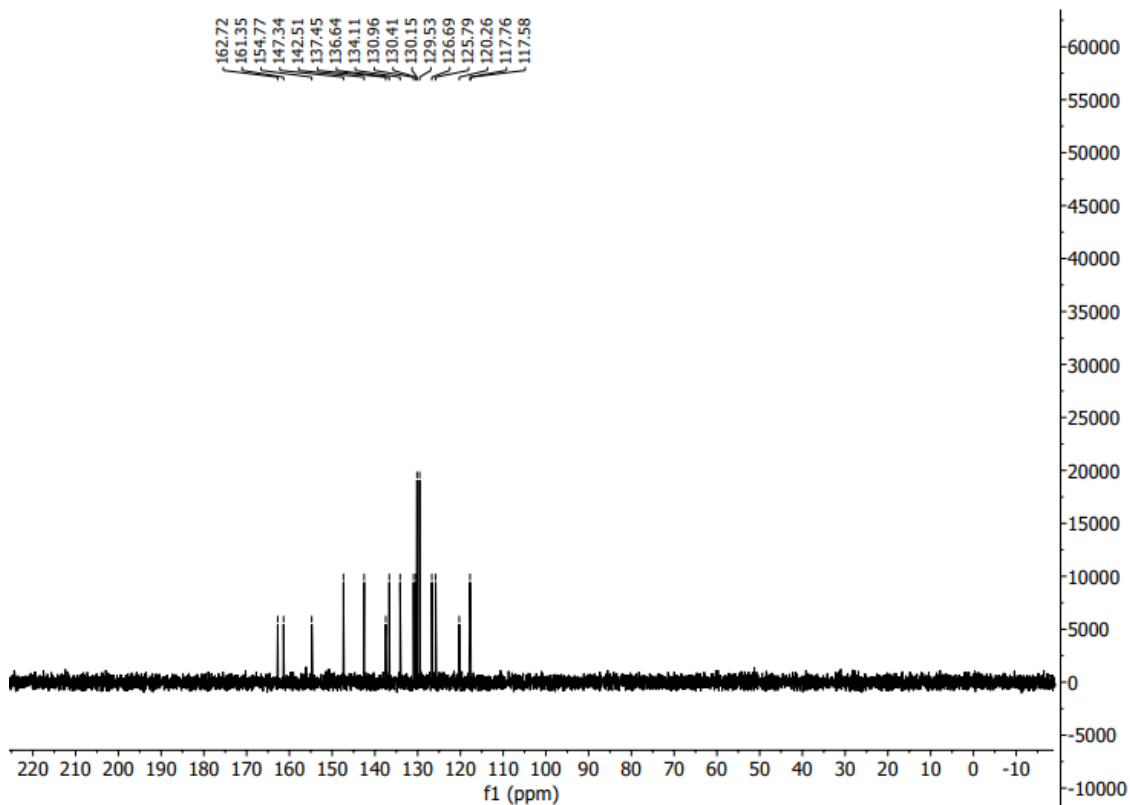


Figure S63. ^{13}C -NMR spectrum of compound AM13

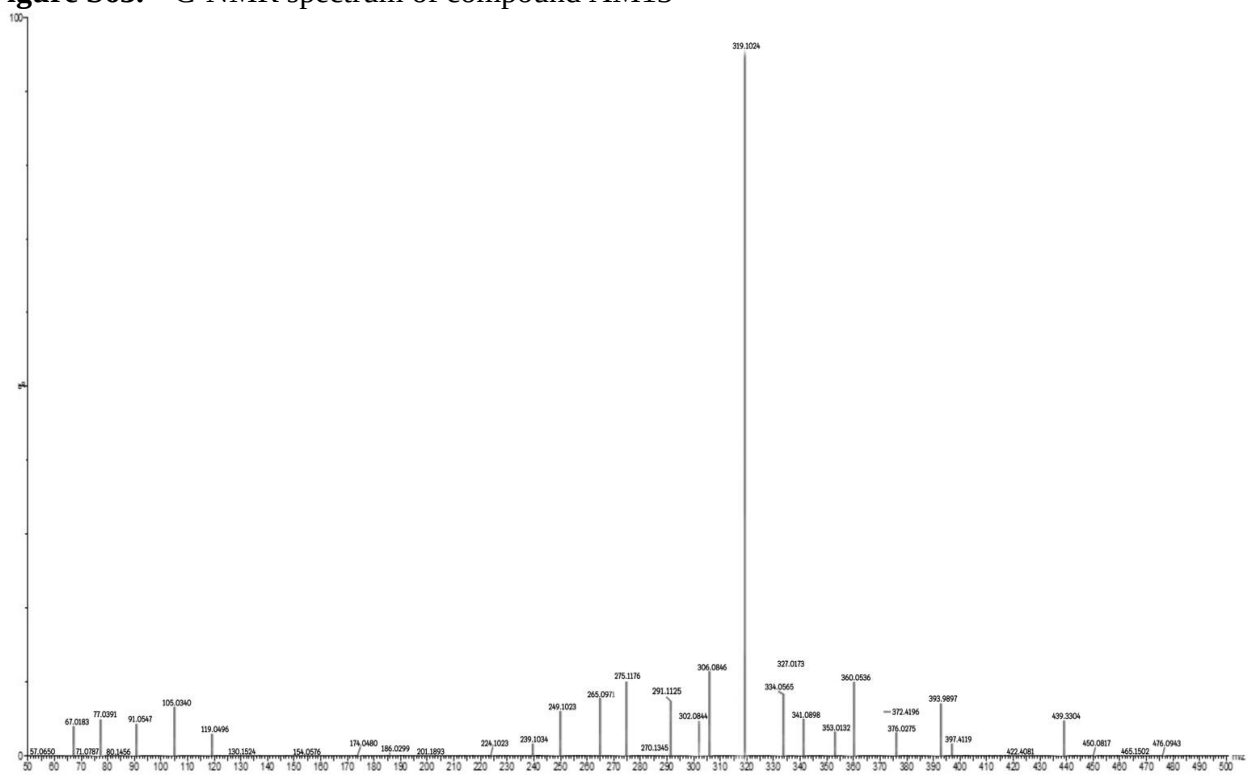


Figure S64. HRMS spectrum of compound AM13

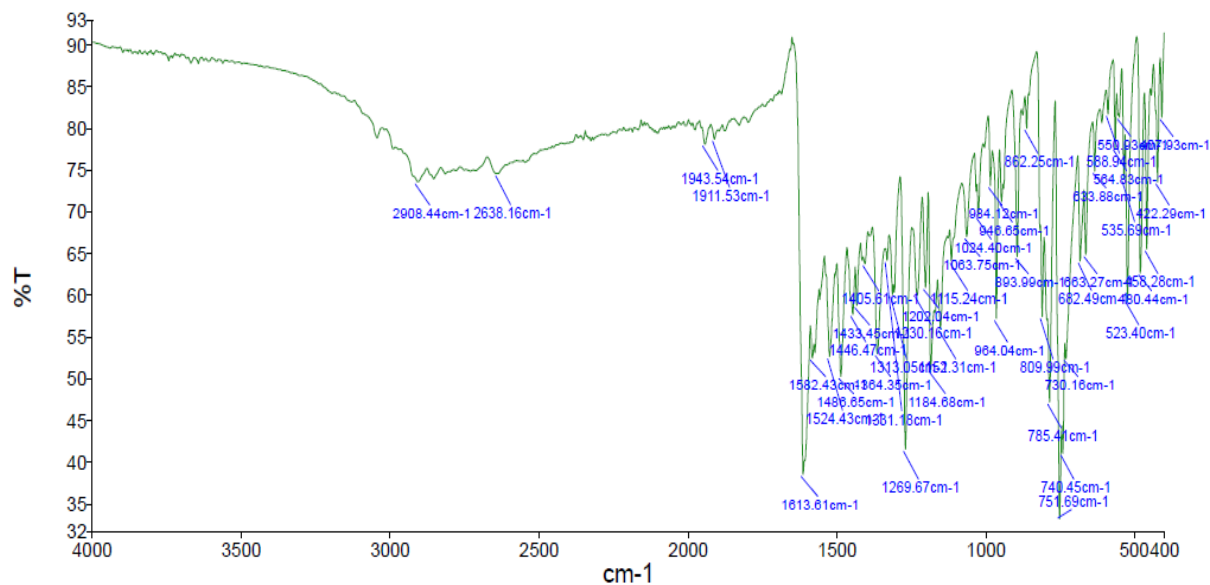


Figure S65. IR spectrum of compound AM14

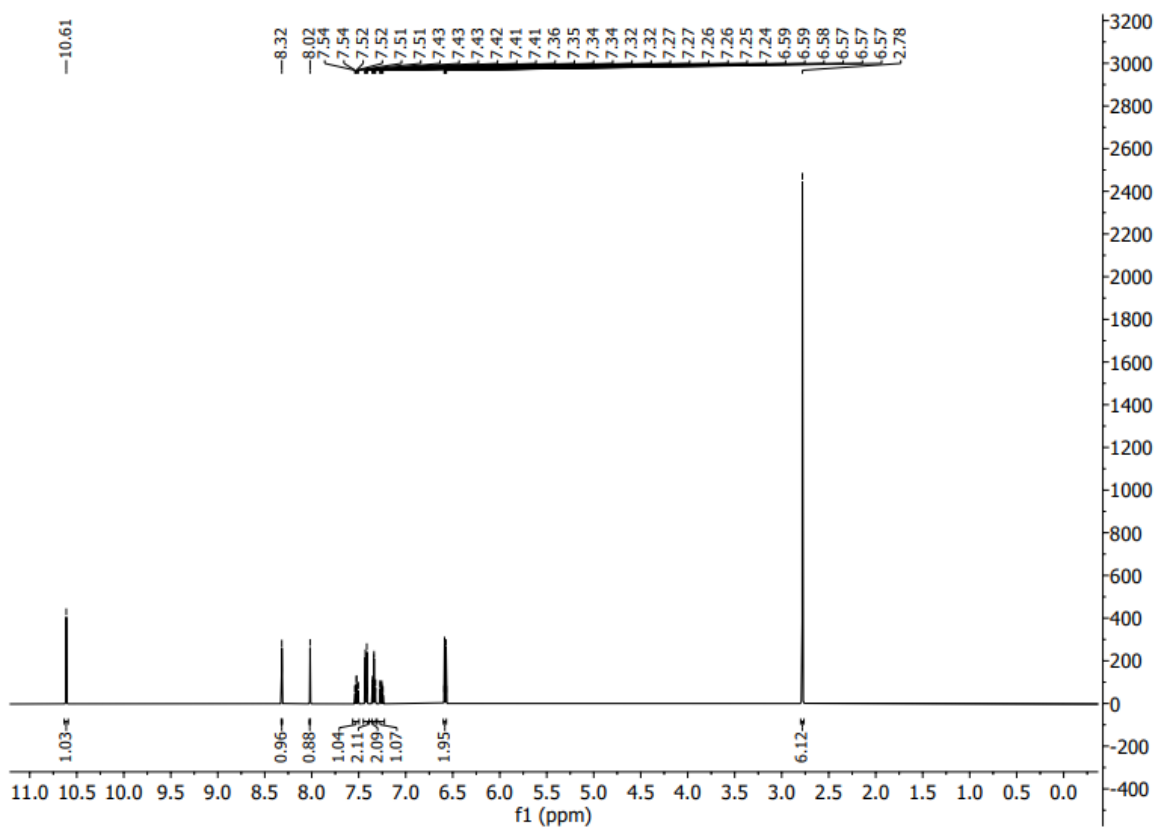


Figure S66. ¹H-NMR spectrum of compound AM14

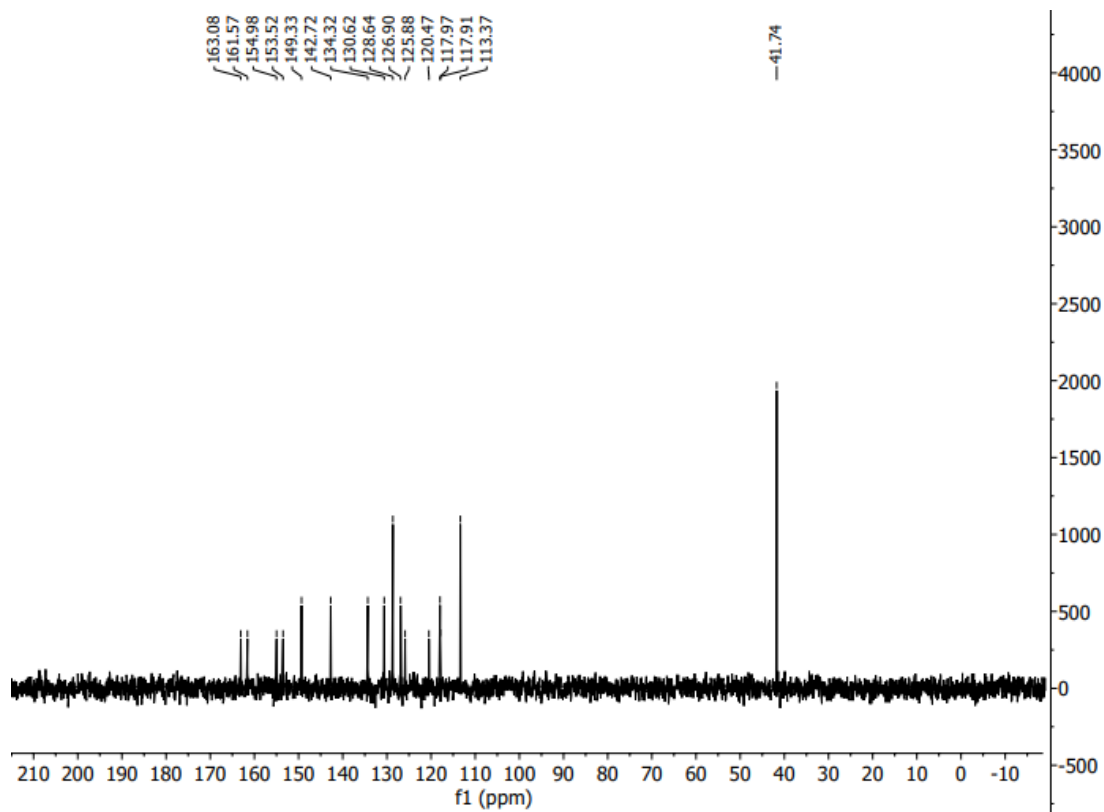


Figure S67. ^{13}C -NMR spectrum of compound AM14

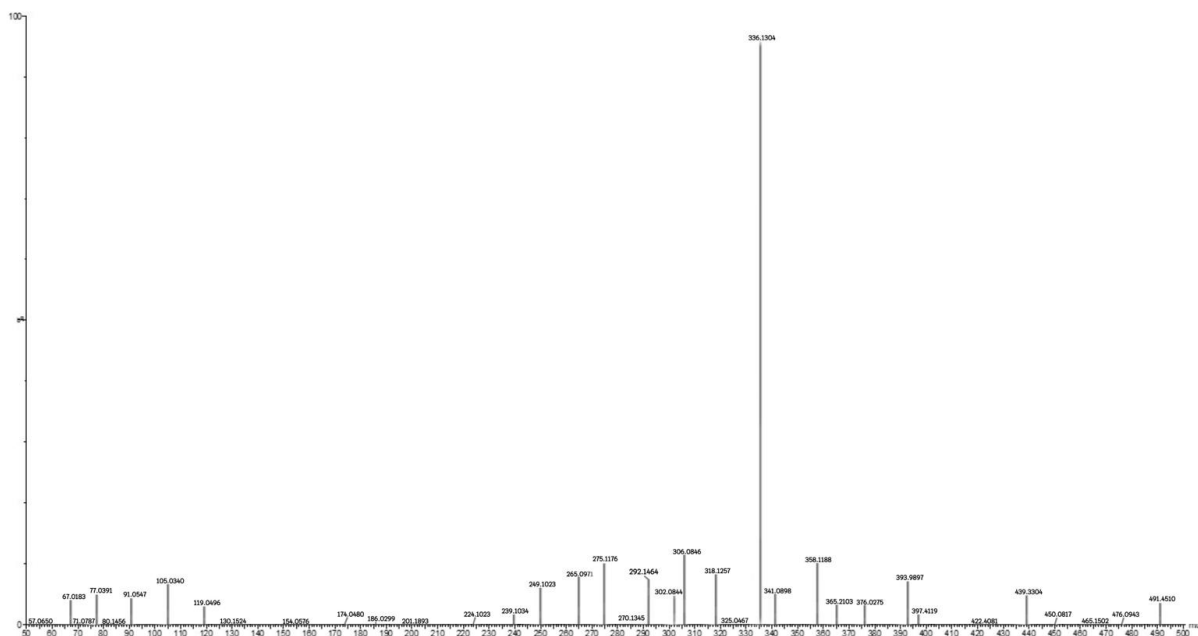


Figure S68. HRMS spectrum of compound AM14

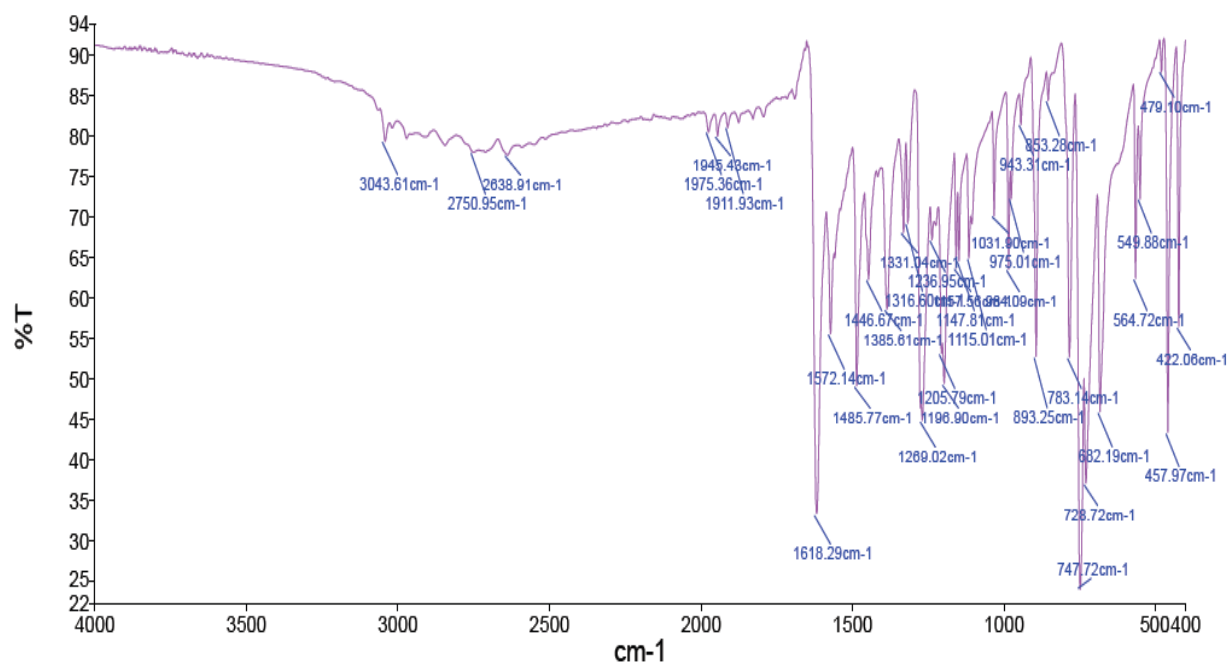
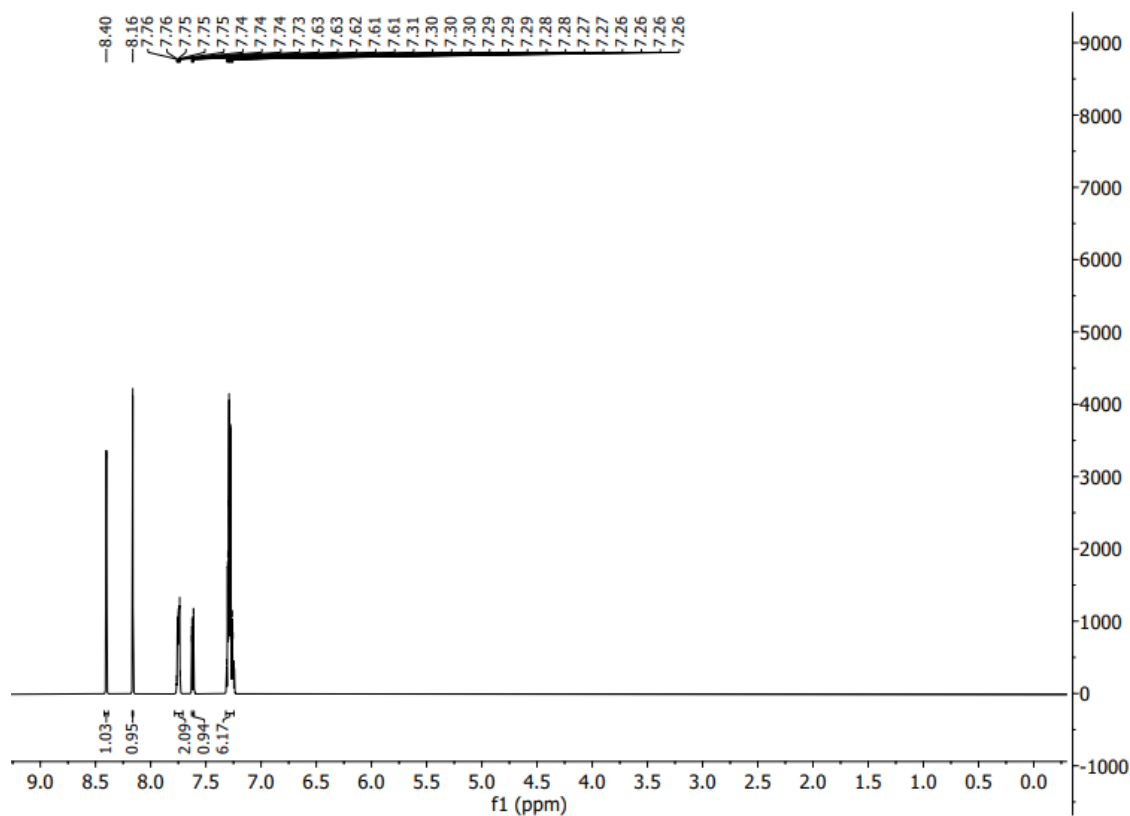


Figure S69. IR spectrum of compound AM15



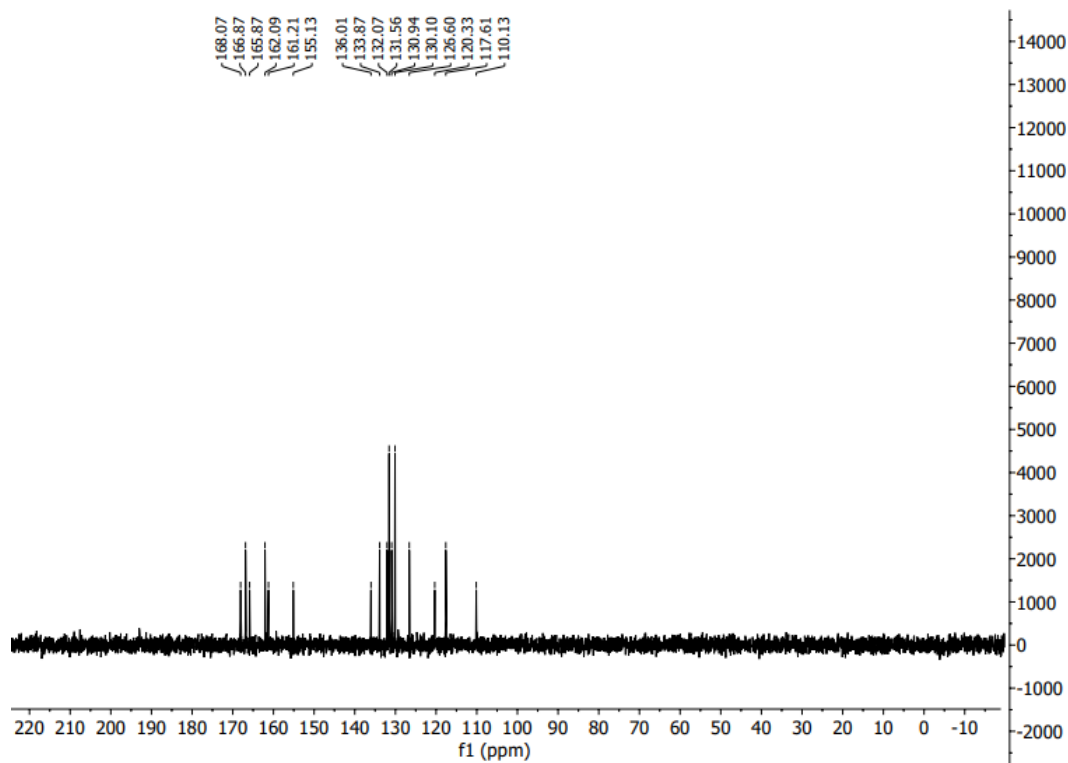


Figure S71. ^{13}C -NMR spectrum of compound AM15

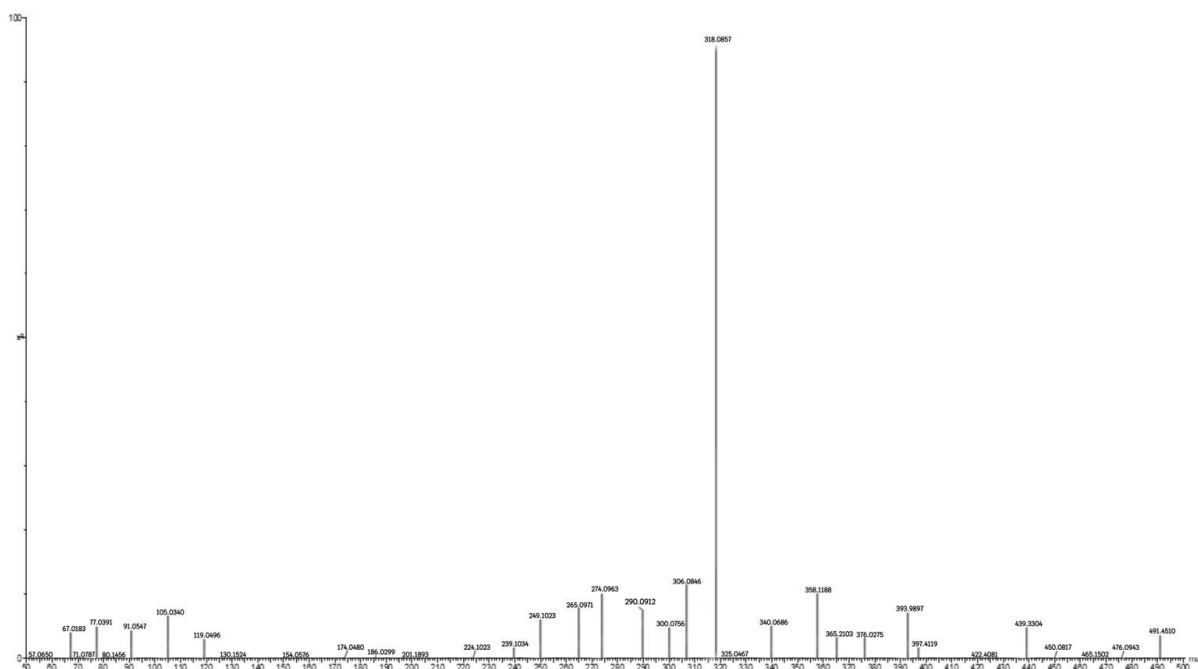


Figure S72. HRMS spectrum of compound AM15

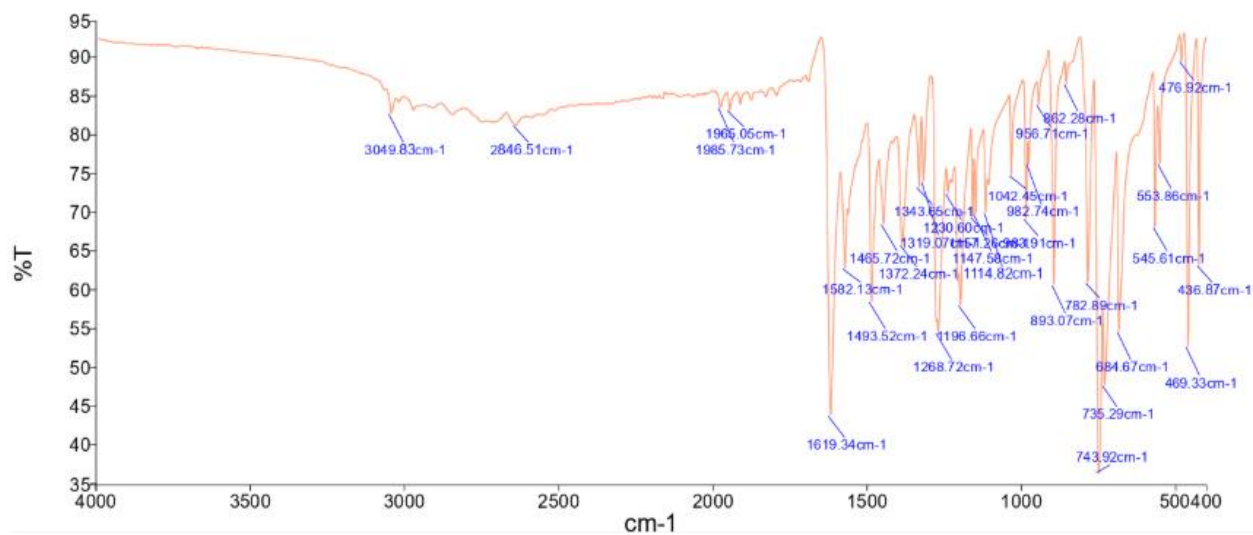


Figure S73. IR spectrum of compound AM16

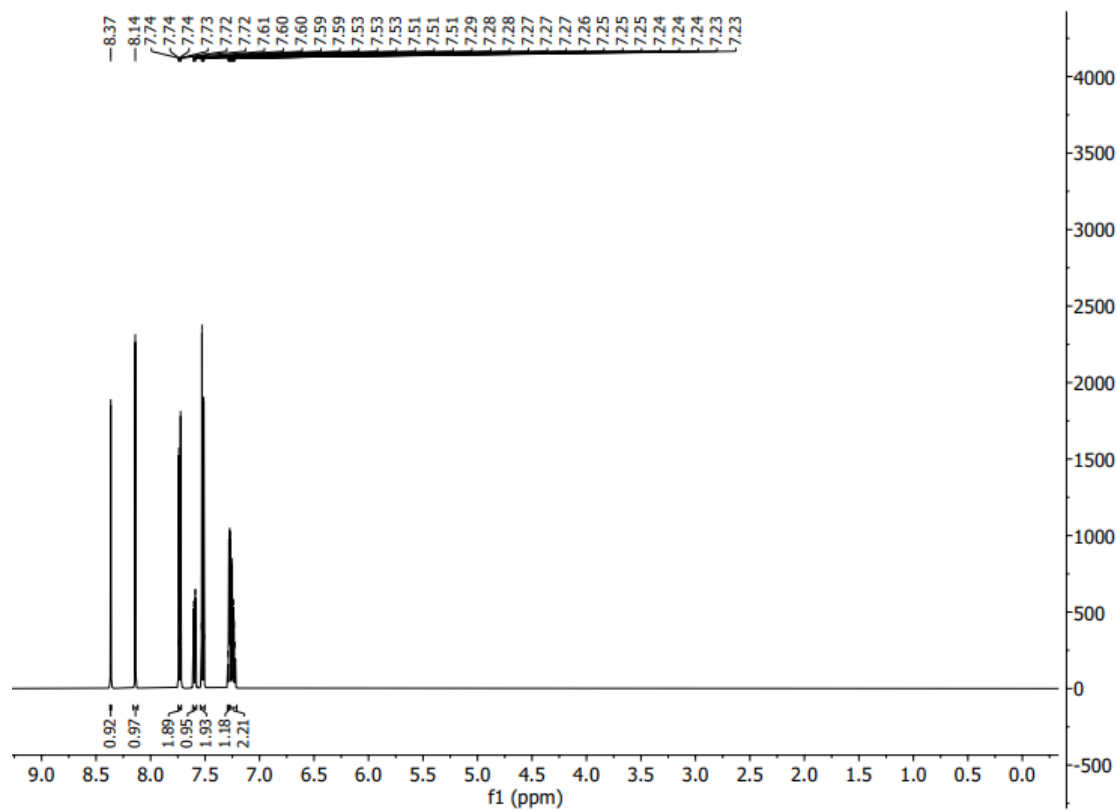


Figure S74. ¹H-NMR spectrum of compound AM16

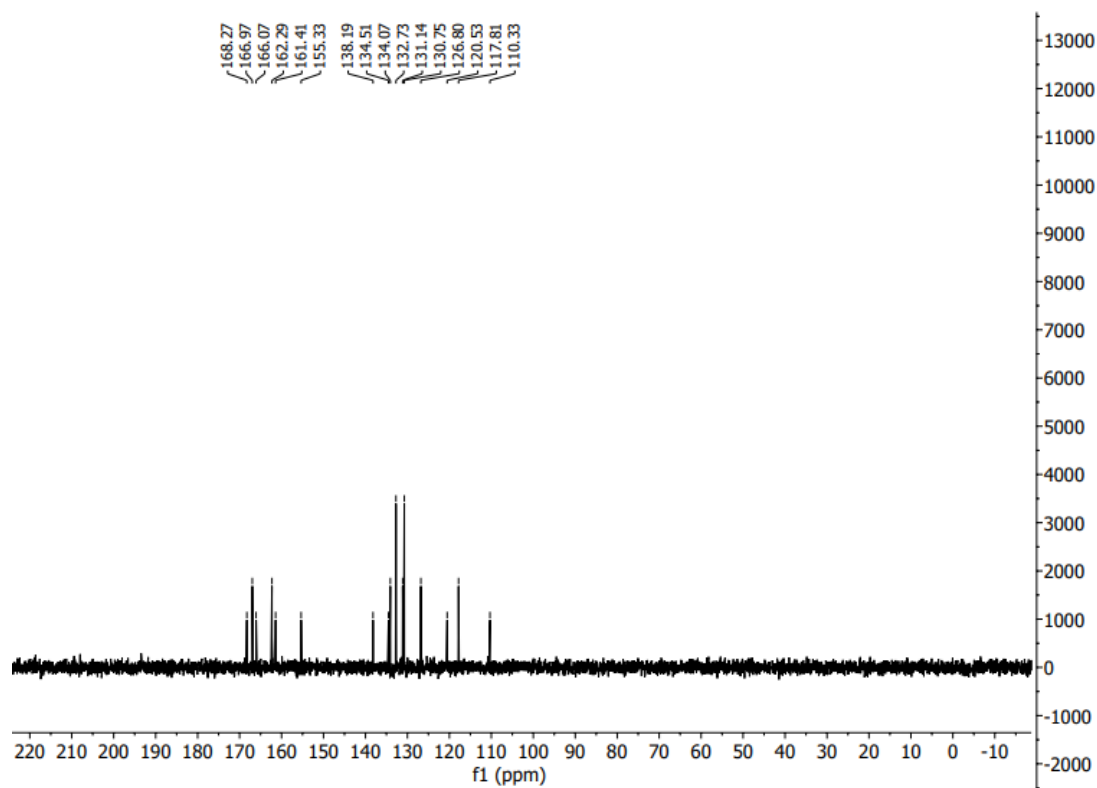


Figure S75. ^{13}C -NMR spectrum of compound AM16

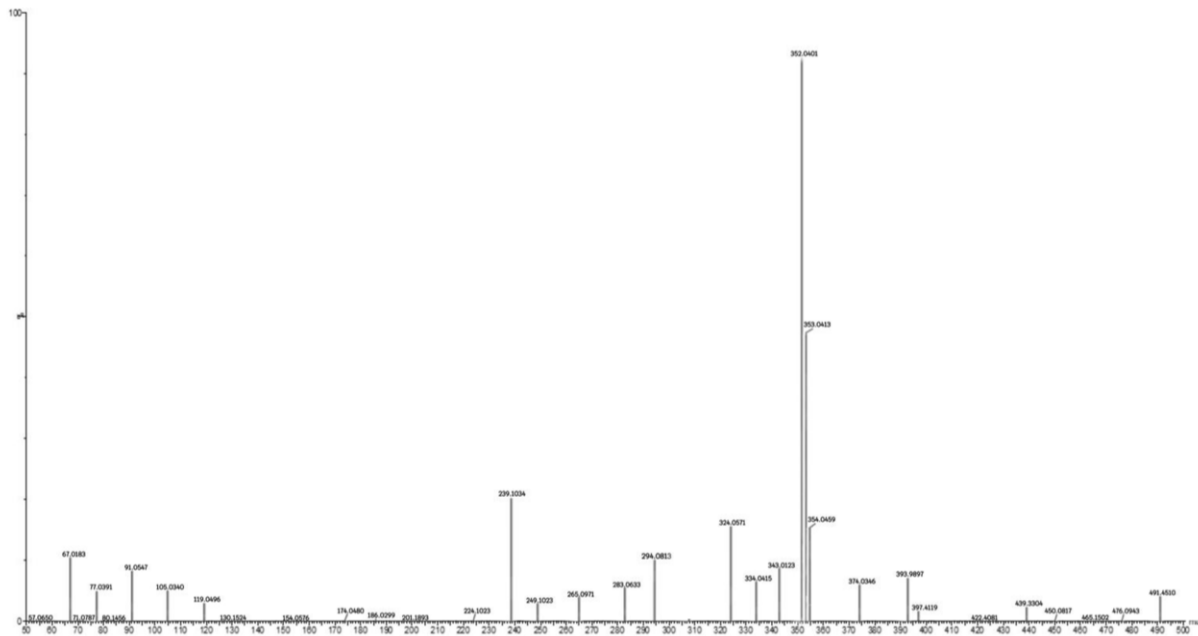


Figure S76. HRMS spectrum of compound AM16

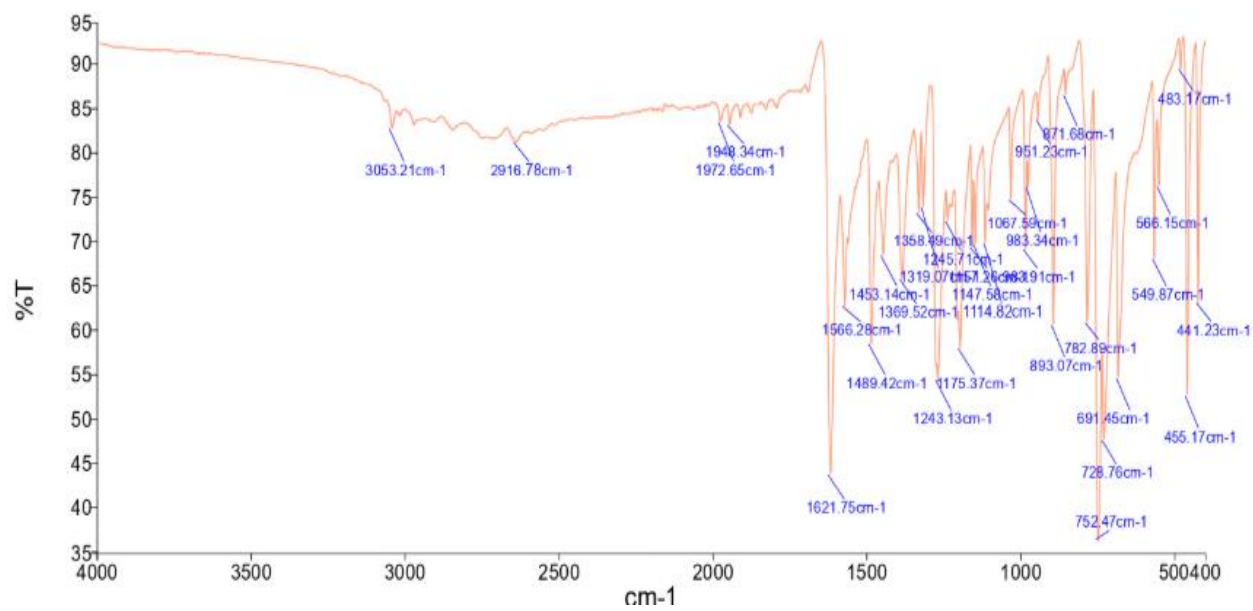


Figure S77. IR spectrum of compound AM17

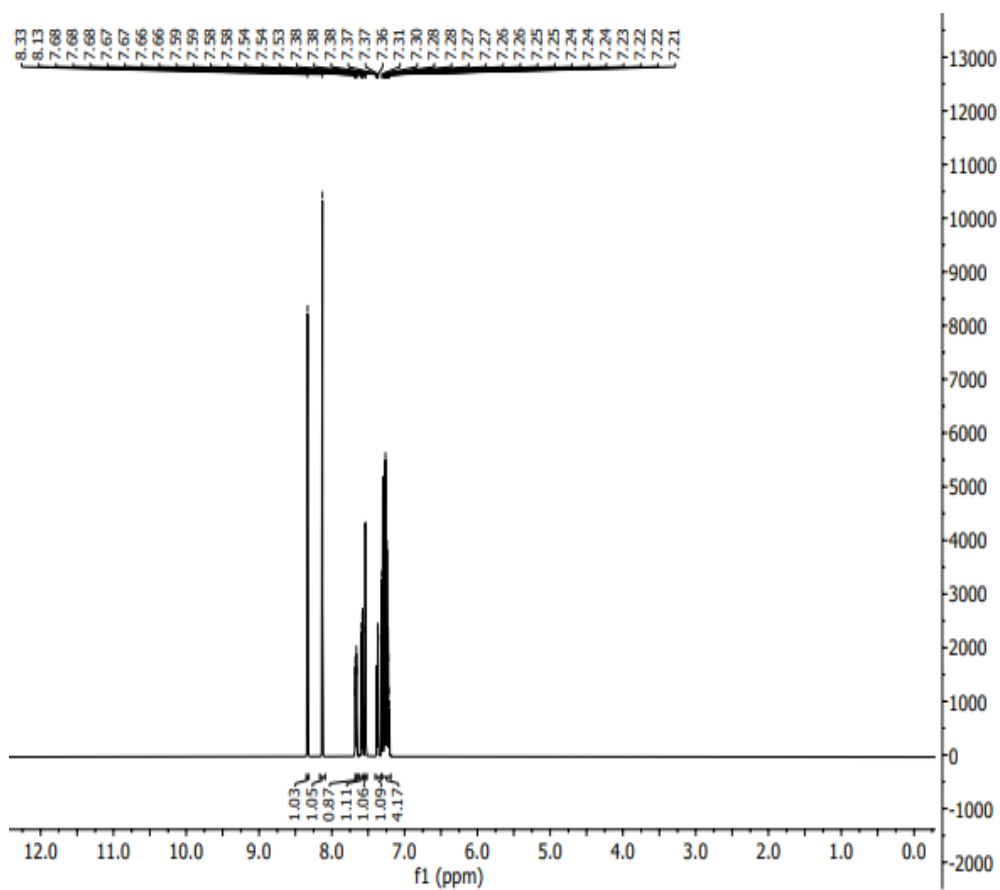


Figure S78. ¹H-NMR spectrum of compound AM17

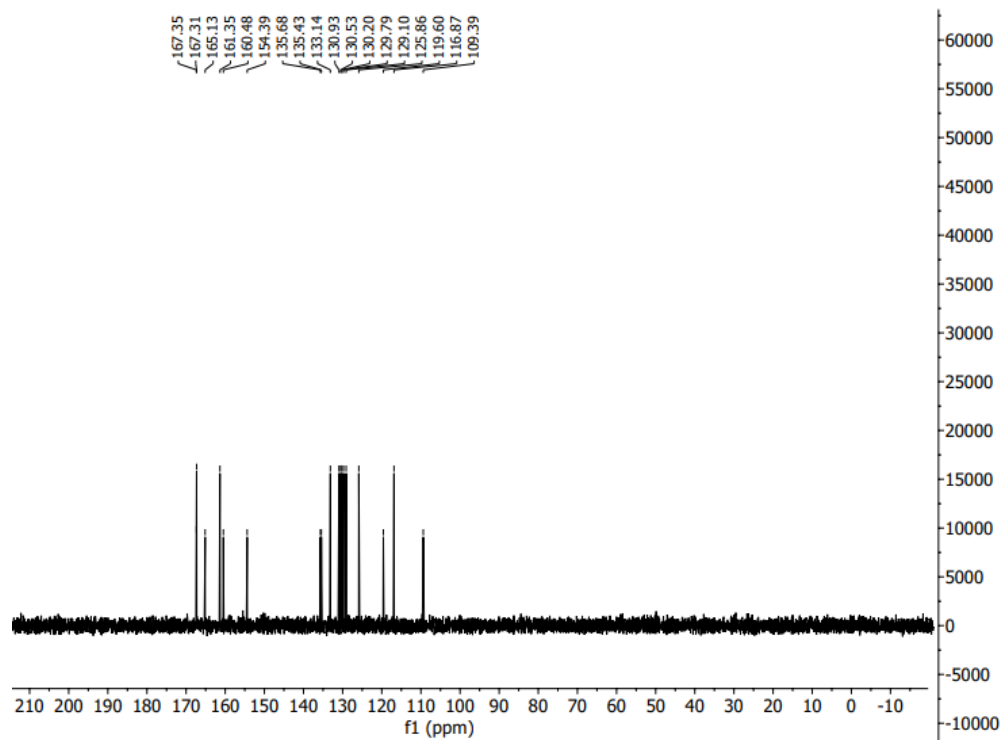


Figure S79. ^{13}C -NMR spectrum of compound AM17

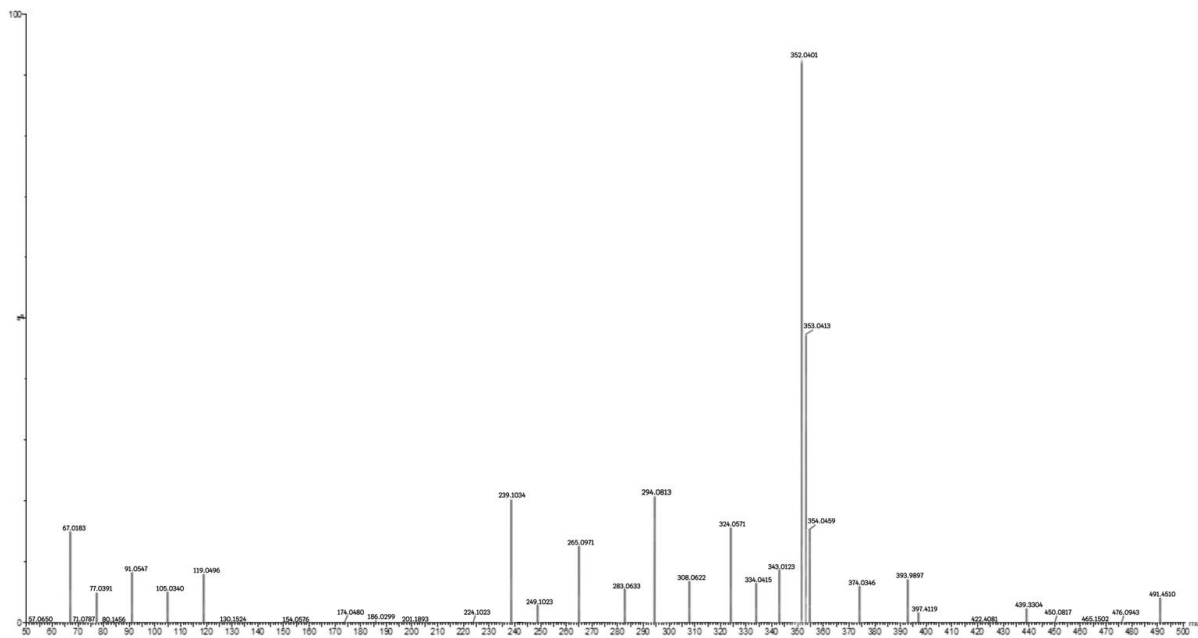


Figure S80. HRMS spectrum of compound AM17

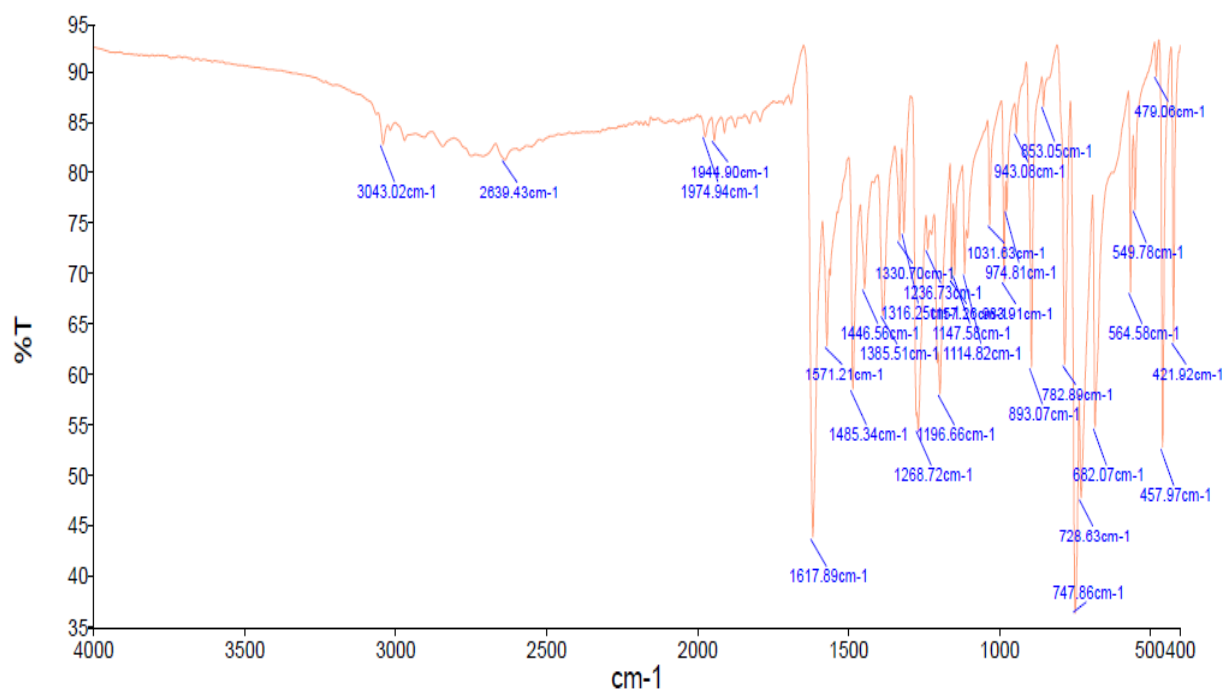


Figure S81. IR spectrum of compound AM18

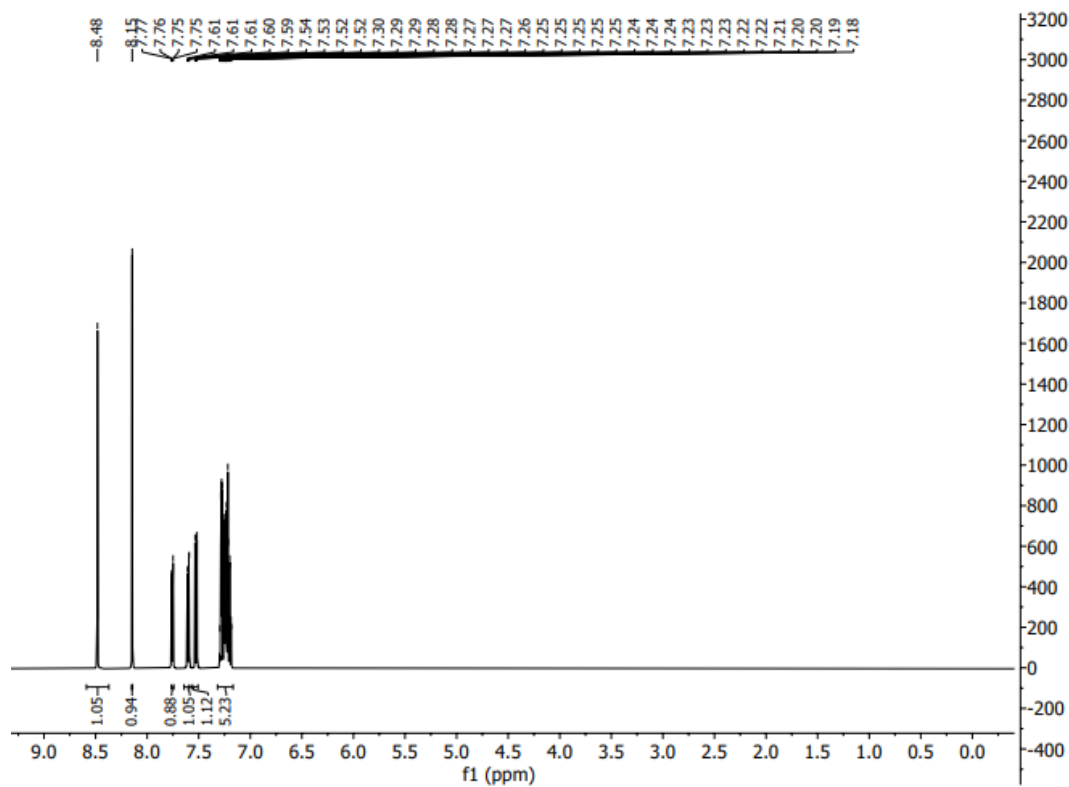


Figure S82. ¹H-NMR spectrum of compound AM18

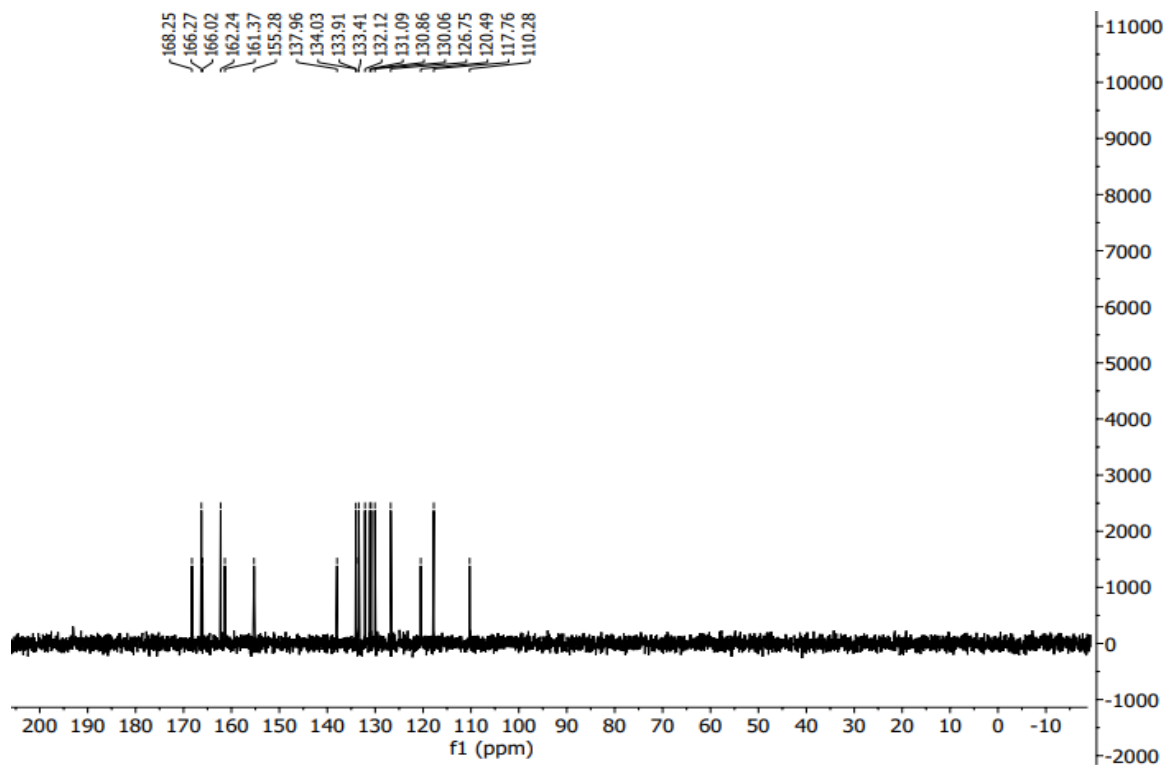


Figure S83. ^{13}C -NMR spectrum of compound AM18

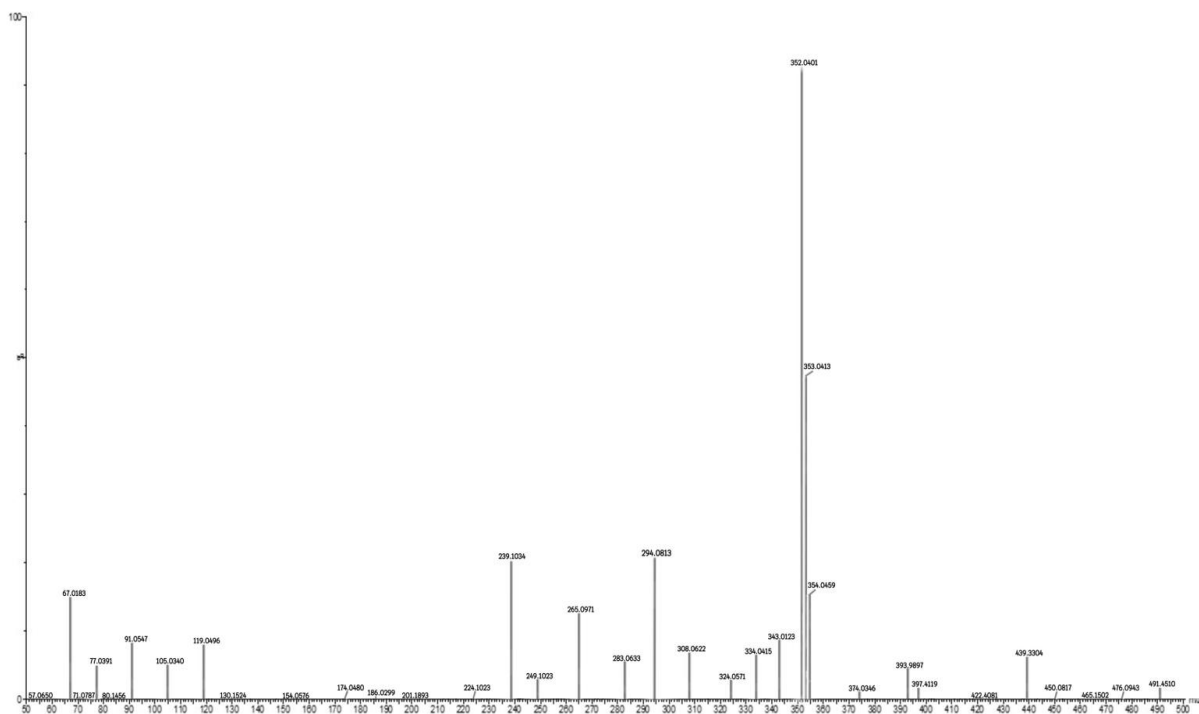


Figure S84. HRMS spectrum of compound AM18

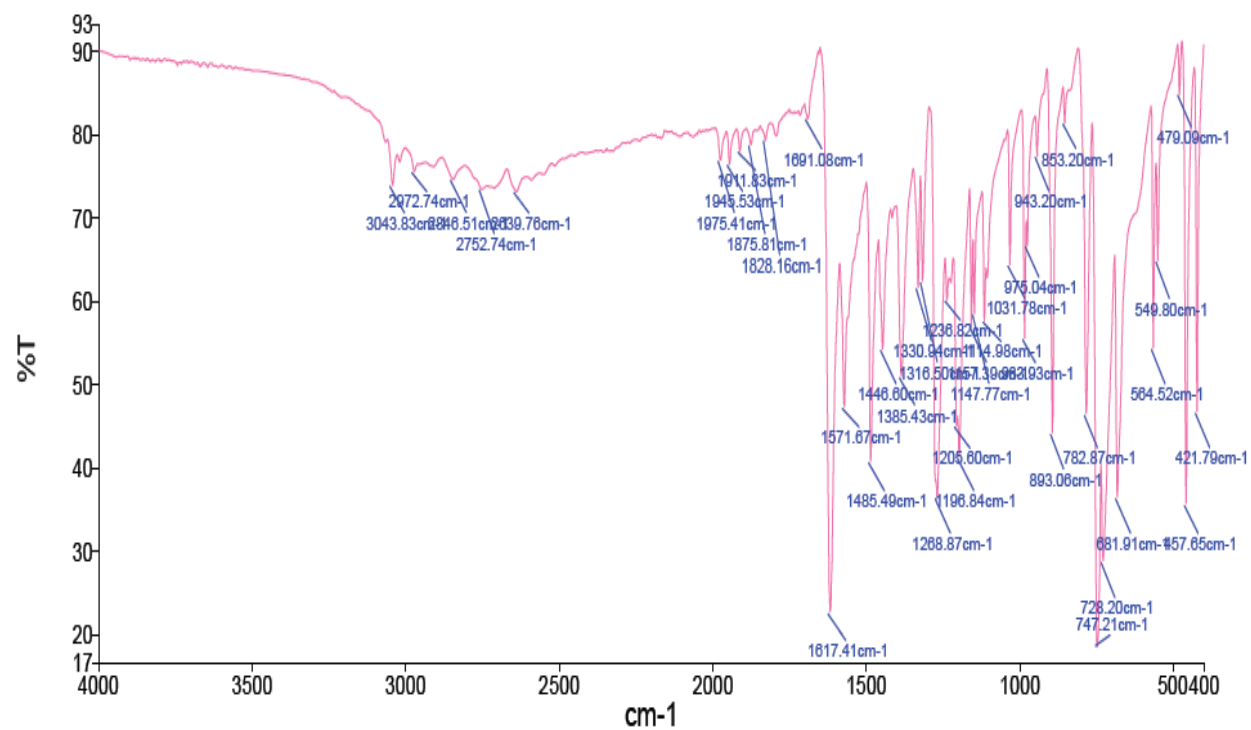


Figure S85. IR spectrum of compound AM19

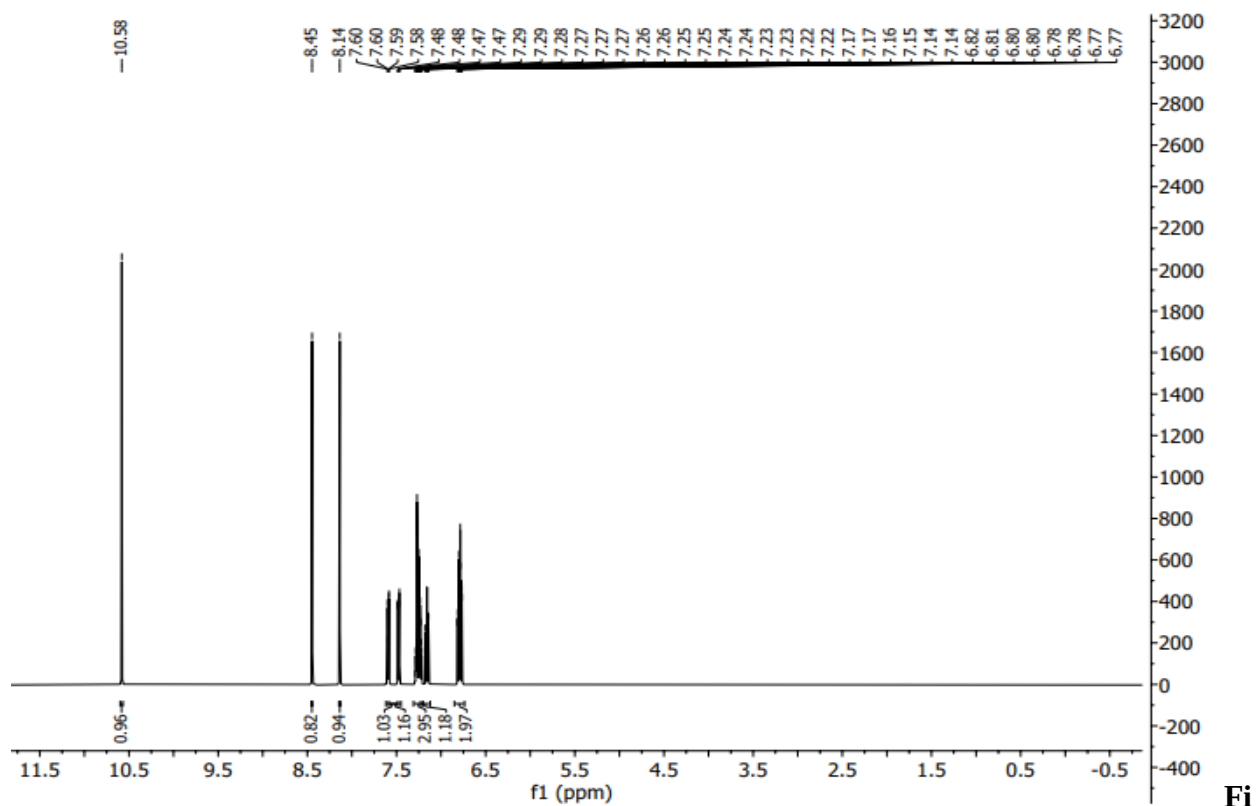


Figure S86. ¹H-NMR spectrum of compound AM19

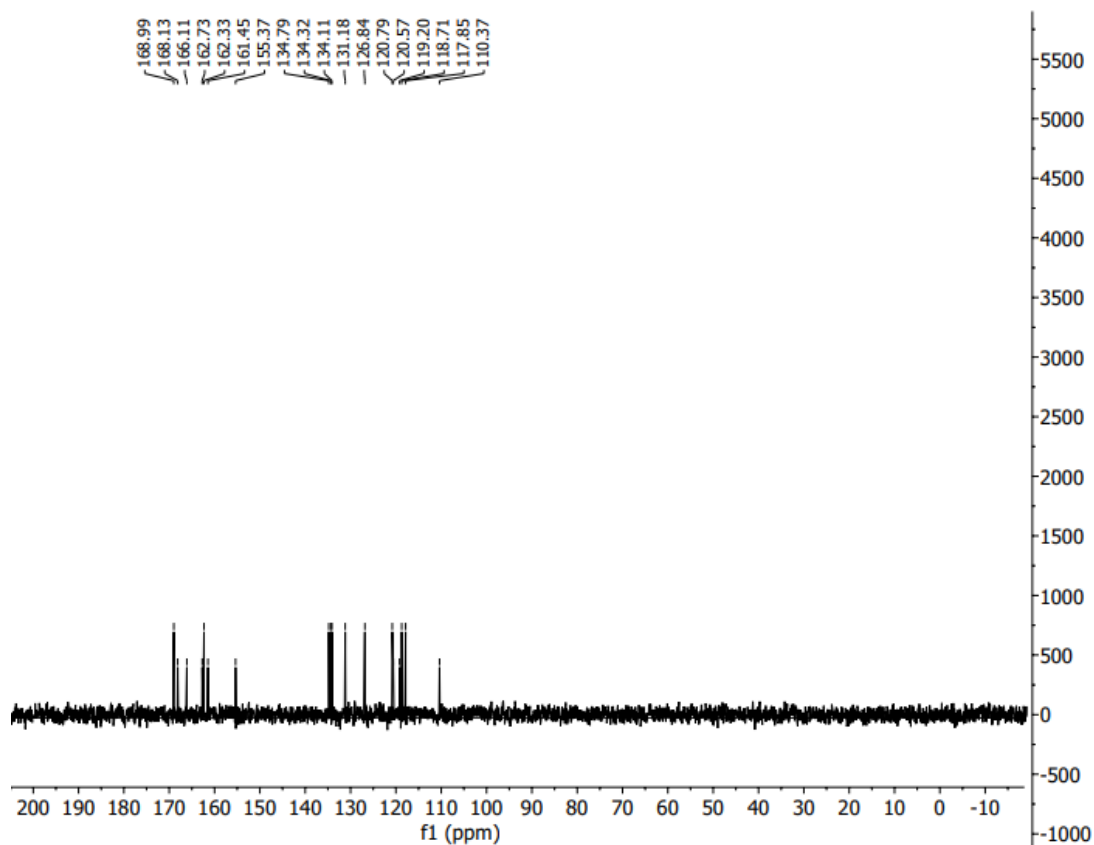


Figure S87. ^{13}C -NMR spectrum of compound AM19

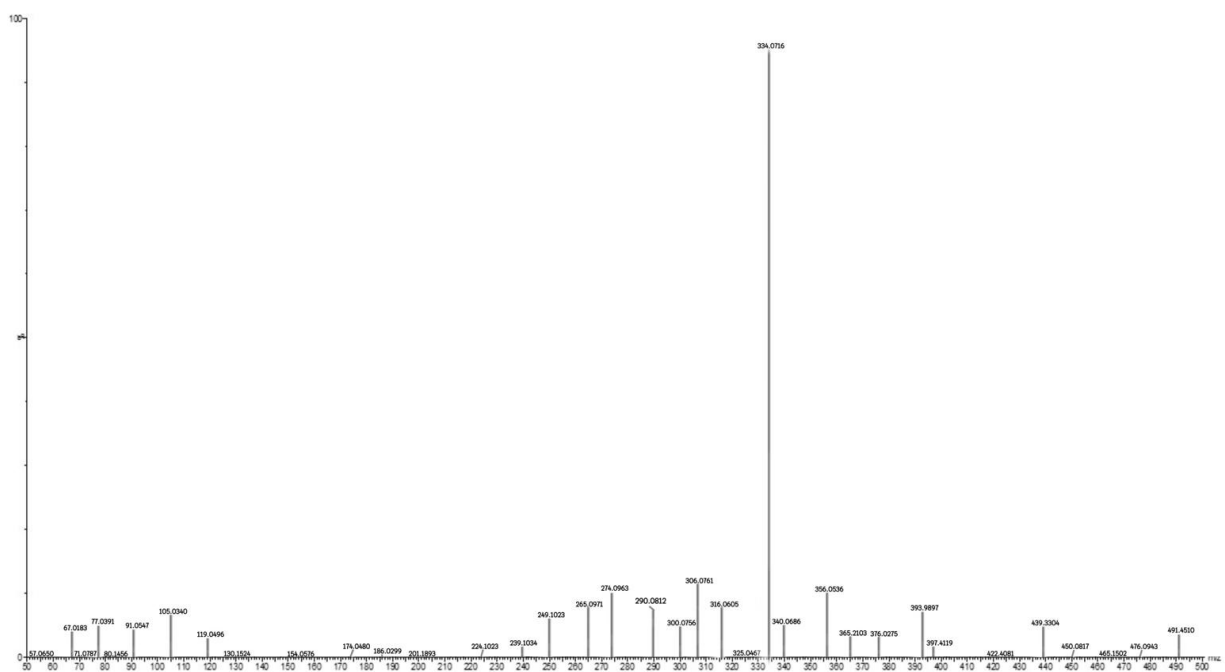


Figure S88. HRMS spectrum of compound AM19

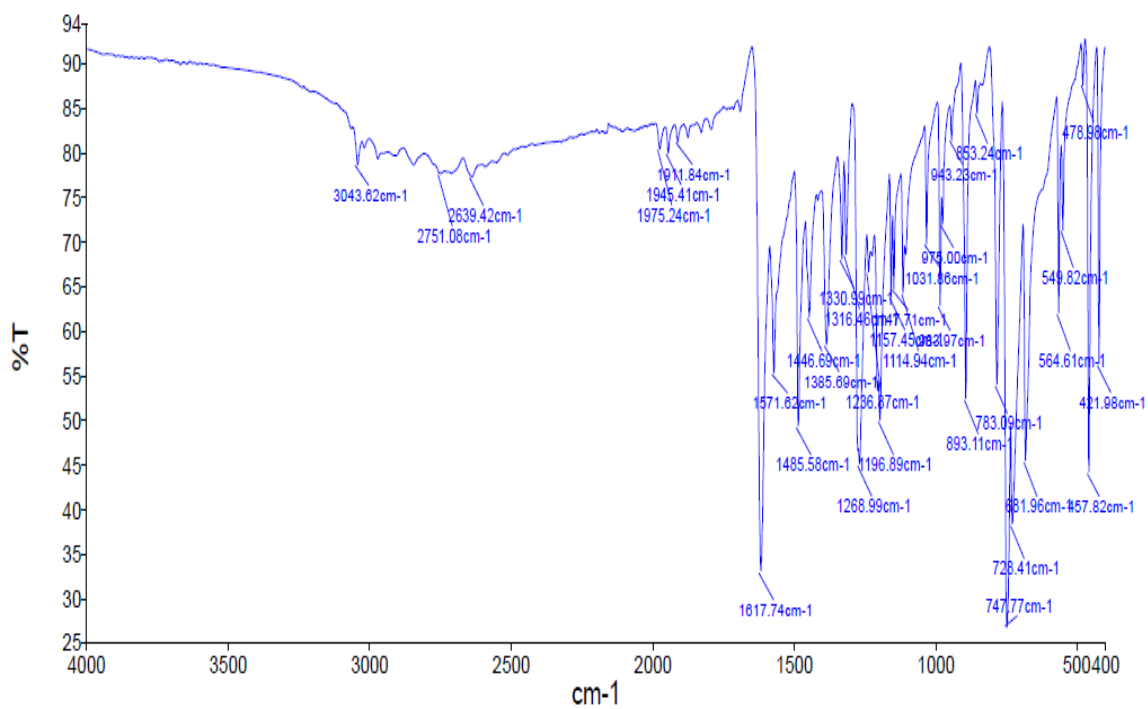


Figure S89. IR spectrum of compound AM20

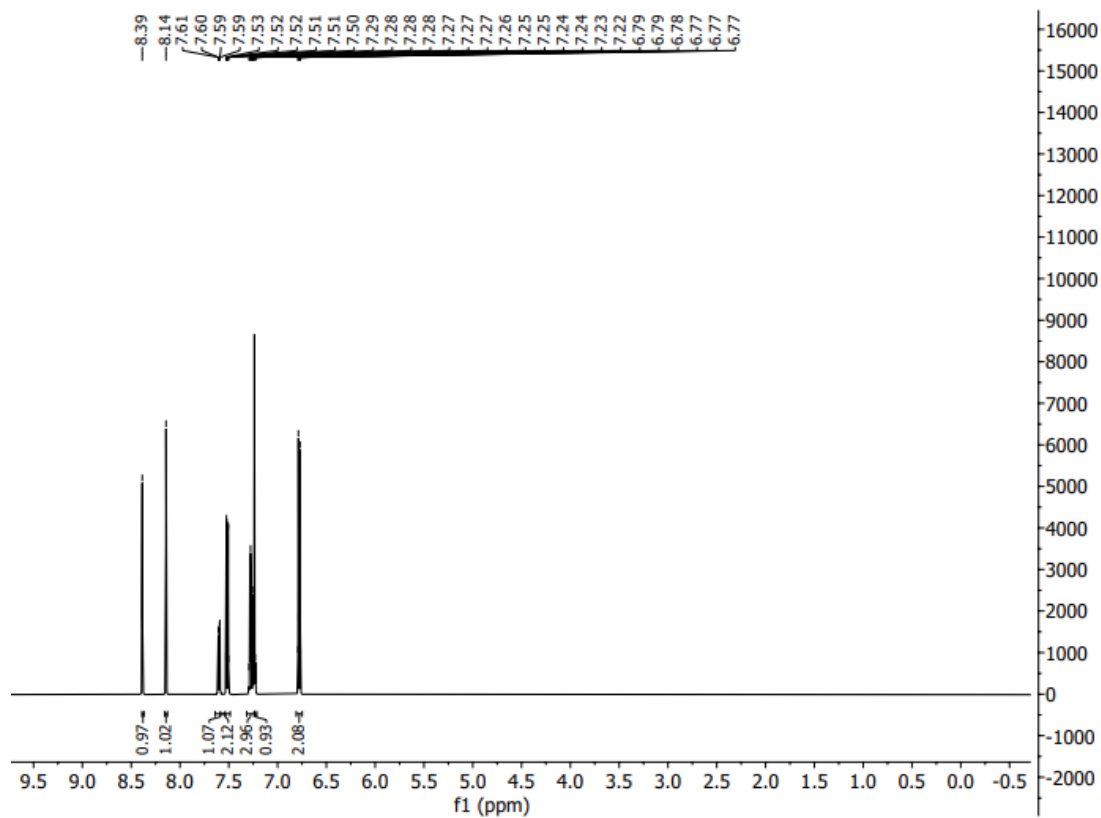


Figure S90. ¹H-NMR spectrum of compound AM20

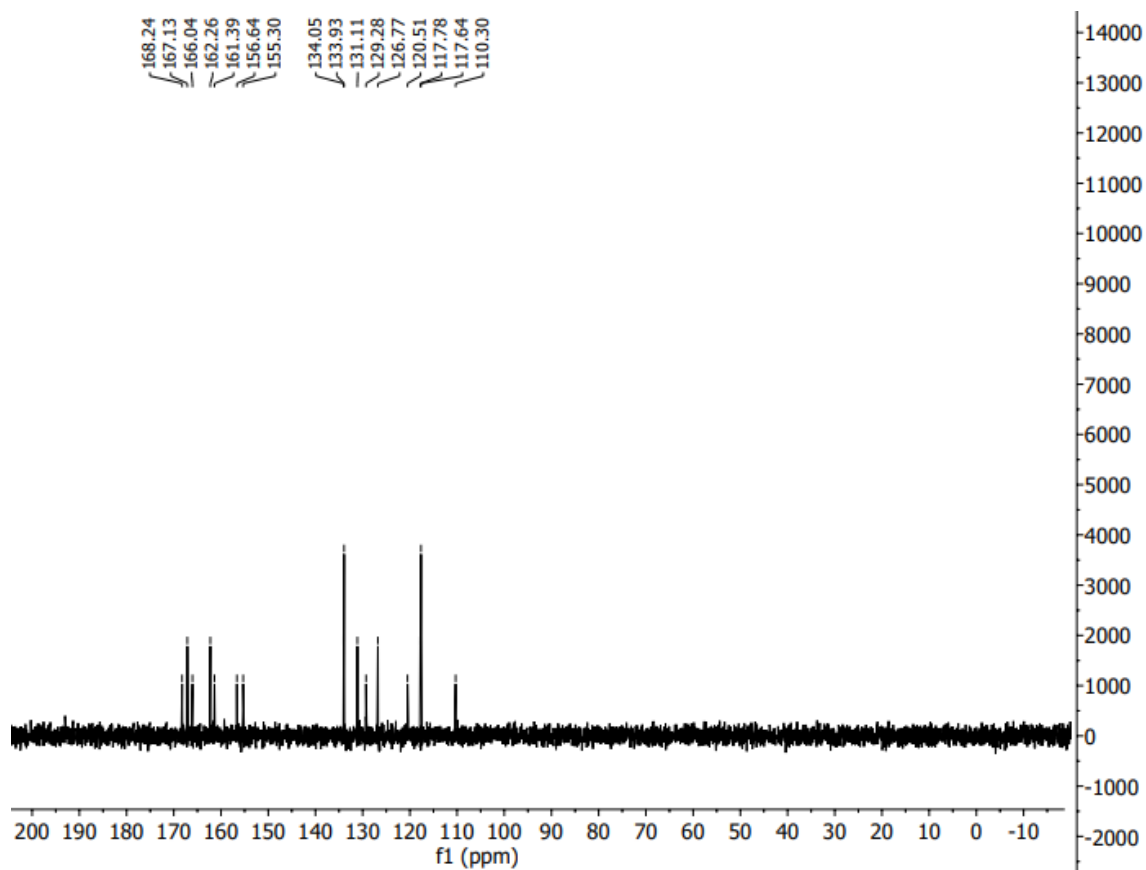


Figure S91. ^{13}C -NMR spectrum of compound AM20

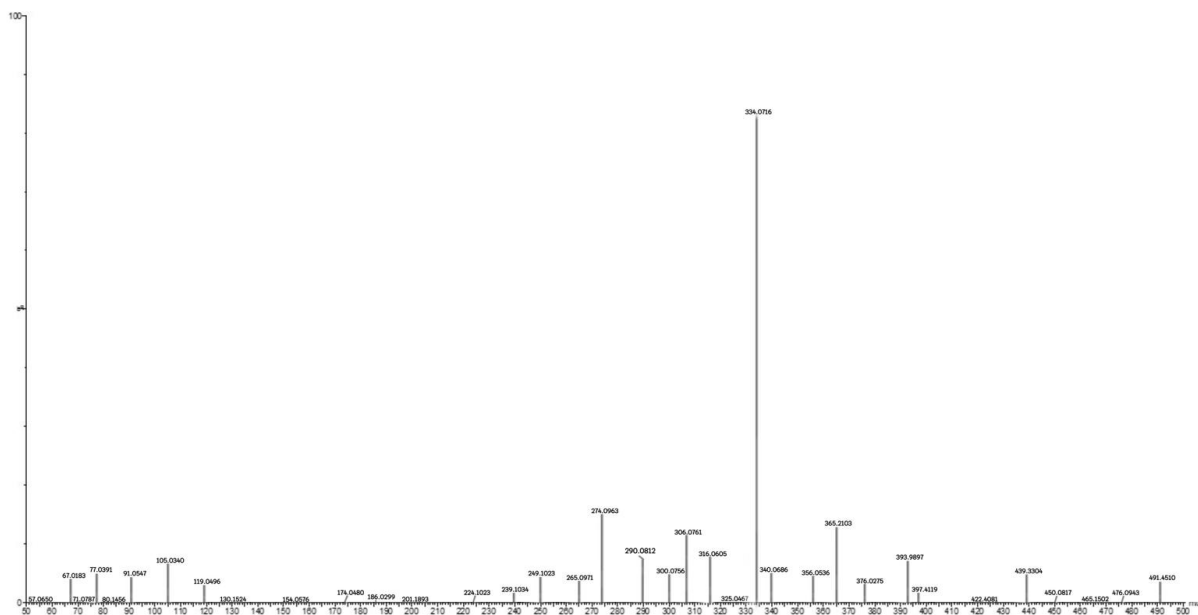
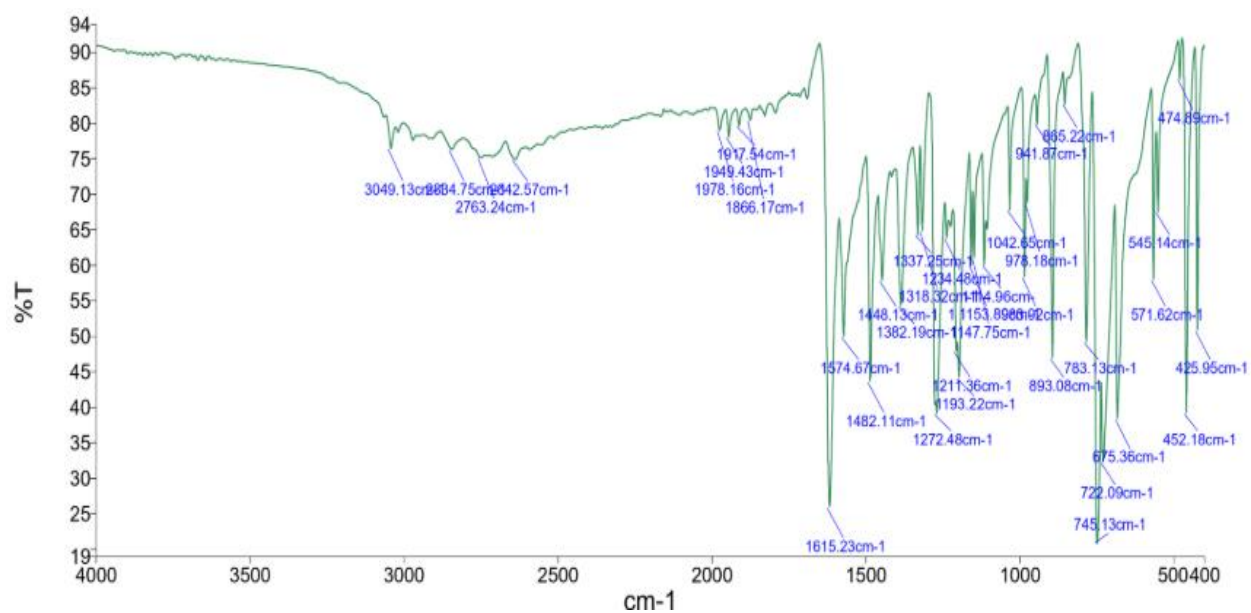


Figure S92. HRMS spectrum of compound AM20



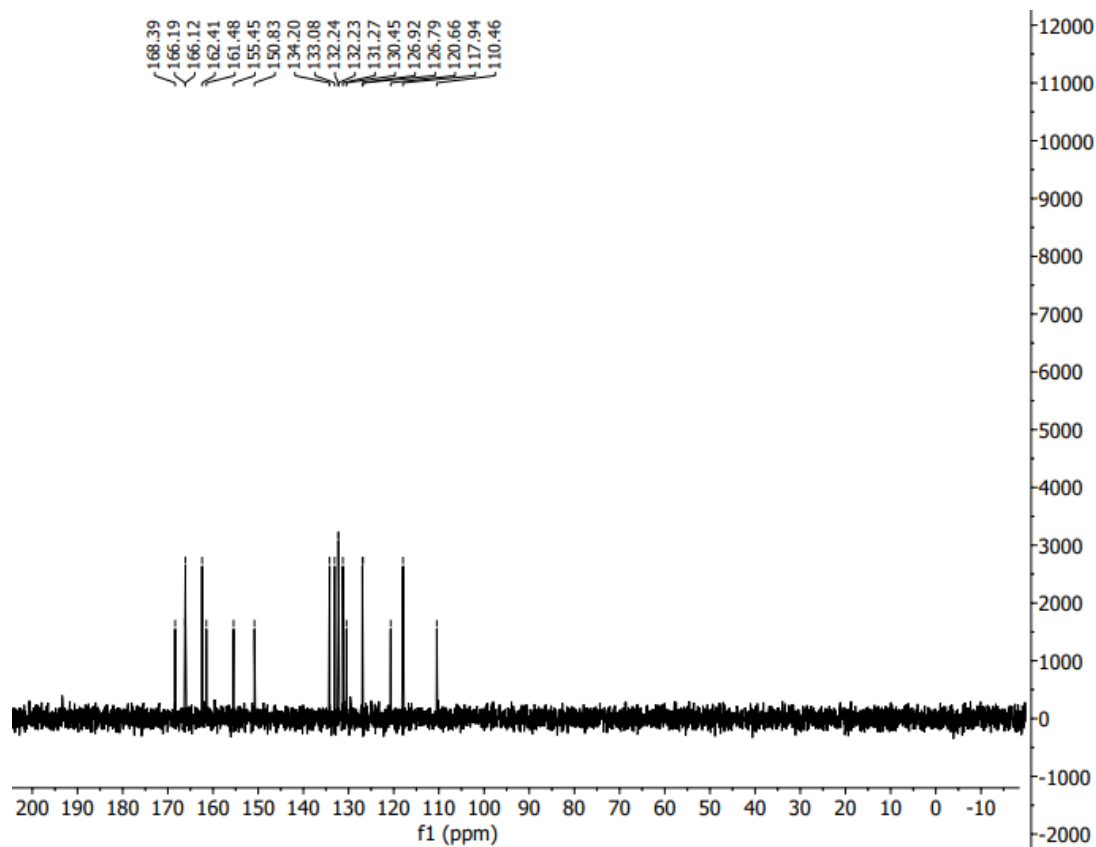


Figure S95. ^{13}C -NMR spectrum of compound AM21

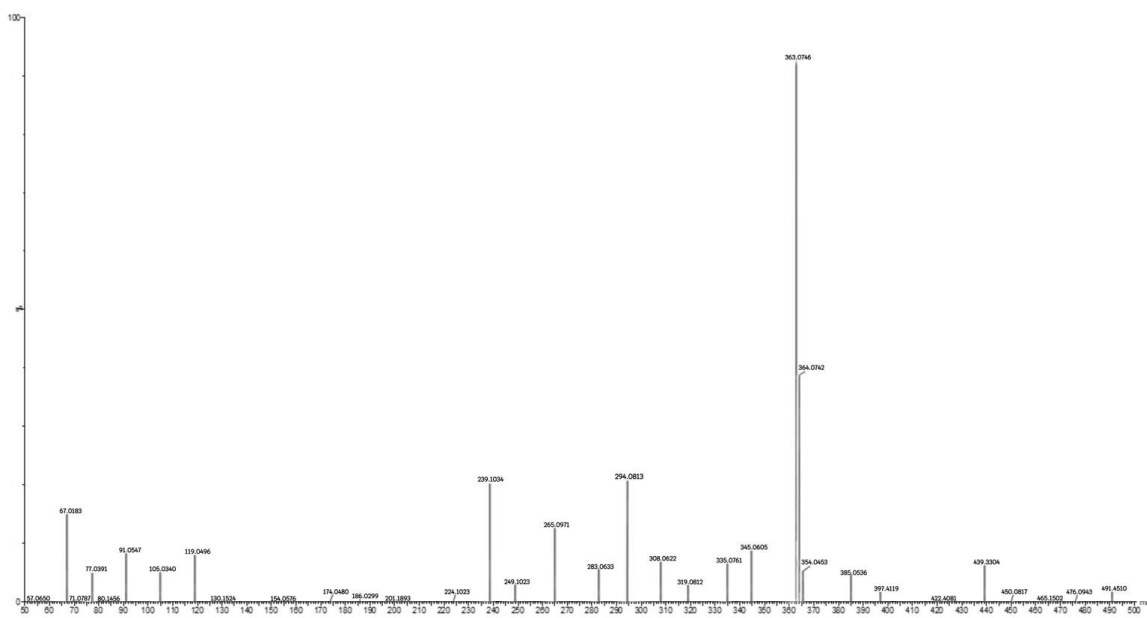


Figure S96. HRMS spectrum of compound AM21

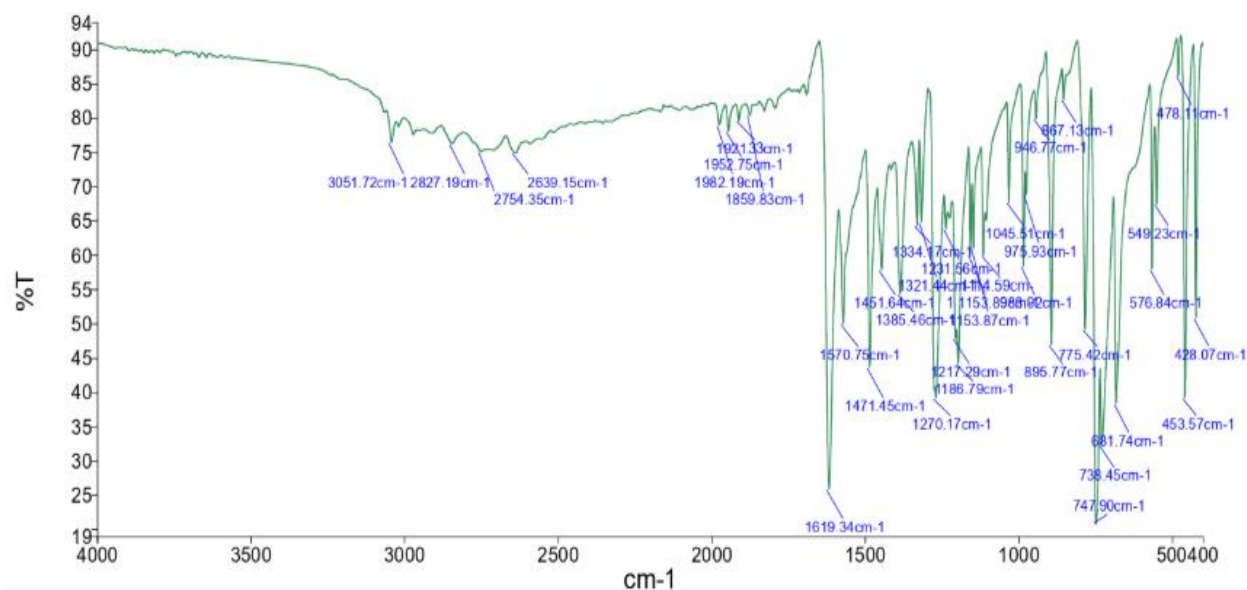


Figure S97. IR spectrum of compound AM22

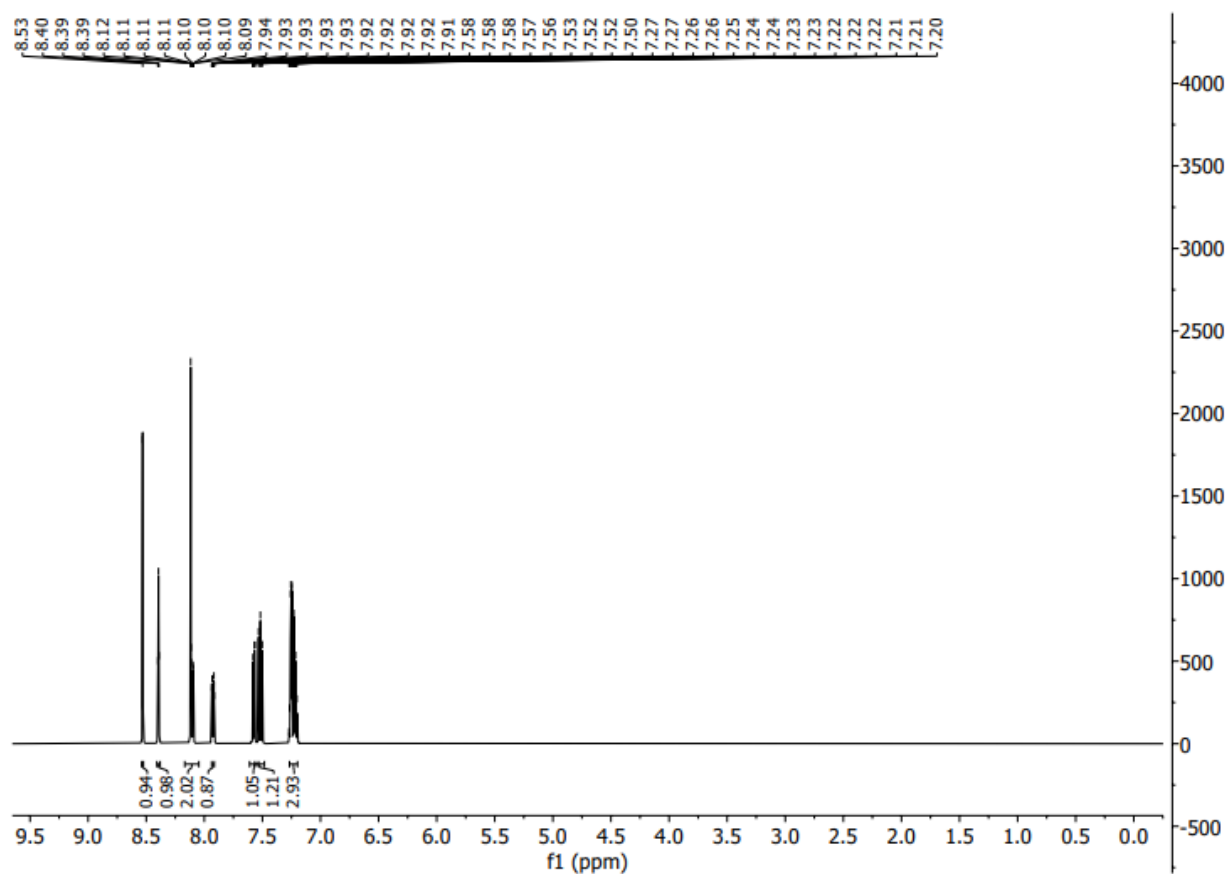


Figure S98. ¹H-NMR spectrum of compound AM22

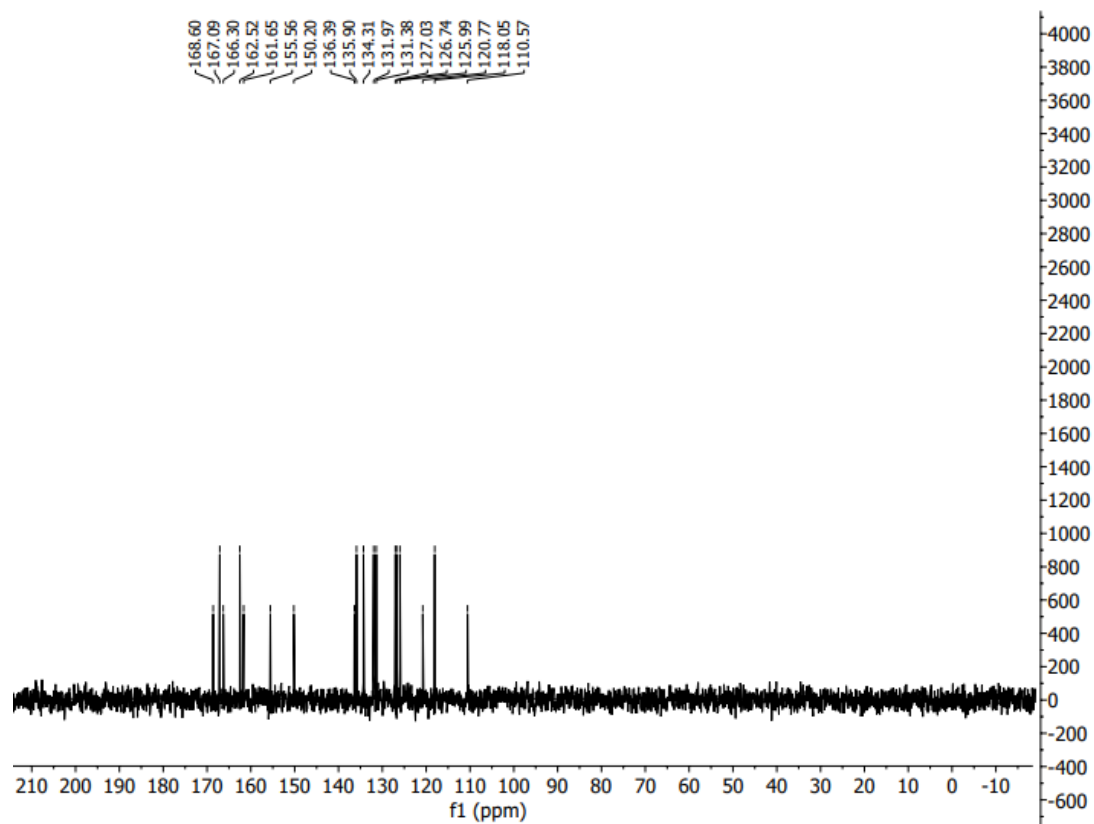


Figure S99. ^{13}C -NMR spectrum of compound AM22

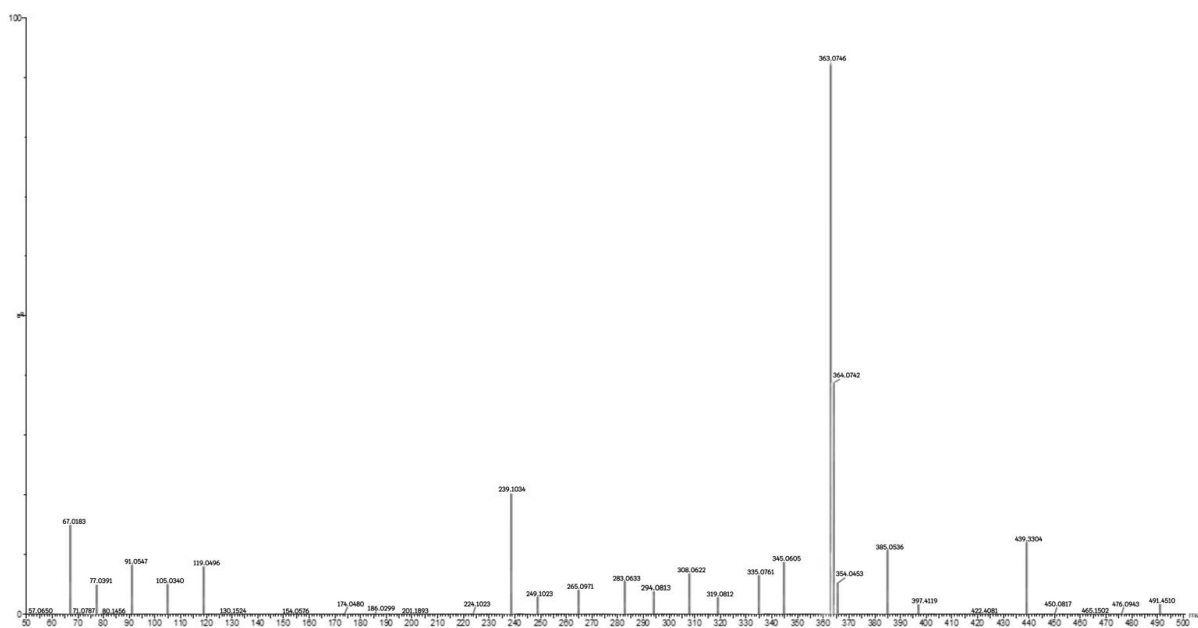


Figure S100. HRMS spectrum of compound AM22

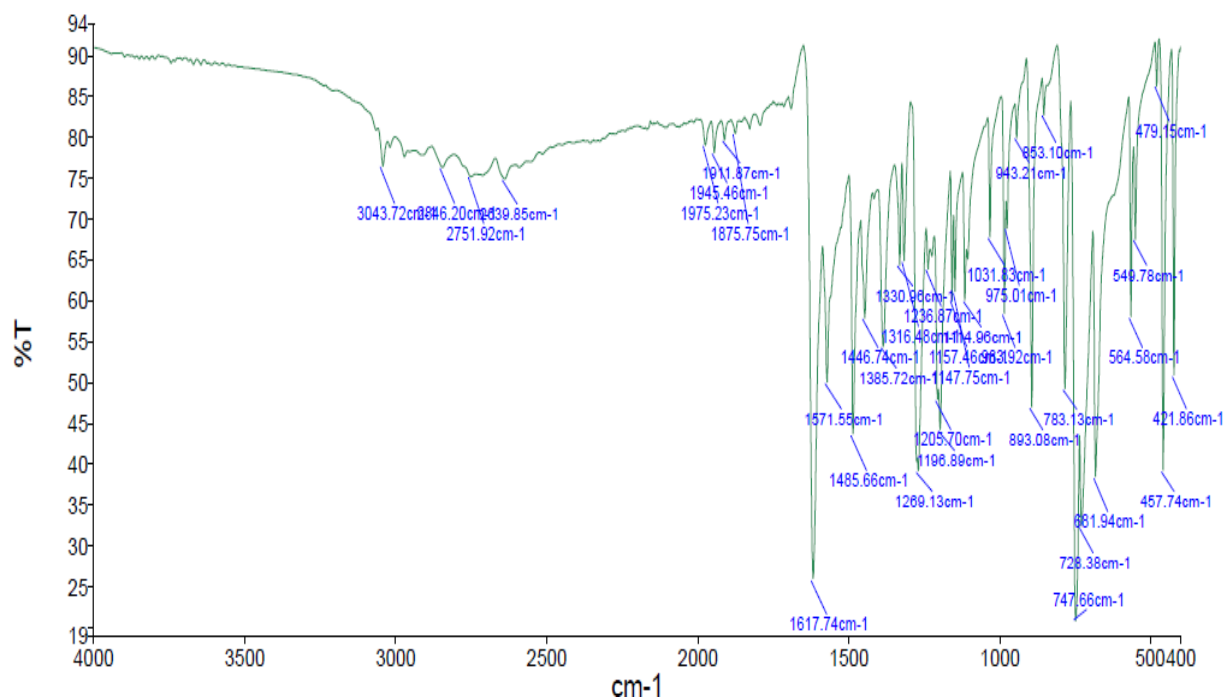


Figure S101. IR spectrum of compound AM23

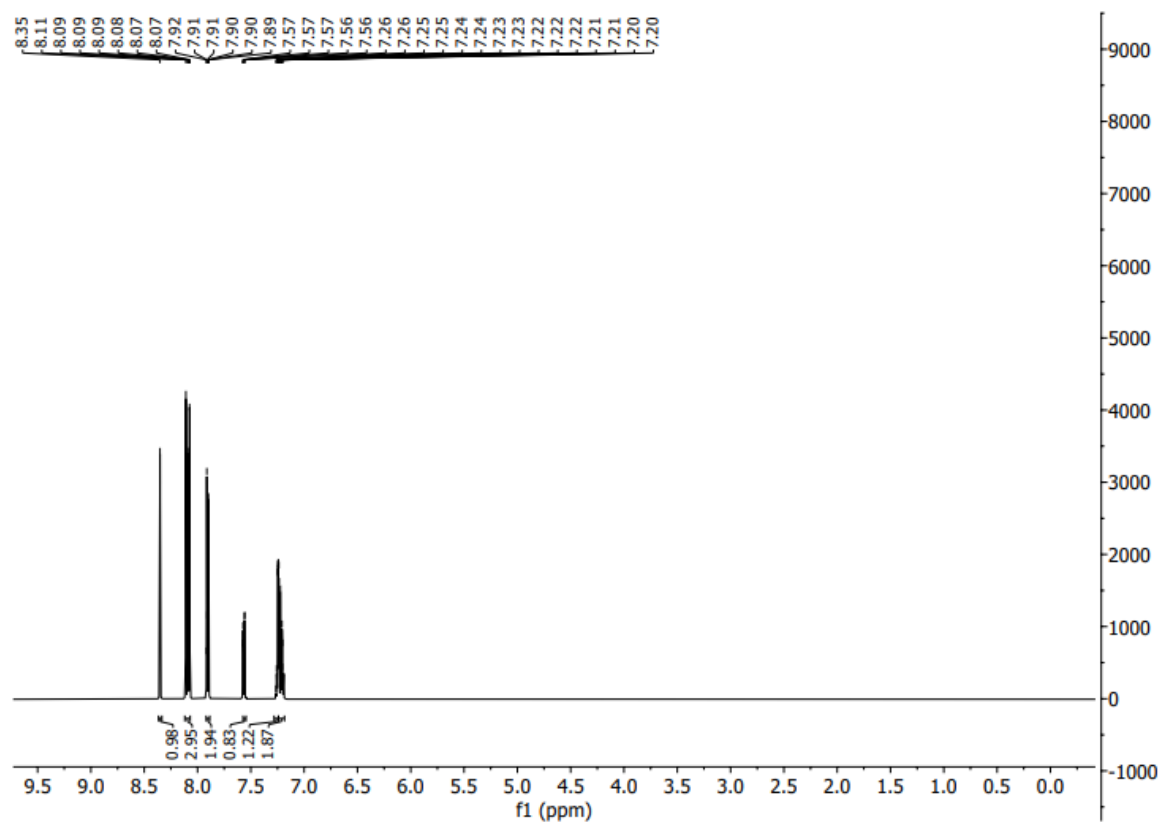


Figure S102. ¹H-NMR spectrum of compound AM23

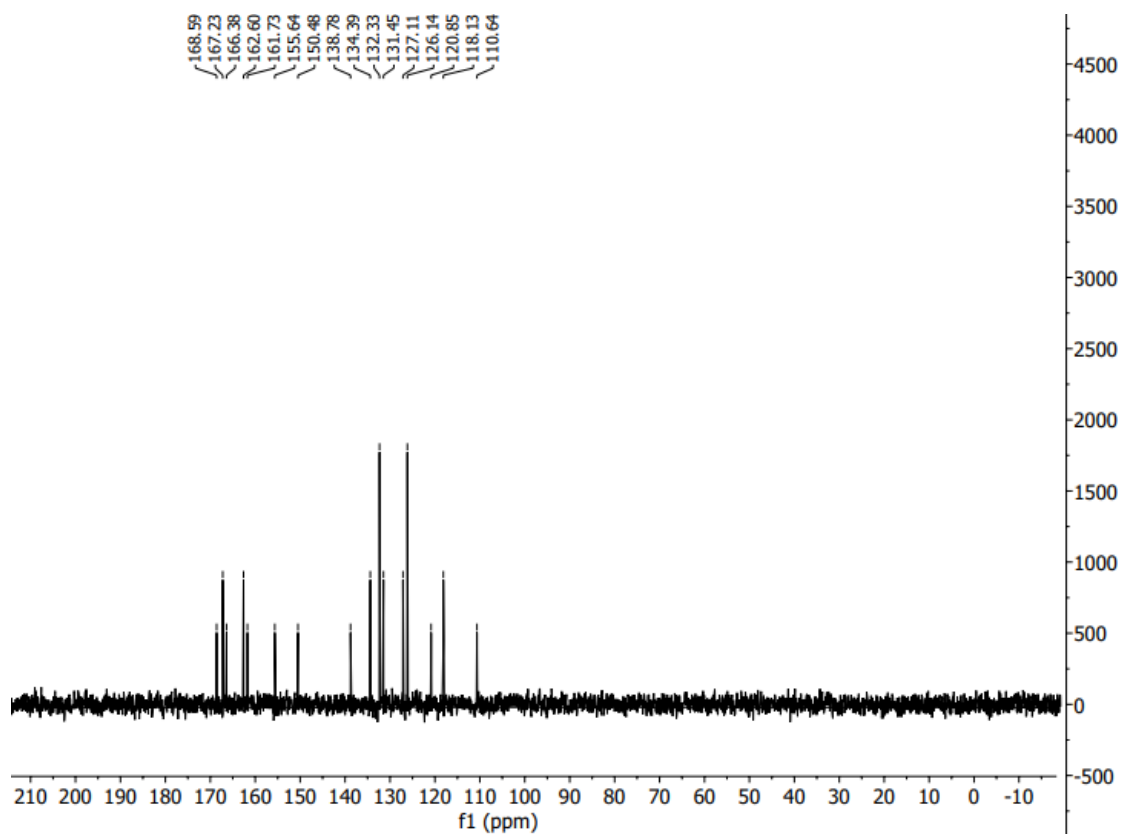


Figure S103. ^{13}C -NMR spectrum of compound AM23

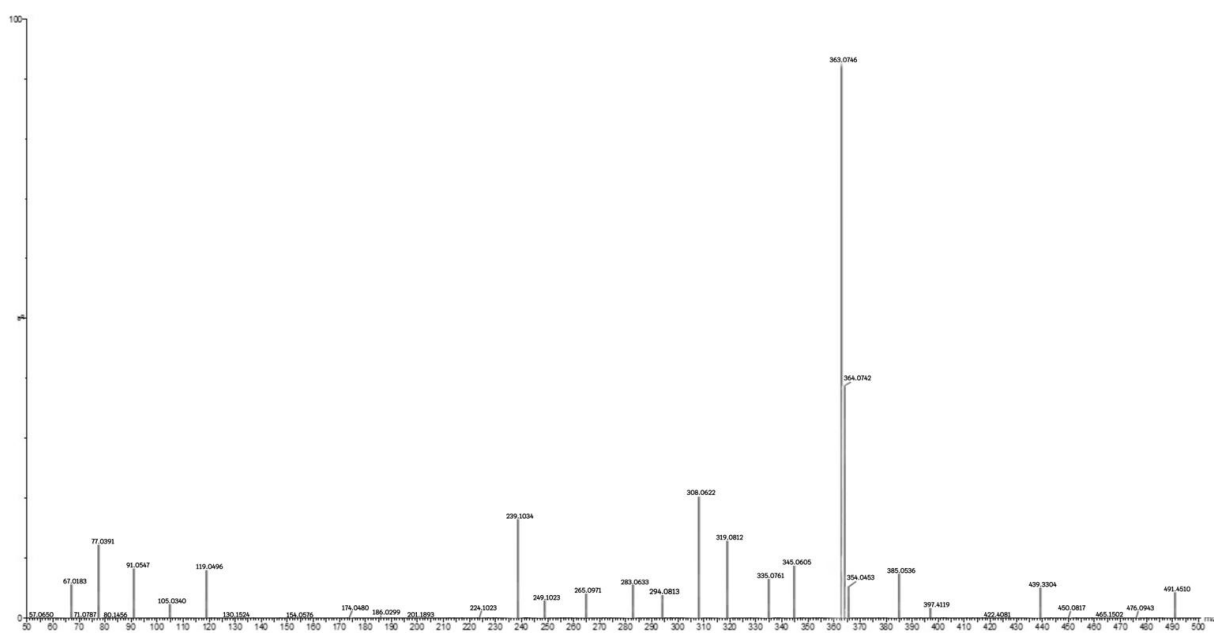


Figure S104. HRMS spectrum of compound AM23

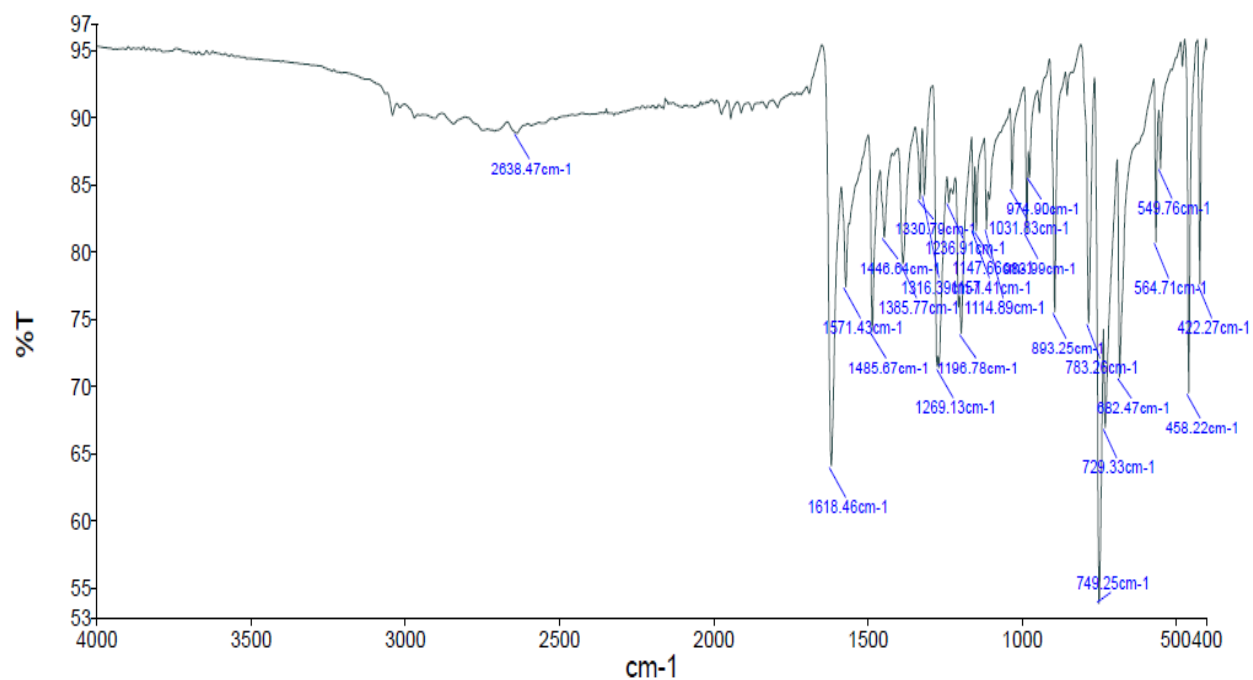


Figure S105. IR spectrum of compound AM24

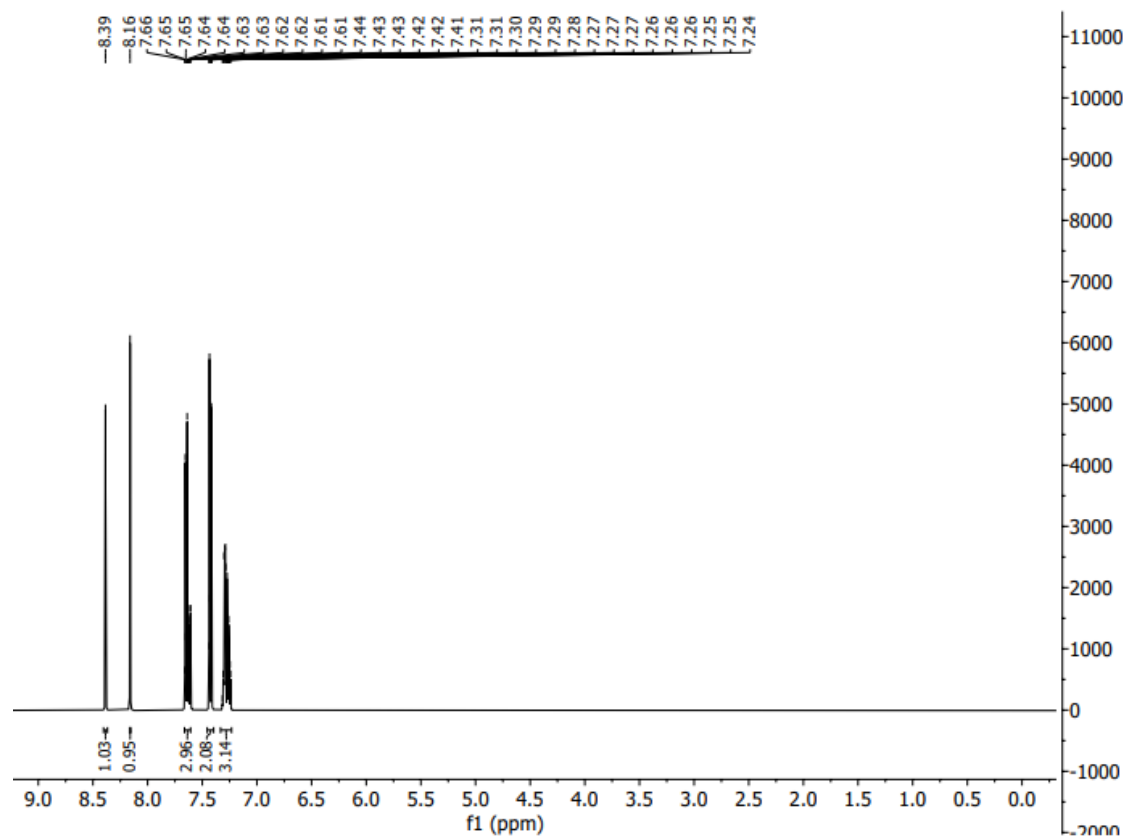


Figure S106. ¹H-NMR spectrum of compound AM24

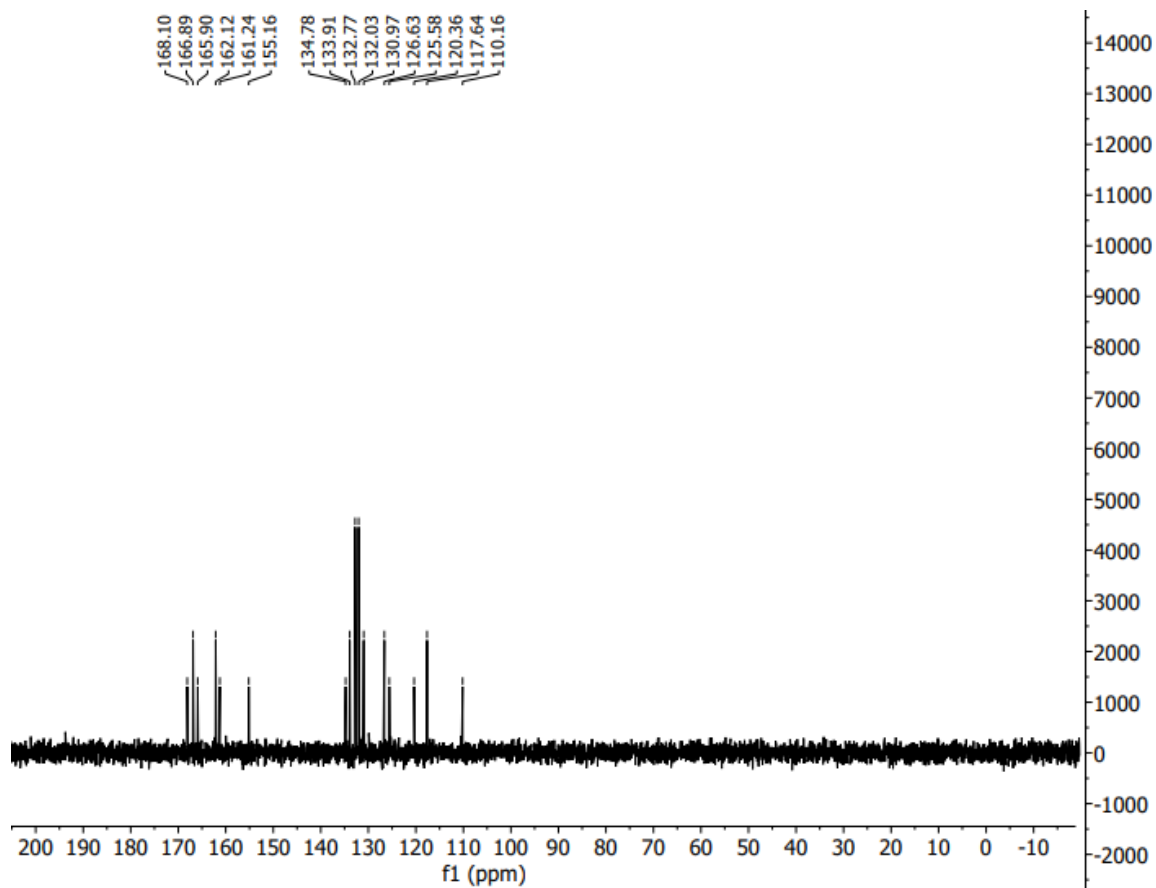


Figure S107. ^{13}C -NMR spectrum of compound AM24

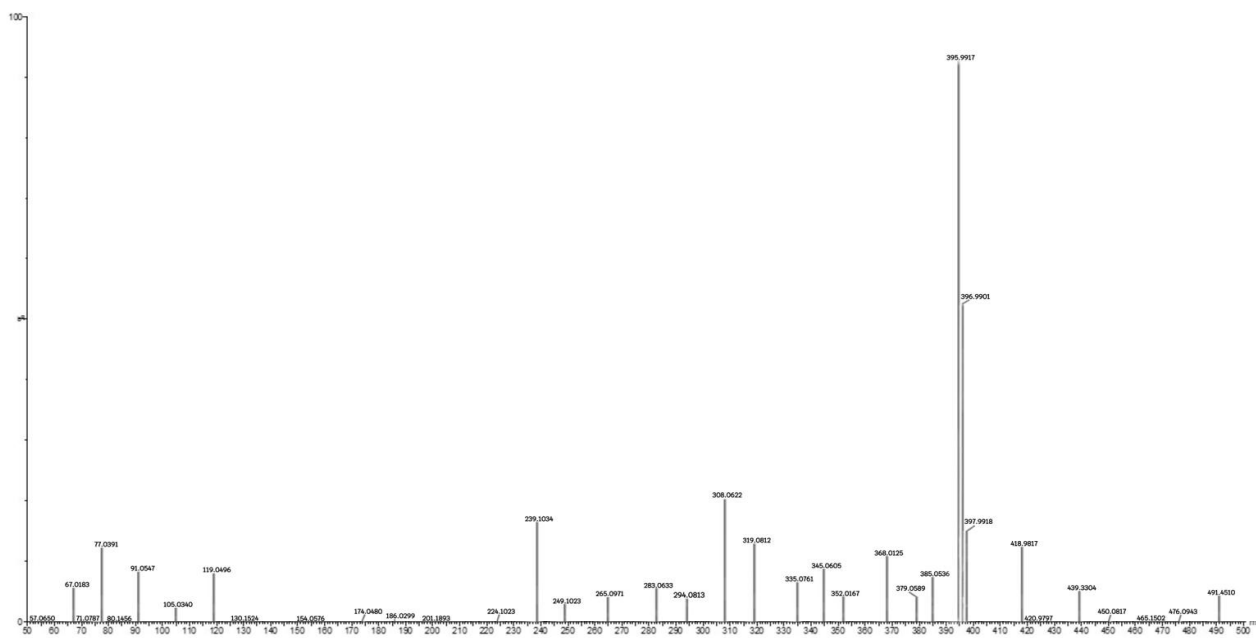
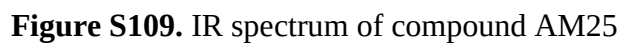


Figure S108. HRMS spectrum of compound AM24



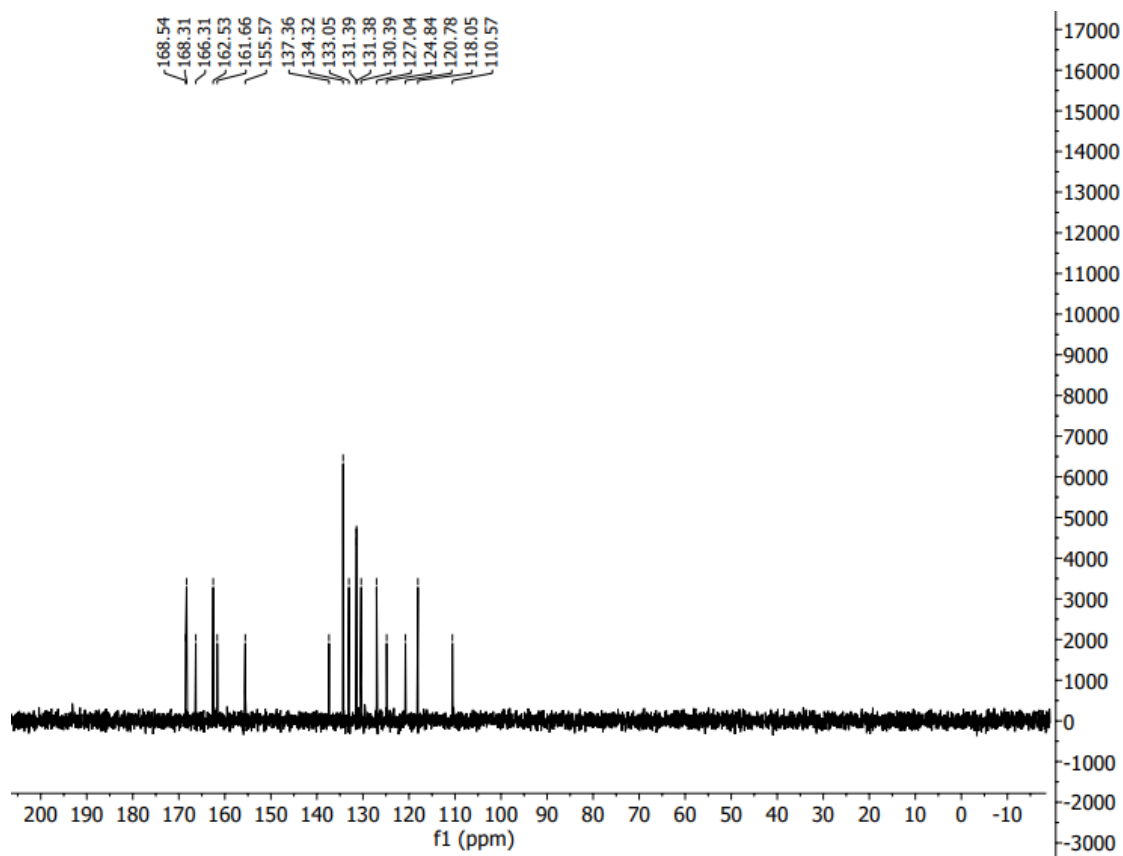


Figure S111. ^{13}C -NMR spectrum of compound AM25

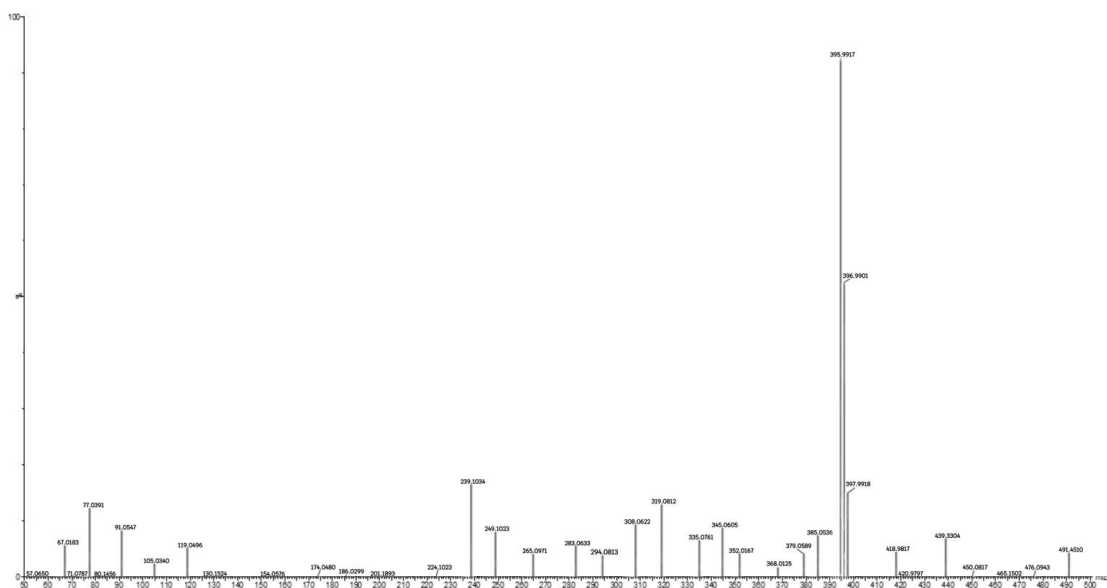


Figure S112. HRMS spectrum of compound AM25

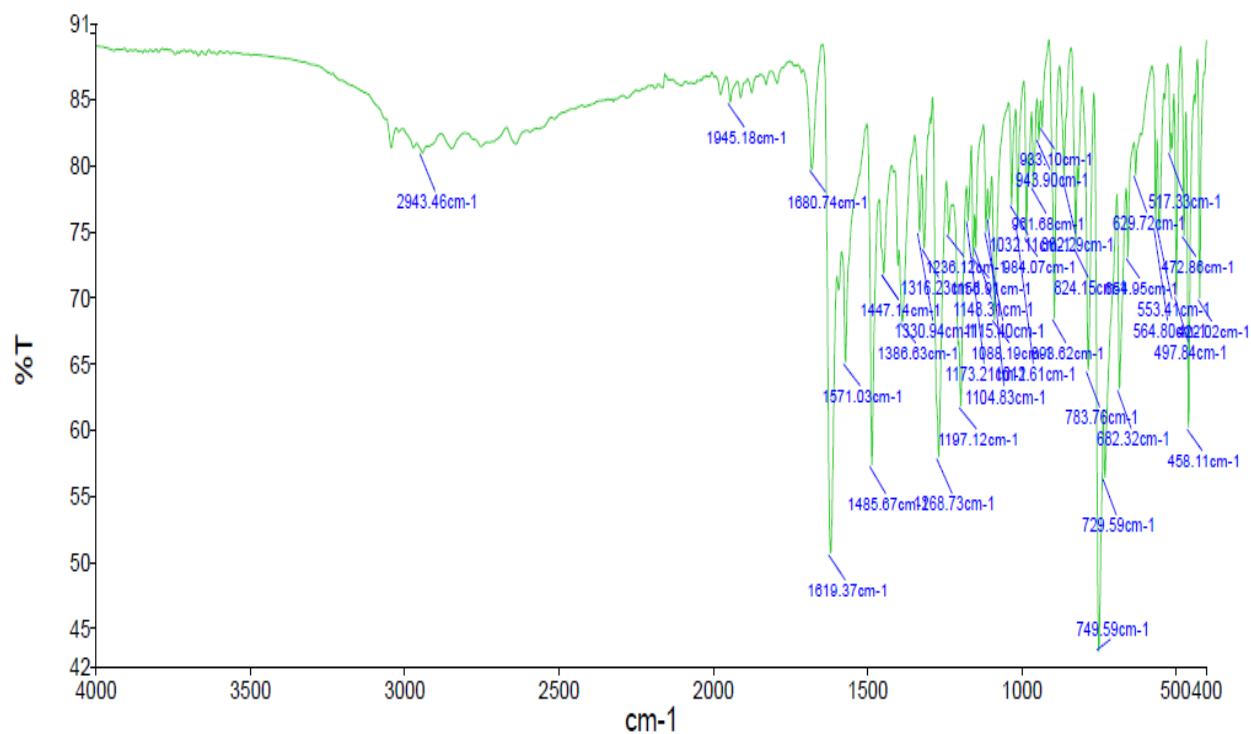


Figure S113. IR spectrum of compound AM26

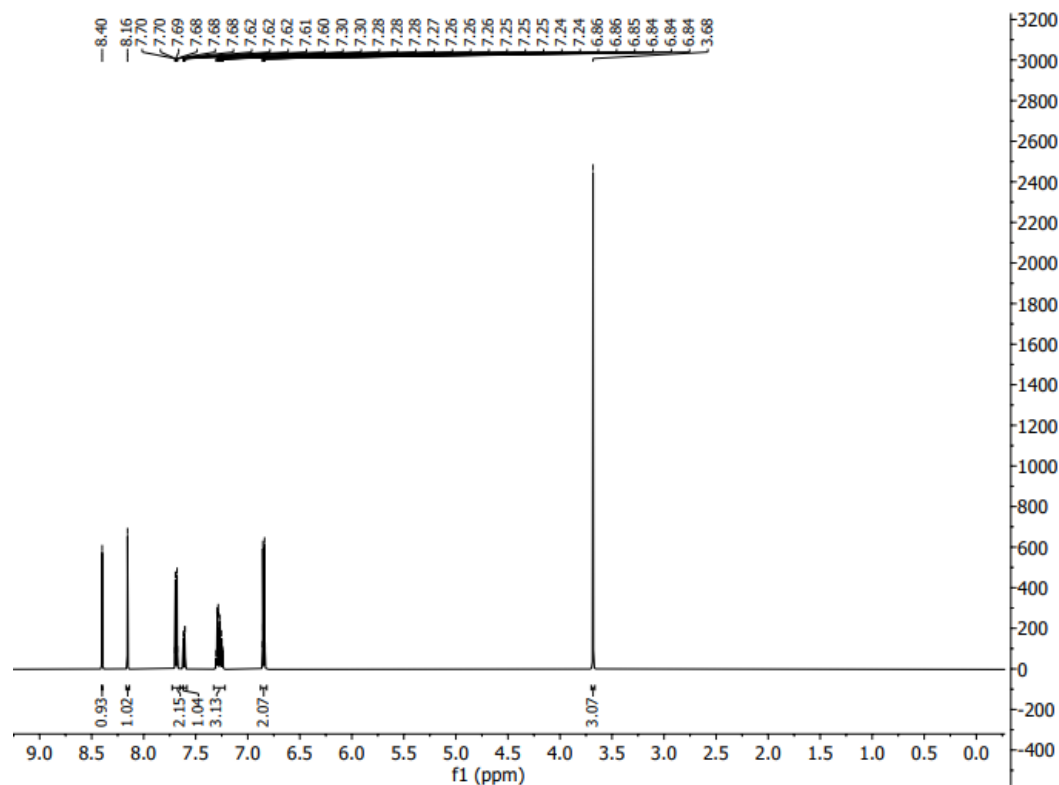


Figure S114. ^1H -NMR spectrum of compound AM26

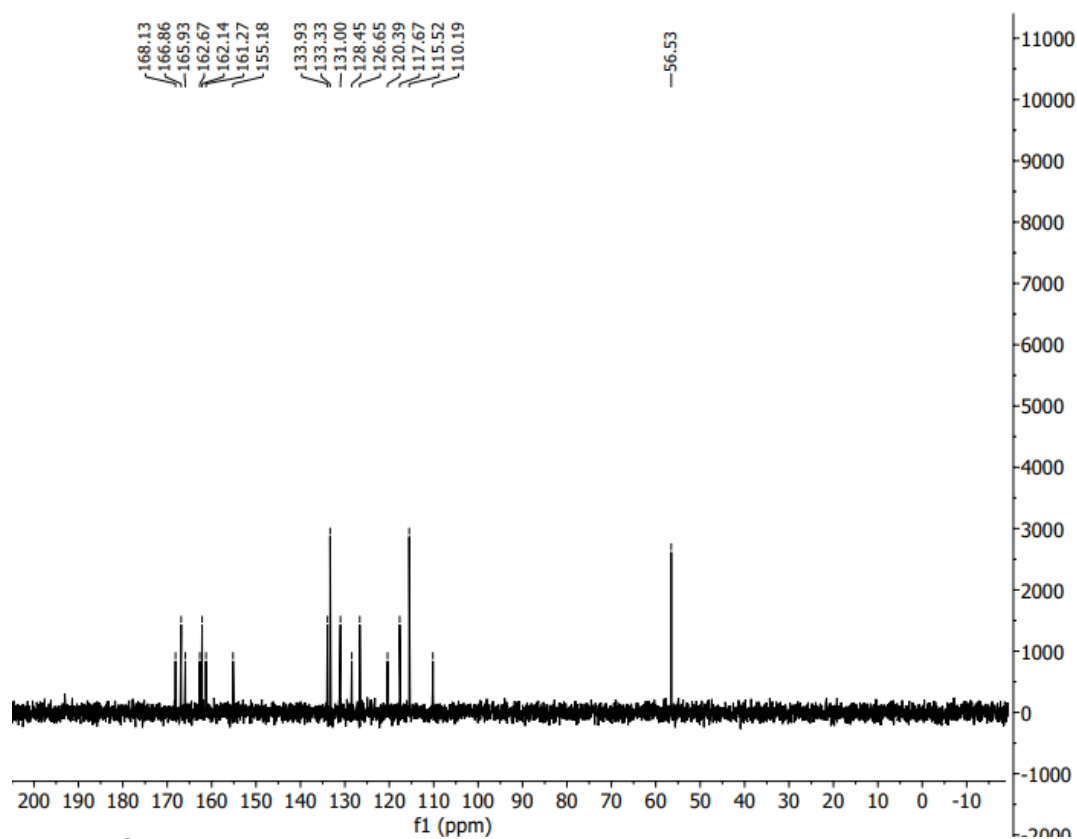


Figure S115. ^{13}C -NMR spectrum of compound AM26

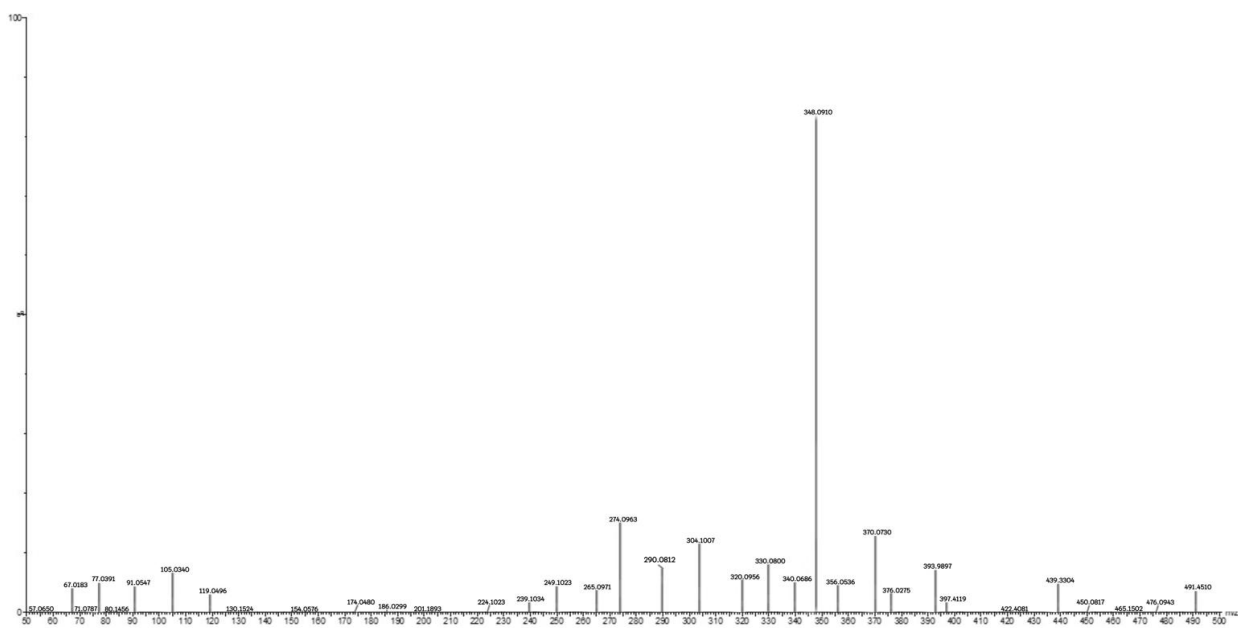


Figure S116. HRMS spectrum of compound AM26

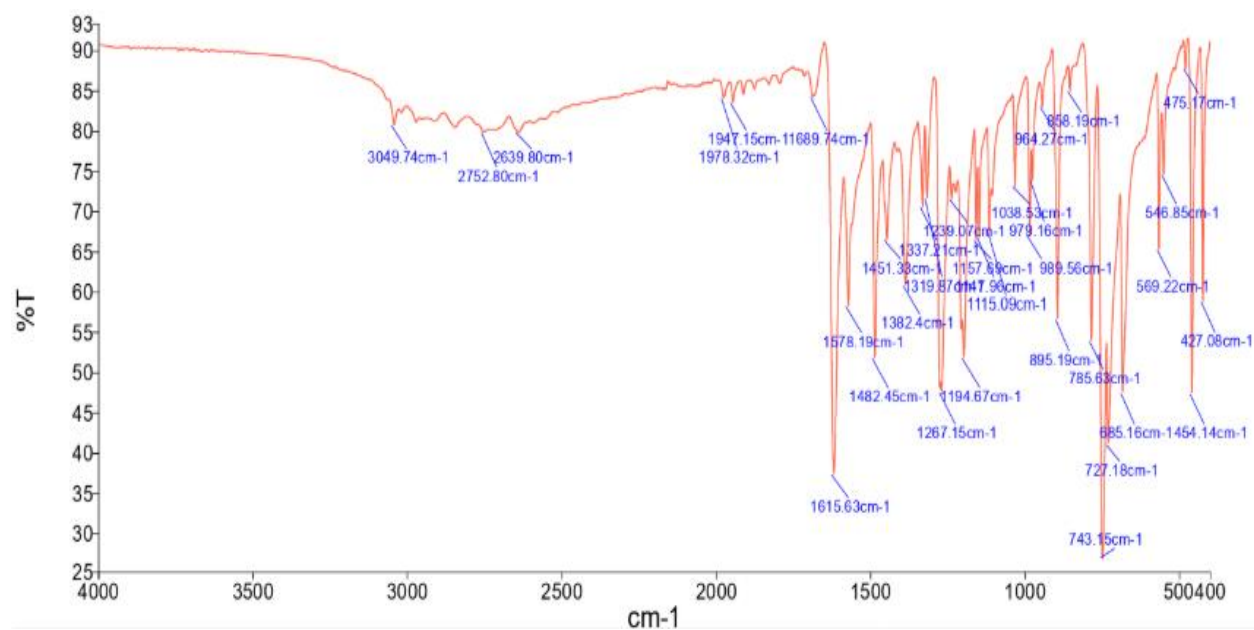


Figure S117. IR spectrum of compound AM27

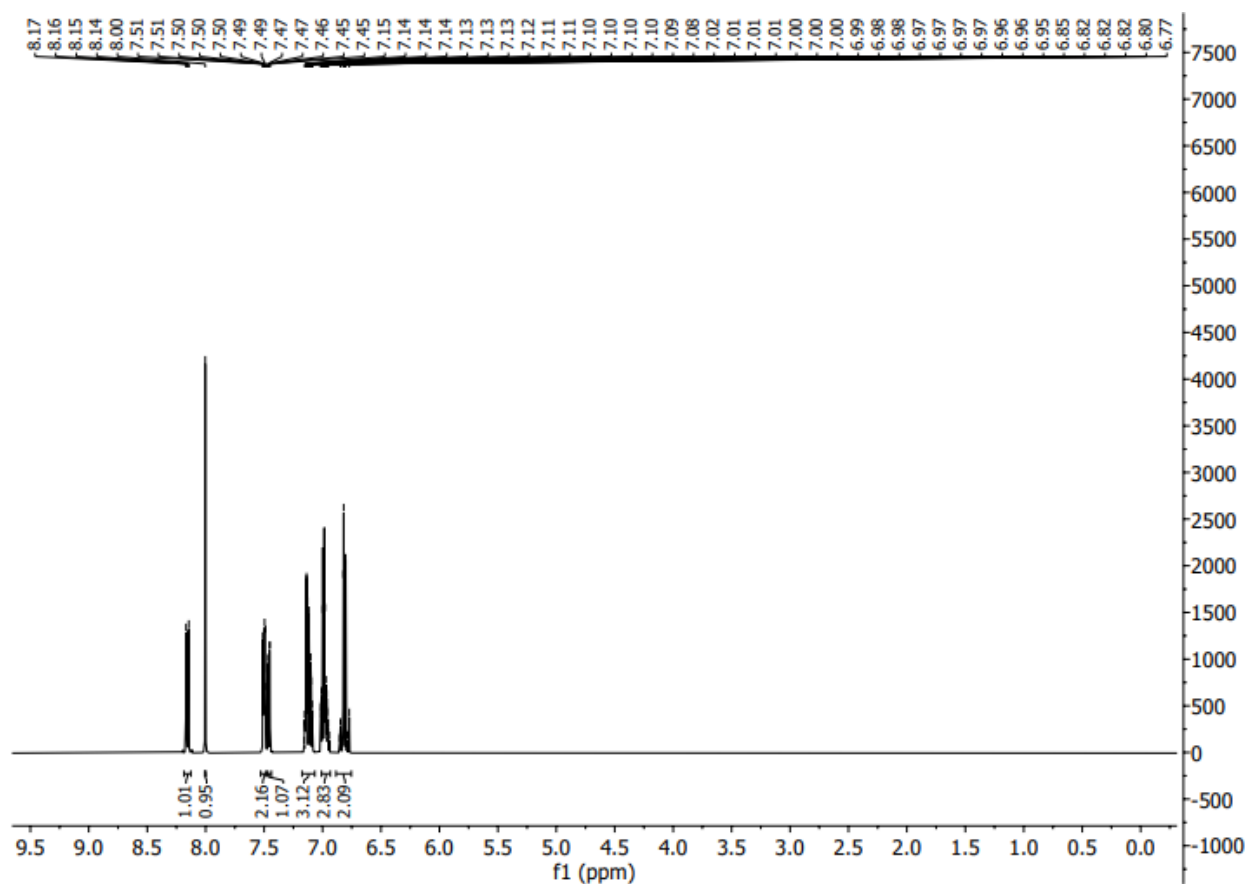


Figure S118. ¹H-NMR spectrum of compound AM27

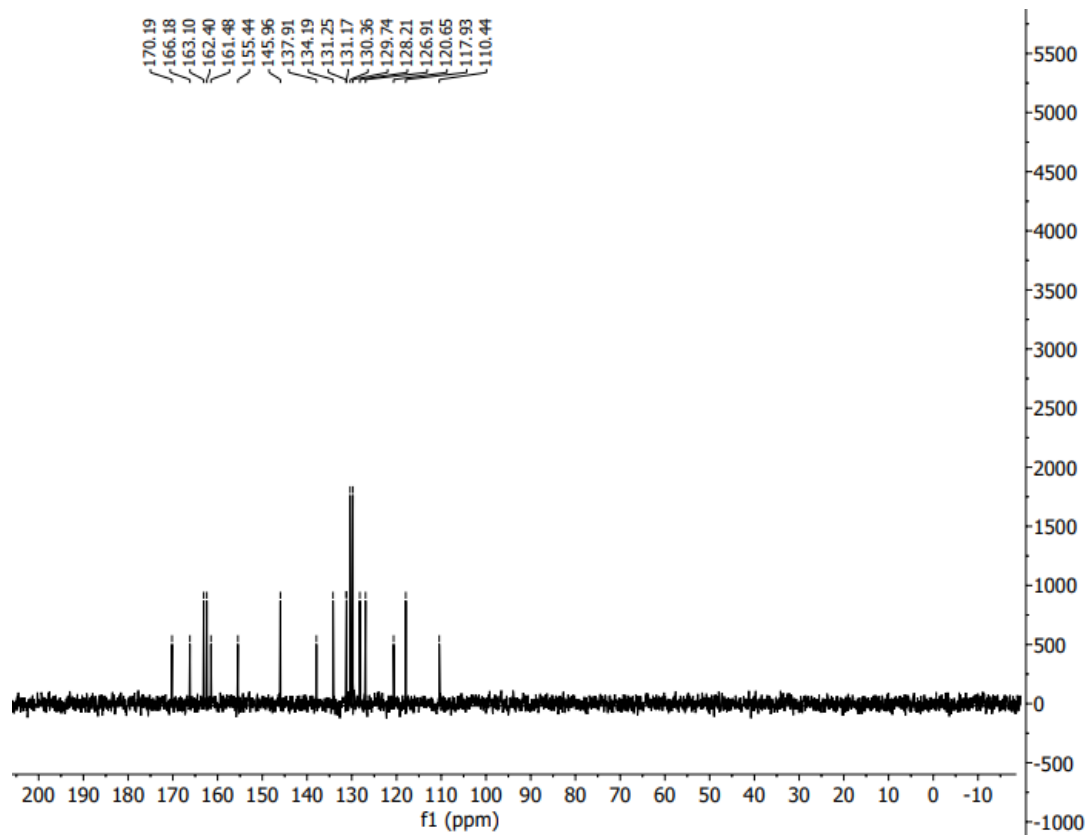


Figure S119. ^{13}C -NMR spectrum of compound AM27

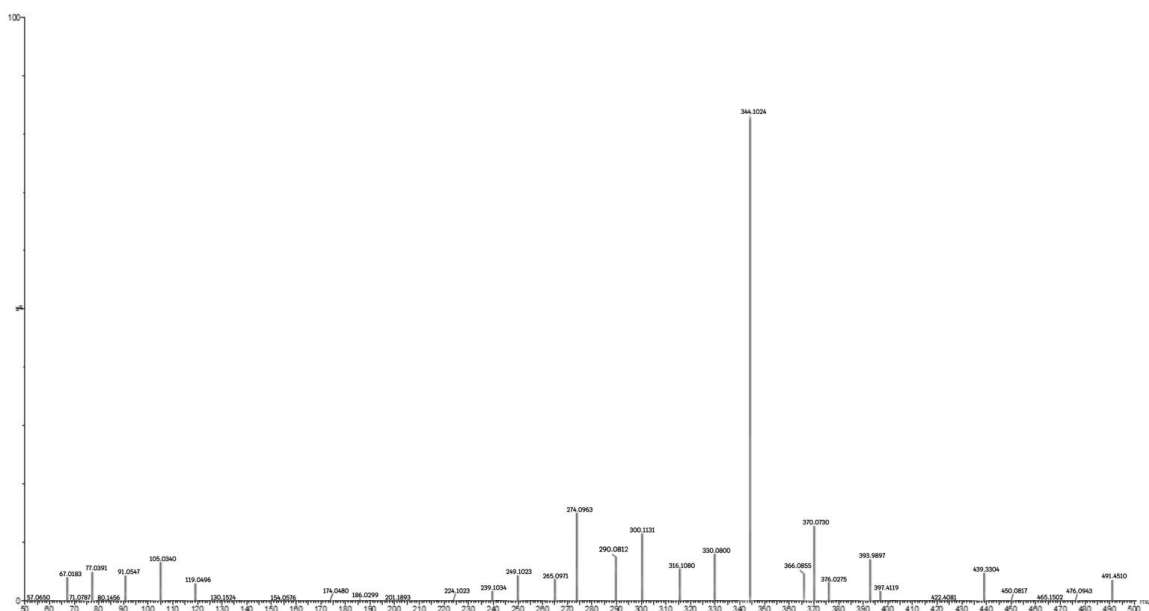


Figure S120. HRMS spectrum of compound AM27

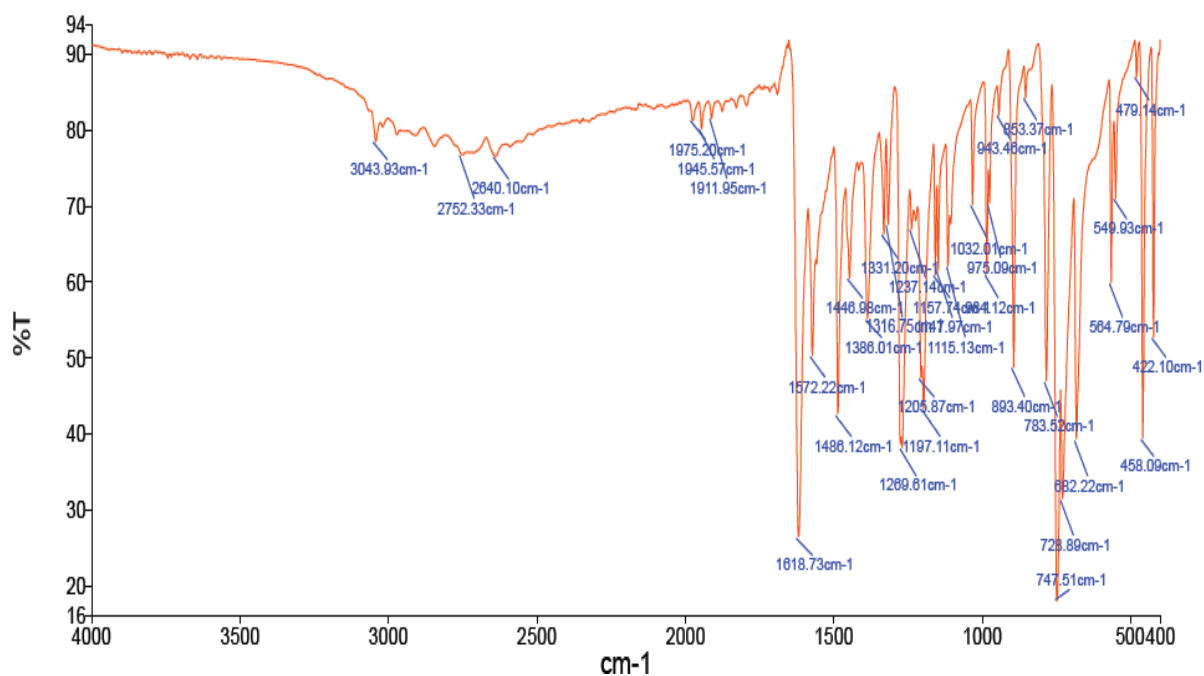


Figure S121. IR spectrum of compound AM28

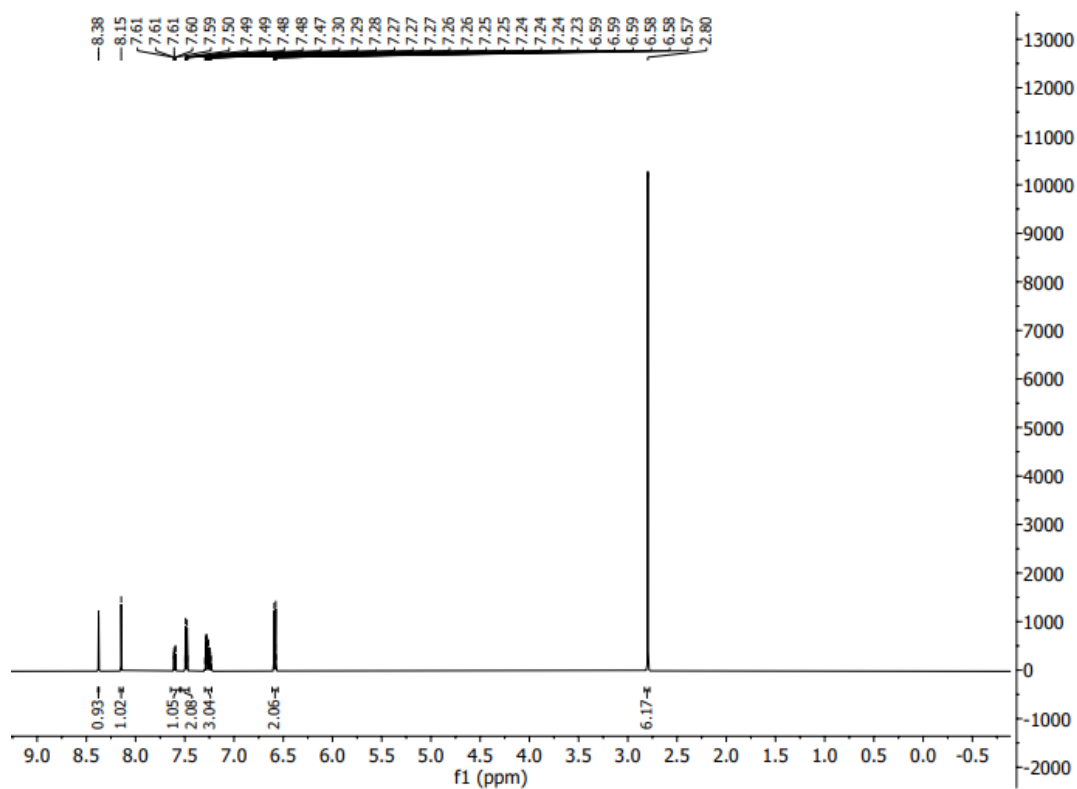


Figure S122. ^1H -NMR spectrum of compound AM28

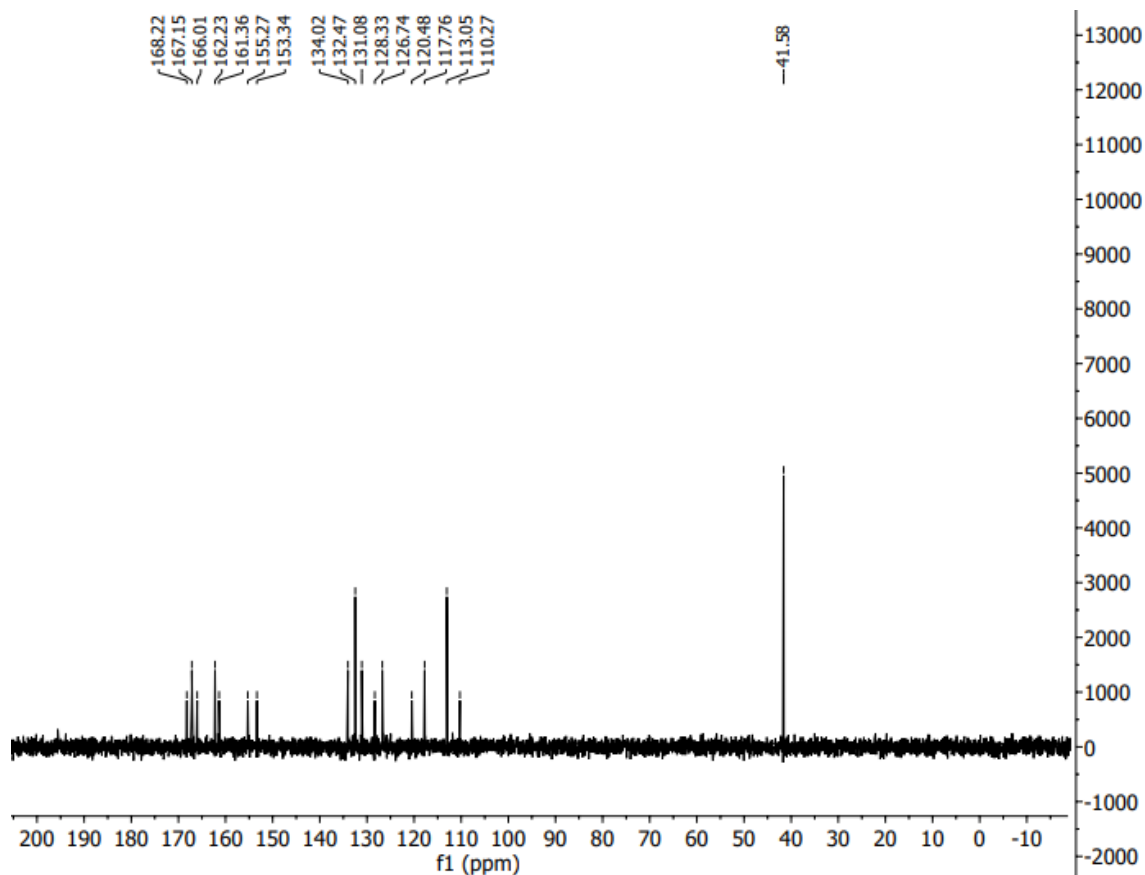


Figure S123. ^{13}C -NMR spectrum of compound AM28

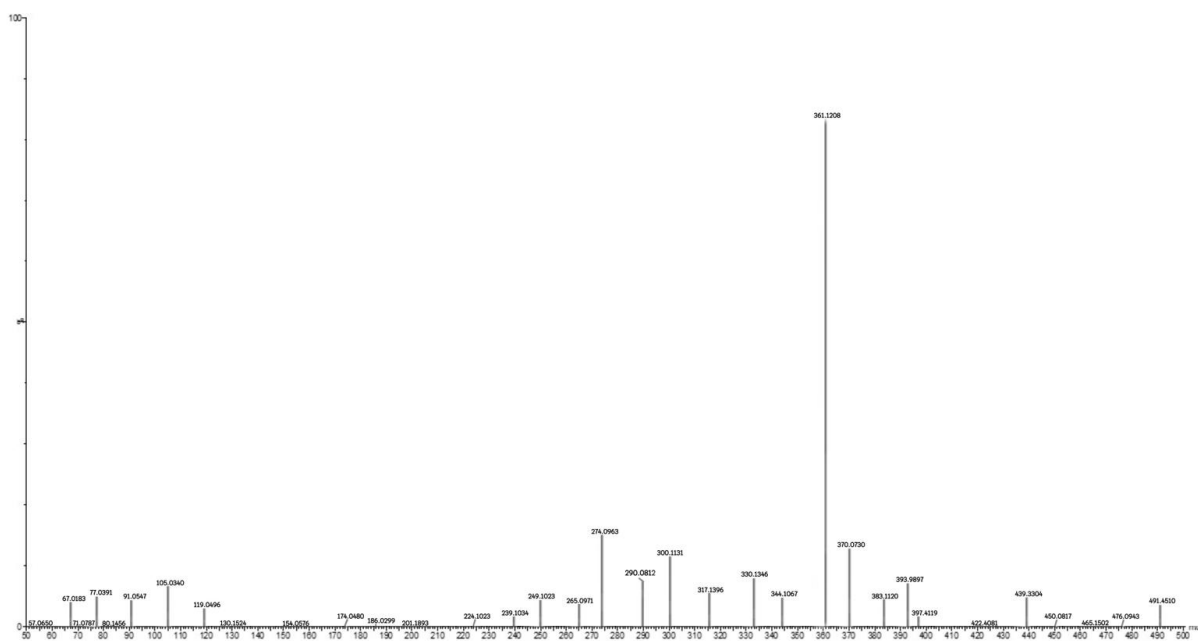


Figure S124. HRMS spectrum of compound AM28

LIST OF PUBLICATIONS

Sr. No.	Title	Category	Indexed	Status of Publication
1	Insights to the emerging potential of glucokinase activators as antidiabetic agent	Review Article	Scopus	Published
2	Molecular design and virtual screening of novel heterocyclic derivatives as Glucokinase activators	Research Papers	Scopus	Published
3	Prediction of Protein-Drug Interactions, Pharmacophore Modeling, and Toxicokinetics of Novel Leads for Type 2 Diabetes Treatment	Research Papers	Scopus	Published
4	Molecular Docking, Pharmacophore Mapping, and Virtual Screening of Novel Glucokinase Activators as Antidiabetic Agents	Research Papers	Scopus	Published
5	Molecular Docking, Pharmacophore Modeling, and ADMET Prediction of Novel Heterocyclic Leads as Glucokinase Activators	Research Papers	Scopus	Published
6	In Silico Approaches to Predict Drug Metabolism: Pharmacophore Mapping and Virtual Screening of Heterocyclic Antidiabetic Agents	Research Papers	Scopus	Published

Insights to the emerging potential of glucokinase activators as antidiabetic agent

Anuradha Mehra^a, Shubham Kumar^a, Anu Mittal^b, Ruchi Kohli^c and Amit Mittal^{*,a}

^aDepartment of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Jalandhar-Delhi G.T. Road, Punjab, 144411, India; ^bDepartment of Chemistry, Guru Nanak Dev University College, Patti, Distt. Tarn Taran, India; ^cDepartment of Chemistry, Guru Nanak Dev University College, Narot Jaimal Singh, 145026, Punjab, India

ABSTRACT

The glucokinase enzyme (belongs to the hexokinase family) is present in liver cells and β -cells of the pancreas. Glucokinase acts as a catalyst in the conversion of glucose-6-phosphate from glucose which is rate-limiting step in glucose metabolism. Glucokinase becomes malfunctioned or remains inactivated in diabetes. Glucokinase activators are compounds that bind at the allosteric site of the glucokinase enzyme and activate it. This article highlights the patent and recent research papers history with possible SAR from year 2014–2023. The data comprises the discussion of novel chemotypes (GKAs) that are being targeted for drug development and entered into clinical trials. GK activators have attracted massive interest since successful results have been reported from clinical trials data.

ARTICLE HISTORY

Received 6 January 2024
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KEYWORDS

GKAs; glucokinase activator; glucokinase enzyme; metabolic syndrome; Type II diabetes mellitus

1. Introduction

One of the persistent diseases globally is diabetes which occurs either due to dysfunction of pancreatic β -cell which leads to the reduction in the production of insulin or body cells not being able to use the insulin produced efficiently [1–3]. The blood sugar level is maintained by insulin which helps in the uptake of glucose by body cells to generate ATP (Adenosine triphosphate) [4,5]. Diabetes is characterized by hyperglycemia, leads to severe damage to the body's system such as blood vessels and nerves [6–8]. Diabetes can be classified into different types.

1.1. Prediabetes

It is a persistent metabolic state which is diagnosed by a raise in the upper threshold than normal but below the threshold for considering under diabetes [9,10]. The prediabetes condition is manifested by the rise in blood sugar level as compared with the normal blood sugar level (70 mg/dL–100 mg/dL) but not in the range of rising such that can be considered under the class of diabetes [11,12]. Prior to diabetes mellitus, prediabetes

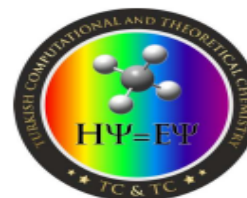
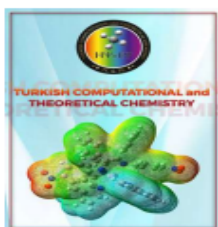
that can be modified back to normal by changing the lifestyle such as loss of weight, increase in physical activity and dietary changes [17–19]. The blood glucose level in different conditions as discussed below.

1.1.1. Gestational diabetes

In the pregnant women manifested with the increased blood sugar level which comes into existence due to some reasons such as insulin resistance, autoimmune disorders [20–22]. Obesity can be the major cause of the development of gestational diabetes mellitus. It leads to a rise in a foetus and maternal complications [23–25]. It is observed in 5% of the gestation cases but this number is continuously increasing due to unhealthy lifestyles such as obesity, lack of physical exercise, junk foods and increased sweet foods intake [26–28].

1.1.2. Diabetes type 1

It is a chronic metabolic state which is characterized by the destruction of the β -cell of the pancreas that leads to either little or no insulin production [29,30]. It is determined by the rise in glucose level of blood (hyperglycemia) due to lack of insulin. The type 1 diabetes



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Research Article

Molecular design and virtual screening of novel heterocyclic derivatives as Glucokinase activators

Anuradha Mehra^a, Amit Mittal^{a, 1}, Pankaj Wadhwa^a, Aryan Mehra^b

^aDepartment of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Lovely Professional University, Jalandhar-Delhi G.T. Road (NH-1), Phagwara (Punjab) 144411, India

^bDepartment of Mechanical Engineering, Lovely Professional University, Jalandhar-Delhi G.T. Road (NH-1), Phagwara (Punjab) 144411, India

Abstract: Deficiency of insulin signaling in type 2 diabetes results from insulin resistance or defective insulin secretion and induce hyperglycemia. Diabetes is a global threat that continues to increase day by day at a very high rate in both developing and developed countries. Glucokinase activators (GKA) can be a novel target used for better management of type 2 diabetes. Recently novel GKA Dorzagliatin received market approval by Japan FDA for treatment of type 2 diabetes. The purpose of designing glucokinase activators was to develop novel therapeutic molecules with minimum side effects. A docking study was conducted using AutoDock Vina 1.5.6, and the structures were created using ChemBiodraw Ultra. The Swiss ADME algorithm was used for online log p prediction. Among all the molecules designed, AM35 had the highest binding affinity to GK receptors. For good absorption and elimination, Log P values range from 2-3.08, indicating good lipophilic properties. The new lead molecules were designed as glucokinase activators, which had a better pharmacokinetic profile and higher binding affinity.

Keywords: Type 2 diabetes, Glucokinase activator, molecular docking, AutoDock Vina.

1. Introduction

Type 2 Diabetes Mellitus is marked by several distinctive features, which encompass heightened production of glucose in the liver, reduced sensitivity to insulin, compromised insulin release, and irregular fat processing [1]. During the initial phases of the condition, the pancreas' beta cells augment their insulin release as a response to the emerging insulin resistance, thereby maintaining relatively normal glucose tolerance [2, 3]. Nevertheless, in some individuals, the pancreatic islets eventually struggle to sustain this state of heightened insulin production and resistance as compensatory mechanisms wane. This progression results in impaired glucose tolerance, evident through elevated post-meal glucose levels [4, 5]. As the disease advances, overt diabetes becomes apparent, characterized by elevated fasting blood sugar due to decreased insulin secretion and heightened hepatic glucose synthesis [6, 7]. The

escalating global menace of diabetes continues to surge, with its prevalence steadily increasing across the world, spanning both developed and developing nations [8]. According to the Tenth Edition of the IDF Diabetes Atlas 2021, a staggering 537 million adults aged 20 to 79 are grappling with diabetes, with the year 2021 witnessing a distressing 6.7 million diabetes-related fatalities [9, 10]. Projections indicate that the number of individuals affected by diabetes will soar to 643 million by 2030 and an even higher 783 million by 2045 [11, 12]. A significant majority, about 81%, of adults afflicted by diabetes are situated in low and middle-income economies. Over the last 15 years, diabetes has accounted for at least USD 966 billion (a 316% increase) in healthcare expenditures [13, 14]. Of utmost concern is the fact that nearly half, specifically 46.5%, of adults with diabetes remain undiagnosed. Glucokinase (GK) is an enzyme located in the cytoplasm that facilitates the

¹ Corresponding Authors

e-mail: amitmittal77@yahoo.com, amit.31641@lpu.co.in

RESEARCH ARTICLE

Prediction of Protein-Drug Interactions, Pharmacophore Modeling, and Toxicokinetics of Novel Leads for Type 2 Diabetes Treatment

Anuradha Mehra¹, Amit Mittal^{1,*} and Prakhar Kumar Vishwakarma¹

¹Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Lovely Professional University, Jalandhar-Delhi G.T. Road (NH-1), Phagwara (Punjab), 144411, India

Abstract: Background: Small heterocyclic compounds have been crucial in pioneering advances in type 2 diabetes treatment. There has been a dramatic increase in the pharmacological development of novel heterocyclic derivatives aimed at stimulating the activation of Glucokinase (GK). A pharmaceutical intervention for diabetes is increasingly targeting GK as a legitimate target. Diabetes type 2 compromises Glucokinase's function, an enzyme vital for maintaining the balance of blood glucose levels. Medicinal substances strategically positioned to improve type 2 diabetes management are used to stimulate the GK enzyme using heterocyclic derivatives.

Objective: The research endeavor aimed to craft novel compounds, drawing inspiration from the inherent coumarin nucleus found in nature. The goal was to evoke the activity of the glucokinase enzyme, offering a tailored approach to mitigate the undesired side effects typically associated with conventional therapies employed in the treatment of type 2 diabetes.

Methods: Coumarin, sourced from nature's embrace, unfolds as a potent and naturally derived ally in the quest for innovative antidiabetic interventions. Coumarin was extracted from a variety of botanical origins, including *Artemisia keiskeana*, *Mallotus resinosa*, *Jatropha integerrima*, *Ferula tingitana*, *Zanthoxylum schinifolium*, *Phebalium clavatum*, and *Mammea siamensis*. This inclusive evaluation was conducted on Muiybridge's digital database containing 53,000 hit compounds. The presence of the coumarin nucleus was found in 100 compounds, that were selected from this extensive repository. Utilizing Auto Dock Vina 1.5.6 and ChemBioDraw Ultra, structures generated through this process underwent docking analysis. Furthermore, these compounds were accurately predicted online log P using the Swiss ADME algorithm. A predictive analysis was conducted using PKCSM software on the primary compounds to assess potential toxicity.

Results: Using Auto Dock Vina 1.5.6, 100 coumarin derivatives were assessed for docking. Glucokinase (GK) binding was significantly enhanced by most of these compounds. Based on superior binding characteristics compared with Dorzagliatin (standard GKA) and MRK (co-crystallized ligand), the top eight molecules were identified. After further evaluation through ADMET analysis of these eight promising candidates, it was confirmed that they met the Lipinski rule of five and their pharmacokinetic profile was enhanced. The highest binding affinity was demonstrated by APV16 at -10.6 kcal/mol. A comparison between the APV16, Dorzagliatin and MRK in terms of toxicity predictions using PKCSM indicated that the former exhibited less skin sensitization, AMES toxicity, and hepatotoxicity.

Conclusion: Glucokinase is most potently activated by 100 of the compound leads in the database of 53,000 compounds that contain the coumarin nucleus. APV12, with its high binding affinity, favorable ADMET (adjusted drug metabolic equivalents), minimal toxicity, and favorable pharmacokinetic profile warrants consideration for progress to in vitro testing. Nevertheless, to uncover potential therapeutic implications, particularly in the context of type 2 diabetes, thorough investigations and in-vivo evaluations are necessary for benchmarking before therapeutic use, especially experiments involving the STZ diabetic rat model.

ARTICLE HISTORY

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RESEARCH ARTICLE

Molecular Docking, Pharmacophore Mapping, and Virtual Screening of Novel Glucokinase Activators as Antidiabetic Agents



Anuradha Mehra¹, Amit Mittal^{1,*} and Divya Thakur¹

¹Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Lovely Professional University, Jalandhar-Delhi G.T. Road (NH-1), Phagwara (Punjab), 144411, India

Abstract: Background: A pivotal impetus has led to the development of numerous small molecules to develop therapeutic strategies for type 2 diabetes. Novel heterocyclic derivatives are now available with expansive pharmacological activity designed specifically to activate Glucokinase (GK) in the body. This target is of particular significance in antidiabetic drug design since it is a newly validated target. Individuals with type 2 diabetes are unable to maintain blood glucose homeostasis due to impaired glucokinase function. The novel approach to managing type 2 diabetes relies on utilizing heterocyclic derivatives to activate the GK enzyme, also known as a metabolic enzyme.

Objective: In this research endeavor, the primary objective was to improve drug delivery while minimizing adverse effects by using molecules that activate glucokinase.

Methods: There are 53,000 compounds included in Maybridge's online repository, which has been subjected to rigorous scrutiny. Eight two compounds that encompass the specific oxadiazole core were selectively extracted from this extensive collection. ChemBioDraw Ultra was used for structural drawing, and AutoDock Vina 1.5.6 was used to perform docking analysis. For the online prediction of log P, the SwissADME algorithm was employed. A PKCSM software program was used to predict toxicity for leading compounds.

Results: Among all of the compounds, AD80 and AD27 displayed the highest affinity for GK receptors. These compounds, by adhering to Lipinski's Rule of Five, exhibited good absorption and excretion profiles through the gastrointestinal (GI) tract. Lipinski's Rule of Five refers to physicochemical properties that favor good oral bioavailability, and these specifications are zero to five hydrogen bond donors, zero to ten hydrogen bond acceptors, molecular weight below 500, and log P no more than five. These criteria ensure that the compounds of the invention have acceptable solubility and permeability, which are vital prerequisites when given orally, to be absorbed *via* the gastrointestinal wall, metabolized, and found in the urine. Therefore, the chance of drug candidates exhibiting favorable pharmacokinetic characteristics is increased, enhancing their chances of being developed for oral administration. In comparison with standard drugs Dorzagliatin as a glucokinase activator (GKA) and MRK (co-crystallized ligand), these compounds exhibit no skin sensitization, AMES toxicity, or hepatotoxicity.

Conclusion: The recently designed lead molecules exhibit an improved pharmacokinetic profile, enhanced binding affinity, and minimal toxicity based on the computational study, potentially making them suitable candidates for further optimization as glucokinase activators.

Keywords: Glucokinase activators, diabetes, docking, oxadiazole derivatives, binding affinity, drug design, virtual screening, pharmacophore, molecular docking, AutoDock Vina.

1. INTRODUCTION

Diabetes Mellitus (DM) constitutes a substantial public health challenge, with an impact on over 537 million adults globally [1]. This metabolic disorder is characterized by the

gradual development of chronic, life-threatening complications affecting macrovascular, microvascular, and neuropathic systems. The highest prevalence of diabetes occurs among individuals aged between 40 to 59 years [2]. The incidence and prevalence of type 2 Diabetes Mellitus (T2DM) exhibit geographical variations, with over 80% of affected individuals residing in low-to-middle-income countries. This distribution adds complexity to the challenge of providing effective treatment [3]. The epidemic of T2DM is influenced by a multifaceted interplay of genetic and epige-

*Address correspondence to this author at the Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Lovely Professional University, Jalandhar-Delhi G.T. Road (NH-1), Phagwara, Punjab-144411, India; E-mails: amitmittal77@yahoo.com; amit.31641@lpu.co.in

RESEARCH ARTICLE

Molecular Docking, Pharmacophore Modeling, and ADMET Prediction of Novel Heterocyclic Leads as Glucokinase Activators

Anuradha Mehra¹, Amit Mittal^{1,*} and Shivangi Singh¹

¹Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Lovely Professional University, Jalandhar, Delhi G.T. Road (NH-1), Phagwara, Punjab, 144411, India

Abstract: Background: A pivotal impetus has driven the development of numerous small molecules aiming to improve therapeutic strategies for type 2 diabetes. Glucokinase (GK) activation has been offered a new realm of therapeutic antidiabetic activity with novel heterocyclic derivatives. In the context of antidiabetic drug design, GK is an interesting and newly validated target. A key enzyme needed for blood glucose homeostasis is Glucokinase, which is dysfunctional in individuals with type 2 diabetes. Heterocyclic derivatives are utilized in this innovative approach to activate GK enzymes as medicinal agents that will significantly improve type 2 diabetes management.

Objective: To address type 2 diabetes, as well as minimize unwanted side effects, this research endeavor aimed to develop activators of glucokinase.

Methods: A rigorous scrutiny was conducted of the Maybridge online repository, which houses a formidable collection of 53,000 lead compounds. A collection of 125 compounds that contain the thiazolidinedione core was selected from this extensive collection. The structures were generated using ChemDraw 2D, stabilized conformation with ChemBioDraw Ultra, and docked using Auto Dock Vina 1.5.6 in this methodology. In addition, log P was predicted online using the Swiss ADME algorithm. The PKCSM software was used to predict the toxicity of the leading compounds.

Results: The highest binding affinity was found for AS72 and AS108 to GK receptors. GI absorption and excretion of these compounds were efficient due to Lipinski's Rule of Five compliance. When compared with the standard drugs Dorzagliatin (GKA) and MRK (co-crystallized ligand), these substances demonstrated a notable lack of AMES toxicity, skin sensitization, and hepatotoxicity.

Conclusion: In recent studies, lead molecules that possess enhanced pharmacokinetic profiles, increased binding affinity, and lower toxicity were developed to act as glucokinase activators.

Keywords: Diabetes, docking, thiazolidinedione derivatives, binding affinity, drug design, mechanism.

1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) has become one of the most common worldwide health issues of the 21st century. It is characterized by persistent hyperglycaemia and glucose intolerance. It develops when the body is unable to respond properly to insulin, which is followed by an increase in insulin production and an ensuing insulin shortage. Its widespread presence and incidence have increased most quickly in adults. T2DM is also increasingly affecting children and adolescents [1]. It may also be characterized by β -cell dysfunction [2].

Although there are several anti-diabetic treatments available, many patients do not meet and maintain treatment goals, which raises their long-term risk of microvascular problems, which may include retinopathy and nephropathy, and macrovascular problems, such as cerebrovascular and cardiovascular diseases [3]. Retinopathy occurs when adipose tissue carries a high level of gene, *i.e.*, ACVR1C, which has been associated with retinal tumors, hyperkeratosis (inflammation in stratum corneum), obesity, and T2DM [4]. As a result, there is still a pressing medical need for new therapies. One of the novel therapies presently under study has the potential to help patients better manage their blood sugar levels, which includes small molecules that activate the glucokinase (GK) enzyme [5].

*Address correspondence to this author at the Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Lovely Professional University, Jalandhar-Delhi G.T. Road (NH-1), Phagwara, Punjab, 144411, India; E-mails: amitmittal77@yahoo.com

RESEARCH ARTICLE

In Silico Approaches to Predict Drug Metabolism: Pharmacophore Mapping and Virtual Screening of Heterocyclic Antidiabetic Agents

Anuradha Mehra¹, Amit Mittal^{1,*} and Harmandeep Singh¹

¹Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Lovely Professional University, Jalandhar-Delhi G.T. Road (NH-1), Phagwara (Punjab), 144411, India

Abstract: Introduction: An emerging impetus has driven the development of various small-molecule compounds for the management of type 2 diabetes. With the advent of novel heterocyclic derivatives, an expansive field of pharmacological endeavors has opened up to stimulate Glucokinase (GK) activation. Recent evidence has validated GK's legitimacy as a potential target for pharmaceutical intervention in diabetes. Glucokinase, an enzyme critical to maintaining blood glucose equilibrium, malfunctions in individuals with type 2 diabetes. A key component of this innovative approach is the use of heterocyclic derivatives to stimulate the GK enzyme, thereby serving as powerful pharmaceutical agents to improve type 2 diabetes management substantially.

Methods: Maybridge's digital archive contained 53,000 compounds, known for their efficacy, which were subjected to thorough examination. From this extensive repository, 422 compounds with an indole core were identified. The structures generated by ChemBioDraw Ultra through this method were meticulously docked using AutoDock Vina 1.5.6. Online log P predictions were also made possible by the Swiss ADME algorithm. PKCSM software was used to assess the potential toxicity of the leading compounds.

Results: Auto Dock Vina 1.5.6 was used to dock 422 indole derivatives. Compounds with the greatest binding affinity to glucokinase (GK) were found in the majority of the compounds. Based on the superior binding capabilities of the top eight molecules compared to Dorzagliatin (the standard drug) and MRK (the co-crystallized ligand). Following the enhancement of their pharmacokinetic profiles and compliance with Lipinski's rule of five, these eight candidates were further evaluated using ADMET analysis. As a result, AH249 displayed the greatest binding affinity with -11.5 kcal/mol. In contrast to the standard drugs Dorzagliatin (GKA) and MRK (co-crystallized ligand), AH249 showed no skin sensitization, AMES toxicity, or hepatotoxicity using PKCSM.

Discussion: This research highlights the importance of *in silico* techniques in discovering antidiabetic medicines, as AH249 outperformed 421 other indole derivatives in terms of glucokinase affinity. According to SwissADME results, both compounds, AH249 and AH102, are drug-like. Analysis through molecular docking revealed interactions that can be tested and verified using experiments.

Conclusion: There are 422 selected lead compounds containing an indole base in the 53,000 compound database that demonstrated the best activation of glucokinase. According to the findings of this computational drug design analysis, the most promising drug candidates deserve consideration for advancement to the *in vitro* stage, particularly AH249, which exhibits the greatest binding affinity, a favorable pharmacokinetic profile, and negligible toxicity. For a better understanding of the therapeutic implications of the drug, particularly within the field of type 2 diabetes, more thorough investigation and evaluation are essential, especially the use of *in-vivo* models such as Streptozotocin-induced diabetic rats.

Keywords: Glucokinase activators, docking, type 2 diabetes, indole derivatives, drug design, binding affinity, pharmacophore mapping.

*Address correspondence to this author at the Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Lovely Professional University, Jalandhar-Delhi G.T. Road (NH-1), Phagwara, Punjab-144411, India; E-mails: amitmittal77@yahoo.com, amit.31641@lpu.co.in

LIST OF ORAL PRESENTATIONS

Sr. No.	Details of Presentations
1	Oral presentation at ICP2022 held on 9 th & 10 th November 2022 at Lovely Professional University
2	Oral presentation at ICRAHS-2024 held on 27 April, 2024 at Lovely Professional University
3	Oral presentation at ICRAAS-2025 held on 28 th & 29 th March, 2025 at Maharishi Markandeshwar University, Mullana



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Certificate of Merit

This is to certify that Prof./Dr./Mr./Ms. ANURADHA MEHRA has Presented Poster/
Oral Presentation on Insilico screening of novel heterocyclic derivatives
as Glucokinase Activator and awarded for best poster/ oral presentation in the 3rd International
Conference of Pharmacy (ICP-2022) on the Theme of "Practice, Promotion & Publication of Innovation : A Way of
Transforming Health" held on 09th & 10th November 2022 organized by School of Pharmaceutical Sciences in a
collaboration with Indian Pharmaceutical Association (IPA) at Lovely Professional University, Punjab.

Mr. Suresh Khanna
National Hon. Gen.
Secretary, IPA

Dr. Bimlesh Kumar
Organizing Secretary

Dr. T.V. Naryana
National President
IPA

Dr. Monica Gulati
LOC Chairperson



Sri Herbasia Biotech



Tishk International University, Iraq



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Mullana-Ambala, Haryana
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(Accredited by NAAC with Grade 'A++')



Certificate OF PARTICIPATION

Presented to

ANURADHA

In recognition of the Participation / ☒ Oral / ☐ Poster Presentation on

Design, Molecular docking and ADMET studies of
novel heterocyclic derivatives as antidiabetic agent

In the 3rd International Conference on Recent Advances in Applied Sciences (ICRAAS-2025) on dated 28th & 29th March, 2025
organized by the Department of Chemistry, MMEC, Maharishi Markandeshwar (Deemed to be University), Mullana, India.

Dr. Nirankar Singh
Co-Convenor
MMEC, MM(DU)

Dr. Bhawna Pareek
Convenor, HOD Chemistry
MMEC, MM(DU)

Dr. Vishal Bharti
Principal
MMEC, MM(DU)

Dr. Sumit Mittal
Registrar, MM(DU)



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**PHARMACEUTICAL
SCIENCES**

Certificate No. 328005

Certificate of Participation

This is to certify that **Prof./Dr./Mr./Ms. Anuradha** has successfully participated as **Delegate and Oral Presenter** on topic **Rationale design and virtual screening of novel heterocyclic derivatives as Antidiabetic agent** in online mode at **"International Conference on Recent Advances in Health Sciences" (ICRAHS-2024)** under the Theme **"Innovations in Sustainable Health Sciences and Research"** held on **27th April 2024**, organized by School of Pharmaceutical Sciences, Lovely Professional University, Punjab.

Date of Issue : 16-05-2024

Place : Phagwara (Punjab), India

Prepared by
(Administrative Officer-Records)

Organizing Secretary
Dr. Vikas Sharma
School of Pharmaceutical Science

Head of School
Dr. Monica Gulati
School of Pharmaceutical Science

Ethical approval certificate



सी.एस.आई.आर.-केन्द्रीय औषधि अनुसंधान संस्थान, लखनऊ
(वैज्ञानिक तथा औद्योगिक अनुसंधान परिषद्)

CSIR-Central Drug Research Institute, Lucknow
(Council of Scientific & Industrial Research)

पोस्ट बॉक्स नं. 173, सेक्टर 10, जानकीपुरम विस्तार, सीतापुर रोड, लखनऊ - 226031 (भारत)
Post Box No. 173, Sector 10, Jankipuram Extension, Sitapur Road, Lucknow-226031 (INDIA)

दूरभाष- Phone: +91-522-2772450, 2772550(PABX), फैक्स Fax: +91-0522-2771941,
टी-फैक्स T-Fax: 2771942, 2772793, ग्राम Gram: CENDRUG बेब Web: www.cdriindia.org



Activity report

Sample source: Anuradha Mehra, Asst. Professor, Lovely Professional University, Phagwara, Punjab,
India

Number of samples : 02

Test requested: Antihyperglycemic activity in Streptozotocin-induced diabetic rat

Model: Streptozotocin-induced diabetic SD rat

Methodology: Sprague Dawley male rats, 7-8 weeks old, weighing 160 ± 20 g, bred at the animal facility of the institute were used in the present study. The animals (5/cage) were housed in polypropylene cages and maintained at temperature $23 \pm 2^\circ\text{C}$; humidity 50-60%; light 300 lux at floor level with regular 12 h light/dark cycle. The animals were provided standard pellet diet and drinking water *ad libitum*. The work with these animals was cleared by the Institutional Animal Ethics Committee (IAEC) of the CSIR-Central Drug Research Institute, Lucknow (Ref. No. IAEC/2022/6/ Renew-3/ Sr. No-12, dated 30.06.2025) and was conducted by the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) formed by the Government of India. Rats were made diabetic by injecting streptozotocin (55 mg/kg in 0.1M citrate buffer pH 4.5) intraperitoneally after overnight-fasting. Blood glucose was checked 72 h later and animals showing blood glucose value above 280 mg/dl were included in the experiment and considered as diabetic.

Date: 20/08/2025

Dr. Akhilesh K. Tamrakar
Division of Biochemistry & Structural Biology

Copyright Details
Graphical Abstract Submission Form

Title of Graphical abstract: Design and development of novel heterocyclic derivatives as antidiabetic agent

Authors name: Anuradha, Amit Mittal

Name of Scholar: Anuradha **Registration Number:** 12208835

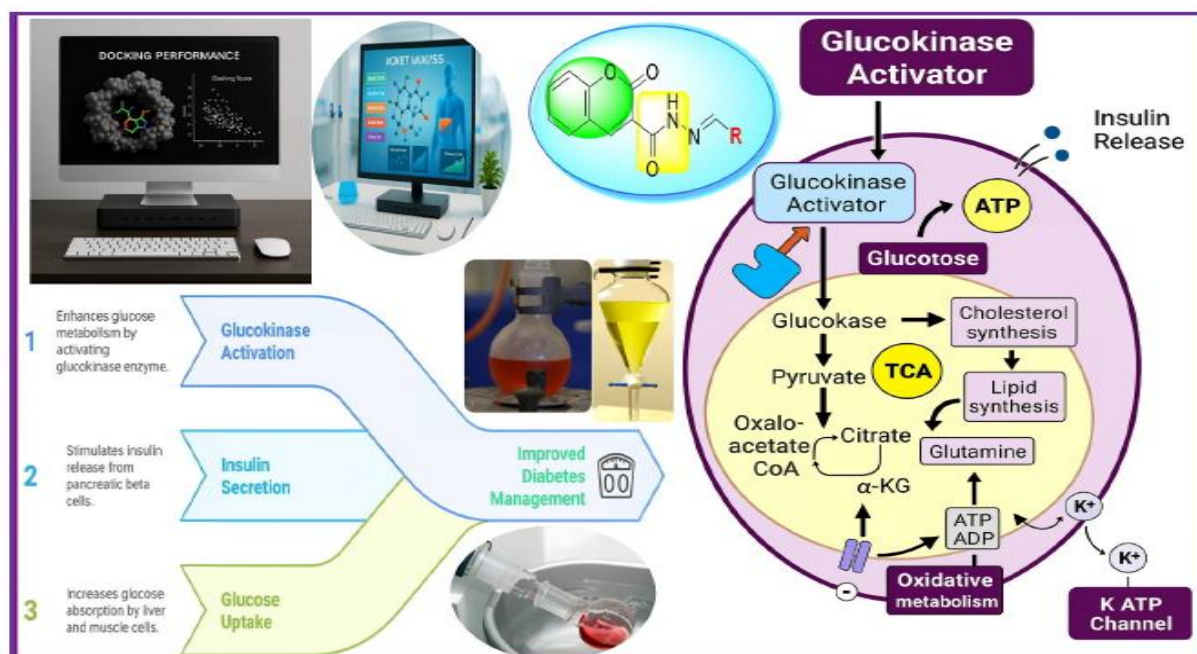
Program Name: Ph.D in Pharmaceutical Chemistry

Name/ UID of Supervisor: Dr. Amit Mittal (31641)

School: Pharmaceutical Sciences

Summary of graphical abstract: The aim of this research is to create and discover new heterocyclic compounds that have good antidiabetic effects and work by targeting the Glucokinase enzyme. The workflow begins with the edifice of the rational design of new molecular entities using ligand-based design strategies. Having applied virtual screening, the strongly binding candidates are highlighted from the designed set of derivatives. These chosen molecules are synthesized and their structures are identified by spectral analysis. The *in vitro* evaluation is performed to analyze the GK activation. The compound that stands out the most active in *in vitro* biological tests, is advanced to *in vivo* evaluation, directing to confirm its efficacy as a novel antidiabetic agent through GK activation.

Graphical abstract:



Caption for graphical abstract: Heterocyclic scaffolds as antidiabetic agent