Synthesis, Characterization and Molecular Docking Studies of Some Newer Thiazolidine-2,4-dione Derivatives

Thesis submitted in partial fulfillment for the requirement of the

Degree of Master of Pharmacy

Faculty of Engineering and Technology

by

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2023

Certificate of Approval

This is to certify that **Pankaj Nahata** (Examination Roll No: M4PHC23026, Registration No: 160237 of 2021-22) has carried out the project work on the subject entitled "***Synthesis, Characterization & Molecular Docking Studies of Some Newer Thiazolidine-2,4-dione Derivatives***" under the supervision of **Prof. (Dr.) Tapan Kumar Maity**, Professor, Department of Pharmaceutical Technology, Jadavpur University. This project work is submitted by him in partial fulfilment of the requirements for the degree of **Master of Pharmacy (Pharmaceutical Chemistry)** of Jadavpur University. He has carried out his work independently and with proper care and attention to our entire satisfaction.

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Declaration of the Originality and Compliance of Academic Ethics

I hereby declare that this thesis contains a literature survey and original research part of my work on "***Synthesis, Characterization & Molecular Docking Studies of Some Newer Thiazolidine-2,4-dione Derivatives*".**

All the information in this document has been obtained and presented in accordance with academic rules and ethical conduct.

I also declare that as required by these rules and conduct, I have fully cited and referred all the information and results that are not original to this work.

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Dedicated to

my guide, family and well wishers

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CHAPTER 1

INTRODUCTION

1. Introduction:

Nitrogen and sulphur-containing heterocyclic compounds, especially those belonging to the thiazole family, have attracted particular interest in their synthetic chemistry because of their pharmacological effectiveness [1]. The heterocyclic nucleus thiazolidine-2,4-dione (TZD) has been intensively studied for the development of innovative medications for several pathophysiological illnesses, including melanoma, diabetes complications, cancer, arthritis, and inflammatory diseases. Apart from medications, TZD is also helpful in inhibiting corrosion of mild steels, as a highly sensitive reagent for heavy metals and as a brighter in the electroplating industry. One of them is the extensively researched anti-hyperglycemic impact of TZD derivatives, which has also led to the development of clinically utilized "glitazone" medications such as rosiglitazone, pioglitazone, lobeglitazone, and troglitazone (**Figure 1**) [2,3].

Diabetes is a fatal metabolic condition characterized by persistent hyperglycemia. Type 1 diabetes mellitus (T1DM), which is caused by a partial or total loss of insulin secretion, or Type 2 diabetes mellitus (T2DM), is caused by insulin irresponsiveness to its target tissues. People with diabetes are more likely to develop significant, life-threatening complications, which raise medical care expenses, lower quality of life, and shorten life expectancy. Some patients with diabetes don't receive a diagnosis until complications such as a foot ulcer, kidney failure, or changes in vision [4].

Approximately 451 million individuals worldwide, or 1 in 11 of the adult population, have type 2 diabetes (T2D). By 2045, the International Diabetes Federation predicted that there would be 693 million persons with diabetes globally. If we consider India, its proportion will be around 101.2 million by 2030.

The first line of treatment for high blood sugar is metformin, which is contraindicated in cases of lactic acidosis. Other commonly used groups of antihyperglycemic

medications include meglitinides, thiazolidinediones, alpha-glucosidase inhibitors, and DPP4 inhibitors. These medications can be used alone or in conjunction with metformin. For the treatment of T2D, numerous new novel pharmacological targets have recently been identified, including 5′ AMP-activated protein kinase, fructose-1, 6-bisphosphatase, glucagonlike peptide-1, glucokinase, G protein-coupled receptor 119, glycogen synthase kinase 3, PTP1B, SGLT2, etc. [5].

In my present work, we have synthesized some newer TZD derivatives and studied their binding modalities through molecular docking studies with two key targets (alpha-amylase and alpha-glucosidase) for management of T2D. Our future perspective is to evaluate this synthesized compound for inhibitory activity on various enzymes, viz. alpha-amylase, alpha-glucosidase and PTP1B.

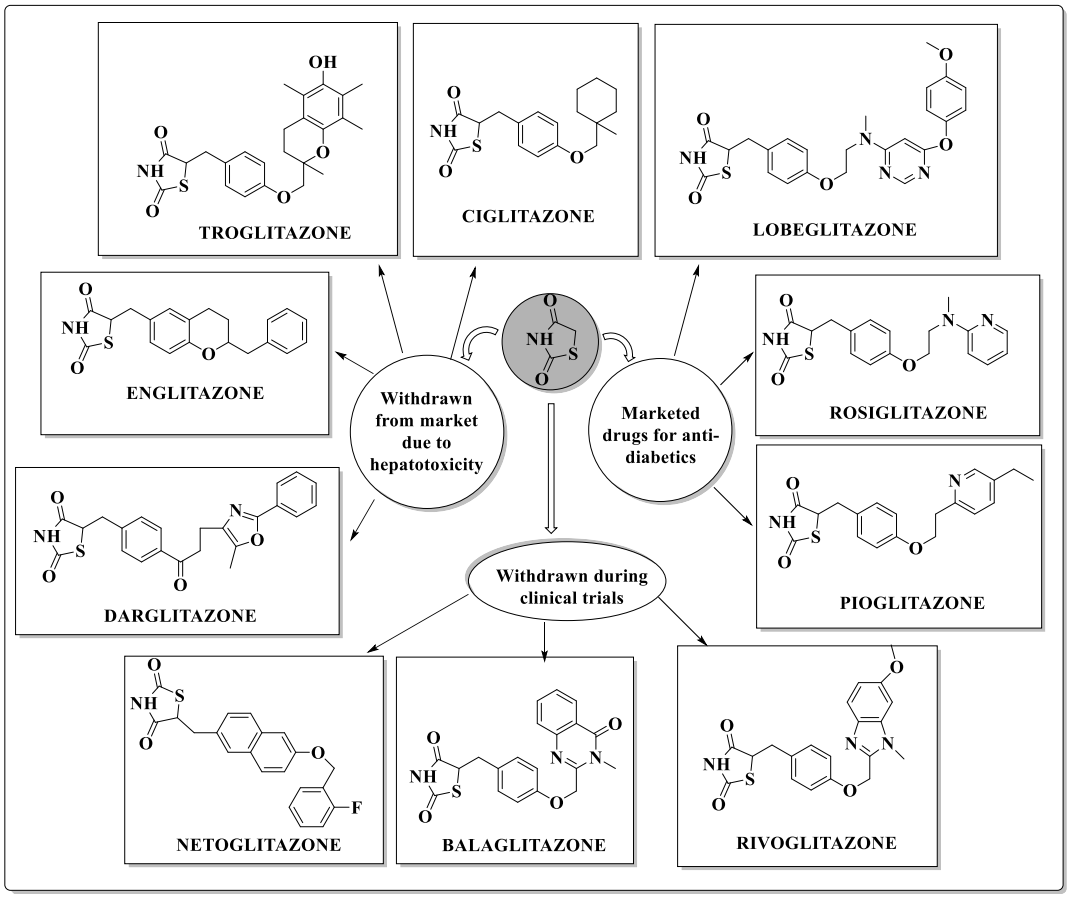


Figure 1: Representatives of antidiabetic drugs containing TZD moiety.

1.1. Brief history of thiazolidine-2,4-dione (TZD) scaffold:

Thiazolidine-2,4-dione (TZD) is a pentacyclic moiety that captured the attention when an Italian scientist, "Vistentini", reported the first pharmacological assessment, i.e., anti-TB activity, of any TZD derivative in 1954. In the same year, Marshall and Vallance documented the anti-convulsing action of various TZD derivatives. Later, in the 1960s and 1970s, other research groups evaluated different pharmacological and toxicological effects of TZD compounds [2]. The first marketed molecule for anti-diabetics was ciglitazone, which caused hepatotoxicity and was withdrawn later. After that, Shankyo Company developed another drug containing TZD scaffold, namely troglitazone, in 1988 and also caused hepatotoxicity. In 1999, two pharmaceutical companies, Takeda and Pfizer, developed two similar molecules, pioglitazone and englitazone, respectively. Since pioglitazone did not show a sign of liver toxicity, it made its position in the market. However, englitazone was discontinued from the market due to the same adverse phenomena. However, at the same time of that particular year, SmithKline and Pfizer developed two other drugs of this class, viz. rosiglitazone and darglitazone. Whereas darglitazone was stopped immediately from the market in 1999, with rosiglitazone, there was some incident of fluid retention and heart failure reported in 2001 and restricted by the Food and Drug Administration. (FDA) in 2010 and later, due to a lack of evidences, the FDA withdrew the restrictions (**Figure 2**) [6].

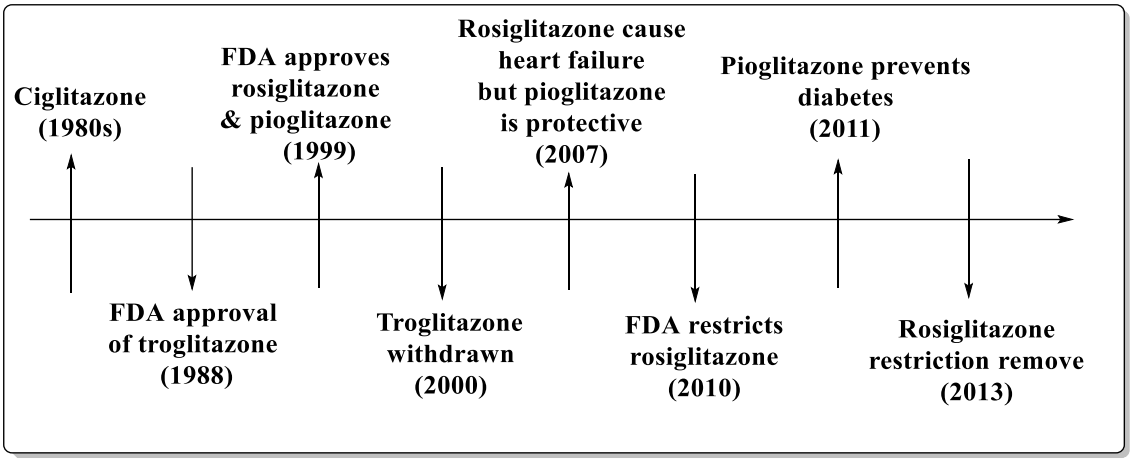


Figure 2: The history of TZDs.

1.2. Chemistry and reactivity of thiazolidine-2,4-dione (TZD):

1.2.1. Chemistry of TZDs:

Thiazolidine-2,4-dione (TZD) is a five-member heterocyclic ring bearing one sulphur, nitrogen, methylene, and carbonyl group. The molecular formula of TZD is $C_3H_3NO_2S$. TZD is an attractive scaffold that has drawn a lot of attention, and extensive research has been carried out due to its diverse pharmacological activities. The TZD nucleus can be synthesized using thiosemicarbazone, thiourea and thiocarbamate as starting compounds. However, the most common way of synthesizing thiazolidinedione is by refluxing α-chloroacetic acid with thiourea for 12 hours. One added advantage of this protocol is that the reaction rate can be accelerated with the help of a microwave-assisted technique (**Figure 3**) [7]. pK_a value of TZD was determined to be 6.82 [3]. The melting point range of TZD is between 120-122 ^oC [3]. In the TZD nucleus, two carbonyl groups and one α hydrogen are present. Because of this, TZD undergoes different tautomerism-amide-idol and keto-enol **Figure 4.**

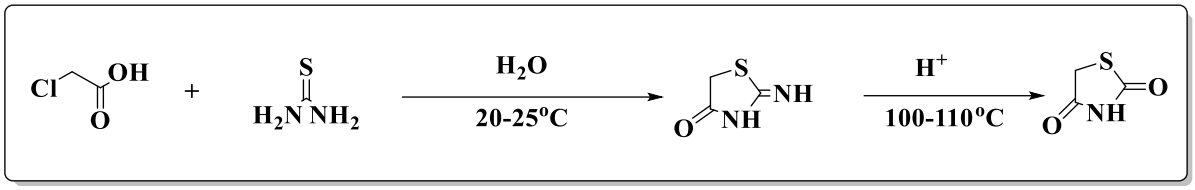


Figure 3: Synthesis of thiazolidine-2,4-dione (TZD) using thiourea and α-chloroacetic acid.

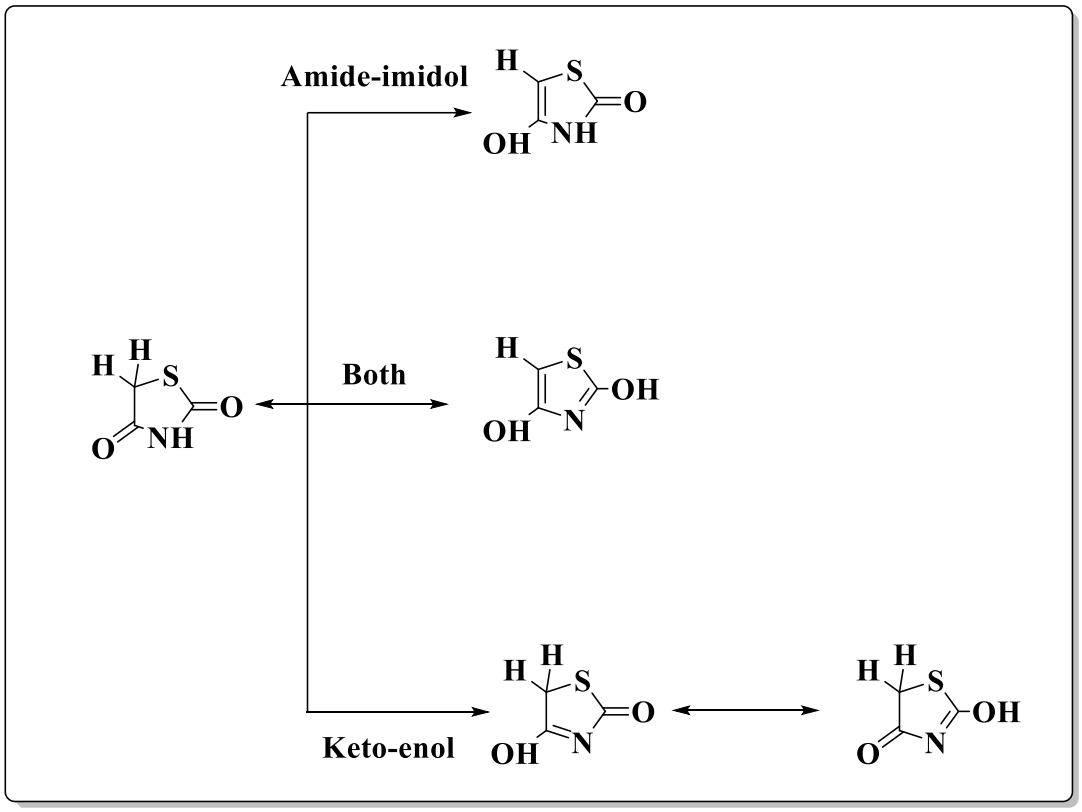


Figure 4: Tautomeric structure of TZD.

1.2.2. Reactivity of TZDs:

There are two positions in the TZD core where substitution can be done.

1.2.2.1. Reactivity at the -NH group of TZD core:

Substitution at NH- group can be done by aryl or alkyl halides in the presence of a base and a solvent. As base potassium carbonate, tetrabutylammonium iodide or sodium hydride can be used, and for solvent, DMF or acetone can be taken.

1.2.2.2. Reactivity -CH_2 moiety of TZD core:

The methylene moiety can be substituted by aromatic aldehydes or ketones and the formation of arylidene derivative of TZD. This reaction is known as 'Knoevenagel condensation'. This particular condensation can be carried out in different conditions. The most commonly used reagents are piperidine (as base) and ethanol or methanol as solvent. Also, anhydrous sodium acetate in glacial acetic acid was another option for Knoevenagel condensation of TZD with aldehyde. In the case of condensation with ketone, ammonium acetate or piperidinium acetate in toluene or ethyl acetate were used (**Figure 5**) [2].

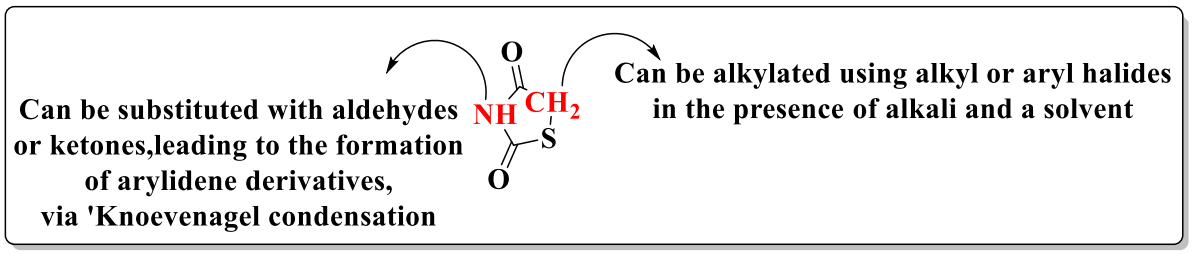


Figure 5: Reactivity of TZD at -CH_2 and -NH.

Dynamic alterations occur at positions 3 and 5, although replacement at position 2 causes the most structural and property changes in TZD. Sulphur replaces oxygen at position 2 to produce rhodanine derivatives, whereas oxygen removes sulphur at position 1 to produce oxazolidinedione derivatives (**Figure 6**) [8].

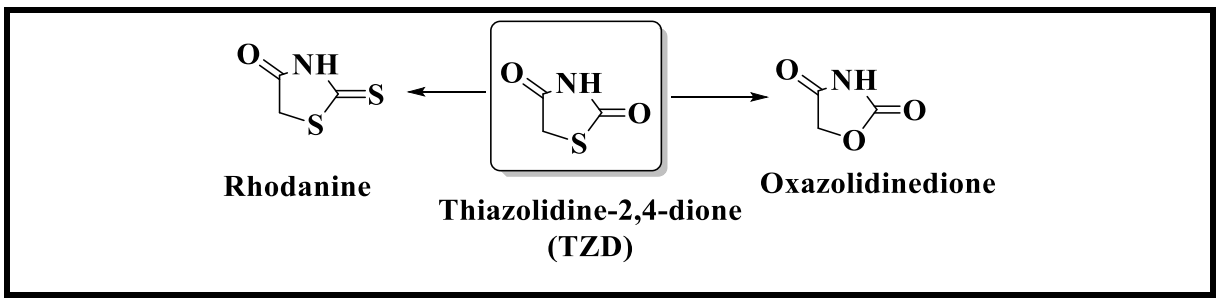


Figure 6: Dynamic alterations of TZD.

1.3. Therapeutic targets in the management of type 2 diabetes mellitus:

1.3.1. Role of PTP1B as a target in diabetes:

Protein tyrosine phosphatases are a group of enzymes that remove phosphate groups from phosphorylated tyrosine residues on proteins. Protein Tyrosine Phosphatase (PTP) play an important role in cell signalling, such as cell growth and differentiation, metabolism, and immune response. PTP1b, a significant member of this group of enzymes, is responsible for the negative regulation of insulin-stimulated signal transduction pathways.

Mechanism of action:

The insulin receptor consists of two subunits: a ligand-binding subunit and a tyrosine kinase subunit, which is responsible for phosphorylating specific tyrosine residues.

Signal propagation happens in a very specific series of steps:

i. Insulin binds to the receptor, and the tyrosine kinase subunit is activated after autophosphorylation of tyrosine residues.

ii. Then, tyrosine kinase phosphorylates other insulin receptor substrate proteins downstream.

iii. This then enables insulin signal propagation, eventually allowing glucose within the cell.

The opposite of tyrosine kinase is tyrosine phosphatase, which removes the phosphates group from the tyrosine residue. Tyrosine kinases and phosphatases work together in regulating signalling within the cell, while kinases are critical in controlling the amplitude of a cellular response; it is widely believed that phosphatases have an essential role in maintaining the

rate and duration of these responses. Now, PTP1B inhibitors will inhibit phosphatase enzyme activity, generally turning off the insulin transduction pathway. This will result in the signal transduction pathway being active for a more extended period of time. Thus allowing more glucose inside the cell [9,10].

1.3.2. Role of alpha-amylase as a target in diabetes:

One of the most important digestive enzymes in humans is pancreatic alpha-amylase (EC 3.2.1.1), which catalyzes the reaction that breaks down starch, amylopectin, amylose, glycogen, and numerous maltodextrins through the hydrolysis of their alpha-1,4 glycosidic linkages. Taka-amylase A is another name for alpha-amylase, honouring the discovery of Takamine. The main digesting enzyme found in saliva is amylase. Amylase becomes inactive in the stomach due to gastric acid. Because of this, the optimal pH for alpha-amylase is slightly alkaline. Since glucose must travel to the brain, big molecules like starch are unable to penetrate the blood-brain barrier. To solve this issue, alpha-amylase breaks the giant starch molecules down into smaller sugar pieces. The job of insulin is to direct cells to metabolise the extra sugar moieties and store them as energy sources, such as glycogen, if there is an excessive amount of starch conversion to sugars, which raises the blood sugar level. But in some circumstances, the amylase enzyme over-activates, there is a lack of insulin, or there is insulin resistance, and this can cause blood sugar levels to rise and lead to hyperglycemia. Thus, by inhibiting the enzyme, we can control diabetes [11,12].

1.3.3. Role of alpha-glucosidase as a target in diabetes:

Multiple digestive mechanisms in the gastrointestinal tract convert complex carbohydrates into monosaccharides, which are absorbed in the small intestine. The

first step in the digestive process is the generation of amylases (EC 3.2.1.1), which catalyse the breakdown of starch into shorter polysaccharides and are produced mainly by the pancreatic and salivary glands. The pancreatic amylases target the α-1,4 links of carbohydrate-releasing dextrins to process partially hydrolysed starch further after it reaches the small intestine. The final stage of glucose metabolism is mediated by α-glucosidases near the brush edge of enterocytes. The enzymes hydrolyse α-glucosidic disaccharide and oligosaccharide linkages thanks to duplicated glycoside hydrolase domains (GH31). The oligosaccharides resulting from α-amylase digestion are finally hydrolysed to monosaccharides by α-glucosidases; maltase glucoamylase and sucrose isomaltose, releasing glucose from non-reducing ends of oligosaccharides. Conversion to monosaccharide is necessary for glucose absorption from brush border cells of the intestine to blood. Thus, inhibiting the enzyme reduces the absorption of glucose and results in the decrease of post-prandial glucose levels [13].

References:

1) Kumar, H., Aggarwal, N., Marwaha, M. G., Deep, A., Chopra, H., Matin, M. M., Roy, A., Emran, T. B., Mohanta, Y. K., Ahmed, R., Mohanta, T. K., Saravanan, M., Marwaha, R. K., & Al-Harrasi, A. (2022). Thiazolidin-2,4-Dione Scaffold: An Insight into Recent Advances as Antimicrobial, Antioxidant, and Hypoglycemic Agents. *Molecules (Basel, Switzerland)*, *27*(19), 6763. https://doi.org/10.3390/molecules27196763
2) Chadha, N., Bahia, M. S., Kaur, M., & Silakari, O. (2015). Thiazolidine-2,4-dione derivatives: programmed chemical weapons for key protein targets of various pathological conditions. *Bioorganic & medicinal chemistry*, *23*(13), 2953–2974. https://doi.org/10.1016/j.bmc.2015.03.071
3) Jain, V. S., Vora, D. K., & Ramaa, C. S. (2013). Thiazolidine-2,4-diones: progress towards multifarious applications. *Bioorganic & medicinal chemistry*, *21*(7), 1599–1620. https://doi.org/10.1016/j.bmc.2013.01.029
4) Kharyal, A., Ranjan, S., Jaswal, S., Parveen, D., Gupta, G. D., Thareja, S., & Verma, S. K. (2022). Research progress on 2,4-thiazolidinedione and 2-thioxo-4-thiazolidinone analogues as aldose reductase inhibitors. In Journal of Molecular Structure (Vol. 1269, p. 133742). Elsevier BV. https://doi.org/10.1016/j.molstruc.2022.133742
5) Rochester CD, Akiyode O. Novel and emerging diabetes mellitus drug therapies for the type 2 diabetes patient. World J Diabetes. 2014 Jun 15;5(3):305-15. doi: 10.4239/wjd. v5.i3.305. PMID: 24936252; PMCID: PMC4058735.
6) Bansal, G., Thanikachalam, P. V., Maurya, R. K., Chawla, P., & Ramamurthy, S. (2020). An overview on the medicinal perspective of thiazolidine-2,4-dione: A remarkable scaffold in treating type 2 diabetes. *Journal of advanced research*, *23*, 163–205. https://doi.org/10.1016/j.jare.2020.01.008
7) Bhat, A. R., Najar, M. H., Dongre, R. S., & Akhter, M. S. (2020). Microwave assisted synthesis of Knoevenagel Derivatives using water as green solvent. In Current Research in Green and Sustainable Chemistry (Vol. 3, p. 100008). Elsevier BV. https://doi.org/10.1016/j.crgsc.2020.06.001
8) Naim, M. J., Alam, M. J., Ahmad, S., Nawaz, F., Shrivastava, N., Sahu, M., & Alam, O. (2017). Therapeutic journey of 2,4-thiazolidinediones as a versatile scaffold: An insight into structure activity relationship. *European journal of medicinal chemistry*, *129*, 218–250. https://doi.org/10.1016/j.ejmech.2017.02.031

9) Proença, C., Ribeiro, D., Freitas, M., Carvalho, F., & Fernandes, E. (2022). A comprehensive review on the antidiabetic activity of flavonoids targeting PTP1B and DPP-4: a structure-activity relationship analysis. *Critical reviews in food science and nutrition*, *62*(15), 4095–4151. https://doi.org/10.1080/10408398.2021.1872483.

10) Kumar, A., Rana, D., Rana, R., & Bhatia, R. (2020). Protein Tyrosine Phosphatase (PTP1B): A promising Drug Target Against Life-threatening Ailments. *Current molecular pharmacology*, *13*(1), 17–30. https://doi.org/10.2174/1874467212666190724150723

11) Kaur, N., Kumar, V., Nayak, S. K., Wadhwa, P., Kaur, P., & Sahu, S. K. (2021). Alpha-amylase as molecular target for treatment of diabetes mellitus: A comprehensive review. *Chemical biology & drug design*, *98*(4), 539–560. https://doi.org/10.1111/cbdd.13909

12) Horii, S., Fukase, H., Matsuo, T., Kameda, Y., Asano, N., & Matsui, K. (1986). Synthesis and alpha-D-glucosidase inhibitory activity of N-substituted valiolamine derivatives as potential oral antidiabetic agents. *Journal of medicinal chemistry*, *29*(6), 1038–1046. https://doi.org/10.1021/jm00156a023

13) Kashtoh, H., & Baek, K. H. (2022). Recent Updates on Phytoconstituent Alpha-Glucosidase Inhibitors: An Approach towards the Treatment of Type Two Diabetes. *Plants (Basel, Switzerland)*, *11*(20), 2722. https://doi.org/10.3390/plants11202722

CHAPTER 2
LITERATURE REVIEW

2. Literature review:

2.1. TZD as alpha-amylase inhibitors:

Manasa and his co-workers synthesized a series of twenty compounds of TZD-phenothiazine derivatives for antidiabetic activity. Synthesized compounds have been tested for alpha-amylase and glucose uptake activity by taking acarbose and metronidazole as positive controls, respectively. After testing the compounds for amylase inhibition with the 3,5-di-nitro salicylic acid (DNS) method, it has been found that four compounds with electron-withdrawing groups on phenylene moiety of TZD enhance inhibition activity reporting IC_{50} value of 83.7-60.8 μM which were more than acarbose (101.7 μM) with **compound 1 (Figure 7)** being the most potent inhibitor. Glucose uptake studies revealed that the electron-releasing group increase glucose uptake activity in yeast. The role of the 3-nitro, 2-chloro-6-fluoro, 4-nitro, and 2,3-dichloro groups on the phenylene moiety of TZD in the inhibition of -amylase enzymes was demonstrated by docking experiments [1].

Chirag et al. developed a series of ten compounds of biphenyl carbonitrile-TZD moiety by using a combination technique by combining two privileged scaffolds, one being a glitazone scaffold and other with PPAR α/γ agonist and PDF inhibition property. It was speculated that the N-atom of biphenyl carbonitrile-TZD moiety developed a better spatial arrangement to bind with the α-amylase active site. From the results of *in vitro* α-amylase studies, it was revealed that **compounds 2** and **3 (Figure 7)** showed high potency with an IC_{50} value of 0.13 μM. Structure-activity relationship (SAR) studies revealed that methoxy and phenoxy were the most potent, and the electron-withdrawing nitro group was the least potent [2].

Sameeh and co-workers developed TZD-containing compounds and synthesized compounds demonstrated low to moderate anti-hyperglycemic potency, with

compound 4 (Figure 7) being the most potent in contrast to standard, according to an evaluation of their radical scavenging and α-amylase inhibitory capabilities. *In vivo,* tests using an alloxan-induced diabetic rat model were carried out, and **compound 4** showed a 69.55% reduction in blood glucose levels. Compounds also demonstrated promising results when measured against several biological indicators. (CH, LDL and HDL) [3].

Addanki et al. designed some phosphorylated derivatives of a TZD, and studied there *in silico* ADMET and molecular docking studies, where results showed drug likeliness and good oral bioavailability. All the compounds were subjected to *in vitro* α-amylase inhibition activity, taking acarbose as standard (IC_{50} = 110.5 µg/ml), and **compound 5 (Figure 7)** was found to be the most potent inhibitor (IC_{50} = 113.1 µg/ml). It was also reported that synthesized compounds are poorly absorbed through the GI tract and unable to cross the BBB, making them unsuitable as P-glycoprotein substrates [4].

Sujatha and co-workers develpoed phosphonates containing TZD moiety as antidiabetic agents. Docking studies revealed that compounds showed better binding than standard rosiglitazone against PPARγ. Compounds were subjected to *in vitro* α-amylase inhibition activity, and **compound 6 (Figure 7)** was found to be most potent among others with IC_{50} = 15.8 µg/ml, which is much less than standard acarbose (47.8 µg/ml) [5].

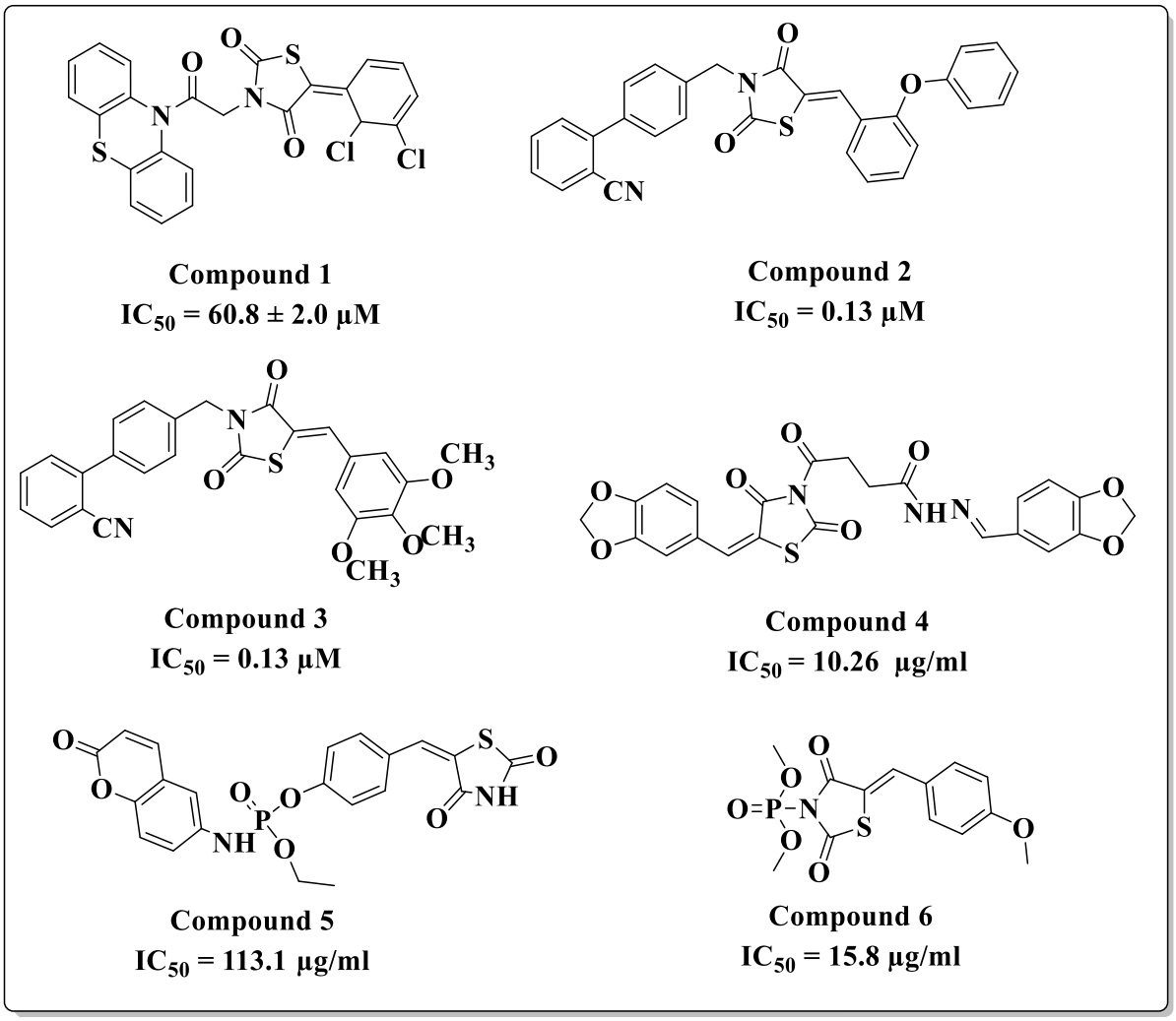


Figure 7: Compounds inhibiting α-amylase.

2.2. TZD as alpha-glucosidase inhibitors:

Hussain et al. synthesized molecules containing TZD and dihydropyrimidine and screened for alpha-glucosidase inhibition properties. From the results, it was found that **compound 7 (Figure 8)** showed the highest potency with IC_{50} of 0.98 µM. *In vivo*, studies on alloxan-induced diabetic mice model confirmed the anti-diabetic potential of the compound. Moreover, toxicity studies ensured the safety of these compounds and SAR studies revealed that N-phenyl, C-phenyl and ethoxy substitution showed better inhibition [6].

TZD, rhodanine, hydantoin, and thiohydantoin-containing novel chemical entities that are connected to benzoxazolyl via meta- and para substitution were developed by Singh

and co-workers. Compounds were tested for alpha-glucosidase inhibitory activity, and **compound 8 (Figure 8),** which is a rhodanine moiety substituted at the meta-position of the phenyl ring, was found to be the most potent inhibitor of α-glucosidase. Further, *in vitro* results were confirmed by correlating with docking results, which showed a good correlation (R^2= 0.883) [7].

Patil et al. developed thirty-two molecules of 5-benzylidine-2,4-thiazolidinedione derivatives to investigate their α-glucosidase inhibitory activity. From *in vitro* studies, it was confirmed that **compound 9 (Figure 8)** is the most potent among others. **Compound 9** has a chlorine group at the 4th position of the phenyl ring and two methyl groups at the 5th and 6th position of the benzothiazole moiety. Out of thirty-two compounds, six were satisfactorily inhibiting the α-glucosidase enzyme when compared with standard acarbose. Docking studies were performed to defend *in vitro* results through which it was found that binding energy was between -7.9 to -9.2 kcal/mol of all ligand-enzyme complexes [8].

Wang et al. developed new series of TZD or rhodanine derivatives for anti-diabetic agents. Compounds were screened for α-glucosidase inhibitory potential, and **compound 10 (Figure 8)** (IC_{50} = 5.44 µM) was found to be most active. Other compounds in the series showed moderate to high inhibitory activity when compared with acarbose (IC_{50} = 817.38 µM). SAR studies revealed that TZD or rhodanine at the 4-position and electron-withdrawing group at the 2-position of phenyl enhances activity [9].

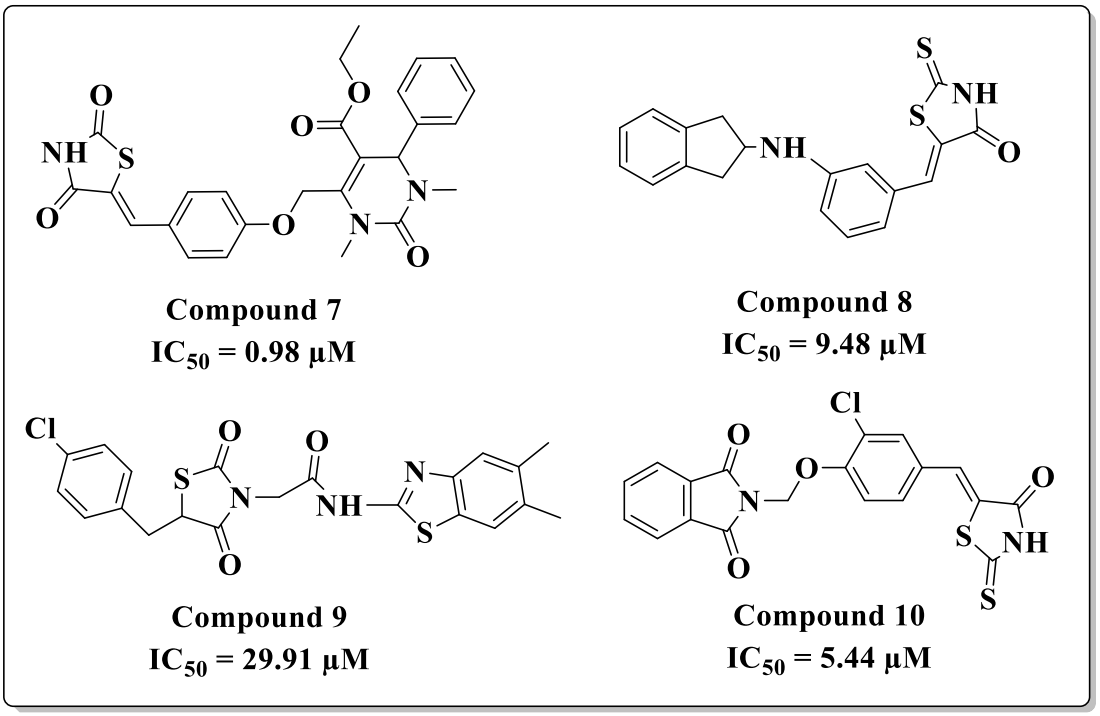


Figure 8: Compounds inhibiting α-glucosidase.

2.3. TZD as dual alpha-glucosidase and alpha-amylase inhibitors:

Arineitwe et al. developed four compounds using a pharmacophore hybridization technique based on N-aryl pyrrole and TZD. Compounds were evaluated for anti-diabetic properties by inhibiting various enzymes, viz. α-amylase, α-glucosidase, aldose reductase, dipeptidyl peptidase-4(DPP4) and PTP1B. It was revealed that **Compound 11 (Figure 9)** was most potent for α-amylase inhibition (18.24 µg/ml) and **Compound 12 (Figure 9)** was most potent for α-glucosidase inhibition (56.82 µg/ml). Furthermore, molecules were docked with PPAR-γ, showing good binding, much like rosiglitazone [10].

Huneif et al. continued their study on a multi-target inhibitor of the thiazolidine-vanillin hybrid molecule by replacing vanillin with a succinimide-based substitute. They developed five molecules by substituting moieties on the N-atom of succimide. Compounds were tested for α-amylase, α-glucosidase, aldose reductase and DPP4 and **compound 13 (Figure 9)** was found to be the most effective anti-diabetic agent.

Furthermore, *in silico, in vivo* and docking results gave promising results, after which it was concluded that the benzyl group of **compound 13** could be further substituted [11]. Srinivasa et al. synthesized some hybrid molecules containing TZD and oxadiazoles that can inhibit α-amylase and α-glucosidase. The compounds were subjected to *in vitro* α-amylase and α-glucosidase inhibitory assays and *in vivo* anti-hyperglycemic activity study. The results showed the good inhibitory potential of the compounds, out of which **compound 14 (Figure 9)** (α-amylase; IC_{50} = 18.42 µM and α-glucosidase; IC_{50} = 17.21 µM) showed the highest potency. *In vivo* studies also revealed a considerable reduction of blood glucose levels after treatment with the compounds on Drosophila melanogaster. SAR studies revealed that methylene linker between TZD and oxadiazole enhances activity. It was also found that withdrawing groups at ortho and para positions and the presence of electronegative groups increased activity. In contrast, activity decreases because of electron-donating and phenyl groups at the para position [12].

A series of compounds containing TZD and quinoline were designed and synthesized by Angajala et al. by Knoevenagel condensation and N-alkylation, a novel one-pot three-component technique was used and catalysed by ANAP (Aspergillus niger from alkaline protease). After performing *in vitro* α-amylase and α-glucosidase inhibitory assays and *in vivo* for evaluating hypoglycemic activity, it was found that **compounds 15** and **16 (Figure 9)** showed better hypoglycemic activity in comparison to the standard pioglitazone [13].

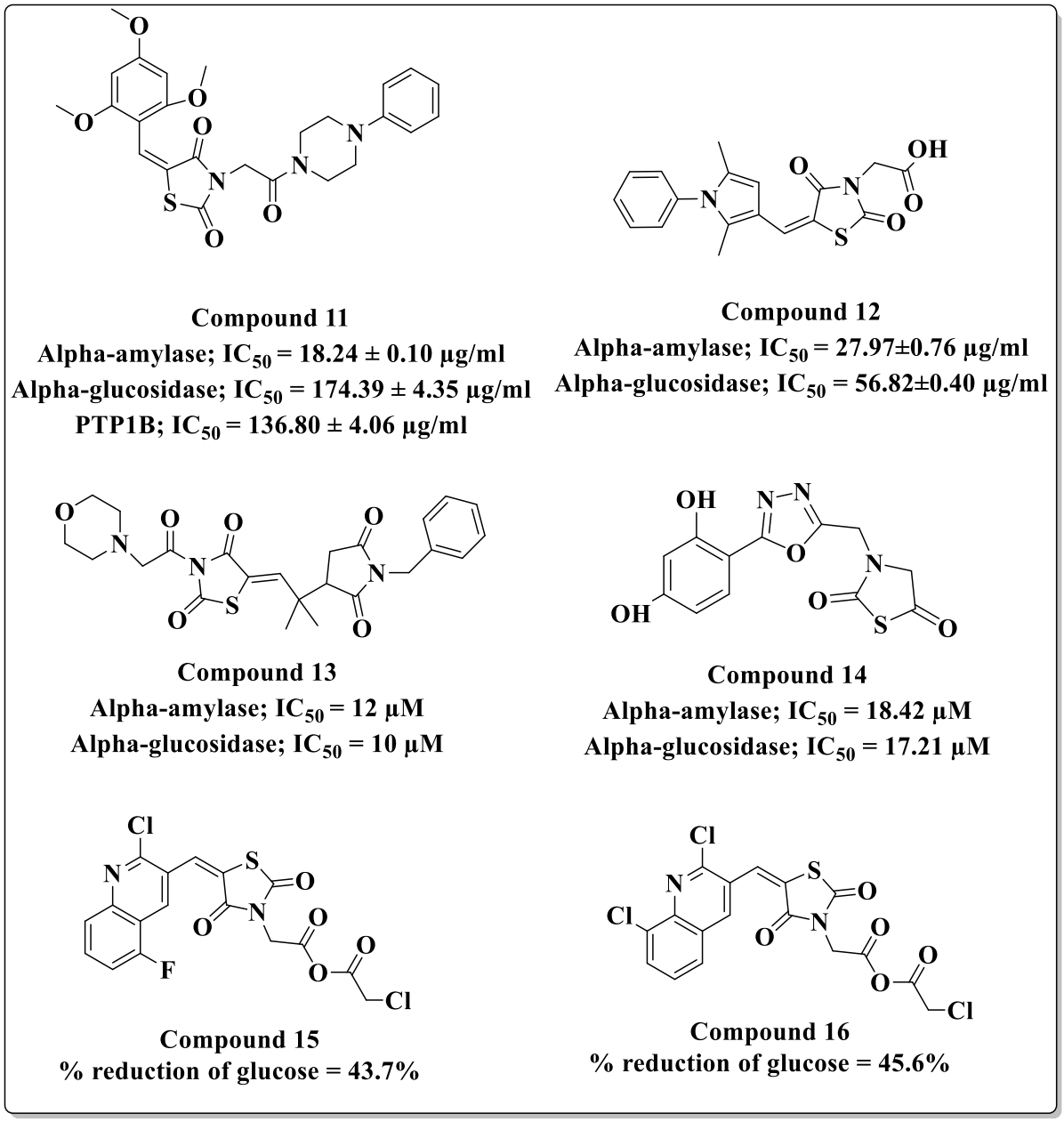


Figure 9: Compounds inhibiting both α-amylase and α-glucosidase.

2.4. Role of TZD in PTP1B inhibition:

Jiang et al. developed and synthesized a variety of TZD and hydantoin compounds with high cell permeability and oral bioavailability that selectively inhibit PTP1B. **Compound 17**, with an IC_{50} value of 0.86 ± 0.29 µM, was the most effective PTP1B inhibitor among the synthesised compounds. In order to ascertain the manner of inhibition and the binding interaction of **compound 17,** kinetic experiments and

molecular docking were conducted. Tests performed on BKS bd mice also revealed **compound 17 (Figure 10)** administration reduced glucose levels and enhanced insulin sensitivity [14].

Mahapatra et al. created a series of thiophene-containing N-alkylated TZD derivatives and synthesized them based on evidence from prior research, where thiophene-containing PTP1B inhibitors showed high potency. The synthesized molecules were tested for their ability to inhibit PTP1B *in vitro*, and the results showed that all but **compound 18** (IC_{50} = 9.96 µM), which had potency comparable to conventional suramin (IC_{50} = 9.76 µM), had weak to moderate action. Furthermore, an *in silico* docking analysis of **compound 18** with the enzyme revealed an important binding interaction in the catalytic domain. The SAR studies showed that there are no benefits by including an alkyl or haloalkyl group to amidic nitrogen other than a small methyl group in **compound 18 (Figure 10)**, which will help in the development of novel PTP1B inhibitors based on TZD [15].

Mahapatra et al. synthesized certain aryl/alkyl sulfonyloxy-arylidene TZD derivatives in light of the large arylidene moiety's function for PTP1B inhibitory action at the 5-position of TZD. An *in vivo* anti-hyperglycemic activity investigation and an *in vitro* PTP1B inhibitory assay were performed on the compounds. With the exception of **compounds 19 (Figure 10)** (IC_{50} = 7.31 µM) and **20 (Figure 10)** (IC_{50} = 8.73 µM), which demonstrated substantial inhibitory potency comparable to standard suramin (IC_{50} = 9.76 µM), the results suggested a compounds moderate inhibitory potency. *In vivo* studies also showed that the compounds significantly reduced blood glucose levels; in the case of **compounds 13** and **16**, the activity was substantially better than pioglitazone. Both compounds demonstrated the necessary binding contact at the catalytic domain in an *in-silico* docking analysis. Although the sulfonyl group produces

no bonding contact, it gives the arylidene group flexibility and length to reach the aryl phosphate-binding site [16].

The discovery of PTP1B as a potential target for synthesising a novel anti-diabetic medication prompted the development of arylidene TZD as an enzyme inhibitor. Mahapatra et al. developed and produced various aryl/alkyl sulfonyloxy moieties containing arylidene TZD derivatives in this study as a follow-up to their earlier work, and they then examined them for their *in vitro* PTP1B inhibitory potency and *in vivo* anti-hyperglycemic activity. Comparing **compounds 21** and **22 (Figure 10)** to standard suramin, the biological activity research revealed that **compounds 21** (IC_{50} = 8.53 µM) and 22 (IC_{50} = 6.89 µM) are potent inhibitors of PTP1B. Additionally, they have identified an essential interaction between the ligand's bidentate binding mechanism and the protein in an *in-silico* analysis [17].

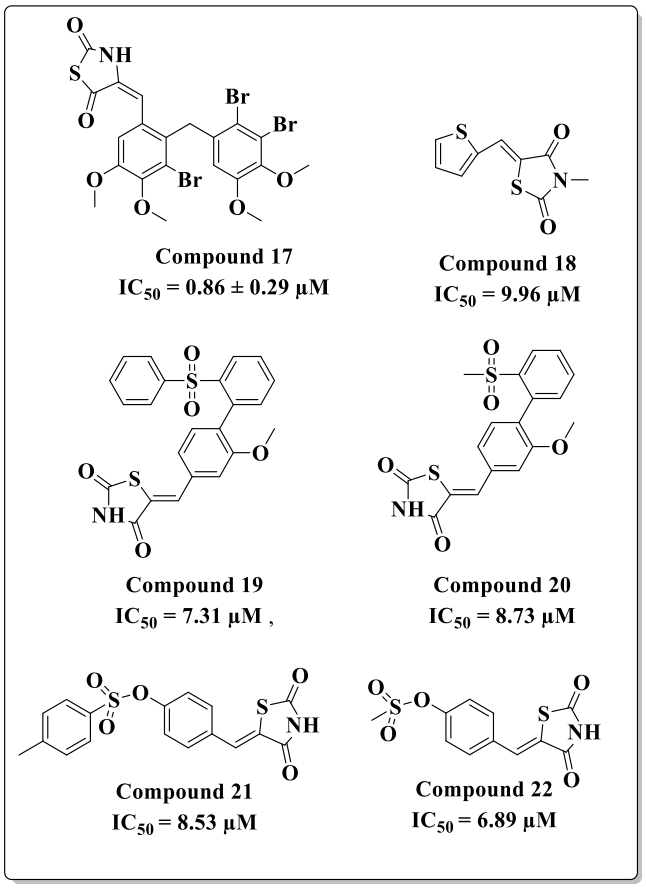


Figure 10: Compounds inhibiting PTP1B.

2.5. Compounds inhibiting both alpha-glucosidase and PTP1B:

Mphahlele and fellow workers synthesized a series of 3,5,7-tricarbo substituted indazoles and tested *in vitro* for the substance's ability to inhibit the activities of α-glucosidase and PTP1B. The compounds have showed a more substantial inhibitory effect against PTP1B, and reduced activity towards α-glucosidase activity. The most effective agents against the PTP1B enzyme are **compound 23 (Figure 11),** whereas the most potent compound against α-glucosidase are **compound 24 (Figure 11)**. The adenocarcinoma human epithelial (A549) cell line and normal green monkey kidney (Vero) cells showed decreased cytotoxicity in response to the most active chemicals. They also studied the kinetics (*in vitro*) and molecular docking (*in silico*) based on the highest derivative of each series to rationalize the drug-receptor interaction mechanism. A lipopolysaccharide (LPS)-induced test for cell-based antioxidant activity assay that produces reactive oxygen species has also been carried out [18].

The stereo pure **compound 25 (Figure 11)** was synthesized by Sadiq et al. and assessed its capacity as a multi-target anti-diabetic agent. The synthetic substance showed promising antidiabetic activity against all *in vitro* targets, showing IC_{50} values of 6.28 μM, 4.58 μM, 0.91 μM, and 2.36 μM in α-glucosidase, α-amylase, PTP1B, and DPPH targets, respectively. Synthesized compounds were also studied *in silico* for their interaction patterns and binding orientations, which supports their research, and it is evident that **compound 25** has the potential to be a multi-target anti-diabetic drug [19].

Yang and co-workers synthesized forty-two derivatives of dammarane triterpenoid (20*S*,24*R*)-epoxy-dammarane-3β,12β,25-triol obtained from *Cyclocarya paliurus*. They synthesized by chemically altering the hydroxyls (C-3 and C-12), rings A and E. These compounds are investigated for their α-glucosidase and PTP1B inhibitory activities.

Four of them strongly inhibited PTP1B, whereas two compounds considerably increased action against α -glucosidase. It was observed that **compound 26 (Figure 11)** acts as a dual target inhibitor with IC_{50} values 467.7 μM (α-glucosidase) and 269.1 μM (PTP1B). Based on an enzyme kinetic analysis, **compound 26** was shown to be a mix-type inhibitor on α-glucosidase and a noncompetitive-type inhibitor on PTP1B. SAR study majorly concluded that (a) ring A plays a crucial role in maintaining α-glucosidase and PTP1B inhibitory activity; (b) The insertion of carboxyl groups at C-3 is beneficial. These findings offer crucial hints for identifying PTP1B and α -glucosidase dual inhibitors [20].

Through an *in vitro* enzymatic test, the 5-acetyl-2-aryl-6-hydroxybenzo[b]furans were examined against targets associated with type 2 diabetes (T2D). The goal is to produce compounds potentially inhibiting PTP1B and α -glucosidase activity simultaneously; Mphahlele et al. synthesized eight compounds. When tested for *in vitro* PTP1B activity, taking sodium vanadate (Na_3VO_4) as a standard compound showed an enhanced activity profile, with IC_{50} values ranging from 11.9 to 31.9 μM. Compounds when examined for yeast α-glucosidase inhibitory activity keeping acarbose as a reference standard with an IC_{50} value of 0.78 μM, the derivative 27 **(Figure 11)** also showed notable inhibitory action against α-glucosidase. Among the test chemicals, **compound 27** proved to have a dual inhibitory impact on the activities against α-glucosidase and PTP1B. According to the SAR study, the biological activity of these compounds against PTP1B and α-glucosidase is significantly elevated by the ortho-hydroxyacetyl benzofuran scaffold [21].

Novel N-aryl-(benzoazol-2-yl)-sulfanylalkanamides have been developed by Wang and fellow scientists, and evaluated their inhibitory effects on PTP1B and α-glucosidase. Ursolic acid was the positive control for dual inhibition against α-glucosidase and

PTP1B. The **compound 28 (Figure 11)** was highly selective for PTP1B and was proven to be an efficient dual α-glucosidase and PTP1B inhibitor with an IC_{50} value of 10.96 µM and13.46 µM respectively. Additionally, they conducted molecular docking, which revealed that **compound 28** had strong contacts with the binding pockets of both α-glucosidase and PTP1B receptors via van der Waals, charge, and pi-cation interactions. The kinetic analysis they demonstrated reveals that the α-glucosidase inhibition of 28 is carried out by a mixed reversible mechanism [22].

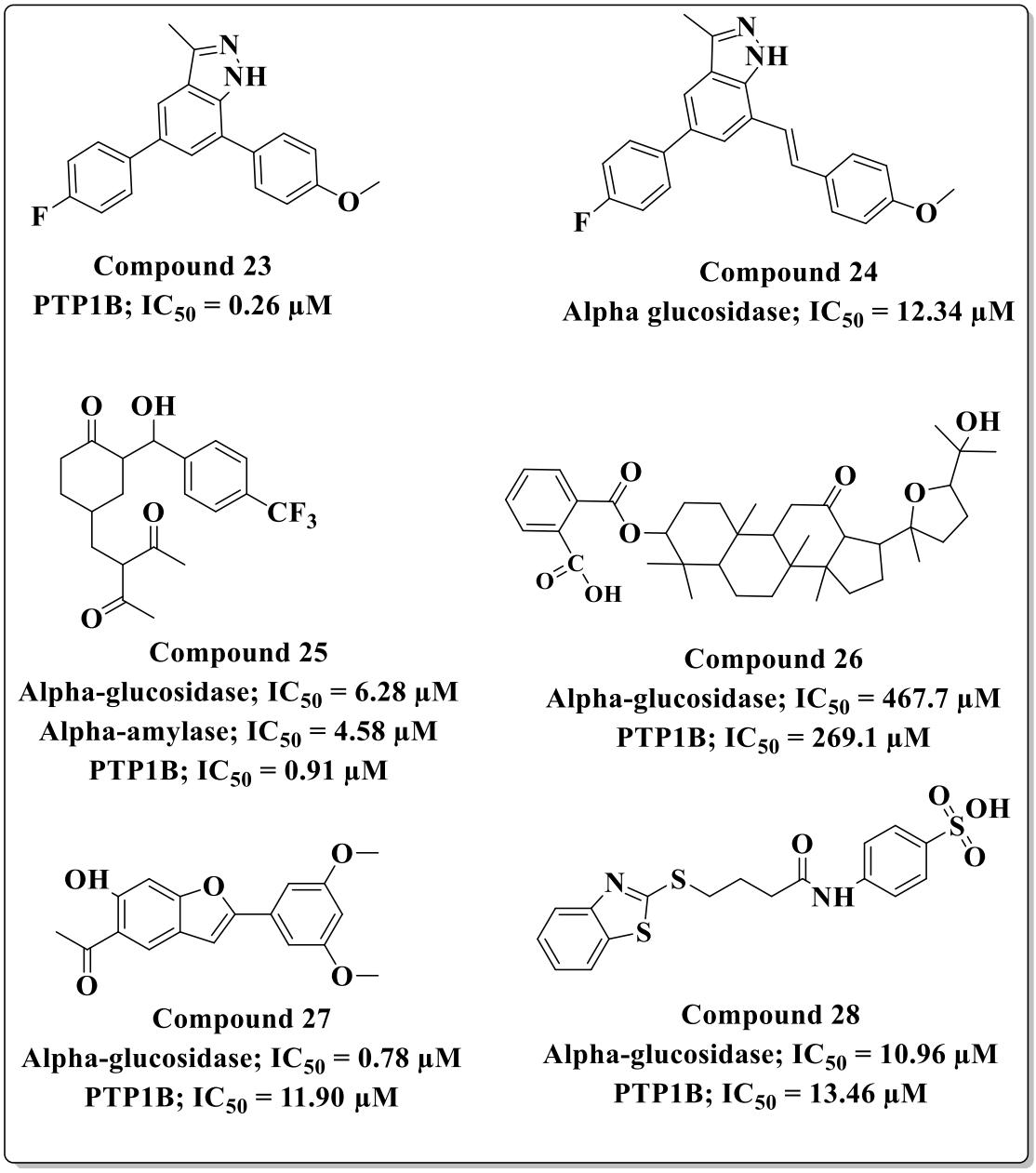


Figure 11: Compounds inhibiting both alpha-glucosidase and PTP1B.

References:

1) Doddagaddavalli, M. A., Kalalbandi, V. K. A., & Seetharamappa, J. (2023). Synthesis, characterization, crystallographic, binding, *in silico* and antidiabetic studies of novel 2,4-thiazolidinedione-phenothiazine molecular hybrids. In Journal of Molecular Structure (Vol. 1276, p. 134625). Elsevier BV. https://doi.org/10.1016/j.molstruc.2022.134625
2) Rathod, C. H., Nariya, P. B., Maliwal, D., Pissurlenkar, R. R. S., Kapuriya, N. P., & Patel, A. S. (2021). Design, Synthesis and Antidiabetic Activity of Biphenylcarbonitrile-Thiazolidinedione Conjugates as Potential α-Amylase Inhibitors. In ChemistrySelect (Vol. 6, Issue 9, pp. 2464–2469). Wiley. https://doi.org/10.1002/slct.202004362
3) Sameeh, M. Y., Khowdiary, M. M., Nassar, H. S., Abdelall, M. M., Alderhami, S. A., & Elhenawy, A. A. (2021). Discovery Potent of Thiazolidinedione Derivatives as Antioxidant, α-Amylase Inhibitor, and Antidiabetic Agent. *Biomedicines*, *10*(1), 24. https://doi.org/10.3390/biomedicines10010024
4) Addanki, H. R., Vallabhaneni, M. R., Chennamsetty, S., Pullagura, P., Sagurthi, S. R., & Pasupuleti, V. R. (2022). An *in silico* ADMET, molecular docking study and microwave-assisted synthesis of new phosphorylated derivatives of thiazolidinedione as potential anti-diabetic agents. In Synthetic Communications (Vol. 52, Issue 2, pp. 300–315). Informa UK Limited. https://doi.org/10.1080/00397911.2021.2024574
5) Sujatha, B., Chennamsetty, S., Chintha, V., Wudayagiri, R., & Prasada Rao, K. (2020). Synthesis and anti-diabetic activity evaluation of phosphonates containing thiazolidinedione moiety. In Phosphorus, Sulfur, and Silicon and the Related Elements (Vol. 195, Issue 7, pp. 586–591). Informa UK Limited. https://doi.org/10.1080/10426507.2020.1737061
6) Hussain, F., Khan, Z., Jan, M. S., Ahmad, S., Ahmad, A., Rashid, U., Ullah, F., Ayaz, M., & Sadiq, A. (2019). Synthesis, *in-vitro* α-glucosidase inhibition, antioxidant, in-vivo antidiabetic and molecular docking studies of pyrrolidine-2,5-dione and thiazolidine-2,4-dione derivatives. *Bioorganic chemistry*, *91*, 103128. https://doi.org/10.1016/j.bioorg.2019.103128
7) Singh, V., Singh, A., Singh, G., Verma, R. K., & Mall, R. (2021). Benzoxazolyl linked benzylidene based rhodanine and analogs as novel antidiabetic agents: synthesis, molecular docking, and *in vitro* studies. In Medicinal Chemistry Research (Vol. 30,

Issue 10, pp. 1905–1914). Springer Science and Business Media LLC. https://doi.org/10.1007/s00044-021-02781-y

8) Patil, V. M., Tilekar, K. N., Upadhyay, N. M., & Ramaa, C. S. (2022). Synthesis, In-Vitro Evaluation and Molecular Docking Study of N-Substituted Thiazolidinediones as α-Glucosidase Inhibitors. In ChemistrySelect (Vol. 7, Issue 1). Wiley. https://doi.org/10.1002/slct.202103848
9) Wang, G. C., Peng, Y. P., Xie, Z. Z., Wang, J., & Chen, M. (2017). Synthesis, α-glucosidase inhibition and molecular docking studies of novel thiazolidine-2,4-dione or rhodanine derivatives. *MedChemComm*, *8*(7), 1477–1484. https://doi.org/10.1039/c7md00173h
10) Arineitwe, C., Oderinlo, O., Tukulula, M., Khanye, S., Khathi, A., & Sibiya, N. (2023). Discovery of Novel Thiazolidinedione-Derivatives with Multi-Modal Antidiabetic Activities *In Vitro* and In Silico. *International journal of molecular sciences*, *24*(3), 3024. https://doi.org/10.3390/ijms24033024
11) Huneif, M. A., Mahnashi, M. H., Jan, M. S., Shah, M., Almedhesh, S. A., Alqahtani, S. M., Alzahrani, M. J., Ayaz, M., Ullah, F., Rashid, U., & Sadiq, A. (2023). New Succinimide-Thiazolidinedione Hybrids as Multi-target Antidiabetic Agents: Design, Synthesis, Bioevaluation, and Molecular Modelling Studies. *Molecules (Basel, Switzerland)*, *28*(3), 1207. https://doi.org/10.3390/molecules28031207
12) Srinivasa, M. G., Paithankar, J. G., Saheb Birangal, S. R., Pai, A., Pai, V., Deshpande, S. N., & Revanasiddappa, B. C. (2023). Novel hybrids of thiazolidinedione-1,3,4-oxadiazole derivatives: synthesis, molecular docking, MD simulations, ADMET study, *in vitro*, and *in vivo* anti-diabetic assessment. *RSC advances*, *13*(3), 1567–1579. https://doi.org/10.1039/d2ra07247e
13) Angajala, G., Aruna, V., Pavan, P., & Guruprasad Reddy, P. (2022). Biocatalytic one pot three component approach: Facile synthesis, characterization, molecular modelling and hypoglycemic studies of new thiazolidinedione festooned quinoline analogues catalyzed by alkaline protease from Aspergillus niger. *Bioorganic chemistry*, *119*, 105533. https://doi.org/10.1016/j.bioorg.2021.105533
14) Jiang, B., Luo, J., Guo, S., & Wang, L. (2021). Discovery of 5-(3-bromo-2-(2,3-dibromo-4,5-dimethoxybenzyl)-4,5-dimethoxybenzylidene)thiazolidine-2,4-dione as a novel potent protein tyrosine phosphatase 1B inhibitor with antidiabetic properties. *Bioorganic chemistry*, *108*, 104648. https://doi.org/10.1016/j.bioorg.2021.104648

15) Mahapatra, M. K., Kumar, R., & Kumar, M. (2017). N-alkylated thiazolidine-2,4-dione analogs as PTP1B inhibitors: synthesis, biological activity, and docking studies. In Medicinal Chemistry Research (Vol. 26, Issue 6, pp. 1176–1183). Springer Science and Business Media LLC. https://doi.org/10.1007/s00044-017-1823-z

16) Mahapatra, M. K., Kumar, R., & Kumar, M. (2017). Synthesis, biological evaluation and *in silico* studies of 5-(3-methoxybenzylidene)thiazolidine-2,4-dione analogues as PTP1B inhibitors. *Bioorganic chemistry*, *71*, 1–9. https://doi.org/10.1016/j.bioorg.2017.01.007

17) Mahapatra, M. K., Kumar, R., & Kumar, M. (2017). Exploring sulfonate esters of 5-arylidene thiazolidine-2,4-diones as PTP1B inhibitors with anti-hyperglycemic activity. In Medicinal Chemistry Research (Vol. 27, Issue 2, pp. 476–487). Springer Science and Business Media LLC. https://doi.org/10.1007/s00044-017-2074-8

18) Mphahlele, M. J., Magwaza, N. M., More, G. K., & Gildenhuys, S. (2022). An *in vitro* and *in silico* α-amylase/α-glucosidase/protein tyrosine phosphatase 1 beta & radical scavenging profiling of the 3,5,7-tricarbo substituted 1H-indazoles. In Medicinal Chemistry Research (Vol. 31, Issue 12, pp. 2132–2151). Springer Science and Business Media LLC. https://doi.org/10.1007/s00044-022-02972-1

19) Sadiq, A., Mahnashi, M. H., Rashid, U., Jan, M. S., Alshahrani, M. A., & Huneif, M. A. (2022). 3-(((1*S*,3*S*)-3-((*R*)-Hydroxy(4-(trifluoromethyl)phenyl)methyl)-4-oxocyclohexyl)methyl)pentane-2,4-dione: Design and Synthesis of New Stereopure Multi-Target Antidiabetic Agent. *Molecules (Basel, Switzerland)*, *27*(10), 3265. https://doi.org/10.3390/molecules27103265

20) Yang, X. T., Li, T. Z., Geng, C. A., Liu, P., & Chen, J. J. (2022). Synthesis and biological evaluation of (20*S*,24*R*)-epoxy-dammarane-3β,12β,25-triol derivatives as α-glucosidase and PTP1B inhibitors. *Medicinal chemistry research: an international journal for rapid communications on design and mechanisms of action of biologically active agents*, *31*(2), 350–367. https://doi.org/10.1007/s00044-021-02836-0

21) Mphahlele, M. J., Choong, Y. S., Maluleka, M. M., & Gildenhuys, S. (2020). Synthesis, *In Vitro* Evaluation and Molecular Docking of the 5-Acetyl-2-aryl-6-hydroxybenzo[b]furans against Multiple Targets Linked to Type 2 Diabetes. In Biomolecules (Vol. 10, Issue 3, p. 418). MDPI AG. https://doi.org/10.3390/biom10030418

22) Wang, M. Y., Cheng, X. C., Chen, X. B., Li, Y., Zang, L. L., Duan, Y. Q., Chen, M. Z., Yu, P., Sun, H., & Wang, R. L. (2018). Synthesis and biological evaluation of novel N-

aryl-ω-(benzoazol-2-yl)-sulfanylalkanamides as dual inhibitors of α-glucosidase and protein tyrosine phosphatase 1B. *Chemical biology & drug design*, *92*(3), 1647–1656. https://doi.org/10.1111/cbdd.13331

CHAPTER 3

MATERIALS AND METHODS

3. Materials and Methods:

3.1. General:

All reagents and solvents were used of laboratory (LR) grade, obtained from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India) and Merck Limited (Mumbai, India), Avra Research Laboratories Pvt. Ltd. (Hyderabad, India). The progress of the reaction and purity of the synthesized compounds were checked on the precoated silica gel F_{254} plates obtained from Merck (Mumbai, India) using hexane and ethyl acetate (1:4) as mobile phase. The iodine chamber and UV lamp (λ = 254 nm) were used to visualise the spots. Melting points were determined in an open capillary tube on the Labtronics melting point apparatus. FT-IR spectra were recorded on a Bruker FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker NMR (300 MHz) in DMSO–d6/$CDCl_3$. Chemical shifts (δ) are given in ppm relative to TMS, and coupling constants (J) are expressed in Hz.

3.2. The rationale of design:

In 2005, Lee et al. isolated *Cuminum cyminum* seed and tested it for α-glucosidase inhibitory activity. The active component of *C. cyminum* seed oil was reported as cuminaldehyde. The IC_{50} value was reported as 0.5 mg/ml. Cuminaldehyde was 1.8 times less active than standard acarbose. Simultaneously, it was also evaluated for aldose reductase inhibition and gave satisfactory results. Finally, it can be concluded that cuminaldehyde can be a lead compound and a new agent for the treatment of diabetes [1].

In 2019, Kostrzewa et al. explored the capacity to inhibit PTP1B enzyme by curcumin and cinnamaldehyde. Both were able to decrease the enzymatic activity of PTP1B. However, curcumin (IC_{50} = 100 µM) was more effective than cinnamaldehyde (IC_{50} =

500 µM). Both compounds appeared as potential agents for developing newer therapeutics [2].

After a thorough search using Sci-Finder, we have found that the compounds we aimed for synthesizing were not screened for anti-diabetic activity except for PN-2, which was evaluated for hypoglycemia on alloxan-induced mice by Leite and co-workers. Reduction in plasma glucose was assessed and at a dose of 30 mg/kg/day, a 40.8% reduction in plasma glucose was reported [3]. So, the rational design for the target compounds has been presented in the following diagram:

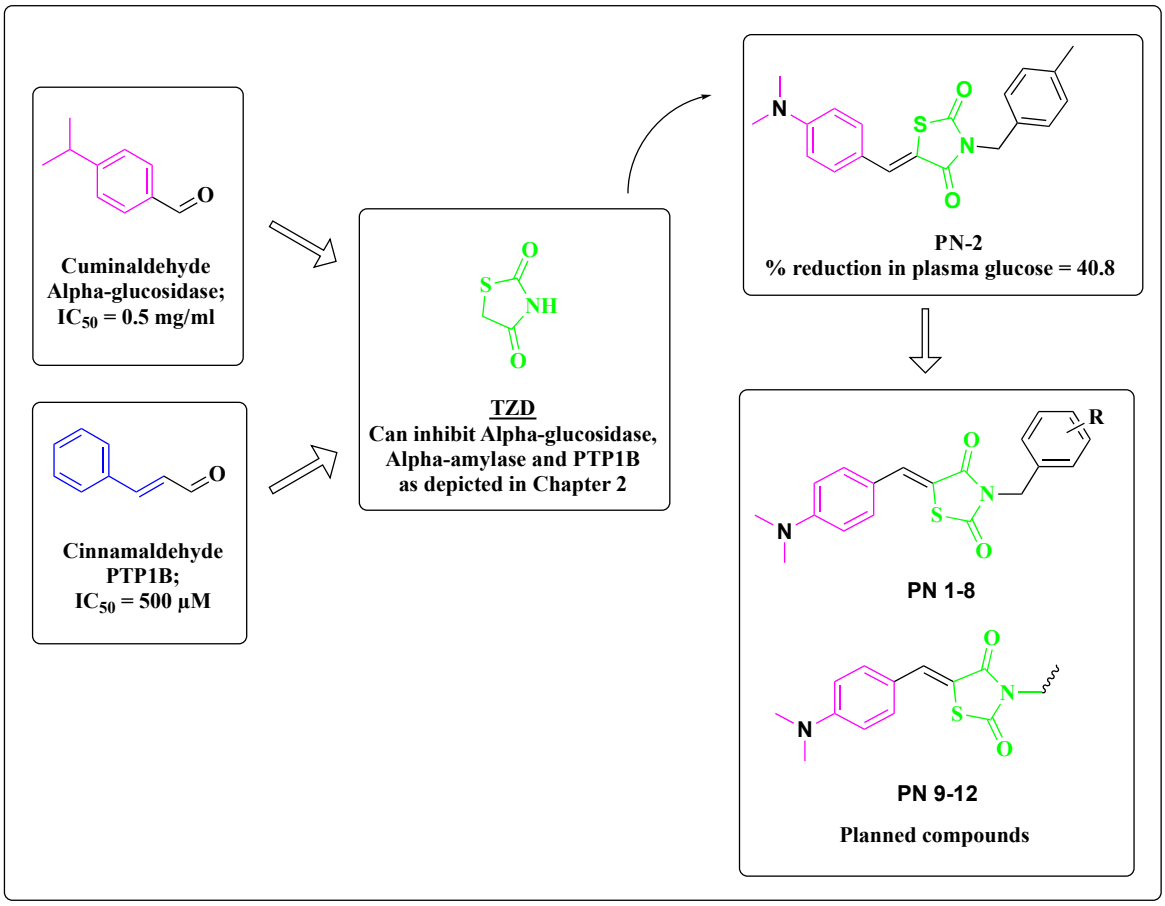
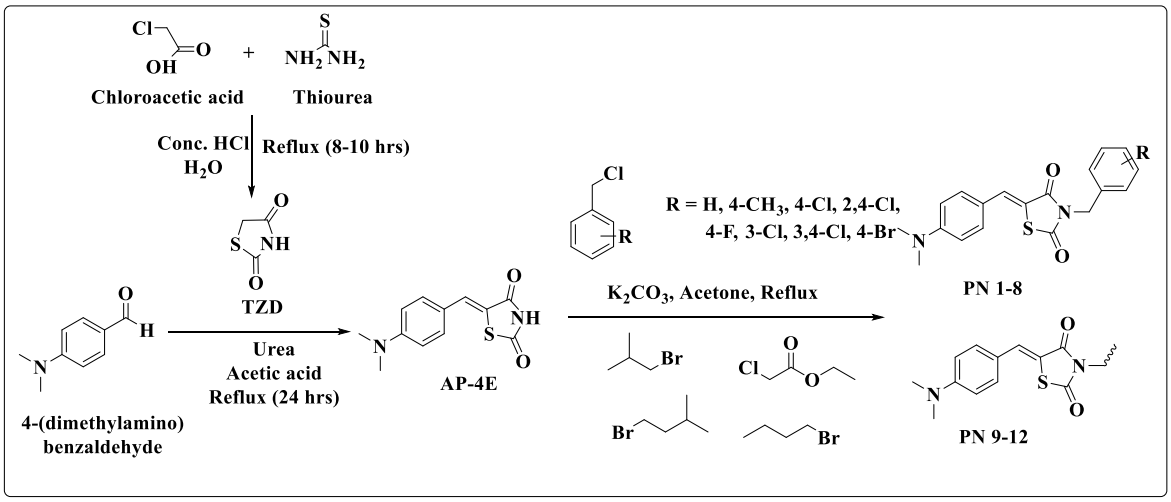


Figure 12: Rationale behind the designing of compounds.

3.3. Synthetic scheme:

The synthetic scheme is depicted below (**Scheme 1**):



Scheme 1: Synthetic scheme of PN-1 to PN-12.

3.4. Novelty search:

Every synthesized compound was searched in Sci-Finder for its novelty and reported activity, which is depicted in **Figure 13** and compiled in **Table 1**:

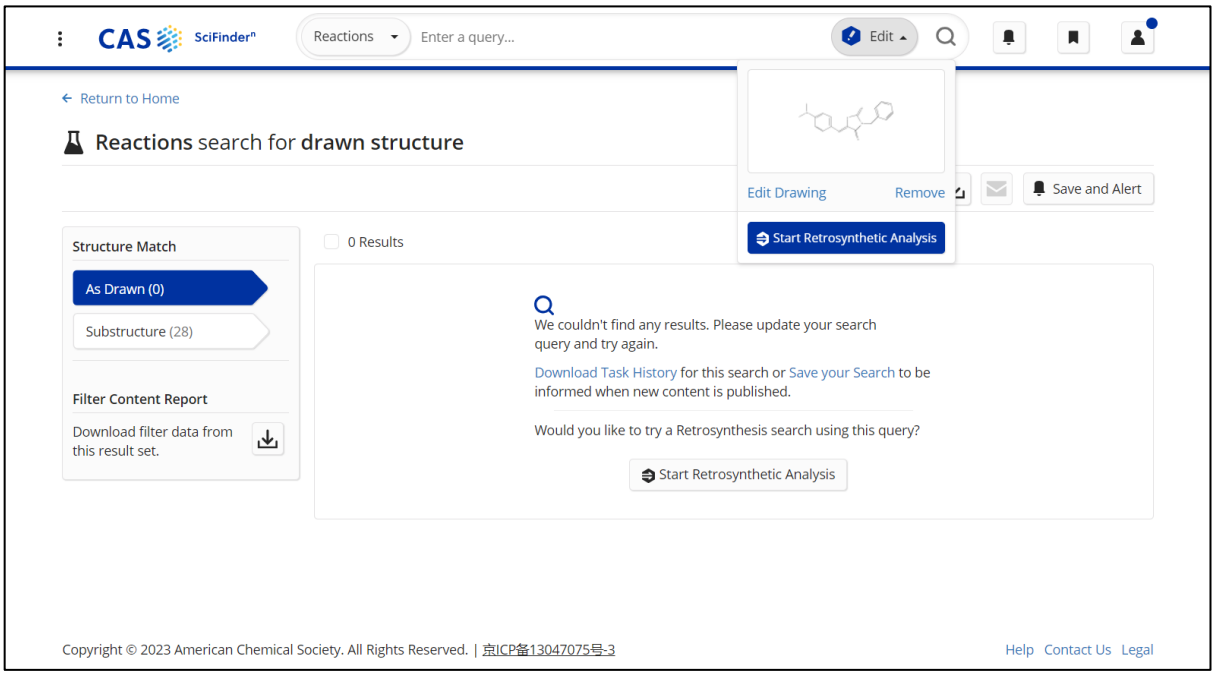
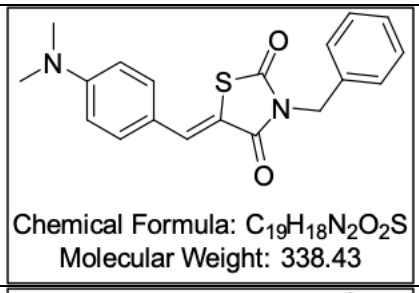
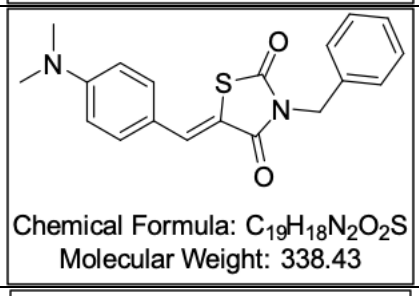
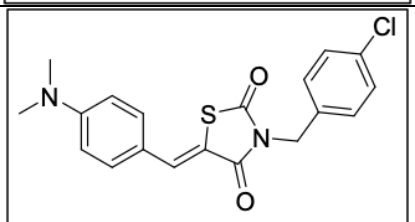
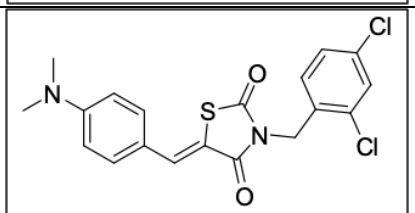
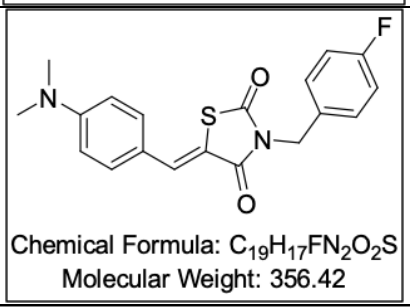
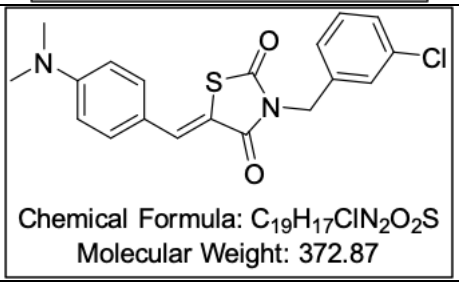
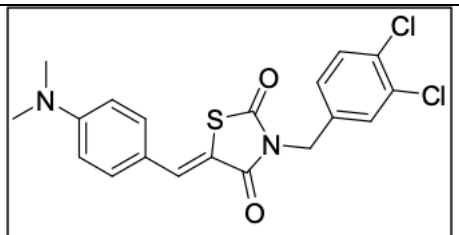
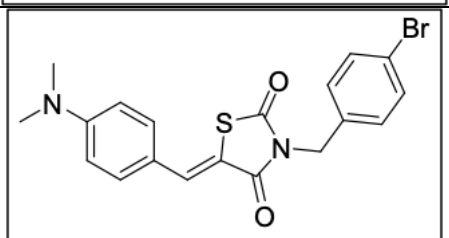
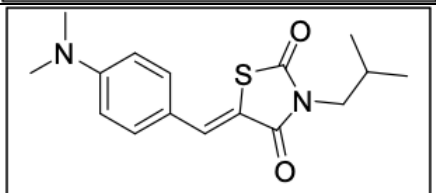
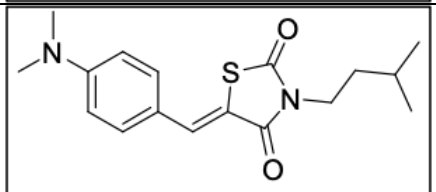
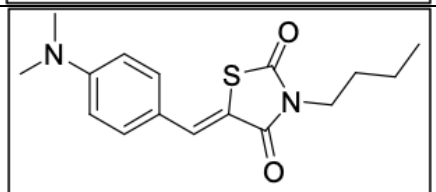
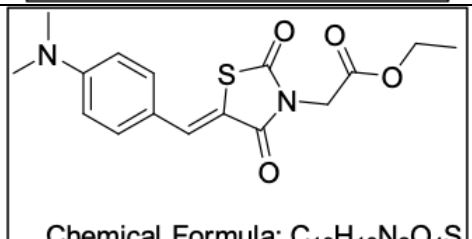


Figure 13: Figure showing no reported activity of PN-1.

Table 1: Sci-Finder novelty report of designed compounds with activity status.

Sl. No.	Compound name	Structure	Status in Sci Finder	Reported activity
1	PN-1	 Chemical Formula: $C_{19}H_{18}N_2O_2S$ Molecular Weight: 338.43	Present	No reported activity
2	PN-2	 Chemical Formula: $C_{19}H_{18}N_2O_2S$ Molecular Weight: 338.43	Present	Hypoglycemic activity Hypolipidemic activity
3	PN-3	 Chemical Formula: $C_{19}H_{17}ClN_2O_2S$ Molecular Weight: 372.87	Present	No reported activity
4	PN-4	 Chemical Formula: $C_{19}H_{16}Cl_2N_2O_2S$ Molecular Weight: 407.31	Present	No reported activity
5	PN-5	 Chemical Formula: $C_{19}H_{17}FN_2O_2S$ Molecular Weight: 356.42	Present	Inhibition of autotaxin
6	PN-6	 Chemical Formula: $C_{19}H_{17}ClN_2O_2S$ Molecular Weight: 372.87	Present	No reported activity

7	PN-7	 Chemical Formula: $C_{19}H_{16}Cl_2N_2O_2S$ Molecular Weight: 407.31	Present	No reported activity
8	PN-8	 Chemical Formula: $C_{19}H_{17}BrN_2O_2S$ Molecular Weight: 417.32	Present	Anti-histamine
9	PN-09	 Chemical Formula: $C_{16}H_{20}N_2O_2S$ Molecular Weight: 304.41	Present	No reported activity
10	PN-10	 Chemical Formula: $C_{17}H_{22}N_2O_2S$ Molecular Weight: 318.44	Present	No reported activity
11	PN-11	 Chemical Formula: $C_{16}H_{20}N_2O_2S$ Molecular Weight: 304.41	Present	No reported activity
12	PN-12	 Chemical Formula: $C_{16}H_{18}N_2O_4S$ Molecular Weight: 334.39	Present	(1) Lung cancer cells (2) Antibacterial and herbicidal (3) Inhibition of lysine biosynthesis (4) Antimicrobial (5) Antihyperglycemic

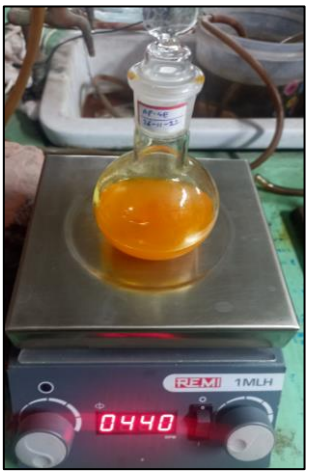
3.5. Chemistry:

In this work, I have synthesized a series of 4-(dimethylamino)benzaldehyde containing TZD moiety from TZD in high yields with simple chemistry.

3.5.1. Methodology

Step 1: An equimolar amount of chloroacetic acid and thiourea will be reacted to carry out the cyclization of chloroacetic acid, and it will give the parent scaffold TZD.

Step 2: The methylene group (-CH_2-) of TZD is substituted with 4-(dimethylamino)benzaldehyde using urea as catalyst and acetic acid as solvent and 5-(4-(dimethylamino))arylidene-2,4-thiazolidinedione was formed.



Step 3: N-3 position of compounds will be substituted by twelve different side chains in the presence of DMF and K_2CO_3 to obtain the target compounds.

3.5.1.1. Synthesis of Thiazolidine-2,4-dione (TZD):

In a 150 mL round-bottomed flask, 28.35 g chloroacetic acid (0.3 mol) and 22.83g thiourea (0.3 mol) were dissolved in 35 mL of water. The mixture was stirred for 1 hr to form a white precipitate, accompanied by considerable cooling. To the contents of the flask, 18 mL of concentrated hydrochloric acid was then added slowly from a dropping funnel, and the flask was then connected with a reflux condenser and gentle heat was applied to produce a complete solution, after which the reaction mixture was stirred and refluxed for 8–10 hrs. at 100–110℃. The progress of the reaction was monitored by using TLC using n-hexane/ethyl acetate 3:2 as an eluent. On cooling the contents of the flask solidified to a cluster of white needles; the product was filtered and washed with double distilled water to remove traces of hydrochloric acid and dried. It was purified by recrystallization from ethyl alcohol.

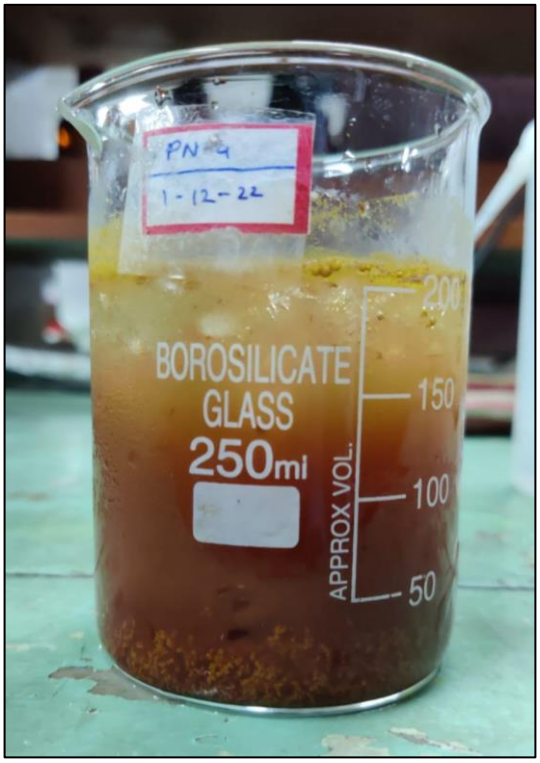
3.5.1.2. Synthesis of 5-(4-(dimethylamino)) benzylidene-2,4-thiazolidinedione:

Acetic acid (10 ml) and 0.423 g urea (0.007 mol) were taken in a 50 ml round-bottomed flask and stirred for 15 mins. To the same flask, 0.550 g 2,4-thiazolidinedione (0.004 mol) was added and again stirred for 15 mins. 0.7 g of 4-dimethylaminobenzaldehyde (0.004 mol) was added and kept for reflux at 100 ºC for 24 hrs. The reaction mixture was then cooled to room temperature (RT) and

then poured into ice-cold water, and the reddish-brown precipitate was recovered by filtering under a vacuum, washing with water several times, and drying at RT.

3.5.1.3. Synthesis of 3-substituted-5-(4-(dimethylamino)benzylidene)thiazolidine-2,4-dione:

0.552 g of potassium carbonate (0.004 mol) and 15 ml of dimethylformamide was taken in a round bottom flask and stirred for 15 mins. 0.497 g of (Z)-5-(4-(dimethylamino)benzylidene)thiazolidine-2,4-dione (0.002 mol) was added and stirred for 20-30 mins. The calculated amount of substituted compound was added dropwise at an interval of 5 mins. The reaction mixture was left steering for 24 hrs. The reaction mixture was then cooled to room temperature (RT) and then poured into ice-cold water, and the precipitate was recovered by filtering under a vacuum, washing with water several times, and drying at RT. The crude material was purified by recrystallization using ethyl acetate/methanol as solvent to achieve shiny crystal.



3.6. Molecular docking studies:

In order to evaluate the binding mode of the ligands at 3top protein and 2qv4 protein, the molecular docking studies were carried out with the help of PyRx software, which mainly works on Auto Dock Vina. This software automatically converts the ligands and protein structures into consecutive energy-minimized BQT format. Then the grid space was then set into x, y, z directions with reference to the ligand binding active site. The native ligands and water molecules were removed manually, and the exhaustiveness was set to 8 in the Auto Dock Vina. For 3top, the grid box details were set as: center-31.2638996215, center 35.0125635836, center z- 26.2301326936, size x 13.6053785155, size y- 17.2948912019, size z 17.820803088, For 2qv4, the grid box parameters were set into: center. x 13.5992716492, centery-47.2139548884, centre z 27.0649018603, size.x- 20.5461645457, size y-15.7026993577, size z 15.4351895482. The docking results were analyzed using the Discovery studio clint. The best docked poses were selected based on binding affinity and RMSD parameters.

References:

1) Lee, H.-S. (2005). Cuminaldehyde: Aldose Reductase and α-Glucosidase Inhibitor Derived from Cuminum cyminum L. Seeds. In Journal of Agricultural and Food Chemistry (Vol. 53, Issue 7, pp. 2446–2450). American Chemical Society (ACS). https://doi.org/10.1021/jf048451g
2) KOSTRZEWA, T., PRZYCHODZEN, P., GORSKA-PONIKOWSKA, M., & KUBAN-JANKOWSKA, A. (2019). Curcumin and Cinnamaldehyde as PTP1B Inhibitors With Antidiabetic and Anticancer Potential. In Anticancer Research (Vol. 39, Issue 2, pp. 745–749). Anticancer Research USA Inc. https://doi.org/10.21873/anticanres.13171
3) da Costa Leite, L. F. C., Veras Mourão, R. H., de Lima, M. do C. A., Galdino, S. L., Hernandes, M. Z., de Assis Rocha Neves, F., Vidal, S., Barbe, J., & da Rocha Pitta, I. (2007). Synthesis, biological evaluation and mmodelling modeling studies of arylidene-thiazolidinediones with potential hypoglycemic and hypolipidemic activities. In European Journal of Medicinal Chemistry (Vol. 42, Issue 10, pp. 1263–1271). Elsevier BV. https://doi.org/10.1016/j.ejmech.2007.02.015

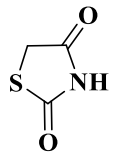
CHAPTER 4

RESULTS AND DISCUSSION

4. Results and discussion:

4.1. Structure and physical appearance of synthesized compounds:

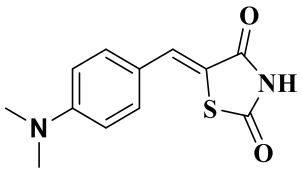
1) Thiazolidine-2,4-dione (TZD):



IUPAC Name: TZD

TLC	Solubility	Melting Point	Recrystallization	Color	Percentage of yield
Hexane: Ethyl acetate (4:1) [Rf = 0.6]	DMSO = Soluble; $CHCl_3$ = Soluble; EtOH = Slightly Soluble	113 - 120 ℃	Ethanol	White	65%

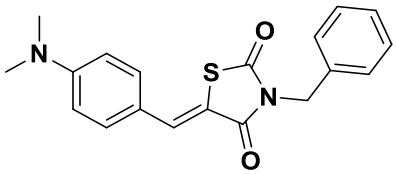
2) AP-4E



IUPAC Name: (E)-5-(4-(dimethylamino)benzylidene)thiazolidine-2,4-dione

TLC	Solubility	Melting Point	Recrystallization	Color	Percentage of yield
Hexane: Ethyl acetate (4:1) Rf= 0.7	DMSO = Soluble; $CHCl_3$ = Soluble; MeOH = Slightly Soluble	223 - 227 ℃	Was not required as crude product was sufficiently pure	Reddish-brown Powder	75.83%

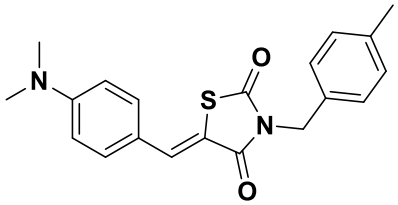
3) PN-1



IUPAC Name: (E)-3-benzyl-5-(4-(dimethylamino)benzylidene)thiazolidine-2,4-dione

TLC	Solubility	Melting Point	Recrystallization	Color	Percentage of yield
Hexane: Ethyl acetate (4:1) Rf= 0.5	DMSO = Soluble; $CHCl_3$ = Soluble; MeOH = Slightly Soluble	173 - 177 ℃	Methanol	Reddish-brown Needle shape	87.64%

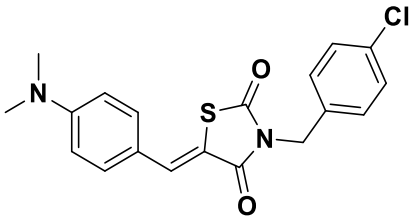
4) PN-2



IUPAC Name: (E)-5-(4-(dimethylamino)benzylidene)-3-(4-methylbenzyl)thiazolidine-2,4-dione

TLC	Solubility	Melting Point	Recrystallization	Color	Percentage of yield
Hexane: Ethyl acetate (4:1) Rf= 0.7	DMSO = Soluble; $CHCl_3$ = Soluble; MeOH = Slightly Soluble EtOAc = Slightly Soluble	170 -175 ℃	EtOAc	Yellow	97.16%

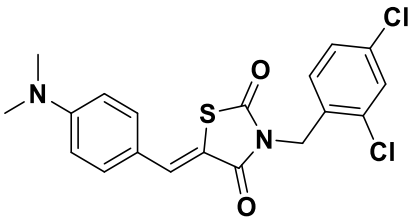
5) PN-3



IUPAC Name: (E)-3-(4-chlorobenzyl)-5-(4-(dimethylamino)benzylidene)thiazolidine-2,4-dione

TLC	Solubility	Melting Point	Recrystallization	Color	Percentage of yield
Hexane: Ethyl acetate (4:1) Rf= 0.8	$CHCl_3$=Soluble; DMSO=Slightly Soluble; MeOH=Slightly Soluble; EtOAc=Slightly Soluble.	173 -177 ℃	EtOAc	Green	92.49%

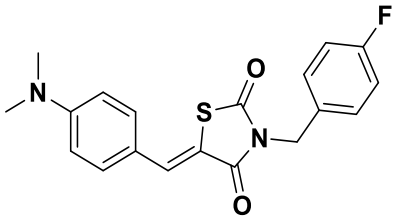
6) PN-4



IUPAC Name: (E)-3-(2,4-dichlorobenzyl)-5-(4-(dimethylamino)benzylidene)thiazolidine-2,4-dione

TLC	Solubility	Melting Point	Recrystallization	Color	Percentage of yield
Hexane: Ethyl acetate (4:1) Rf= 0.6	$CHCl_3$ = Soluble; DMSO = Sparingly Soluble; MeOH = Sparingly Soluble; EtOAc = Slightly Soluble.	208-213 ℃	EtOAc	Yellow	93.9%

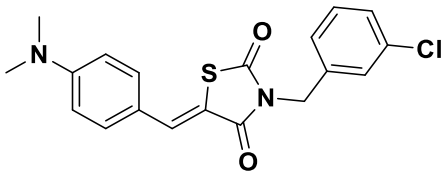
7) PN-5



IUPAC Name: (E)-5-(4-(dimethylamino)benzylidene)-3-(4-fluorobenzyl)thiazolidine-2,4-dione

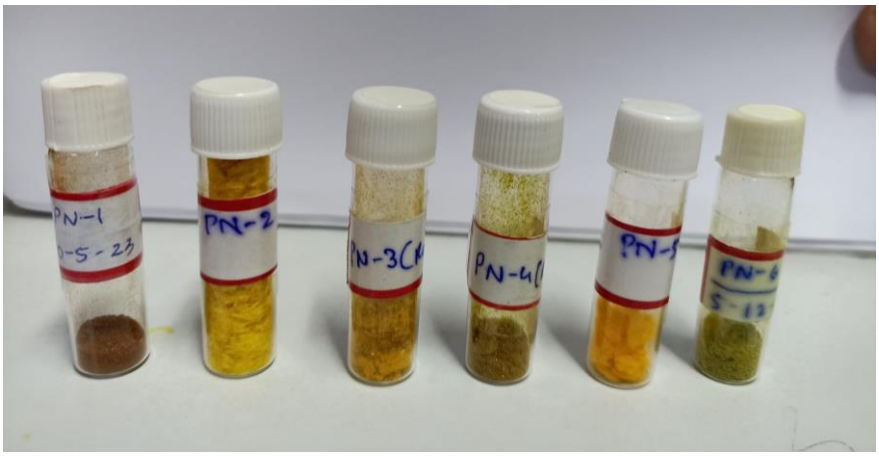
TLC	Solubility	Melting Point	Recrystallization	Color	Percentage of yield
Hexane: Ethyl acetate (4:1) Rf= 0.6	$CHCl_3$ = Soluble; DMSO = Slightly Soluble; MeOH = Slightly Soluble; EtOAc = Soluble after heating	198-200 ℃	EtOAc	Green	91.41%

8) PN-6

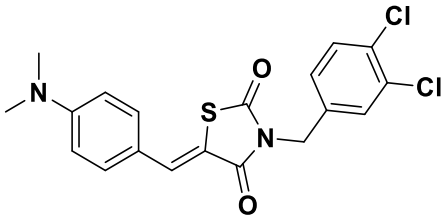


IUPAC Name: (E)-3-(3-chlorobenzyl)-5-(4-(dimethylamino)benzylidene)thiazolidine-2,4-dione

TLC	Solubility	Melting Point	Recrystallization	Color	Percentage of yield
Hexane: Ethyl acetate (4:1) Rf= 0.4	$CHCl_3$ = Soluble; DMSO = Slightly Soluble; MeOH = Slightly Soluble; EtOAc = Soluble after heating	194 -197 ℃	EtOAc	Green	91.88%



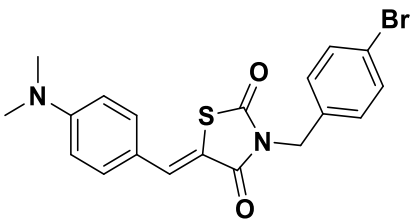
9) PN-7



IUPAC Name: (E)-3-(3,4-dichlorobenzyl)-5-(4-(dimethylamino)benzylidene)thiazolidine-2,4-dione

TLC	Solubility	Melting Point	Recrystallization	Color	Percentage of yield
Hexane: Ethyl acetate (4:1) Rf= 0.6	$CHCl_3$ = Soluble; DMSO = Slightly Soluble; MeOH = Slightly Soluble; EtOAc = Soluble after heating	194 -197 ℃	EtOAc	Yellow	94.94%

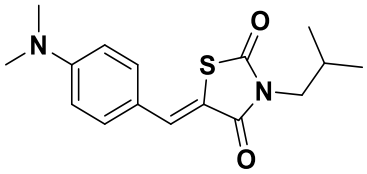
10) PN-8



IUPAC Name: (E)-3-(4-bromobenzyl)-5-(4-(dimethylamino)benzylidene)thiazolidine-2,4-dione

TLC	Solubility	Melting Point	Recrystallization	Color	Percentage of yield
Hexane: Ethyl acetate (4:1) Rf= 0.7	$CHCl_3$ = Soluble; DMSO = Slightly Soluble; EtOAc = Soluble after heating	192-195℃	EtOAc	Green	92.32%

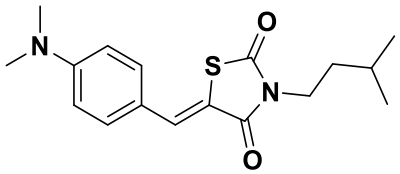
11) PN-9



IUPAC Name: (E)-5-(4-(dimethylamino)benzylidene)-3-isobutylthiazolidine-2,4-dione

TLC	Solubility	Melting Point	Recrystallization	Color	Percentage of yield
Hexane: Ethyl acetate (4:1) Rf= 0.4	$CHCl_3$ = Soluble; MeOH = Slightly Soluble; EtOAc = Soluble after heating	178 -185 ℃	MeOH	Reddish-brown	66.07%

12) PN-10

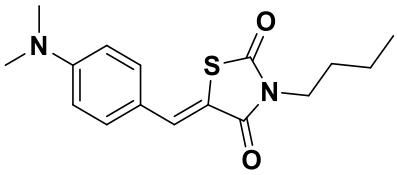


IUPAC Name: (E)-5-(4-(dimethylamino)benzylidene)-3-isopentylthiazolidine-2,4-dione

TLC	Solubility	Melting Point	Recrystallization	Color	Percentage of yield
Hexane: Ethyl acetate (4:1) Rf= 0.6	$CHCl_3$ = Soluble; MeOH = Slightly Soluble;	195 - 201 ℃	MeOH	Yellow	76.83%

	EtOAc = Soluble after heating				

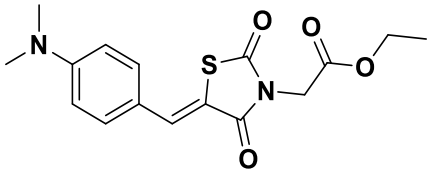
13) PN-11



IUPAC Name: (E)-3-butyl-5-(4-(dimethylamino)benzylidene)thiazolidine-2,4-dione

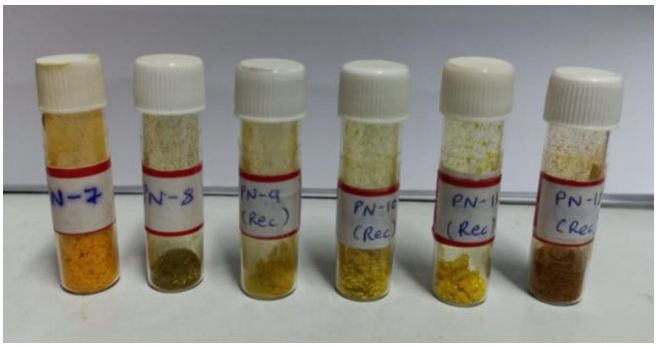
TLC	Solubility	Melting Point	Recrystallization	Color	Percentage of yield
Hexane: Ethyl acetate (4:1) Rf= 0.7	$CHCl_3$ = Soluble; MeOH = Slightly Soluble; EtOAc = Soluble after heating	188 -194 ℃	MeOH	Yellow	83.52%

14) PN-12



IUPAC Name: ethyl (E)-2-(5-(4-(dimethylamino)benzylidene)-2,4-dioxothiazolidin-3-yl)acetate

TLC	Solubility	Melting Point	Recrystallization	Color	Percentage of yield
Hexane: Ethyl acetate (4:1) Rf= 0.7	$CHCl_3$ = Soluble; MeOH = Slightly Soluble; EtOAc = Soluble after heating	191 -197 ℃	MeOH	Reddish-brown	77.85%



4.2. Characterization of synthesized compounds via NMR and FT-IR spectra:

4.2.1. Thiazolidine-2,4-dione (TZD):

^{1}H NMR (DMSO-d6) δ_H: 4.01 (2H, d, *J* = 12.3 Hz) 8.68 (1H, s). FTIR (cm^{-1}); 2978.35,2885.66 (Ar-H), 3543.28 (-NH), 1808.99 (-C=O)

4.2.2. (E)-5-(4-(dimethylamino)benzylidene)thiazolidine-2,4-dione (AP-4E):

^{1}H NMR (DMSO-d6) δ_H: 3.02 (6H, s), 6.79-6.83 (2H, d, *J* = 8.0, 1.1, 0.4 Hz), 7.40-7.66 (3H, 7.44 (ddd, *J* = 8.0, 1.5, 0.4 Hz), 12.33 (1H, s). ^{13}C NMR (DMSO-d6) δ_c: 40.1 (2C, s), 111.0 (2C, s), 112.1(1C, s), 115.7-116.5 (1C, s), 119.5-121.32 (2C, s), 132.0 4-133.9 (1C, s), 151.5-151.7 (1C, s), 165.1 (1C, s), 167.64-168.28 (1C, s). FTIR (cm^{-1}); 3477.51 (-NH), 3285.55, 3146.79, 2983.57 (Ar-H), 2767.94 (N-C-H), 1735.23 (-C=O).

4.2.3. (E)-3-benzyl-5-(4-(dimethylamino)benzylidene)thiazolidine-2,4-dione (PN-1):

^{1}H NMR ($CDCl_3$): δ_H 3.06-3.08 (6H, s), 4.89 (2H, s), 6.76-6.79 (2H, m, *J* = 8.0, 1.1, 0.4 Hz), 7.26-7.31 (2H, m, *J* = 7.9, 1.6, 1.2, 0.6 Hz), 7.33-7.39 (2H, m, *J* = 7.9, 7.7, 1.8, 0.6 Hz), 7.41-7.43 (2H, m, *J* = 7.7, 1.6 Hz)), 7.45 (1H, m, *J* = 8.0, 1.5, 0.4 Hz), 7.83 (1H, s); ^{13}C NMR ($CDCl_3$) δ_c: 76.63-77.05 (2C, s), 77.26-77.48 (1C, s), 112.46 (2C, s), 114.79 (1C, s), 121.45 (1C, s), 128.10-128.69 (3C, 128.10 (s), 128.69 (s)), 128.83 (2C, s), 132.53 (2C, s), 134.85 (1C, s), 135.54 (1C, s), 151.19 (1C, s), 166.61 (1C, s), 168.41 (1C, s). FTIR (cm^{-1}); 3244.41, 3125.20, 2920.30,

2630.20, 2530.26, 2482.33, 2388.91 (Ar-H), 2724.50 (N-C-H), 1823.96,1718.97 (-C=O).

4.2.4. (E)-5-(4-(dimethylamino)benzylidene)-3-(4-methylbenzyl)thiazolidine-2,4-dione (PN-2):

^{1}H NMR ($CDCl_3$): δ_H 2.17-2.32 (3H, s), 3.06-3.07 (6H, s), 4.85 (2H, s), 6.73 (2H, m, *J* = 8.0, 1.1, 0.4 Hz), 6.74-6.77 (2H, m, *J* = 8.0, 1.2, 0.5 Hz), 7.11 (2H, m, *J* = 8.0, 1.3, 0.5 Hz)), 7.14 (2H, m, *J* = 8.0, 1.5, 0.4), 7.38 (2H, m) 7.82 (1H, s). ^{13}C NMR ($CDCl_3$) δ_c: 21.19 (1C, s), 40.28 (2C, s), 44.83 (1C, s), 76.64-77.48 (2C, s), 112.27 (1C, s), 114.72 (1C, s), 121.19 (2C, s), 128.86 (2C, s), 129.34 (2C, s), 132.52-132.62 (1C, s), 134.80 (1C, s), 137.86 (1C, s), 151.31 (1C, s), 166.64 (1C, s), 168.44 (1C, s). FTIR (cm^{-1}); 3879.60, 3649.72, 3557.35 (Ar-H), 2801.16, 2496.56, 2306.14 (Ar-H), 1720.24, 1669.37 (-C=O).

4.2.5. (E)-3-(4-chlorobenzyl)-5-(4-(dimethylamino)benzylidene)thiazolidine-2,4-dione (PN-3):

^{1}H NMR ($CDCl_3$): δ_H 3.06-3.07 (6H, s), 4.84 (2H, s), 6.72-6.78 (2H, m, *J* = 8.0, 1.1, 0.4 Hz), 7.26-7.38 (2H, m, *J* = 8.3, 1.3, 0.6 Hz), 7.40 (2H, m, *J* = 8.3, 1.4, 0.6 Hz), 7.42 (2H, m, *J* = 8.0, 1.5, 0.4 Hz), 7.82(1H, s). ^{13}C NMR ($CDCl_3$) δ_c: 40.3 (2C, s), 44.6 (1C, s), 76.64-77.48 (2C, s), 112.27 (1C, s), 128.86 (1C, s), 130.34 (2C, s), 132.60 (4C, s), 134.02 (1C, s), 134.07 (1C, s), 135.20 (1C, s), 151.41 (1C, s), 166.49 (1C, s), 168.39 (1C, s). FTIR (cm^{-1}); 3888.27, 3732.26, 3648.88, 3603.31, 3558.81, 3451.18 (Ar-H), 3308.19 (=CH), 3261.58, 3019.46 (-N-C-H), 1796.85, 1751.57 (-C=O).

4.2.6. (E)-3-(2,4-dichlorobenzyl)-5-(4-(dimethylamino)benzylidene)thiazolidine-2,4-dione (PN-4):

^{1}H NMR ($CDCl_3$): δ_H 3.06-3.07 (6H, s), 4.98 (2H, s), 6.73-6.76 (2H, m , *J* = 8.0, 1.1, 0.4 Hz), 7.10-7.13 (1H, m, *J* = 8.3, 1.7 Hz), 7.18-7.21 (1H, m, *J* = 1.7, 0.5 Hz), 7.40 (2H,m, *J* = 8.3, 0.5 Hz), 7.42 (2H m, *J* = 8.0, 1.5, 0.4 Hz), 7.85 (1H,s). ^{13}C NMR ($CDCl_3$) δ_c: 40.15 (2C, s), 42.18-42.39 (1C, s), 112.0 (2C, s), 121.9 (1C, s), 125.7 (1C, s), 128.5-128.8 (4C, 128.6 (s), 128.7 (s), 128.7 (s)), 129.6 (1C, s), 132.0 (1C, s), 133.2 (1C, s), 133.8 (1C, s), 136.3 (1C, s), 150.9 (1C, s), 164.6 (1C, s), 169.9 (1C, s). FTIR (cm^{-1}); 3887.48, 3732.67, 3602.61, 3559.06, 3398.28 (Ar-H), 2924.57 (=CH), 2858.61, 2785.96 (- N-C-H), 1795.53, 1749.45 (-C=O).

4.2.7. (E)-5-(4-(dimethylamino)benzylidene)-3-(4-fluorobenzyl)thiazolidine-2,4-dione (PN-5):

^{1}H NMR ($CDCl_3$): δ_H 3.06 (6H, s), 4.85 (2H, s), 6.73-6.76 (2H, m, *J* = 8.0, 1.1, 0.4 Hz), 6.97-7.03 (2H, m, *J* = 8.4, 1.2, 0.6 Hz), 7.26-7.38 (2H, m, *J* = 8.4, 1.3, 0.6 Hz), 7.45 (2H, m, *J* = 8.0, 1.5, 0.4 Hz), 7.82 (1H, s). ^{13}C NMR ($CDCl_3$) δ_c: 40.24 (2C, s), 44.28 (1C, s), 112.23 (2C, s), 114.36 (2C, s), 115.41 (1C, s), 121.02 (1C, s), 130.90 (2C, s), 131.46 (2C, s), 132.58 (1C, s), 135.11 (1C, s), 151.41 (1C, s), 160.92 (1C, s), 164.18 (1C, s), 166.55 (1C, s). FTIR (cm^{-1}); 3887.07, 3732.46, 3604.15(Ar-H), 2893.26 (=CH), 2388.84, 2310.10 (-N-C-H), 1718.63, 1668.57(-C=O).

4.2.8. (E)-3-(3-chlorobenzyl)-5-(4-(dimethylamino)benzylidene)thiazolidine-2,4-dione (PN-6):

^{1}H NMR ($CDCl_3$): δ_H 3.07-3.08 (6H, s), 4.85 (2H, s), 6.75-6.78 (2H, m, *J* = 8.0, 1.1, 0.4 Hz), 7.25 (2H, m, *J* = 8.0, 1.2 Hz), 7.27 (1H, m, *J* = 8.1, 1.4, 1.2 Hz)), 7.41 (3H, m, *J* = 8.0, 0.5 Hz), 7.84 (1H, s). ^{13}C NMR ($CDCl_3$) δ_c: 40.3 (2C, s), 53.5 (1C, s), 112.0 (2C, s), 121.9 (1C, s), 125.7 (1C, s), 127.0-127.1 (2C, 127.0 (s), 127.1 (s)), 127.7 (1C, s), 128.6 (2C, s), 128.7 (1C, s), 130.4 (1C, s), 132.0 (1C, s), 140.2 (1C, s), 150.9 (1C, s), 164.6 (1C, s), 169.9 (1C, s). FTIR (cm^{-1}); 3887.38, 3734.22,

3604.46, 3558.91, 3395.28 (Ar-H), 3110.40 (=CH), 2388.00, 2310.15 (-N-C-H), 1794.20, 1749.98(-C=O).

4.2.9. (E)-3-(3,4-dichlorobenzyl)-5-(4-(dimethylamino)benzylidene)thiazolidine-2,4-dione (PN-7):

^{1}H NMR ($CDCl_3$): δ_H 3.06 (6H, s), 4.82 (2H, s), 6.71-6.74 (2H, m, *J* = 8.0, 1.1, 0.4 Hz), 7.30 (1H, m, *J* = 8.1, 1.4 Hz), 7.38 (2H, m, *J* = 1.4, 0.5 Hz), 7.40 (1H,m, *J* = 8.1, 0.5 Hz), 7.53 (1H, m, *J* = 8.0, 1.5, 0.4 Hz), 7.83 (1H,s). ^{13}C NMR ($CDCl_3$) δ_c: 40.3 (2C, s), 53.5 (1C, s), 112.0 (2C, s), 121.9 (1C, s), 125.7 (1C, s), 128.6 (2C, s), 128.7 (1C, s), 128.9 (1C, s), 129.8 (1C, s), 130.3 (1C, s), 132.0 (1C, s), 132.6 (1C, s), 140.2 (1C, s), 150.9 (1C, s), 164.6 (1C, s), 169.9 (1C, s). FTIR (cm^{-1}); 3774.33, 3696.78, 3534.68, 3403.98, (Ar-H), 2925.05 (=CH), 2538.36, 2174.87 (-N-C-H), 1727.34, 1670.14 (-C=O).

4.2.10. (E)-3-(4-bromobenzyl)-5-(4-(dimethylamino)benzylidene)thiazolidine-2,4-dione (PN:8):

^{1}H NMR ($CDCl_3$): δ_H 3.06-3.07 (6H, s), 4.83 (2H, s), 6.74-6.77 (2H, m, *J* = 8.0, 1.1, 0.4 Hz), 7.30-7.31 (2H, m, *J* = 8.4, 1.3, 0.6 Hz), 7.32-7.34 (2H, m, *J* = 8.4, 1.5, 0.6 Hz), 7.40-7.46 (2H,m, *J* = 8.0, 1.5, 0.4 Hz), 7.82 (1H,s). ^{13}C NMR ($CDCl_3$) δ_c: 40.3 (2C, s), 53.5 (1C, s), 112.0 (2C, s), 121.9 (1C, s), 122.3 (1C, s), 125.7 (1C, s), 128.6 (2C, s), 129.7 (2C, s), 131.7 (2C, s), 132.0 (1C, s), 136.6 (1C, s), 150.9 (1C, s), 164.6 (1C, s), 169.9 (1C, s). FTIR (cm^{-1}); 3895.35, 3776.86, 3679.16, 3656.18, 3610.79 (Ar-H), 3044.51 (=CH), 2396.21, 2311.45 (-N-C-H), 1719.93, 1666.54 (-C=O).

4.2.11. (E)-5-(4-(dimethylamino)benzylidene)-3-isobutylthiazolidine-2,4-dione (PN-9):

^{1}H NMR ($CDCl_3$): δ_H 0.92-0.94 (6H, d, *J* = 6.6 Hz), 2.07-2.21 (1H, se, *J* = 7.0, 6.6 Hz), 3.06 (6H, s), 3.54-3.57 (2H, d, *J* = 7.0 Hz), 6.72-6.75 (2H, d, *J* = 8.0, 1.1, 0.4 Hz), 7.39-7.81 (3H, 7.81 (d, *J* = 8.0, 1.5, 0.4 Hz). ^{13}C NMR ($CDCl_3$) δ_c: 20.4 (2C, s), 25.7 (1C, s), 40.3 (2C, s), 53.3 (1C, s), 112.0 (2C, s), 121.9 (1C, s), 125.7 (1C, s), 128.6 (2C, s), 132.0 (1C, s), 150.9 (1C, s), 164.6 (1C, s), 169.9 (1C, s). FTIR (cm^{-1}); 3889.69, 3761.08, 3632.47, (Ar-H), 3527.73, 3476.07 (Isobutyl) 3286.81 (=CH), 2405.71, 2372.75 (-N-C-H), 1721.60, 1665.01 (-C=O).0

4.2.12. (E)-5-(4-(dimethylamino)benzylidene)-3-isopentylthiazolidine-2,4-dione (PN-10):

^{1}H NMR ($CDCl_3$): δ_H 0.94-0.97 (6H, d, J = 6.6 Hz), 1.50-1.67 (3H, 1.50 (se, J = 7.7, 6.6 Hz), 1.60 (dt, J = 7.7, 7.6 Hz)), 3.06 (6H, s), 3.72-3.77 (2H, t, J = 7.6 Hz), 6.72-6.76 (2H, d, J = 8.0, 1.1, 0.4 Hz), 7.38-7.81 (3H, 7.55 (d, J = 8.0, 1.5, 0.4 Hz). 13C NMR (CDCl3) δc: 22.6 (2C, s), 27.0 (1C, s), 38.4 (1C, s), 40.3 (2C, s), 50.1 (1C, s), 112.0 (2C, s), 121.9 (1C, s), 125.7 (1C, s), 128.6 (2C, s), 132.0 (1C, s), 150.9 (1C, s), 164.6 (1C, s), 169.9 (1C, s). FTIR (cm^{-1}); 3894.83, 3777.09, 3693.94, 3626.26, 3570.57 (Ar-H), 3246.41, 3081.31, 3003.03 (Isopentyl) 2913.61 (=CH), 2856.64, 2305.19 (-N-C-H), 1718.21, 1659.65 (-C=O).

4.2.13. (E)-3-butyl-5-(4-(dimethylamino)benzylidene)thiazolidine-2,4-dione (PN-11):

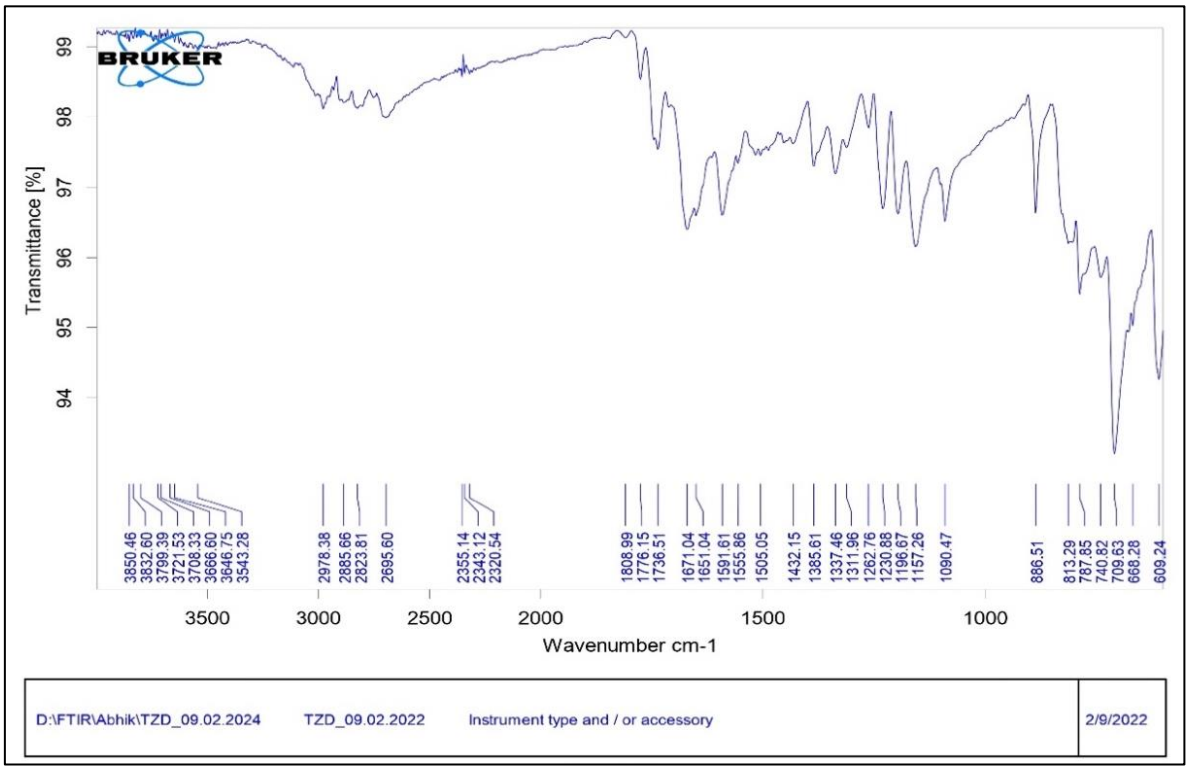
^{1}H NMR ($CDCl_3$): δ_H 0.92-0.96 (3H, t, *J* = 6.6 Hz), 1.33-1.39 (2H, tq, *J* = 7.0, 6.6 Hz), 1.60-1.68 (2H, tt, *J* = 7.7, 7.0 Hz), 3.06 (6H, s), 3.71-3.75 (2H, t, *J* = 7.7 Hz), 6.73-6.76 (2H, ddd, *J* = 8.0, 1.1, 0.4 Hz), 7.39-7.81 (3H, 7.42 (ddd, *J* = 8.0, 1.5, 0.4 Hz)). ^{13}C NMR ($CDCl_3$) δc: 13.62 (1C, s), 19.99 (1C, s), 29.90 (1C, s), 40.13 (2C, s), 41.61 (1C, s), 112.14 (2C, s), 114.87 (1C, s), 121.13 (2C, s), 132.44 (1C, s), 134.43 (1C, s), 151.42 (1C, s), 166.88 (1C, s), 168.55 (1C, s).FTIR (cm^{-1}); 3320.22, 3244.22, 3162.68, (Ar-H), 2911.37, 2866.38, 2803.70, 2682.34 (Butyl) 2585.61.61 (=CH), 2866.38, 2803.70 (-N-C-H), 1662.41 (-C=O).

4.2.14. ethyl (E)-2-(5-(4-(dimethylamino)benzylidene)-2,4-dioxothiazolidin-3-yl)acetate (PN-12):

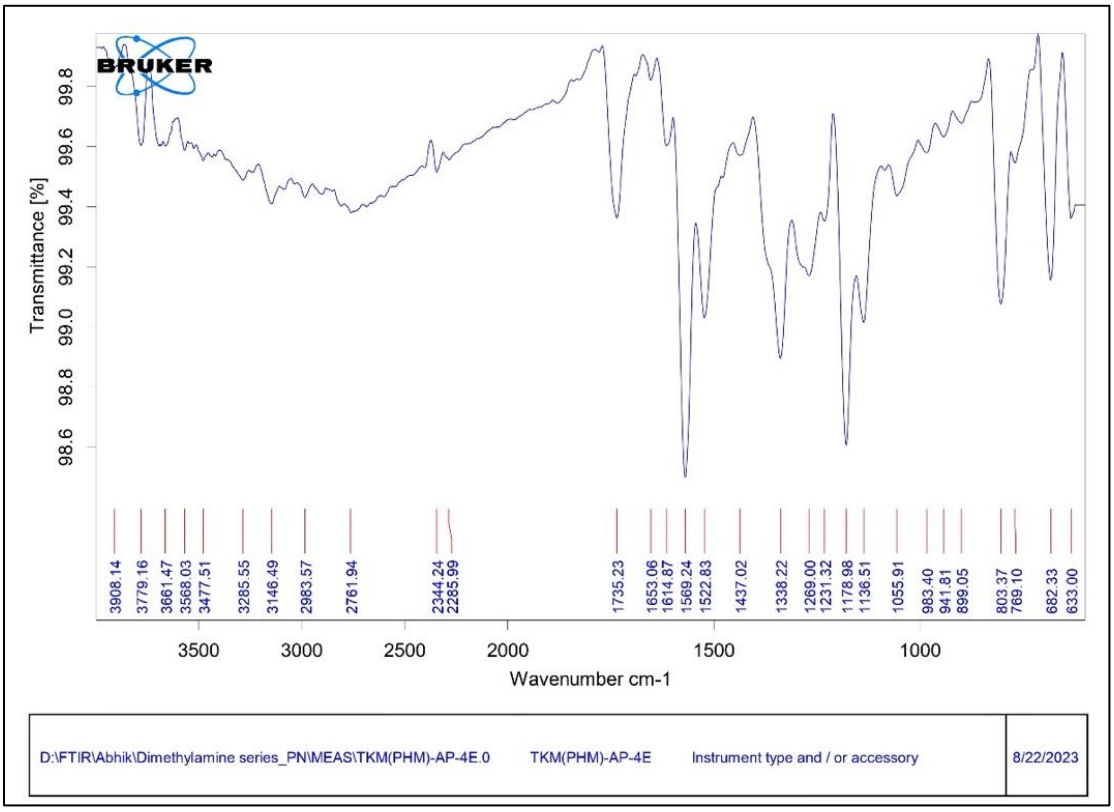
^{1}H NMR ($CDCl_3$): δ_H 1.27-1.30 (3H, t, *J* = 7.1 Hz), 3.06 (6H, s), 4.20-4.26 (2H, q, *J* = 7.1 Hz), 4.46 (2H, s), 6.74-6.76 (2H, d, *J* = 8.0, 1.1, 0.4 Hz), 7.40-7.85 (3H, 7.42 (d, *J* = 8.0, 1.5, 0.4 Hz)). ^{13}C NMR ($CDCl_3$) δc: 14.08 (1C, s), 30.87 (2C, s), 40.13 (1C, s), 42.03 (1C, s), 112.17 (2C, s), 114.05 (1C, s), 120.88 (2C, s), 132.62 (1C, s), 135.49 (1C, s), 151.61 (1C, s), 165.98 (1C, s), 166.53 (1C, s), 168.09 (1C, s). FTIR (cm^{-1}); 3480.70, 3442.73, 3416.34, (Ar-H), 3317.33, 3173.05, 3089.90, 3036.52 (acetate) 2987.99 (=CH), 2892.70, 2809.58 (-N-C-H)

4.3. FT-IR spectra of the synthesized compounds:

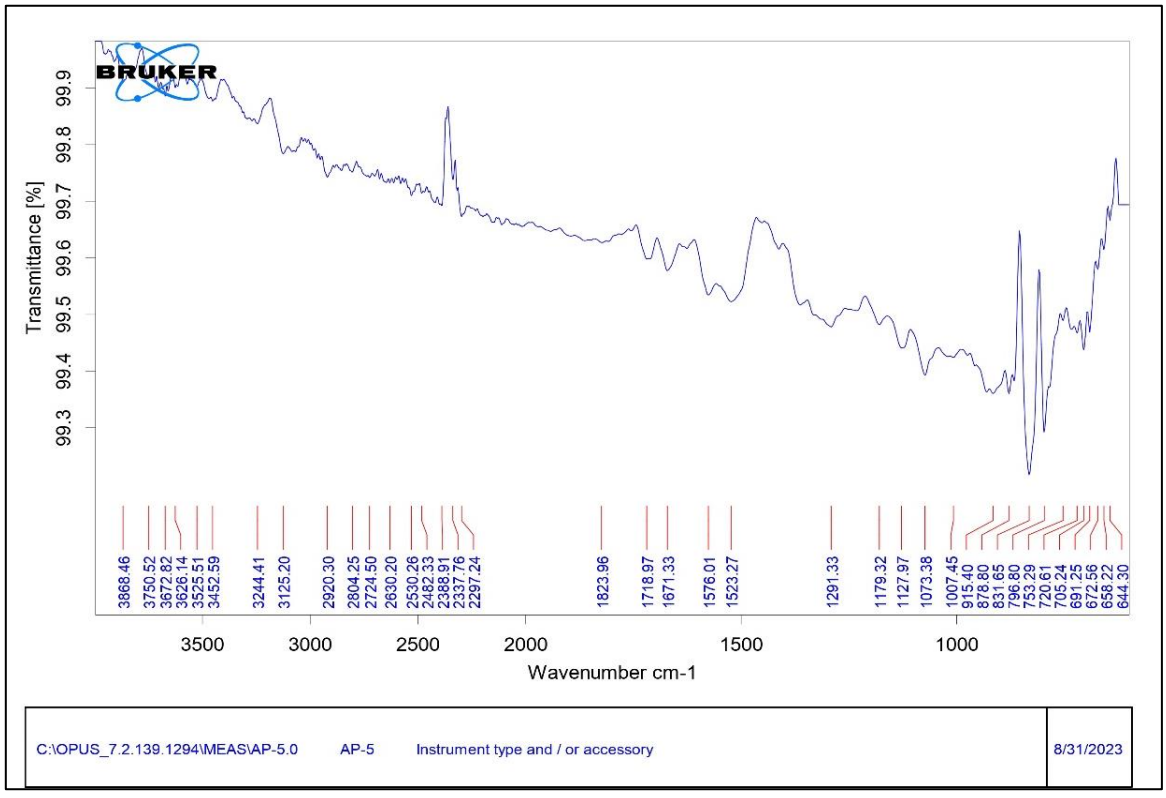
4.3.1. TZD



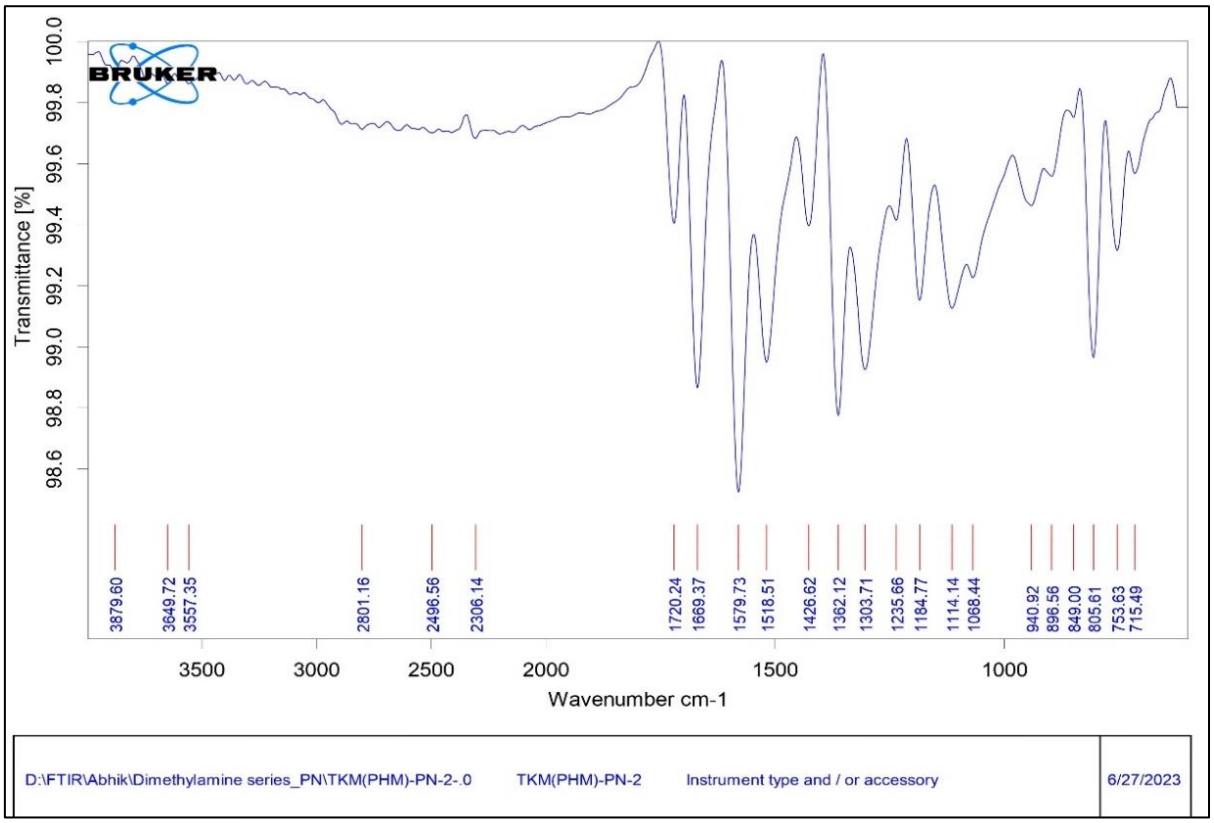
4.3.2. AP-4E:



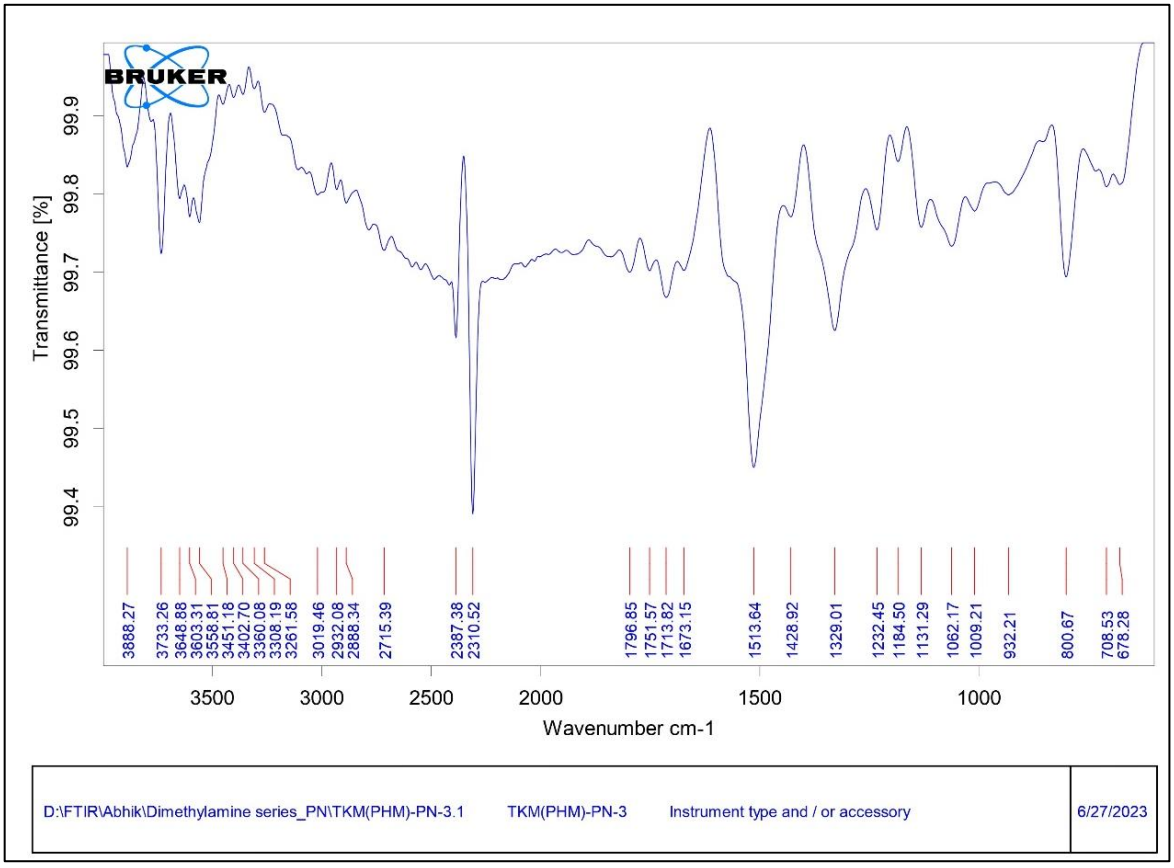
4.3.3. PN-1:



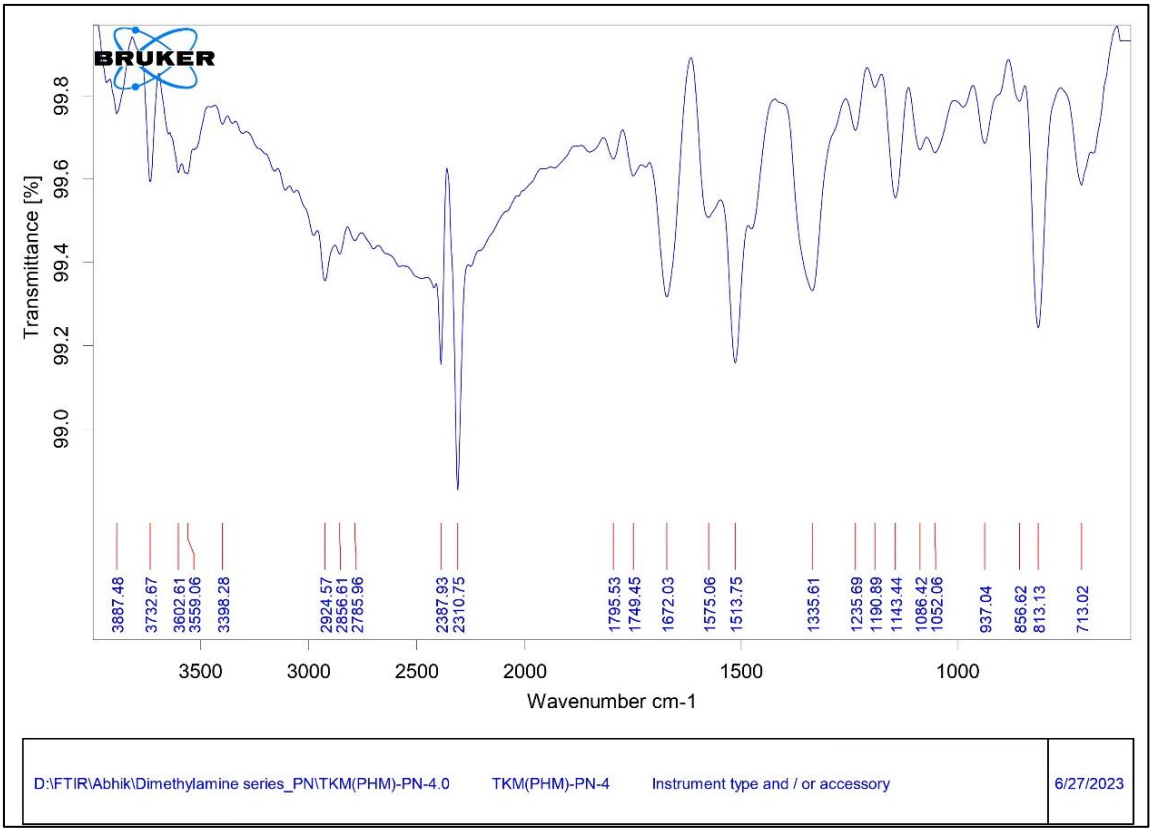
4.3.4. PN-2:



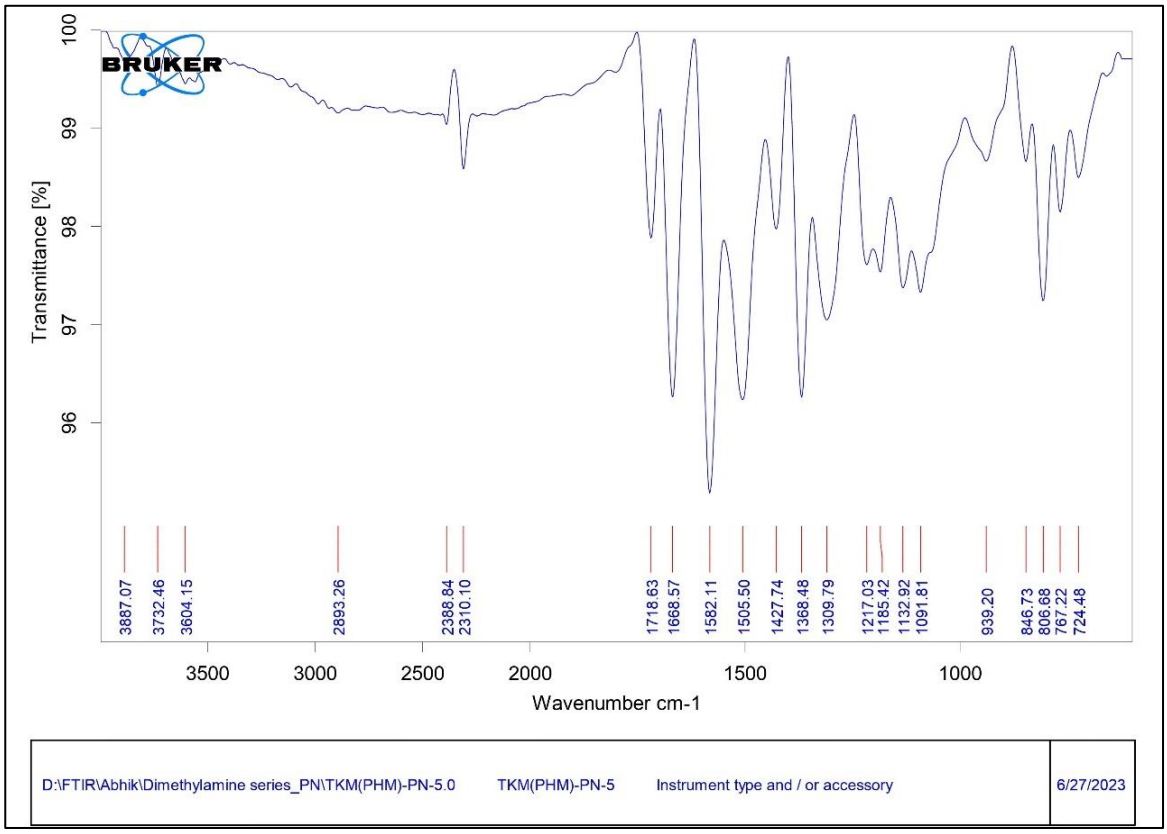
4.3.5. PN-3:



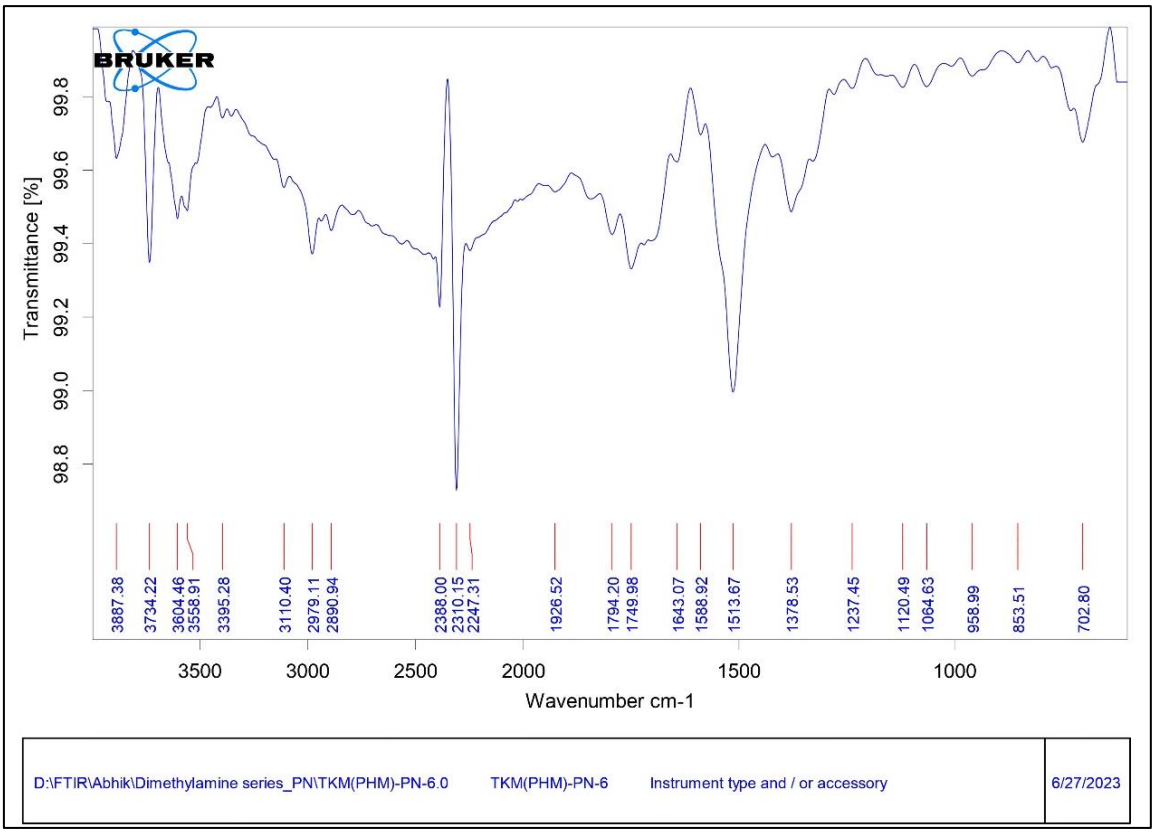
4.3.6. PN-4:



4.3.7. PN-5:



4.3.8. PN-6:


BRUKER
Transmittance [%]
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99.0
98.8
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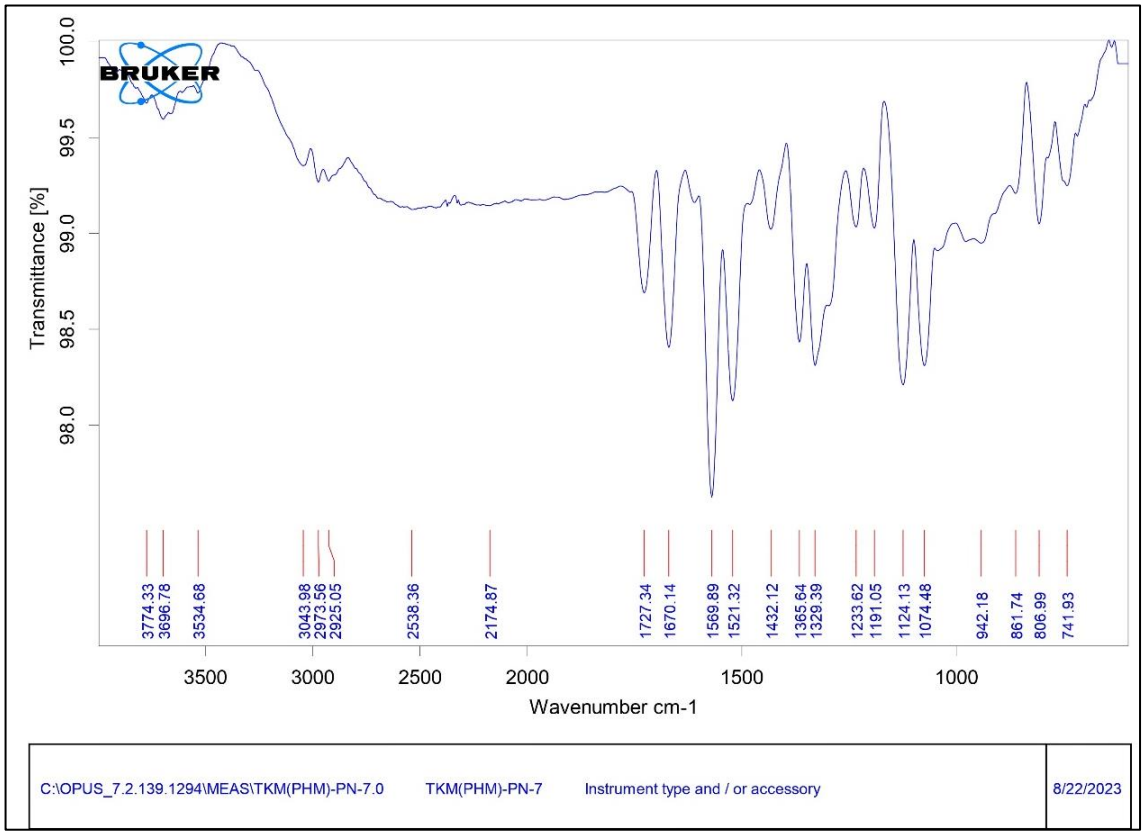
3395.28
3110.40
2979.11
2890.94
2388.00
2310.15
2247.31
1926.52
1794.20
1749.98
1643.07
1588.92
1513.67
1378.53
1237.45
1120.49
1064.63
958.99
853.51
702.80
3500
3000
2500
2000
1500
1000
Wavenumber cm-1
D:\FTIR\Abhik\Dimethylamine series_PN\TKM(PHM)-PN-6.0
TKM(PHM)-PN-6
Instrument type and / or accessory
6/27/2023

4.3.9. PN-7:


BRUKER
Transmittance [%]
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98.5
98.0
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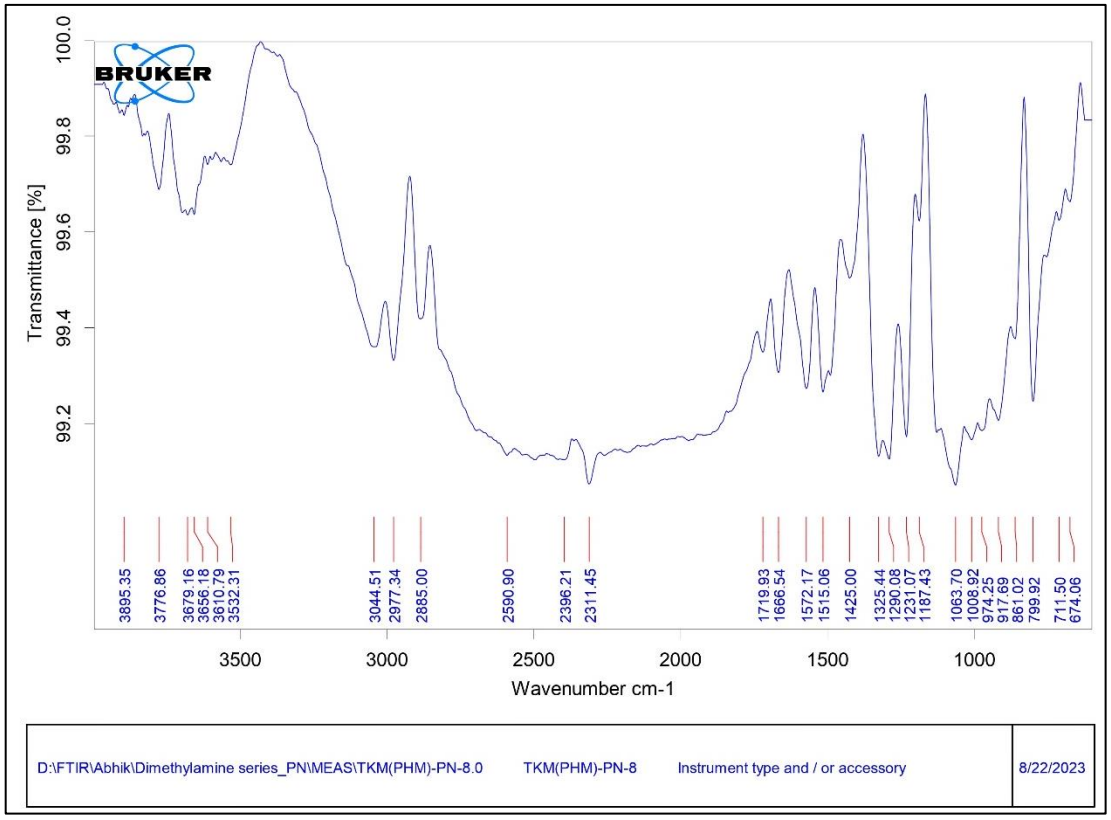
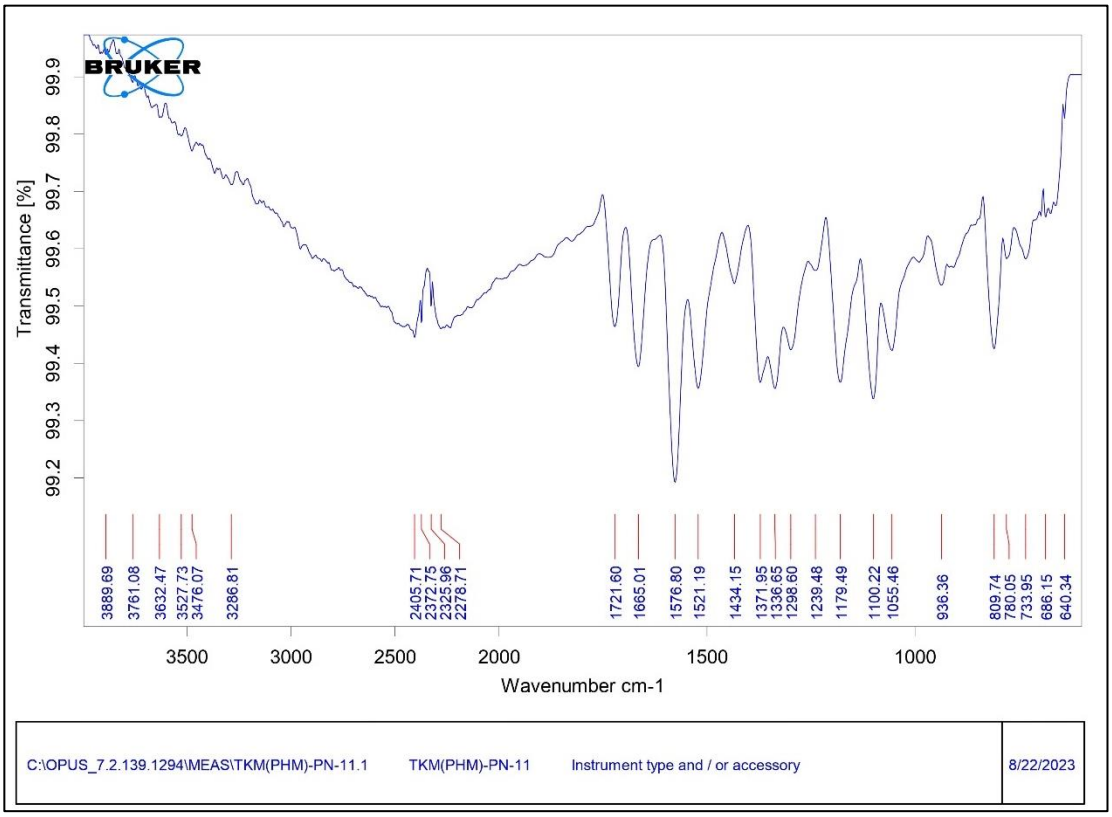
2538.36
2174.87
1727.34
1670.14
1589.89
1521.32
1432.12
1365.64
1329.39
1233.62
1191.05
1124.13
1074.48
942.18
861.74
806.99
741.93
3500
3000
2500
2000
1500
1000
Wavenumber cm-1
C:\OPUS_7.2.139.1294\MEAS\TKM(PHM)-PN-7.0
TKM(PHM)-PN-7
Instrument type and / or accessory
8/22/2023

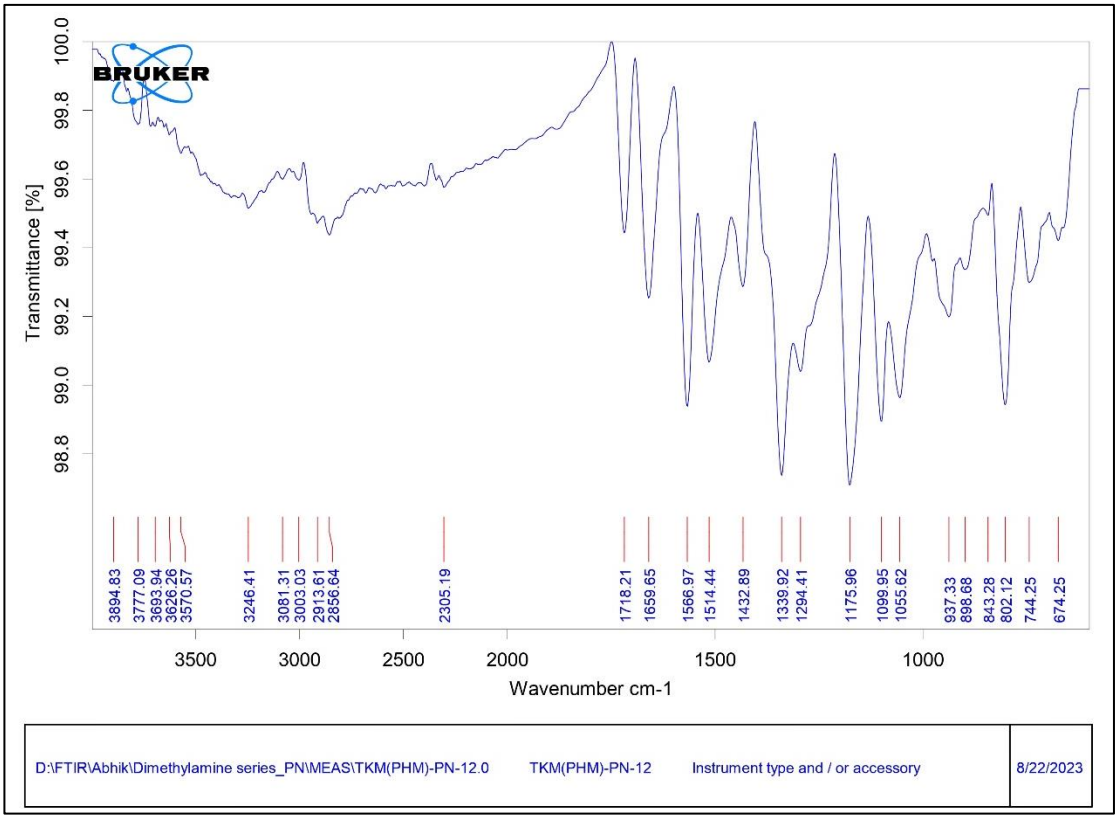
4.3.10. PN-8:



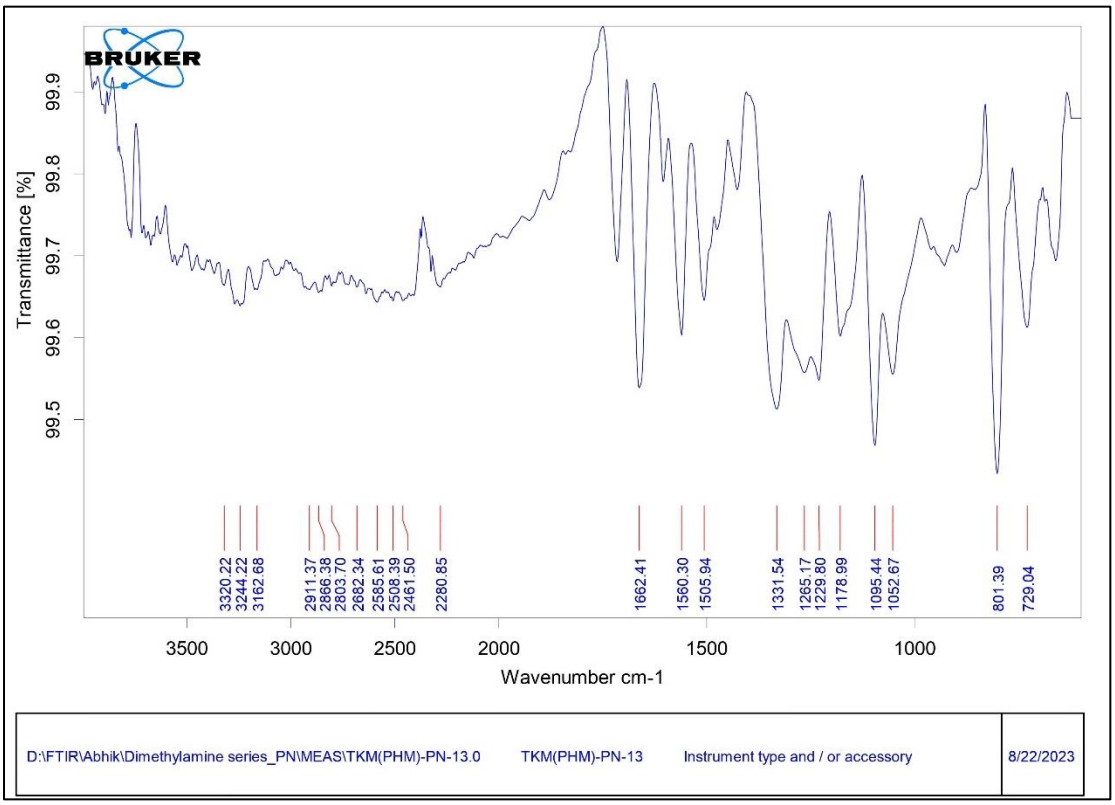
4.3.11. PN-9:



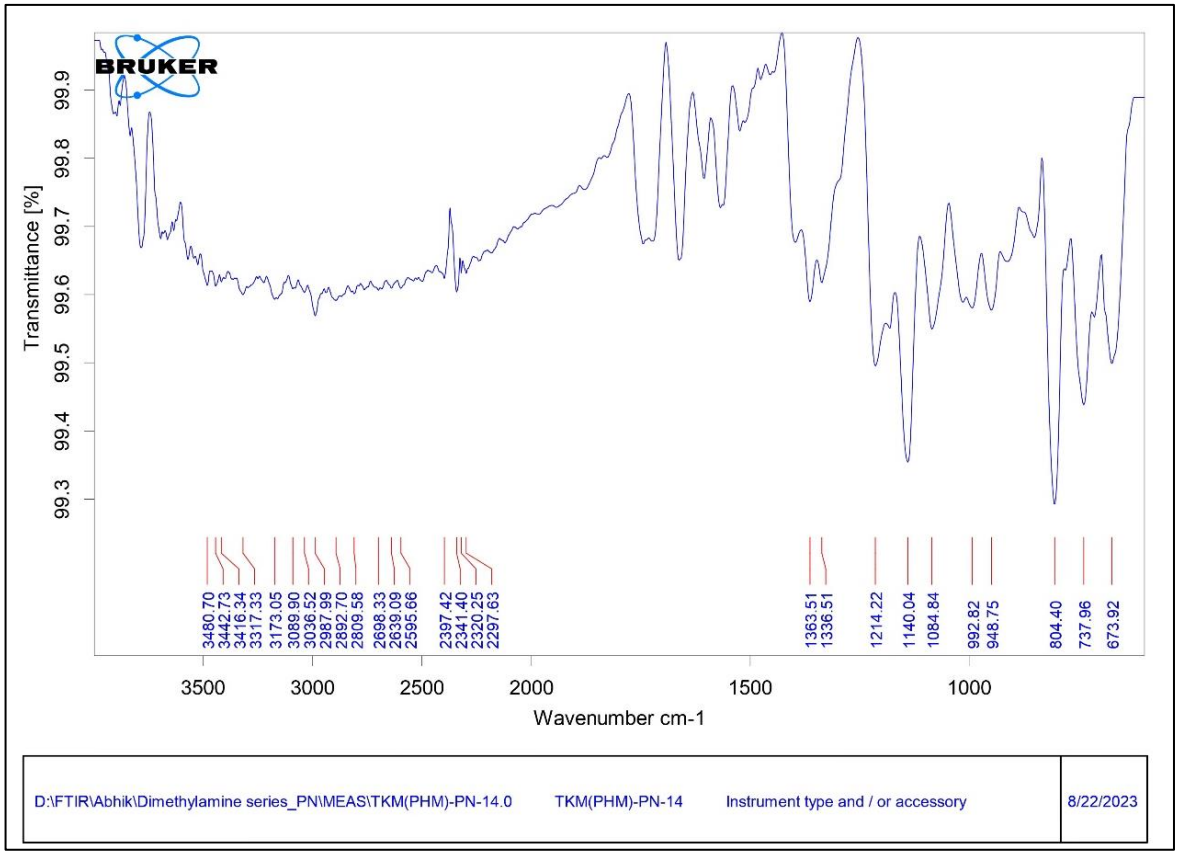
4.3.12. PN-10:



4.3.13. PN-11:

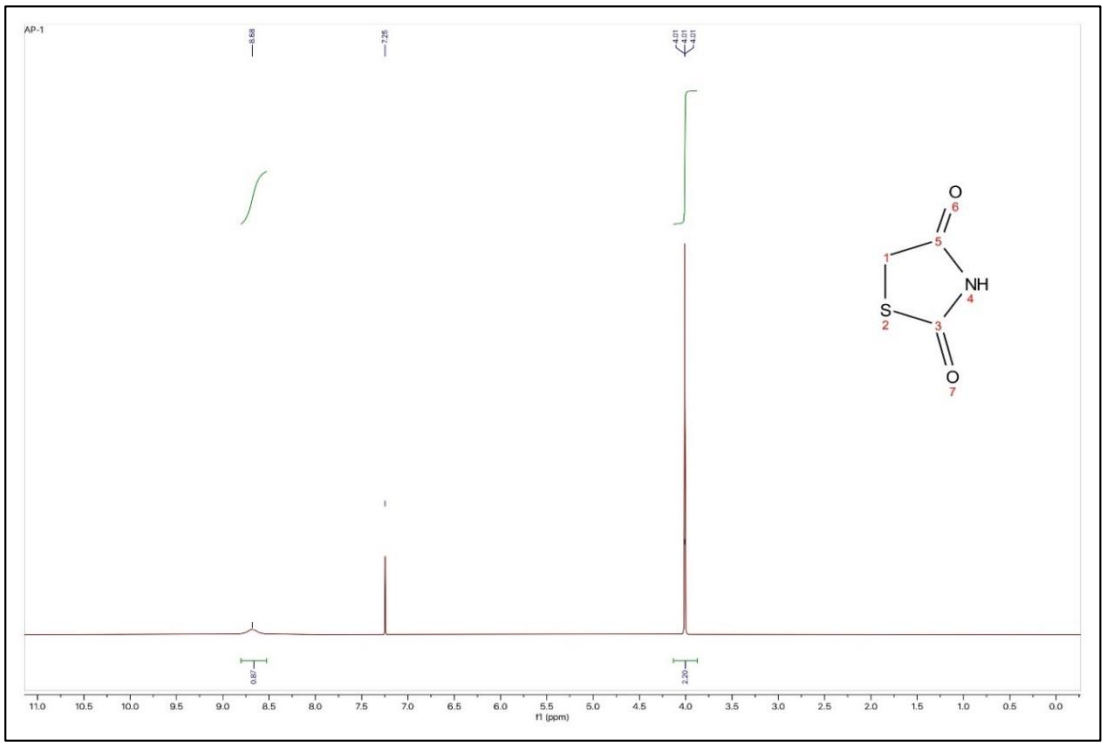


4.3.14. PN-12:

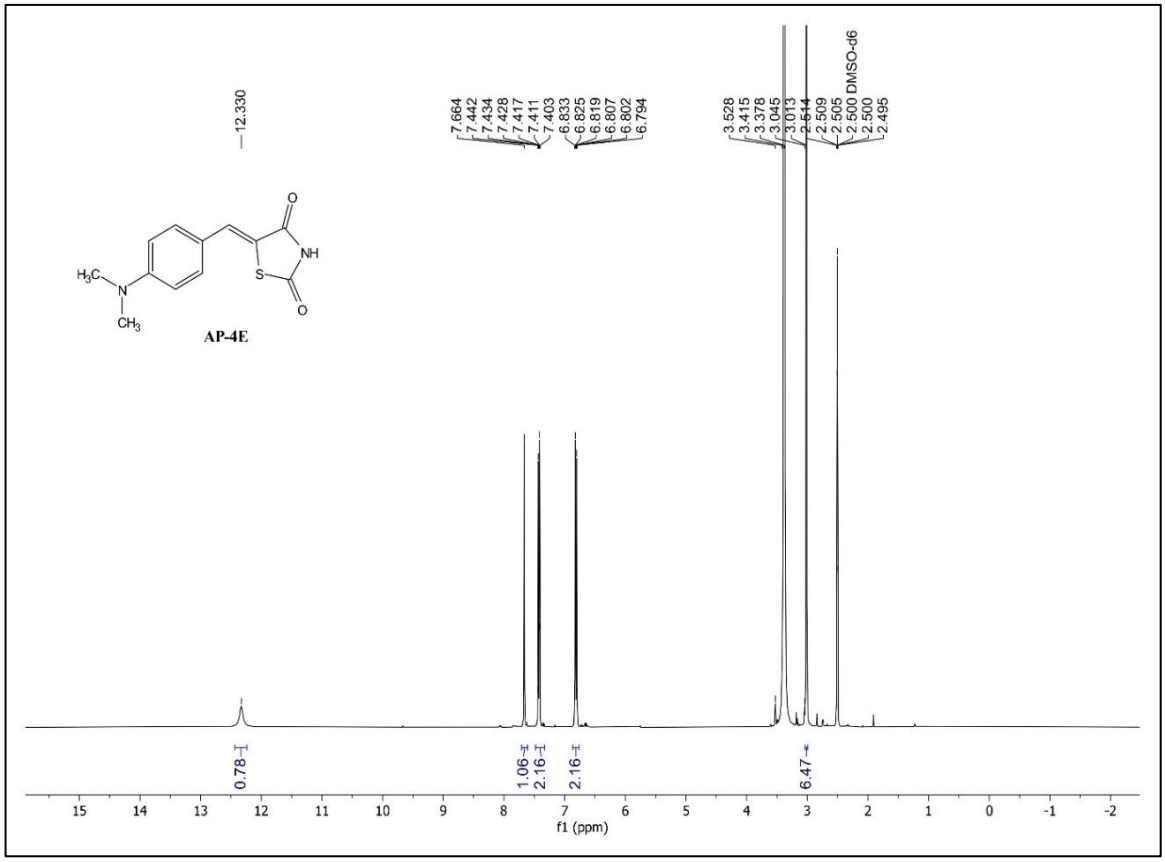


4.4. ^{1}H NMR spectra of the synthesized compound:

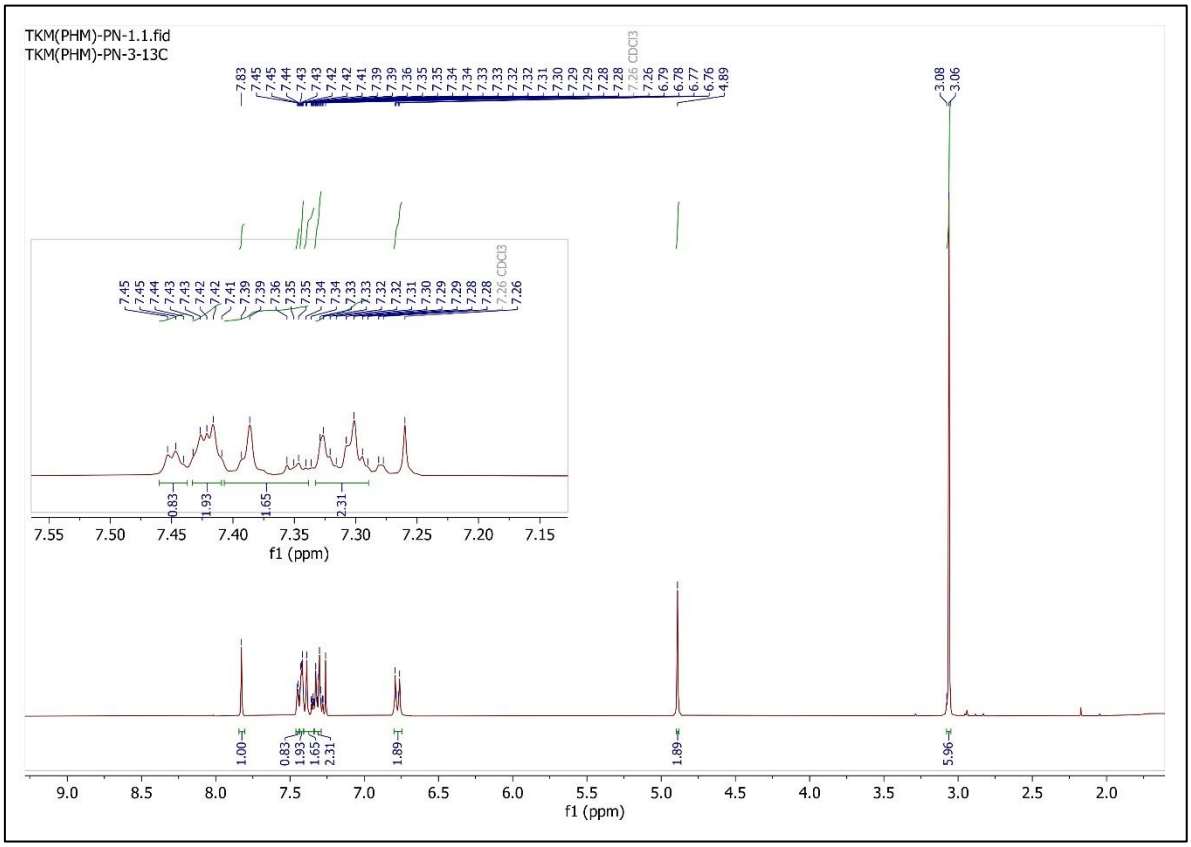
4.4.1. TZD:



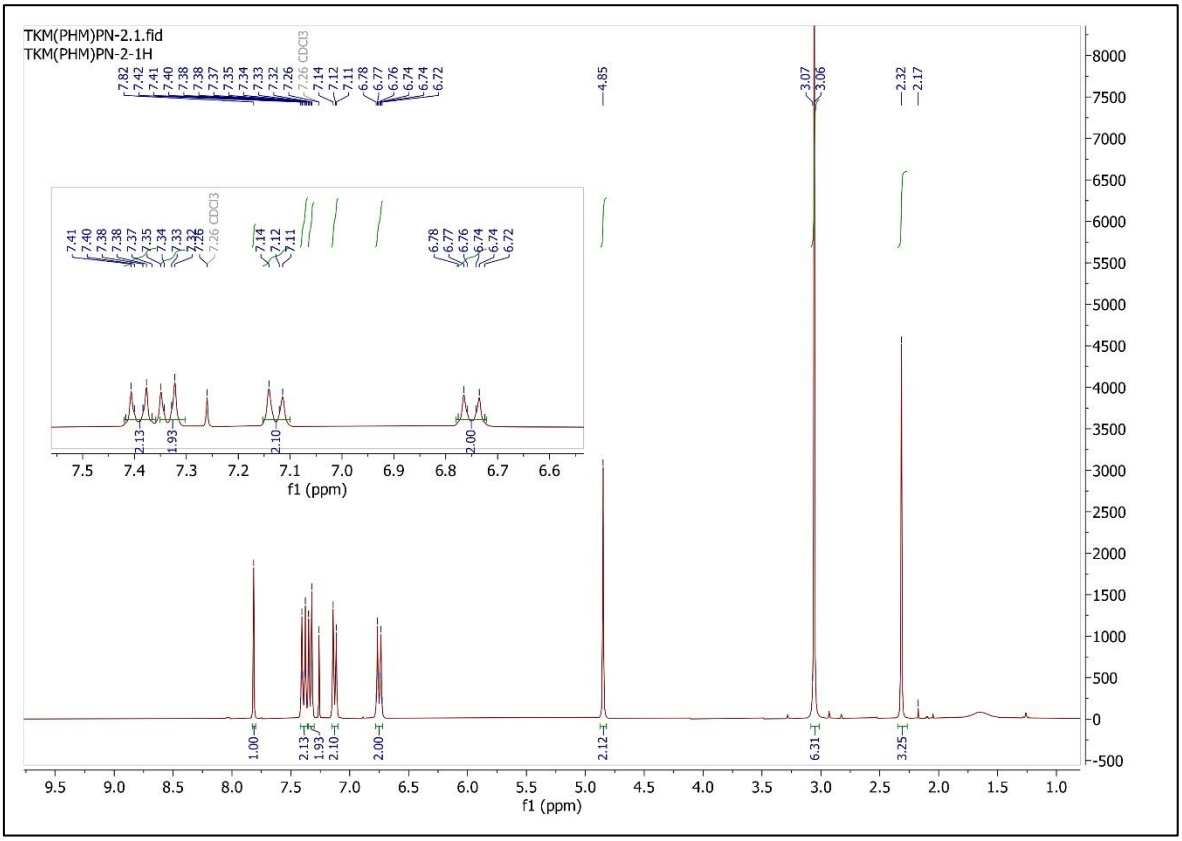
4.4.2. AP-4E:



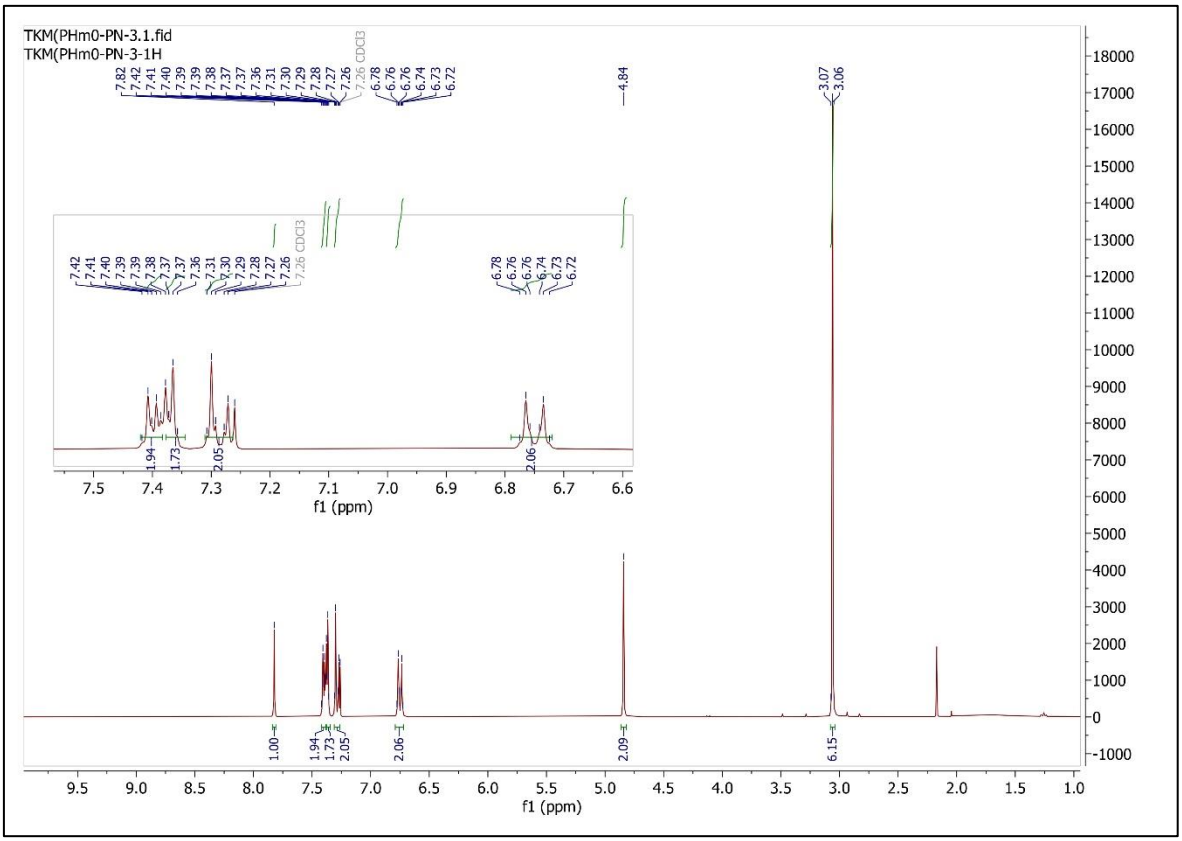
4.4.3. PN-1:



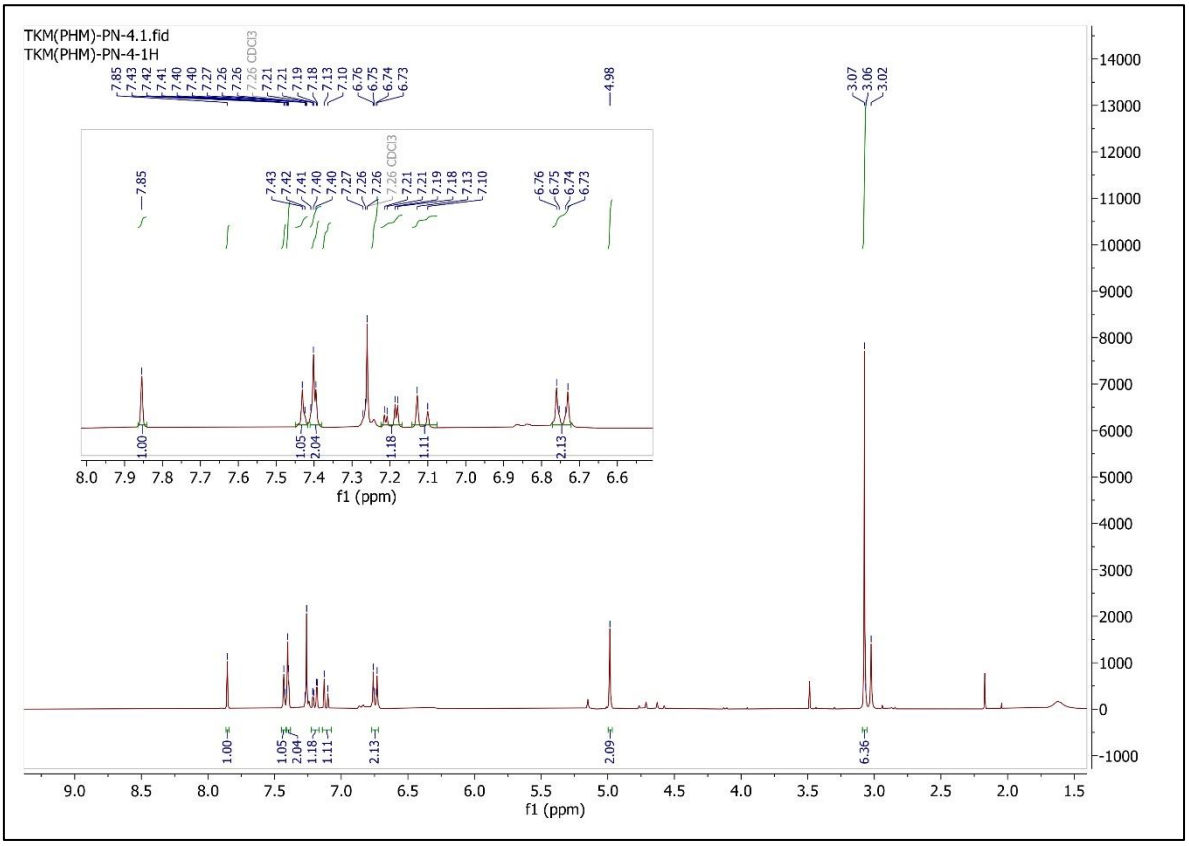
4.4.4. PN-2:



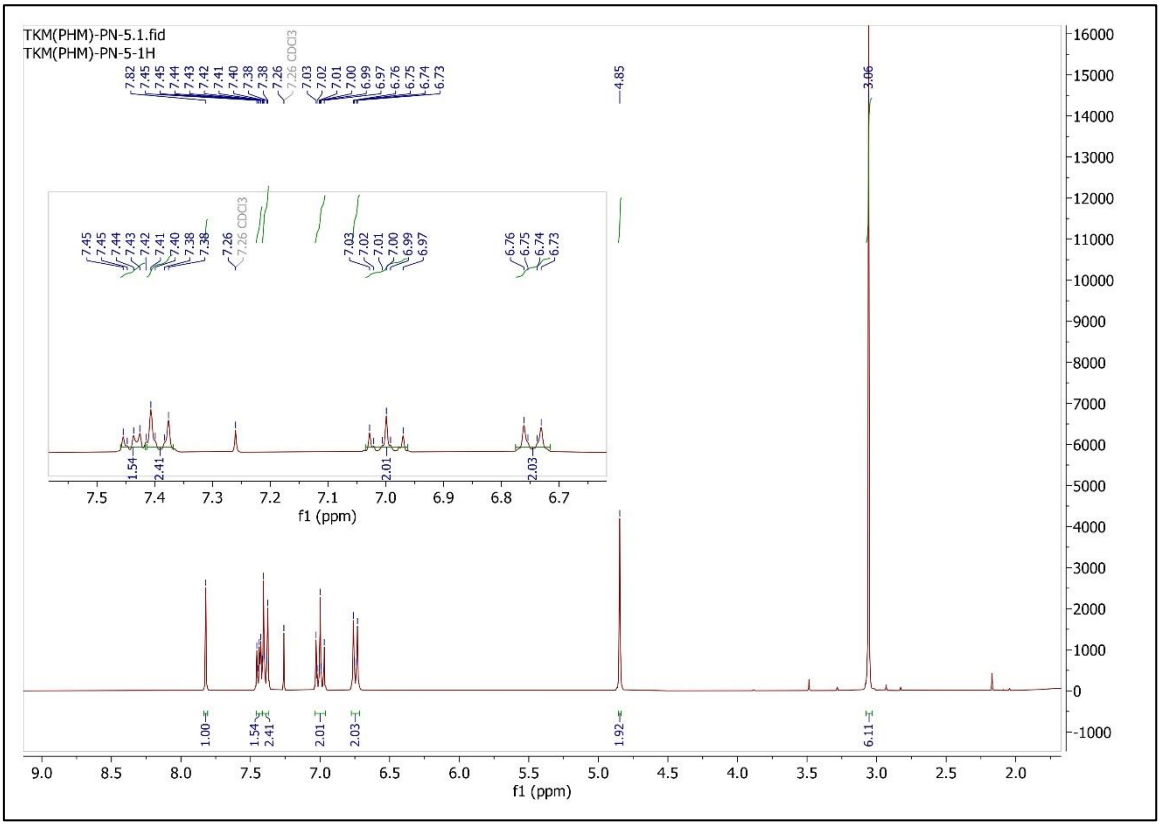
4.4.5. PN-3:



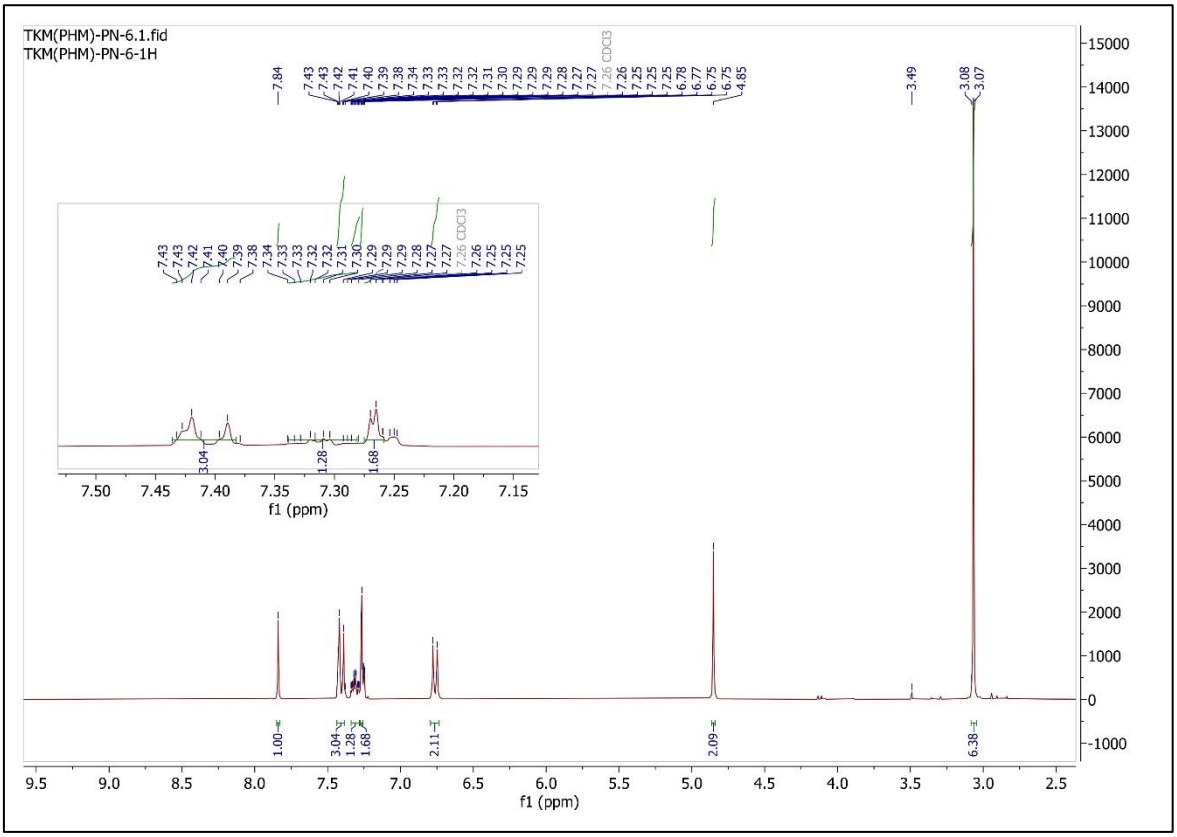
4.4.6. PN-4:



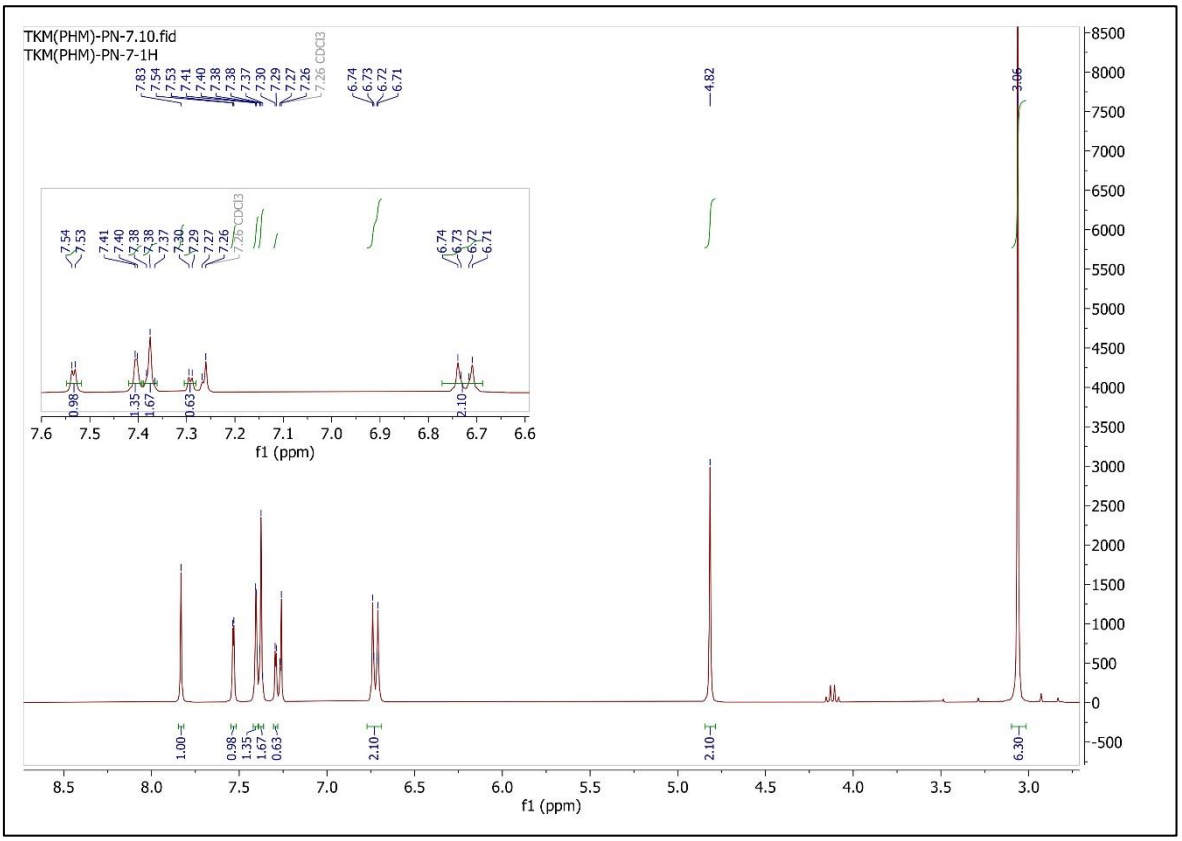
4.4.7. PN-5:



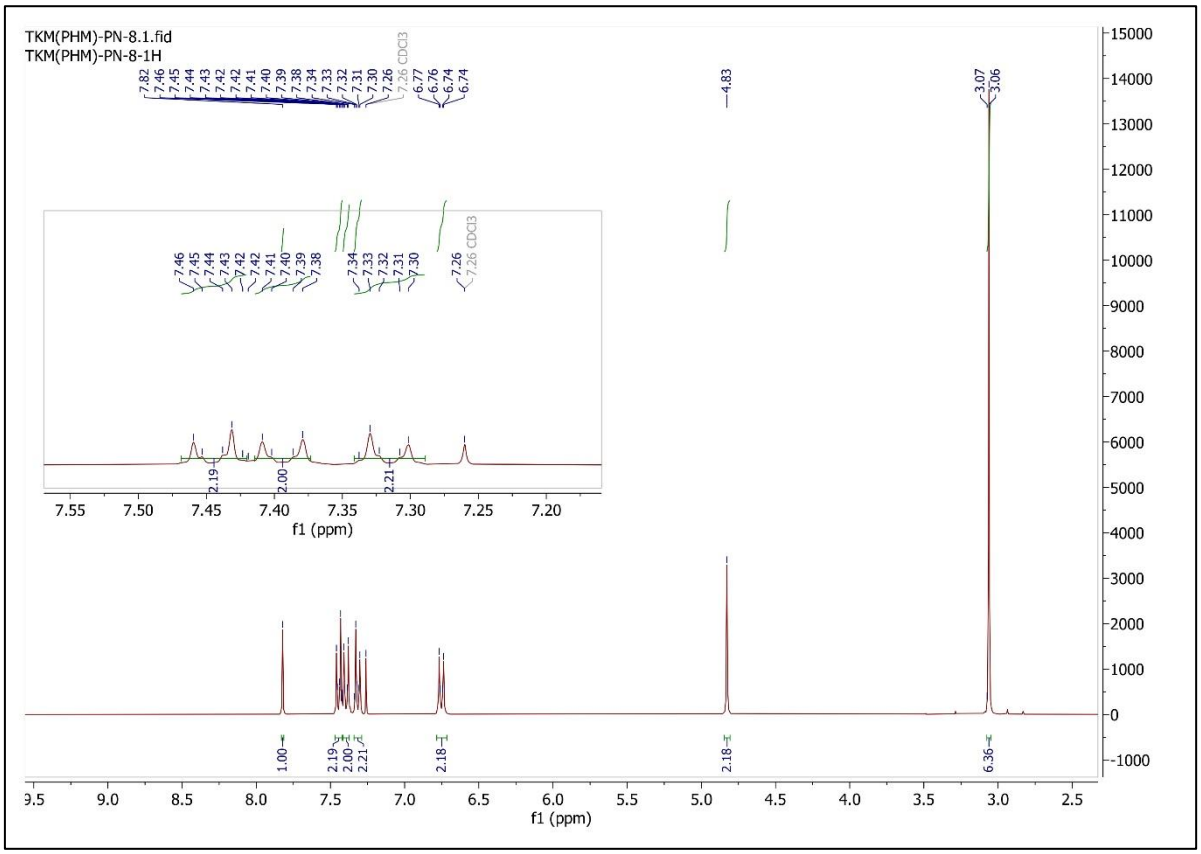
4.4.8. PN-6:



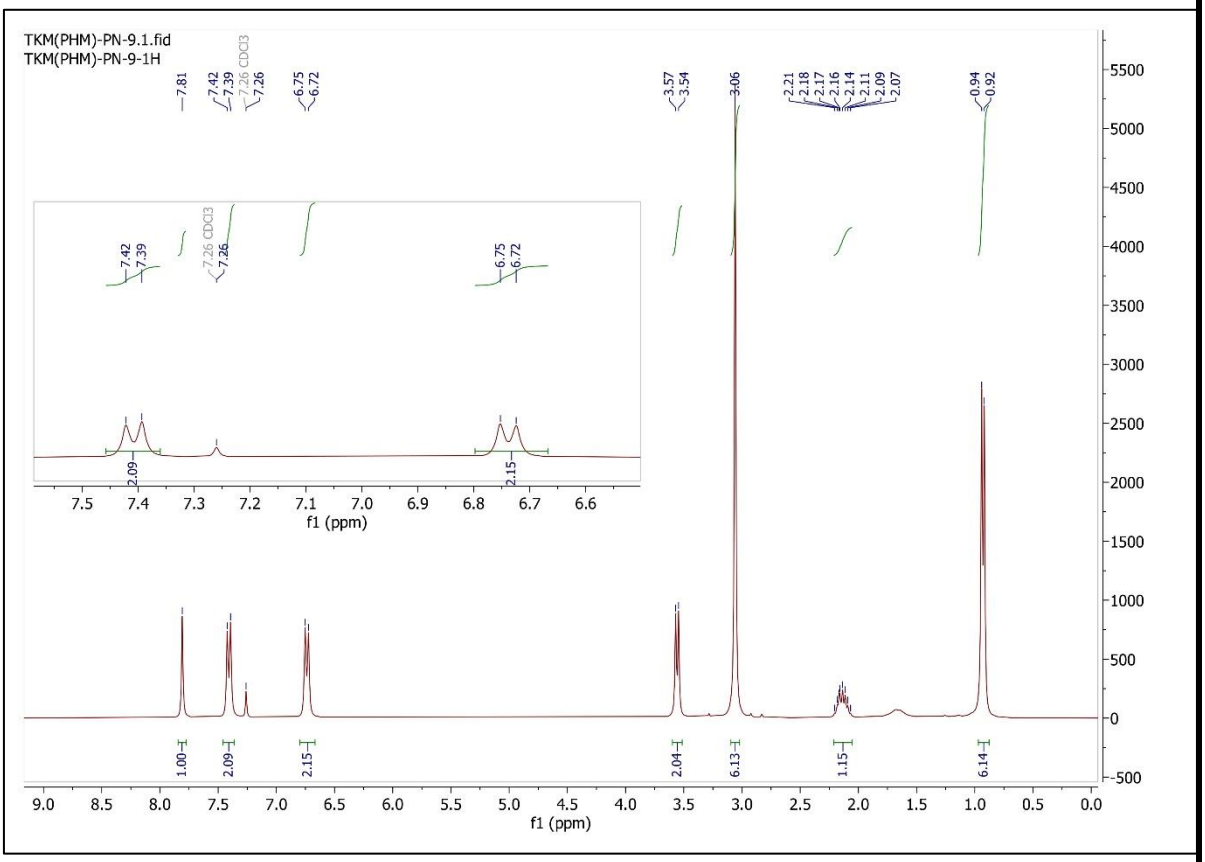
4.4.9. PN-7:



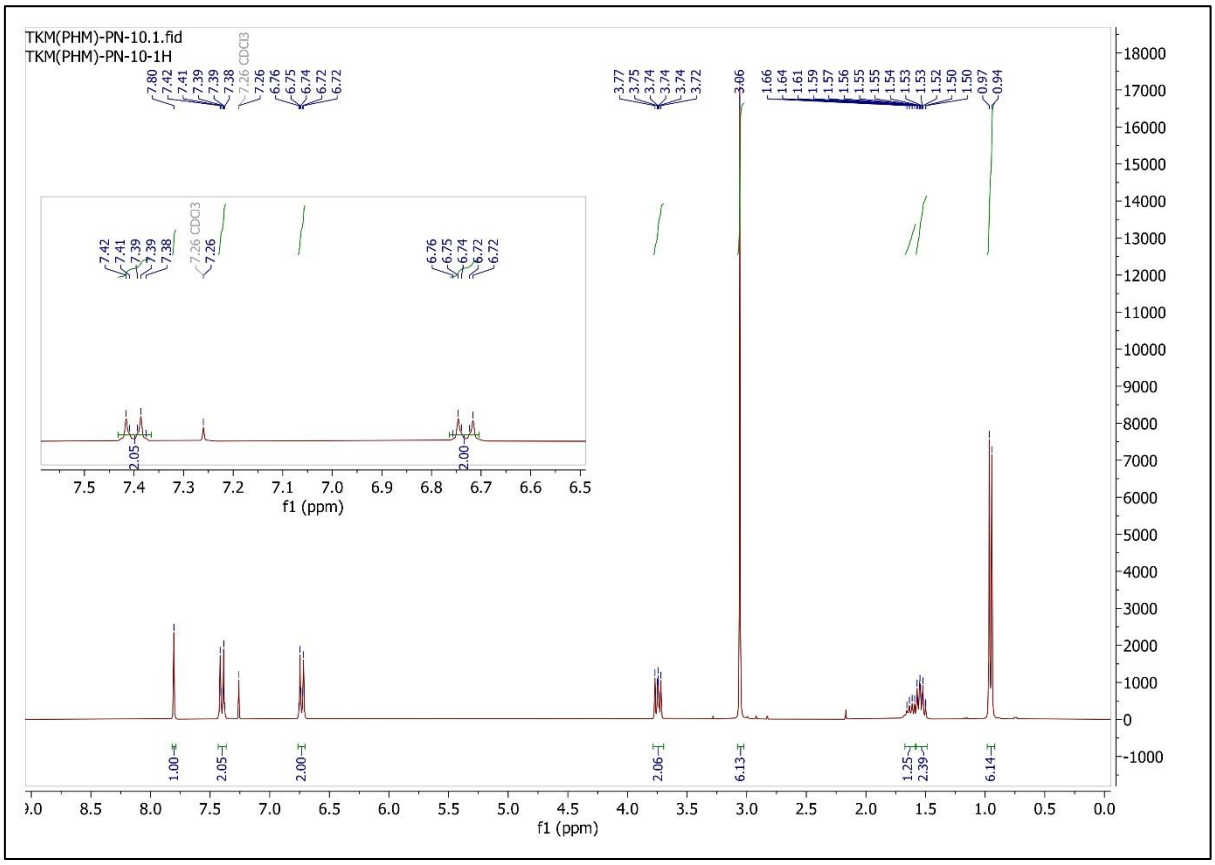
4.4.10. PN-8:



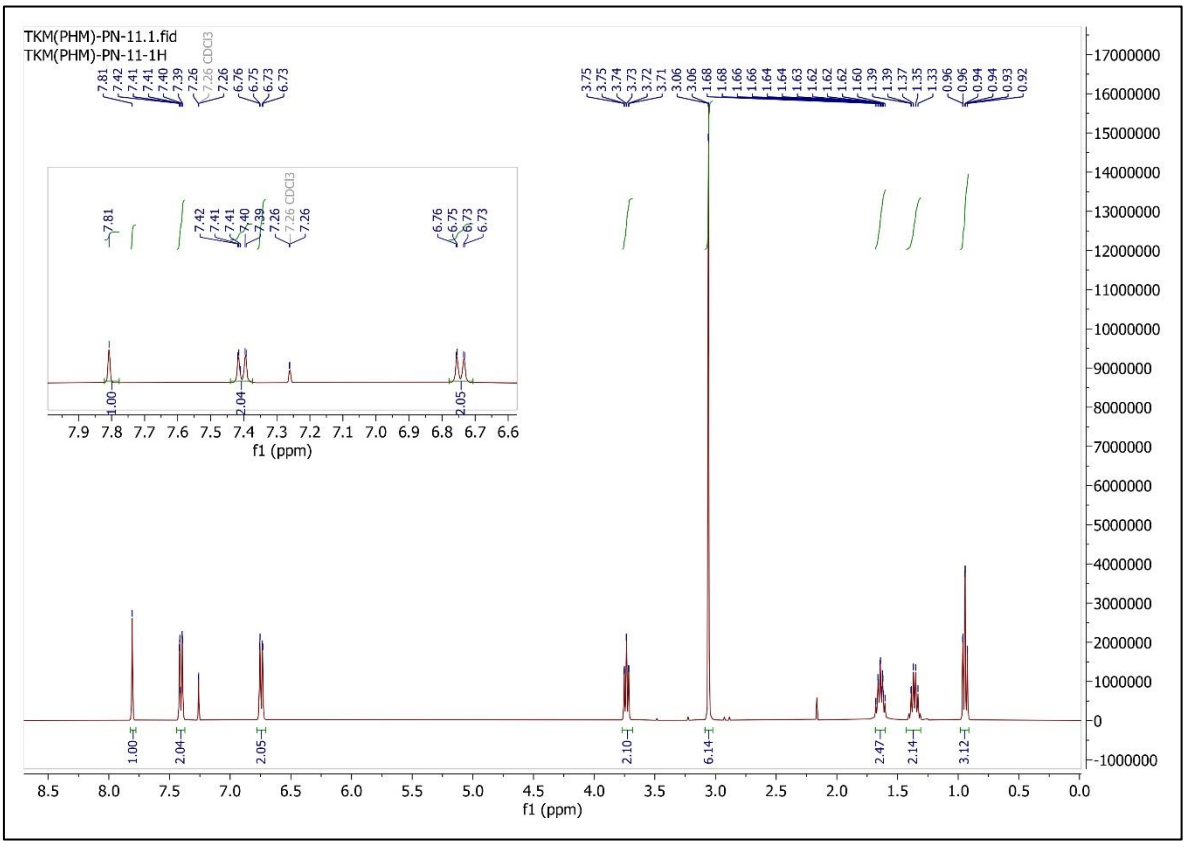
4.4.11. PN-9:



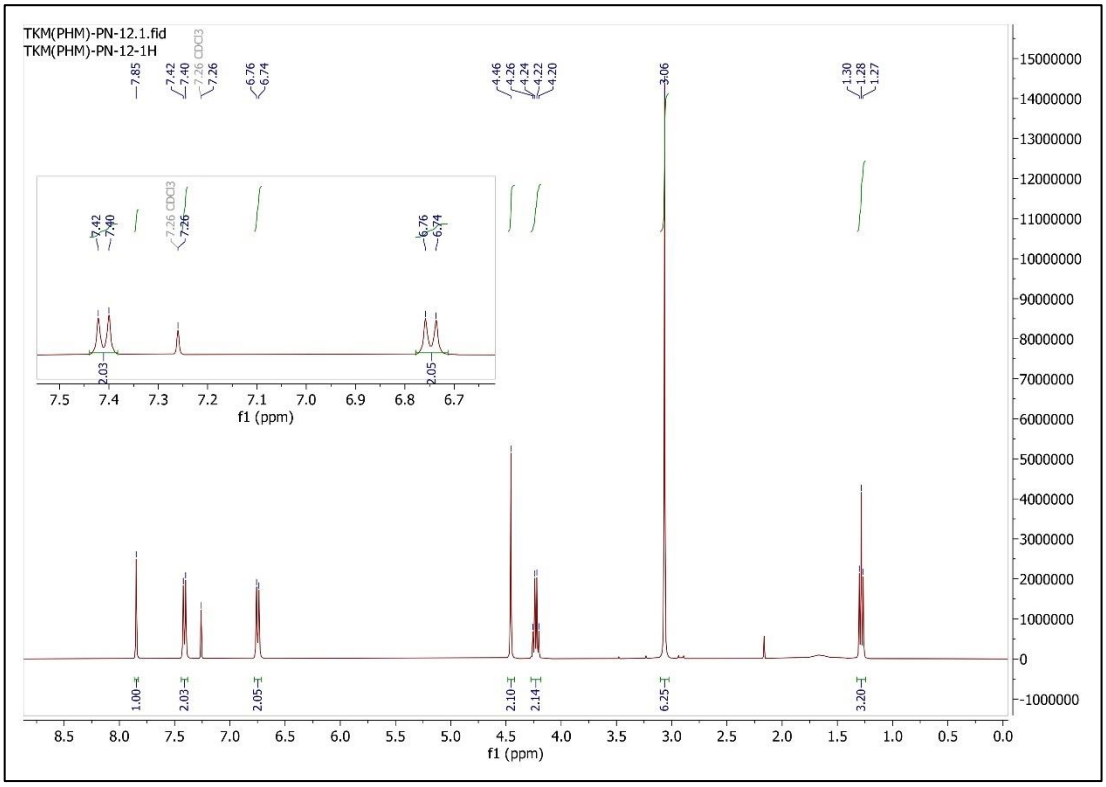
4.4.12. PN-10:



4.4.13. PN-11:

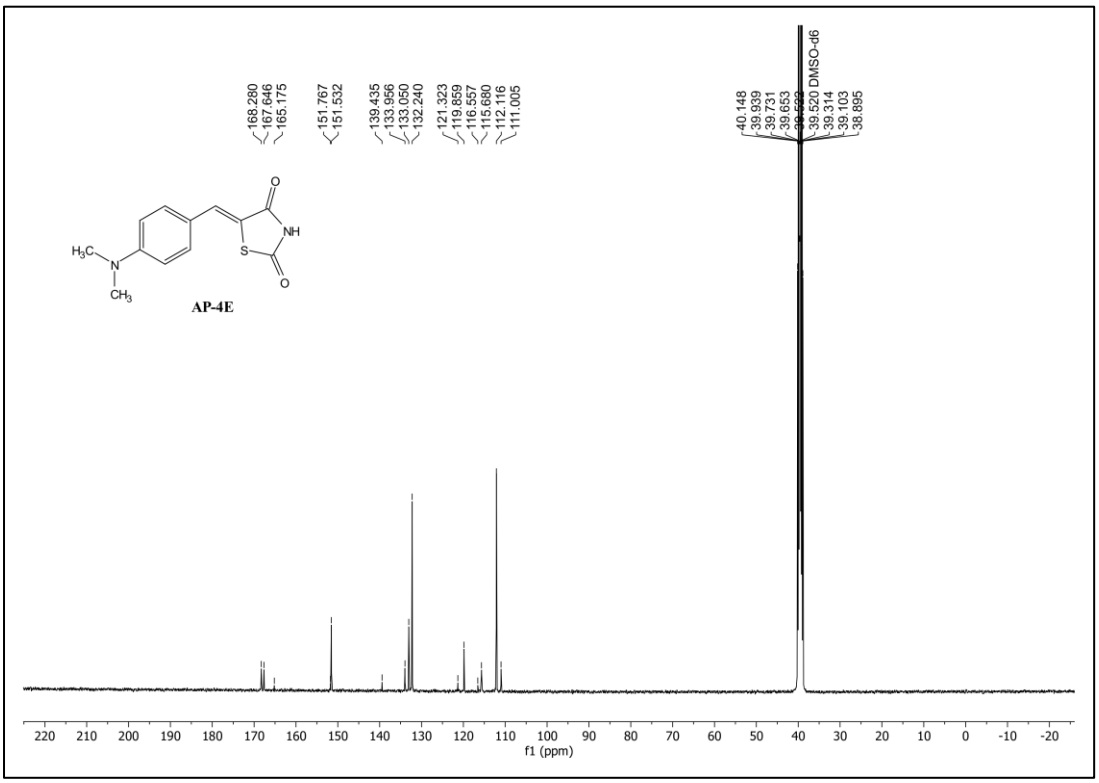


4.4.14. PN-12:

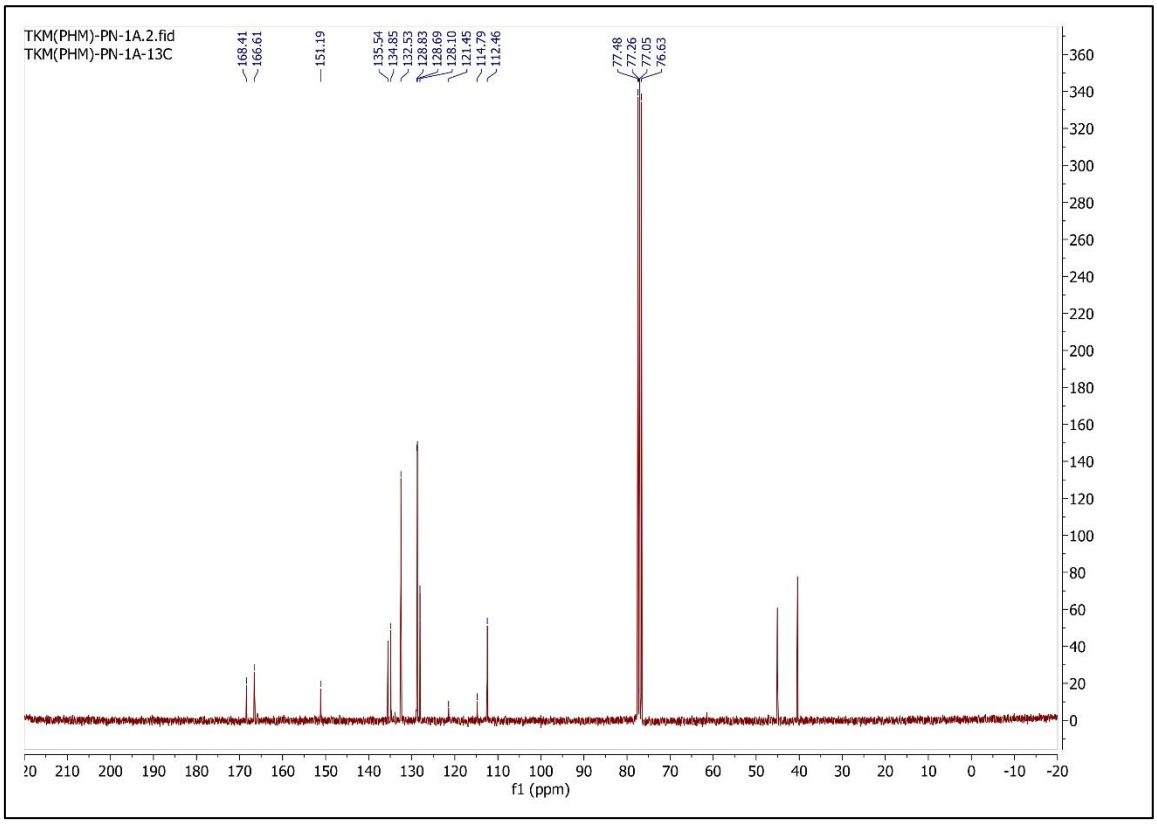


4.5. ^{13}C NMR spectra of the synthesized compound:

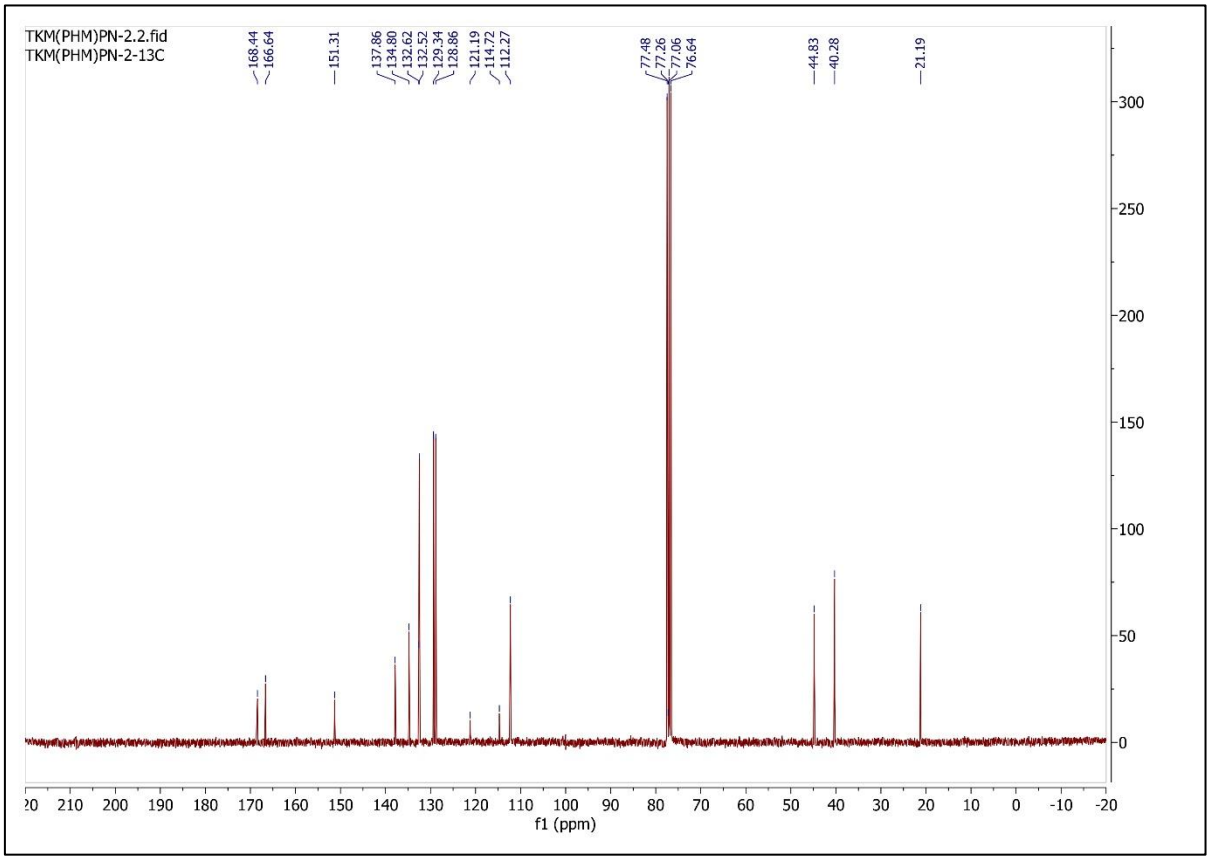
4.5.1. AP-4E



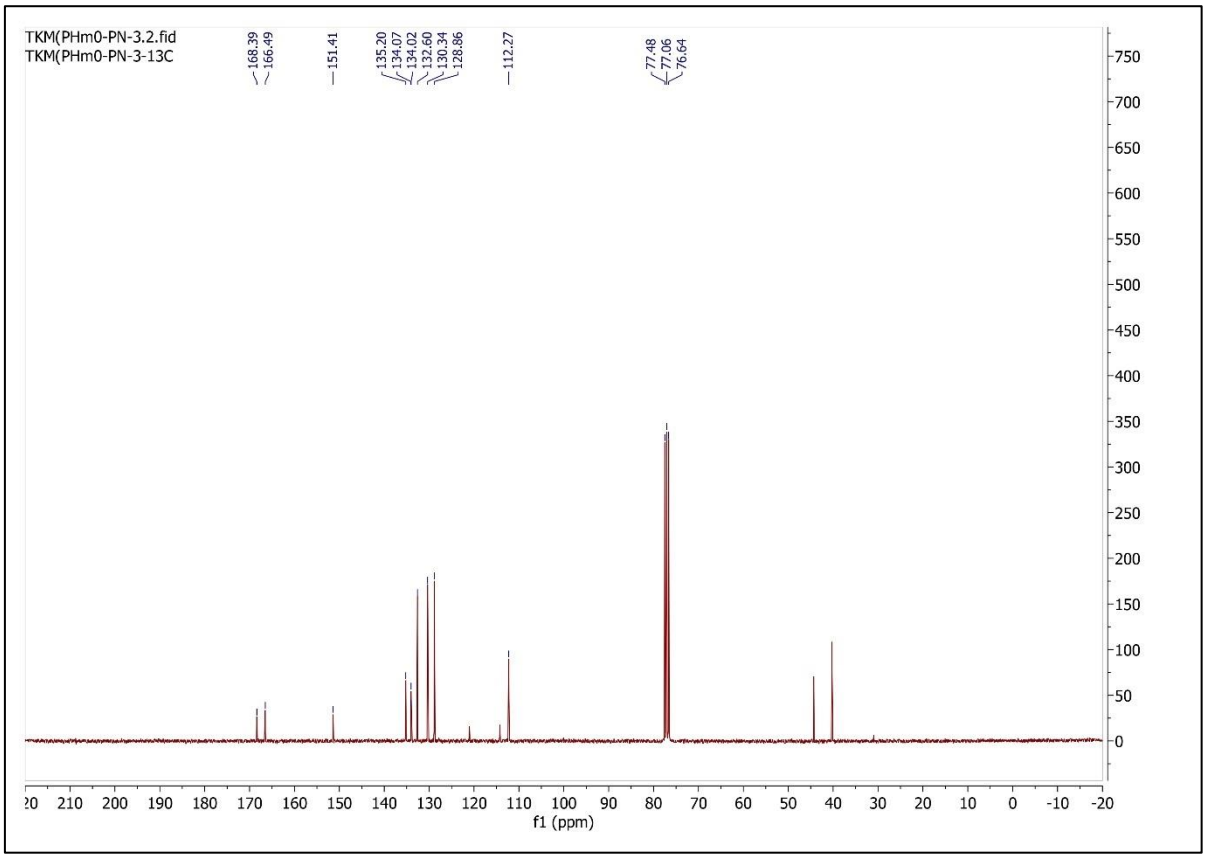
4.5.2. PN-1:



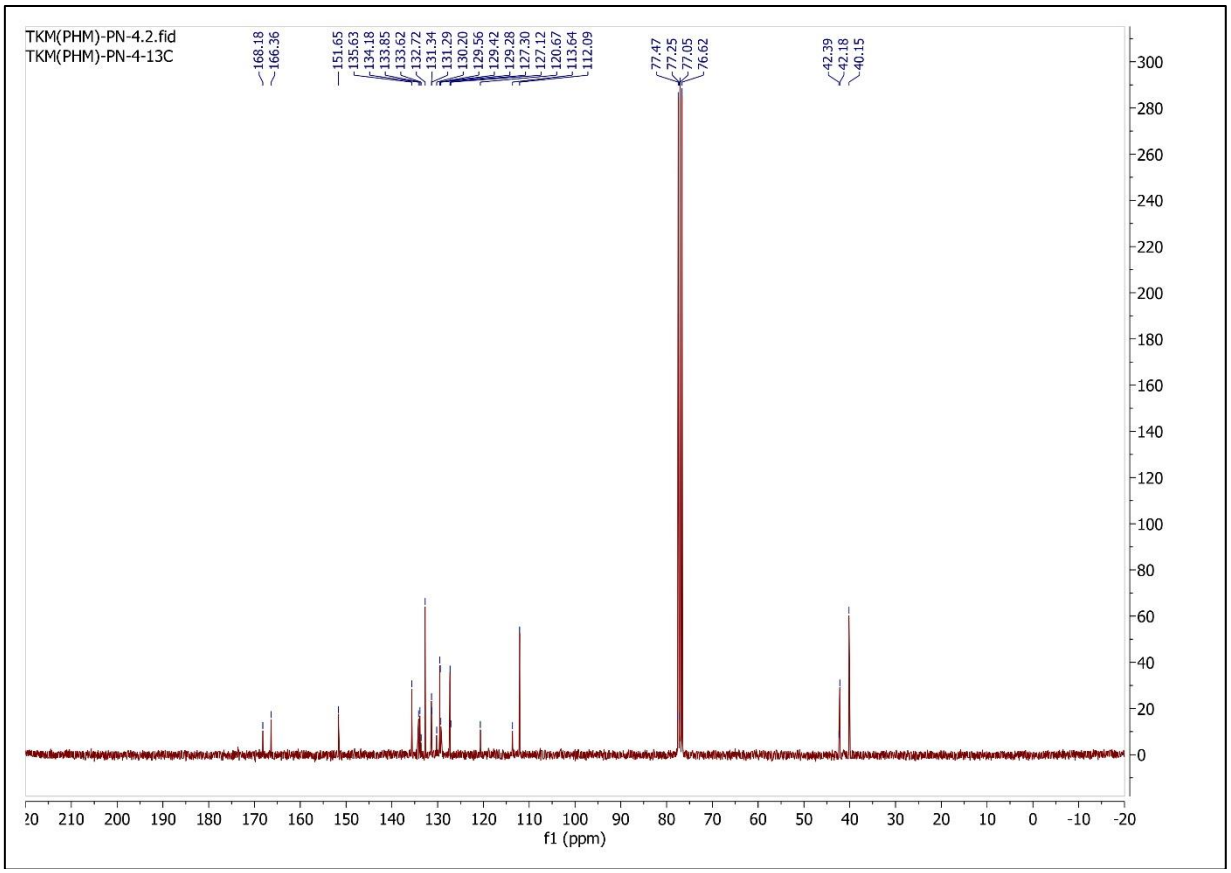
4.5.3. PN-2:



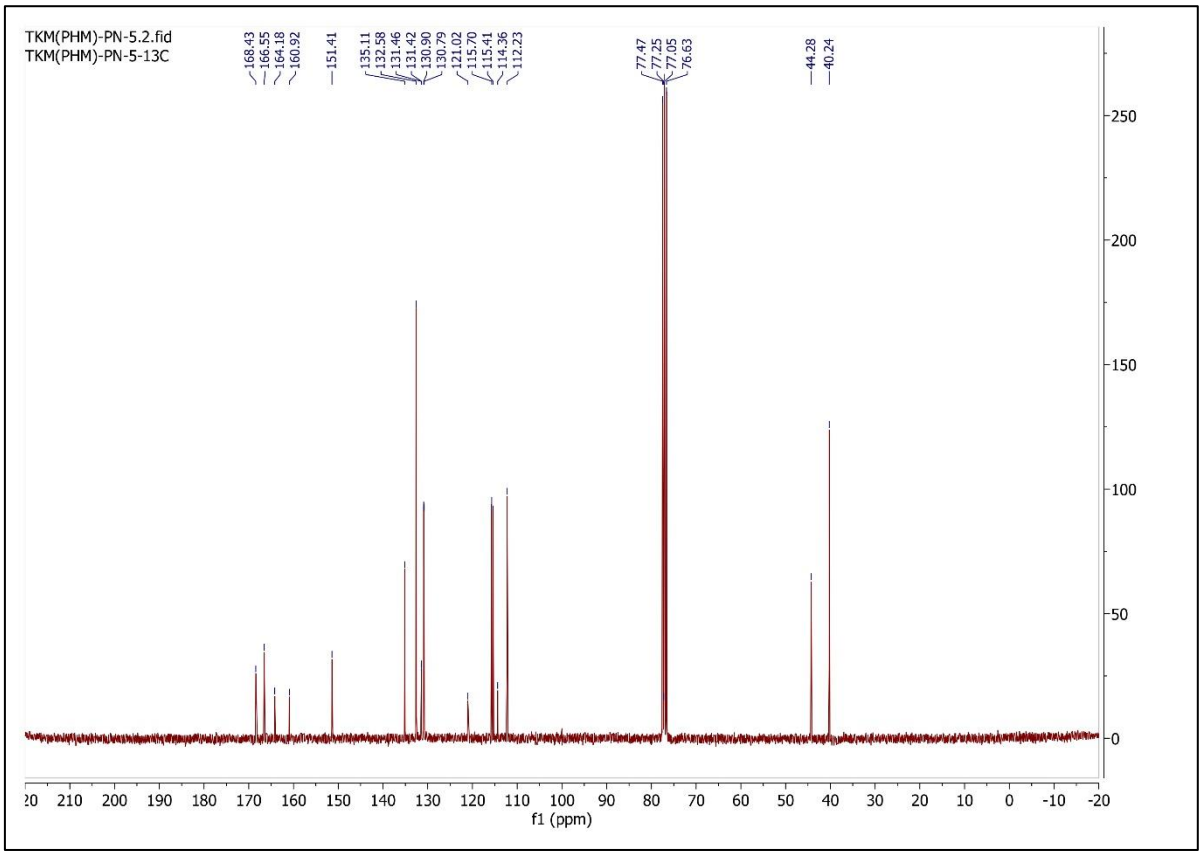
4.5.4. PN-3:



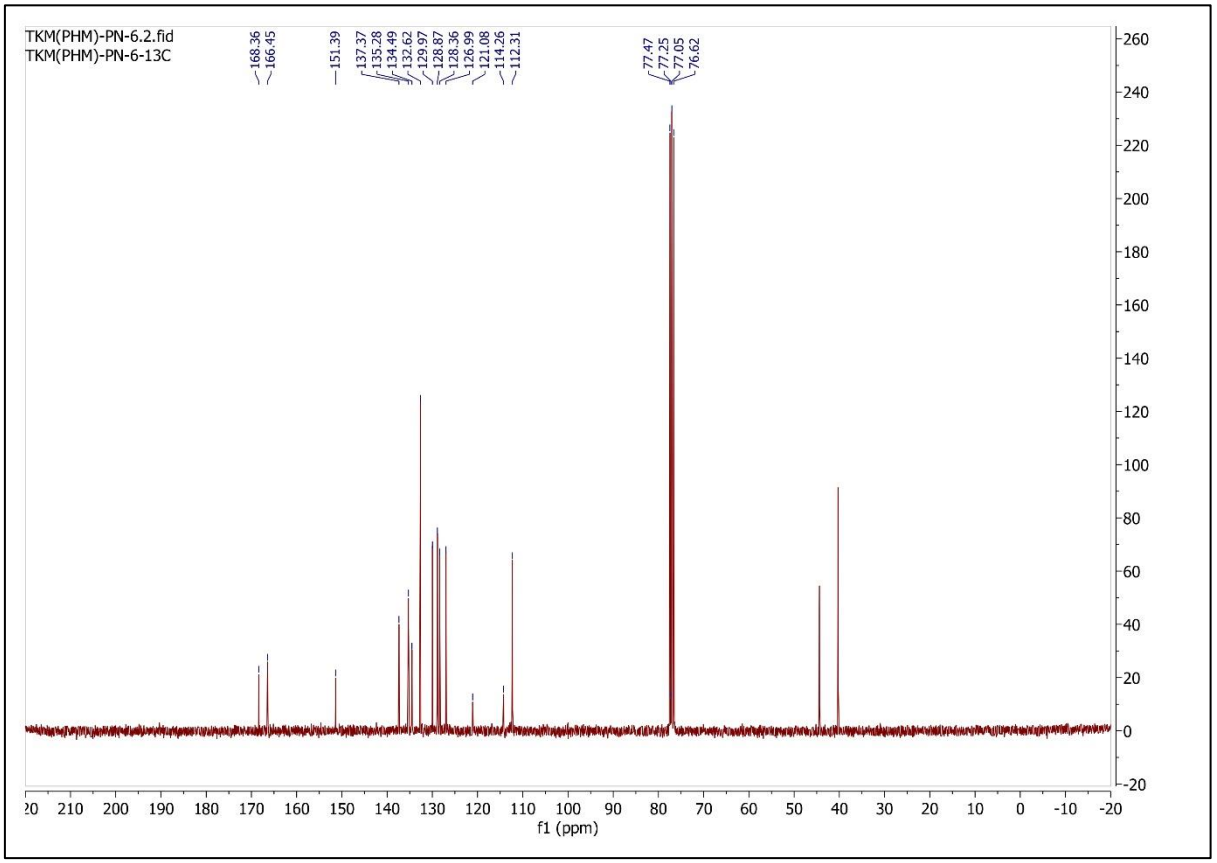
4.5.5. PN-4:



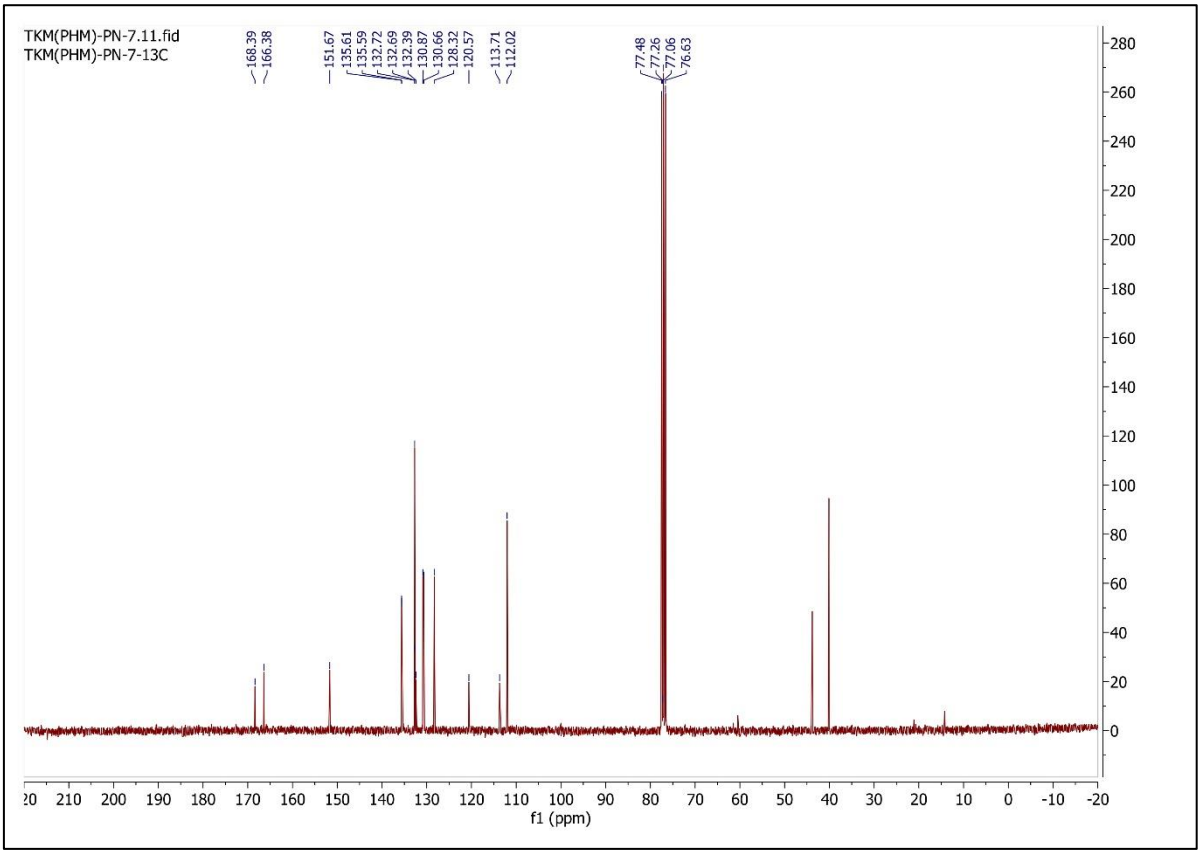
4.5.6. PN-5:



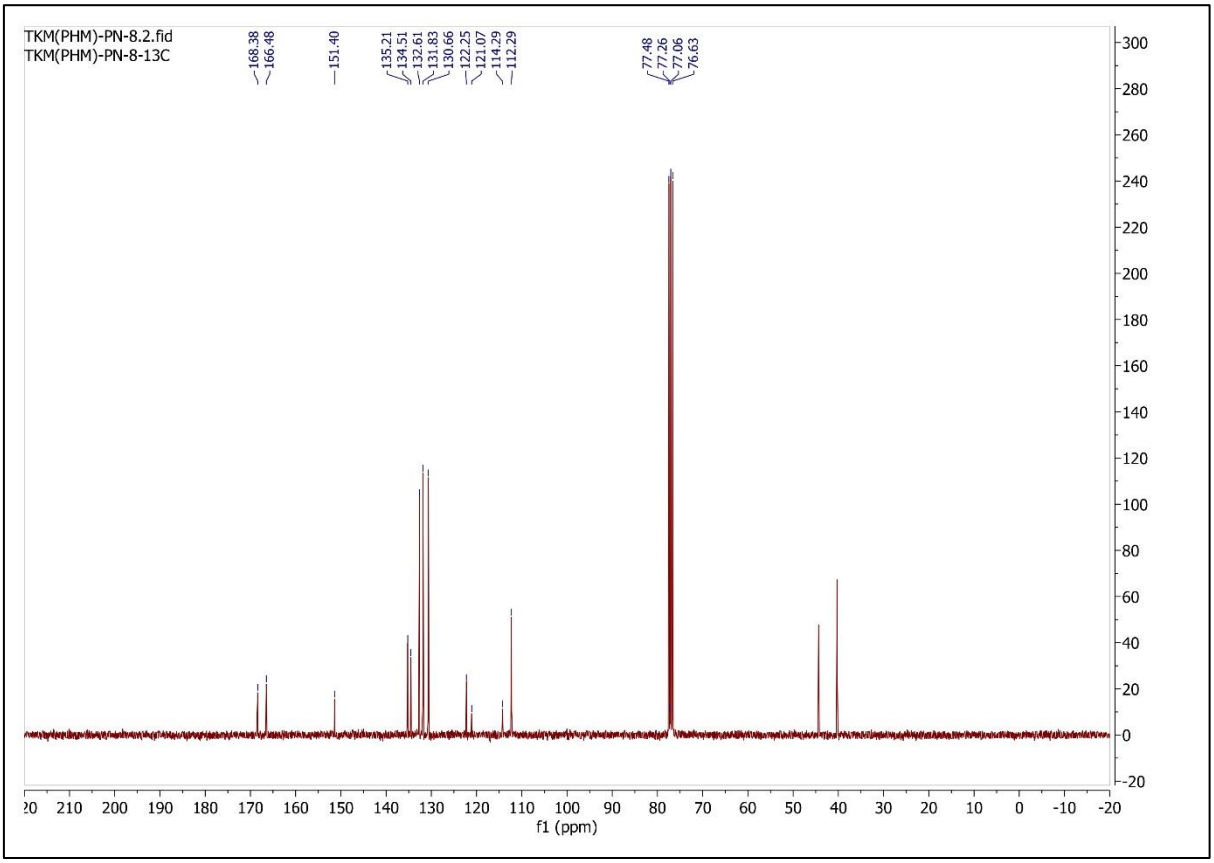
4.5.7. PN-6:



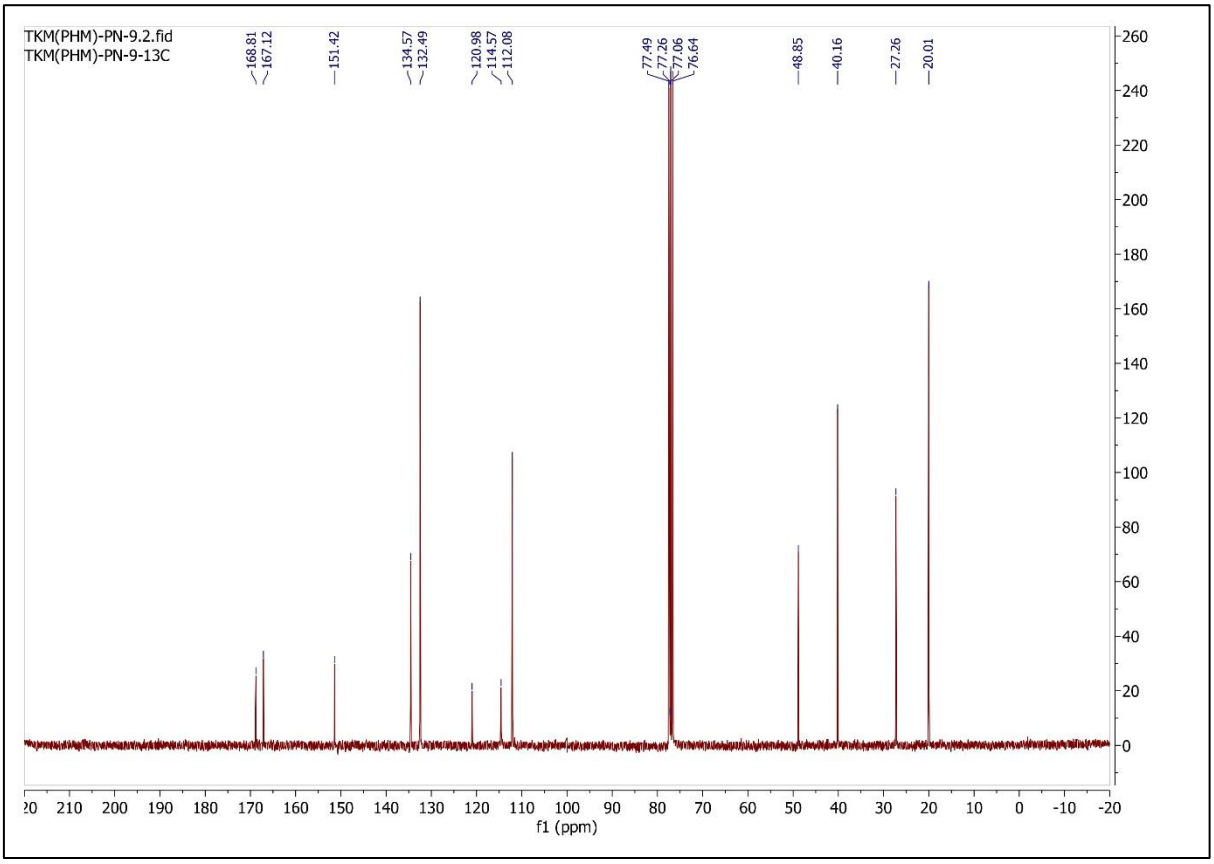
4.5.8. PN-7:



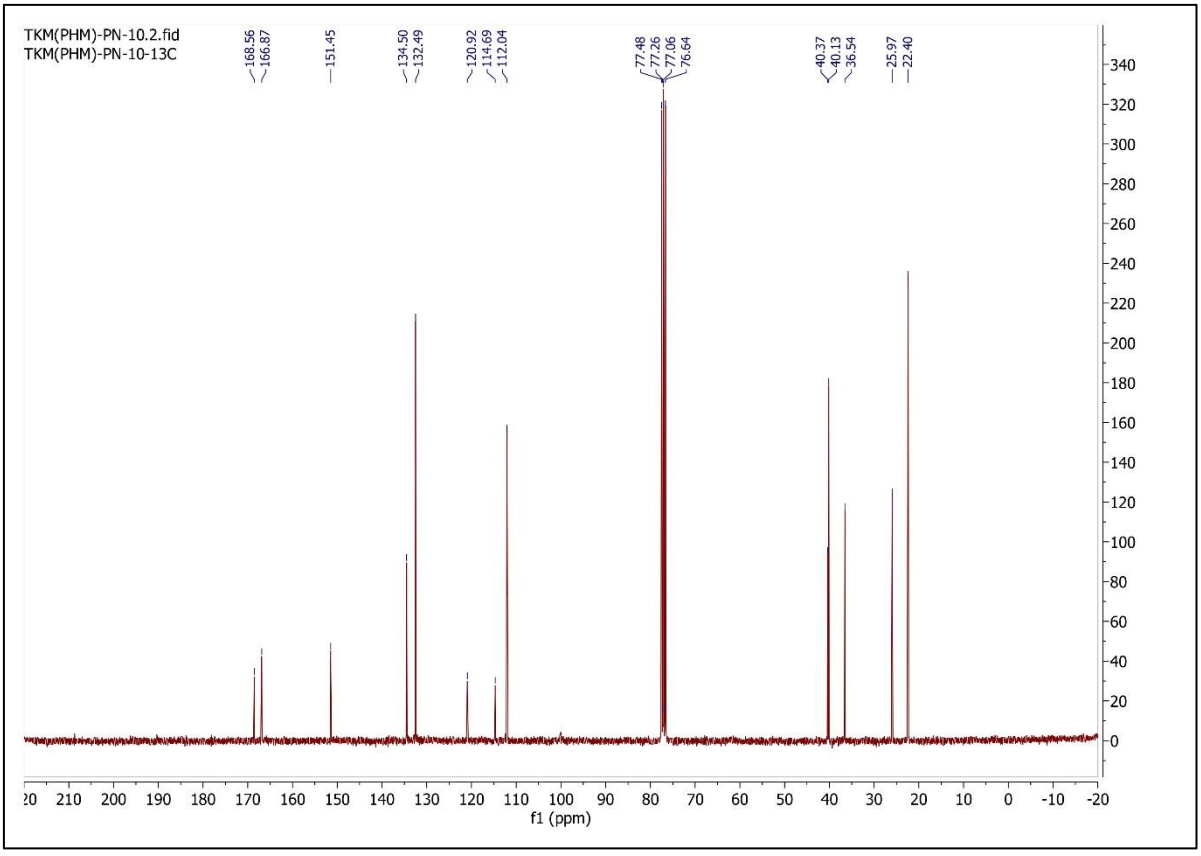
4.5.9. PN-8:



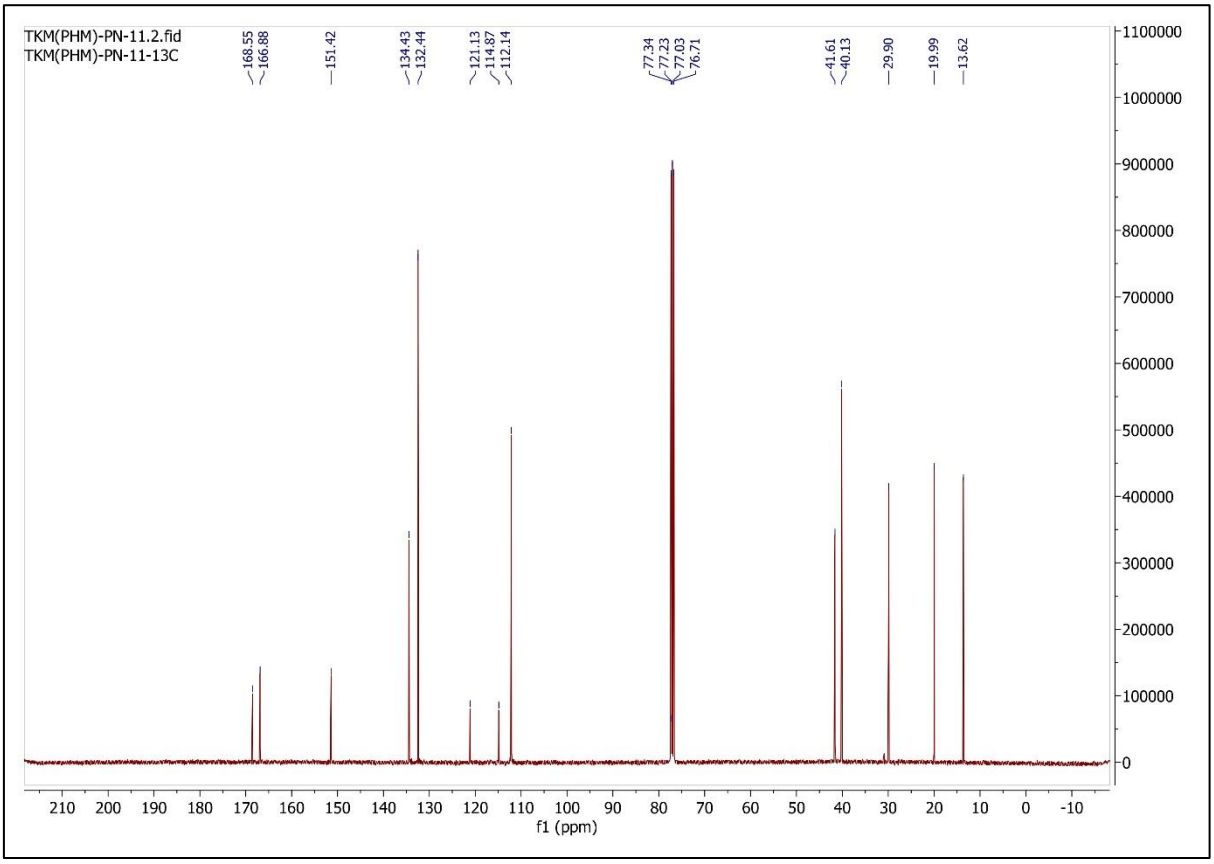
4.5.10. PN-9:



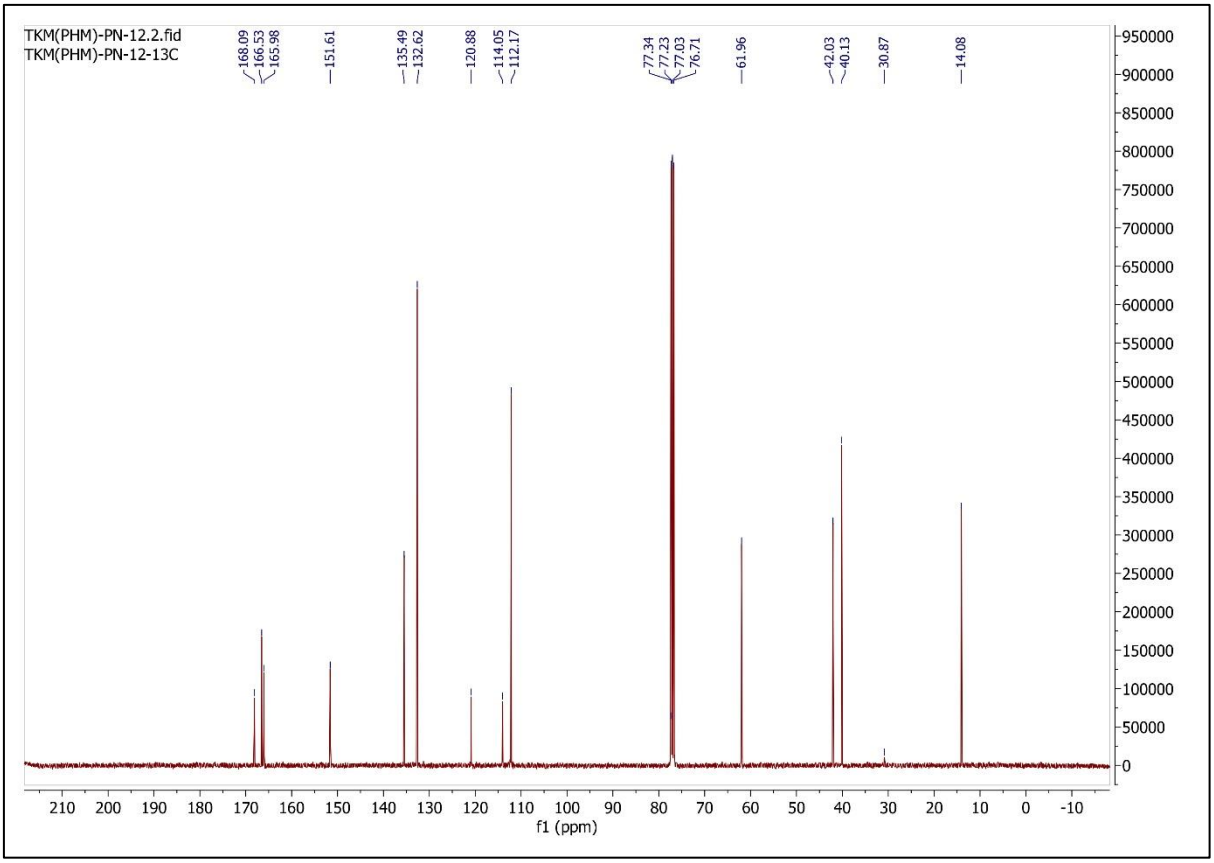
4.5.11. PN-10:



4.5.12. PN-11:

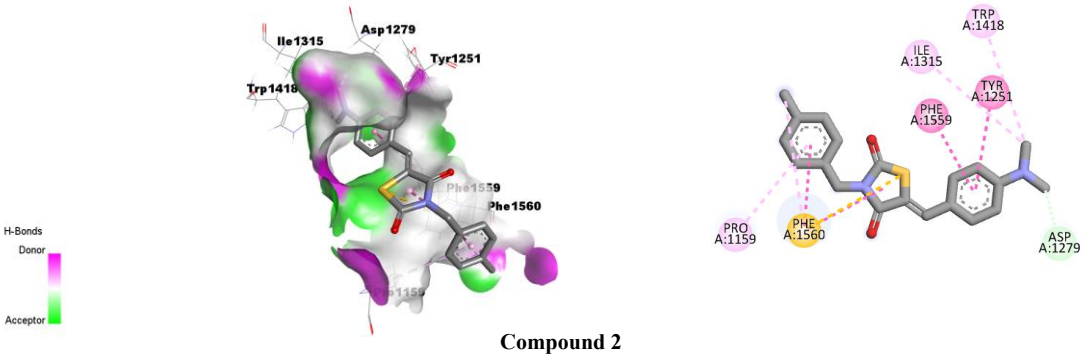


4.5.13. PN-12:

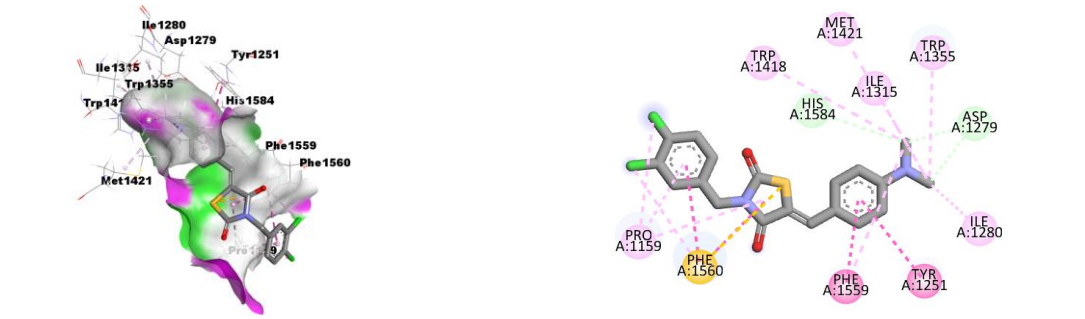


4.6. Docking studies:

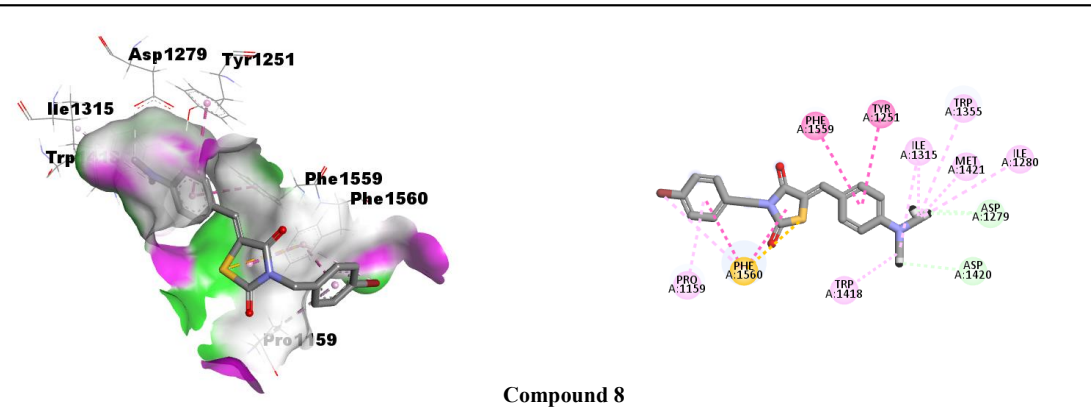
4.6.1. Alpha-glucosidase (PDB ID: 3TOP):



Compound 2
B.A: -9 kcal/mol

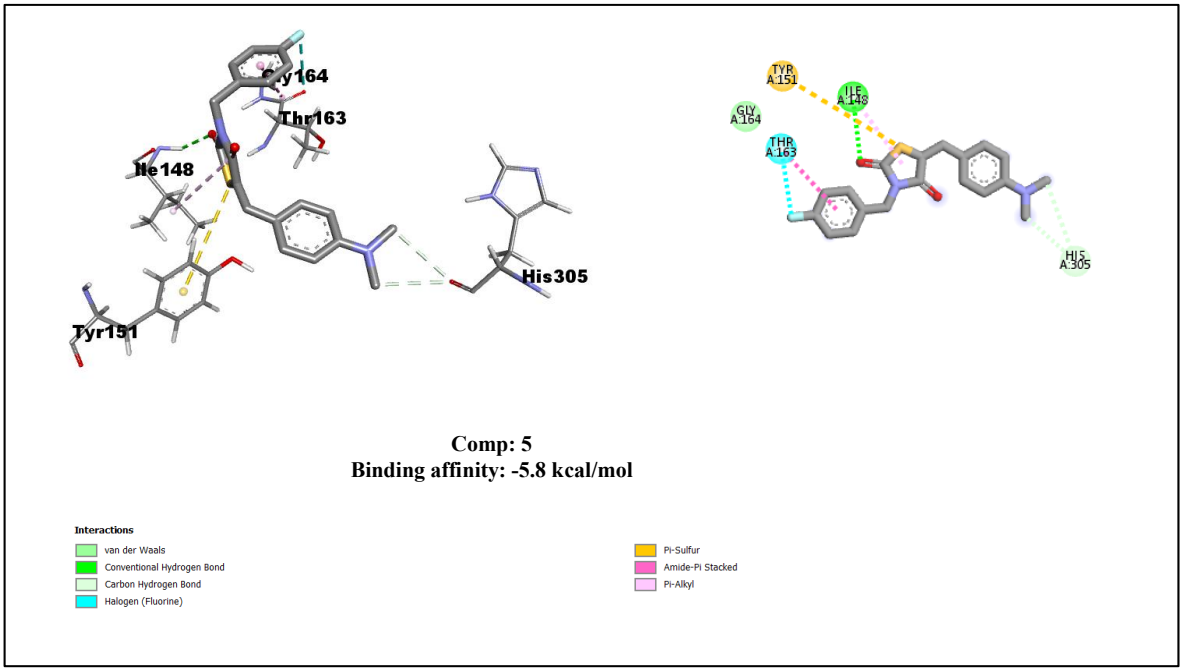


Compound 7
B.A: -8.8 kcal/mol



Compound 8
B.A: -8.8 kcal/mol

4.6.2. Alpha-amylase (PDB ID: 2QV4):



Comp: 5
Binding affinity: -5.8 kcal/mol

CHAPTER 5

CONCLUSIONS AND FUTURE PERSPECTIVES

5. Conclusions and future perspectives:

As a part of my research work, we have synthesized some newer 2,4-thiazolidinedione (TZD) derivatives and identified the compounds through chemical analysis such as TLC, melting points, and recrystallization. All the recrystalized products were characterized and analyzed by IR, ^{1}H NMR and ^{13}C NMR spectroscopy (summarized in chapter 4). Similarly, we have studied molecular interactions of synthesized compounds through molecular docking studies for two key target enzymes such as alpha-amylase and alpha-glucosidase. From the docking studies, we have found that the synthesized compounds are actively binding to the key enzyme and this compounds can give good results in *in vitro* alpha-amylase and alpha-glucosidase inhibitory activity.

During my work we came to know that synthesis with TZD is simple and versatile. The TZD scaffold can be modified by substituting different moieties at the third (-N) and fifth ($-CH_2$) positions to create unique TZD molecules. If we explore third and fifth position with different heterocyclic rings and substituting those with various other moieties viz $-OCH_3$, $-NO_2$ etc. may result into large no. of compounds increasing the chemical space TZD derivatives enormously.

Our future perspective is to study anti-diabetic activities of this synthesized compounds via *in vitro* alpha-amylase and alpha-glucosidase inhibitory assay. Similarly, *in-vivo* studies and prediction of ADME parameters, pharmacokinetic properties, druglike nature will be evaluated to identify best promising compound from this series as alpha-amylase and alpha-glucosidase inhibitor.