

**DESIGN, SYNTHESIS & EVALUATION OF  
COIXOL LIKE DERIVATIVES AS  
ANTIDIABETIC AGENTS**

Thesis Submitted For the Award of the Degree of

**DOCTOR OF PHILOSOPHY**

**In**

**Pharmaceutical Chemistry**

**By**

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## **DECLARATION**

I declare that the thesis entitled “Design, Synthesis & Evaluation of Coixol-Like Derivatives as Antidiabetic Agents” has been prepared by me under the guidance of Dr Paranjeet Kaur and Dr Navneet Khurana, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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## LIST OF ABBREVIATIONS

| S. NO. | Abbreviations     | Full form                                |
|--------|-------------------|--|
| 1      | DM                | Diabetes mellitus                        |
| 2      | NP                | Natural Products                         |
| 3      | FDA               | Food and drug Administration             |
| 4      | WHO               | World health Organization                |
| 5      | USD               | United state dollars                     |
| 6      | TAM               | Therapeutically active moieties          |
| 7      | SAR               | Structure activity relationship          |
| 8      | MBOA              | 6-methoxy benzoxazolinone                |
| 9      | DCM               | Di cholo methane                         |
| 10     | HPLC              | High performance liquid chromatography   |
| 11     | ELISA             | Enzyme-linked immunosorbent assay        |
| 12     | cAMP              | Cyclic Adenosine mono phosphate          |
| 13     | HIV               | Human immunodeficiency virus             |
| 14     | DVS               | Dehydrating value of sulfuric acid       |
| 15     | THF               | Tetra hydro furan                        |
| 16     | Cu O              | Copper oxide                             |
| 17     | K ATPase          | ATP sensitive Potassium channels         |
| 18     | MIN6              | Mouse Insulinoma 6                       |
| 19     | 3T3               | 3-day transfer, inoculum cells           |
| 20     | CADD              | Computer- aided drug designing           |
| 21     | TLC               | Thin layer chromatography                |
| 22     | FTIR              | Fourier transform infra-red Spectroscopy |
| 23     | NMR               | Nuclear magnetic resonance               |
| 24     | Rf                | Retention factor                         |
| 25     | 3D                | 3-dimensional                            |
| 26     | CDH               | Central drug house                       |
| 27     | NaH               | Sodium hydride                           |
| 28     | DMSO              | Di methyl Suphoxide                      |
| 29     | CDCl <sub>3</sub> | Deuterated chloroform                    |

|    |         |  |
|----|---------|--|
| 30 | MD      | Molecular dynamic                                  |
| 31 | MS      | Mass spectroscopy                                  |
| 32 | TBAC    | Tert-Butyl acetate                                 |
| 33 | BSA     | Bovine serum albumin                               |
| 34 | HEPES   | 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid |
| 35 | CMC     | Carboxy methyl cellulose                           |
| 36 | HFD     | High fat diet                                      |
| 37 | NPD     | Normal pellet diet                                 |
| 38 | BWT     | Total body weight                                  |
| 39 | PGL     | Plasma glucose level                               |
| 40 | PTC     | Plasma Total cholesterol                           |
| 41 | PI      | Plasma insulin                                     |
| 42 | i.p.    | Intraperitoneal                                    |
| 43 | GOD-POD | Glucose oxidase peroxidase enzyme                  |
| 44 | S.D.    | Sprague dawley                                     |
| 45 | ADME    | Adsorption, distribution, metabolism and excretion |
| 46 | ANOVA   | Analysis of variance                               |
| 47 | GBM     | Glibenclamide                                      |
| 48 | HBSS    | Hank's balanced salt solution                      |
| 49 | KRB     | Krebs ringer bicarbonate                           |
| 50 | HRP     | Horseradish peroxidase                             |
| 51 | STZ     | Streptozotocin                                     |
| 52 | SD      | Standard deviation                                 |

## ABSTRACT

Diabetes mellitus has been considered a serious metabolic disorder throughout the globe. This disease can be isolated into two groups, type-I and type-II. It has been seen that approximately, 90% of the cases are type-II associated diabetes mellitus, which is either due to resistance towards insulin or insufficiency of insulin. Worldwide the figure of diabetic patients is continuously on the rise and the patient counts are expected to reach nearly 700 million by the year 2045. Specifically in India, diabetes has been observed as the 9<sup>th</sup> leading underlying causes of mortalities as per the report published in 2019. In addition, the International Federation of Diabetes has already declared India as major diabetes-suffering country as compared to another parts of the world and raised an alert that by 2030, Indian citizens may confront the highest number of diagnosed diabetes cases.

Currently available drugs are associated with serious side effects like severe cardiac toxicity, hepatotoxicity and weight gain. Therefore the discovery of novel anti-diabetic molecules with high potency and fewer adverse effects has become a necessity for researchers. Considering the sources of novel therapeutics discovered from 1981 to 2020, half of the drugs approved during this period are based on natural products. These naturally driven products are considered drug-like molecules and continue to exist as the best sources for novel drug leads. Natural products contain great possibilities for structural modifications to achieve the desired biological effect. In recent years, medicines from natural products have already been established as more secure, less expensive, effectively accessible, cheaper and more efficacious as compared to pure synthetic drugs. Therefore, we identified a natural compound coixol which is known to be a potent insulin secretagogue or insulin secretory agent, obtained from the plant parts of *Scoparia dulcis*. It is proposed to act on insulin receptors i.e. ATP sensitive Potassium channels and lead to produce insulin secretion. Despite its small structure, it has been considered as most potent insulin secretagogue as compared to other active constituents with the large structural framework. Thus, this research project aimed to identify modified coixol natural product based derivatives that could potentially emerge as a successful clinical candidate for the treatment of type-II diabetes. As a result, a pharmacophore was developed based on

the structure of coixol and skeleton was optimized by studying the SAR via varying electron donating, electron withdrawing groups and other possible substitutions which resulted in the identification of some more potent antidiabetic agents than coixol.

The designed novel molecules were analysed and docked by the software's, Auto Dock Vina 1.5.6 and PyMOL. Based on the ligands binding scores and their compatibility with the receptor 5y7 (A pancreatic ATP-sensitive potassium channel) and standard drug Glibenclamide, We selected 12 best molecules coded as **DP102 (Coixol)**, **DP104**, **DP105**, **DP106**, **DP151**, **DP154**, **DP260**, **DP263**, **DP322**, **DP330**, **DP422** for the synthesis, Also considering the feasibility of the reactions and availability of the starting material, we proposed total four series to the synthesis of Benzoxazolinone, Benzimidazolinone, Benzimidazole-thiones, aryl ether derivatives and aryl amide derivatives. Further, their *in-silico* toxicity and *in-silico* ADME properties were predicted which confirmed the non-carcinogenic effect of these compounds. The result of *in silico* ADME shown good solubility in water and suppose to have high GI absorption with no penetration into Blood Brain Barrier. Calculated lipophilicity (*I Log P*) was observed in between 0.73 to 1.90. Later, Compounds were synthesized with good yield and characterization was done using various spectroscopic methods. Synthesized compounds were further subjected to *in vitro* studies using rat insulin ELISA assay kit by considering Glibenclamide as control on isolated S.D. rats islets. Percentage of comparative insulin release of the test samples was calculated considering Glibenclamide as standard at 100%. The analysis of assay result revealed that Compound **DP422** and **DP104** had the most potent effect on the membrane receptor ATP-sensitive potassium channel. The insulin release concentration of **DP422** and **DP104** was found to be  $305.46 \pm 12.39$  and  $207.26 \pm 10.73$  respectively as compared to coixol ( $125.62 \pm 4.50$ ) and glibenclamide ( $100.00 \pm 3.33$ ). The synthesized compounds **DP104** and **DP422** along with coixol were further tested for their anti-diabetic effect in diabetes induced model. *In vivo* study result indicated the successfully development of type-II diabetes model incorporating high fat diet and low dose administration of streptozotocin (35mg/kg). The *in vivo* evaluation of coixol and synthesized compounds **DP422** and **DP104** were found to release insulin secretagogue effect by inhibiting ATP sensitive potassium

channels and significant effect in decreasing plasma glucose level and total plasma cholesterol level in diabetic animals. Reportedly, selected compound coixol and synthesized compounds shown better results than the standard drug glibenclamide and also showed positive results in histopathological analysis. Thus, the entire study suggests that synthesized compounds **DP422**, **DP104** and natural product coixol having good potential to be used for the treatment of type-II diabetes

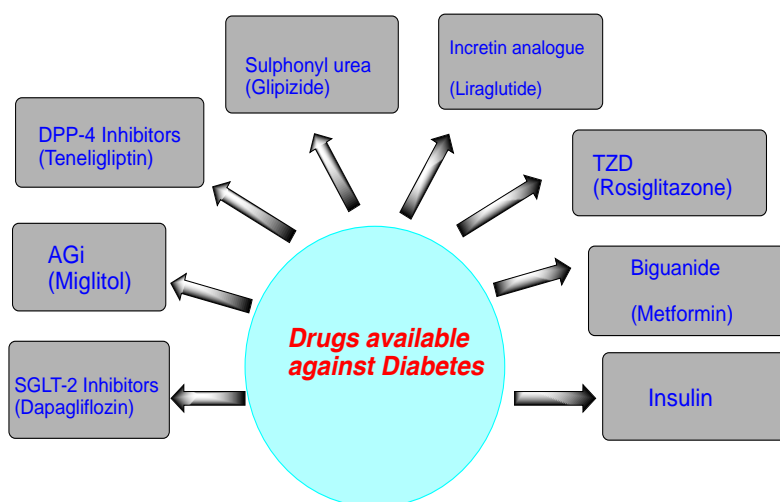


## CHAPTER 1

### INTRODUCTION

Diabetes mellitus has been considered a life-threatening metabolic disorder familiar by a deformity in insulin secretion which leads to various metabolic disorders primarily, hyperglycaemia.<sup>1,2</sup> In the year 2015, approximately 415 million persons reported with type-II diabetes and it's anticipated to rise up to 700 millions by the year 2045.<sup>3,4</sup> In accordance to that, A study published by the ADAS (American Diabetes Association states) stated that by the year 2030, Indian citizens may confront the highest number of diagnosed diabetes cases.<sup>5</sup> Similarly, In the year 2019, among the most underlying causes of mortalities, diabetes was observed as the ninth leading cause in India.<sup>6</sup> As a result, the International Federation of Diabetes declared recently that India has more cases of diabetes as compared to another country. In response to that in April 2021, World health organisation introduced a worldwide initiative sighted for continuous development in the prevention and care of diabetes specifically for providing support to undeveloped and developing nations. This campaign was named as "Global Diabetes Compact". This disease can be isolated into two extensive classes: diabetes dependent with insulin also known as type-1 diabetes and diabetes non-dependent with insulin, commonly called type-II diabetes.<sup>7</sup> It is distinguished with abnormal high level of fasting state glucose in the plasma. Diabetes mellitus causes depletion or disintegration of insulin release.<sup>8</sup> DM with type-I consequences in insulin insufficiency give rise to cells mediated autoimmune demolition of  $\beta$ -cells of pancreas which is mostly seen developing in young age.<sup>9</sup> It has been seen that approximately, 90% of the cases are type-II associated diabetes mellitus, which is either due to peripheral aversion or resistance towards insulin or insufficiency of insulin.<sup>10</sup> Nevertheless, type-II possibly could also develop among younger population, as a report of MODY (Maturity Onset Diabetes of the Young) and it is currently impacted by an excess of genetic or surrounding elements, resulting in improvement of insulin resistance and dysfunction of  $\beta$  cells.<sup>11,12,13</sup> The involvement of hereditary and environmental factors in the development of type-II diabetes cases differs from people to people. On the other aspect, Naturally obtained products with medicinal properties have contributed a major role in the management of several diseases and in the finding of leads to

support drug discovery studies. These naturally driven products are progressively considered as drug like molecules and continue to exist as the best sources for drugs leads.<sup>14</sup> NP's persist as a source of several new compounds with a great possibilities of structural modifications be in possession of compulsive biological activities for the treatment of various ailments. Medications from naturally driven products have already been established as more secure, less expensive, effectively accessible cheaper and every so often more efficacious as compare to pure synthetic drugs. Additionally, in the recent years researchers are in investigation for safer and most potent molecules from natural resources especially from medicinal plants and herbs. Diabetes has been considered as one of the persistence disorders that is related to high mortality risk as well.<sup>15</sup>



**Figure 1:** Available drugs for type-II Diabetes <sup>16</sup>

### 1.1 Diabetes-mellitus and it's supervision:

As the whole globe is moving towards infestations of diabetes-mellitus, Especially the cases of insulin-independent or non-insulin dependent diabetes mellitus (NIDDM) are growing at a disturbing level.<sup>17,18,19</sup> The WHO have accessed that approximately 346 millions of patients around the world have been diagnosed with diabetes and this count will be continue to rise at a multiplying rate by 2030 which has been appeared as a biggest challenge to the health-care systems as of now<sup>20,21</sup> Meanwhile, The existing antidiabetic drugs utilization is linked to several consequential adverse

effects, and henceforth it's high time to find novel potentially active therapeutic drugs with high efficacy and less adverse effects.

**Table 1:** Estimate burden of diabetes in India by 2045. <sup>23</sup>

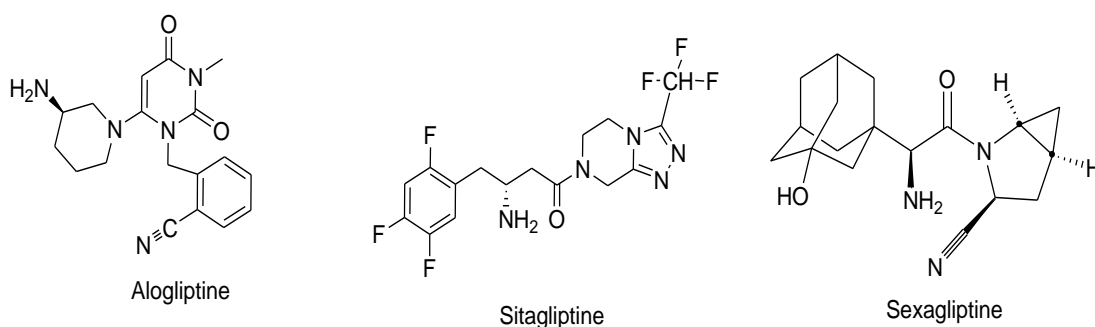
|  | Year |       |
|--|------|-------|
|  | 2019 | 2045  |
| Impaired glucose tolerance [20-80 years]<br>Number of people | 25.2 | 35.2  |
| Rank   | 4    | 3     |
| <b>Diabetes estimates [20-80]</b>                            |      |       |
| Prevalence (%)   | 8.9  | -     |
| Age adjusted prevalence                                      | 10.4 | -     |
| Number of people affected (Million)                          | 77   | 134.2 |
| Rank   | 2    | 2     |
| <b>Diabetes estimates [&gt;65]</b>                           |      |       |
| Number of people affected (Million)                          | 12.1 | 27.5  |
| Rank   | 3    | 2     |
| <b>Undiagnosed Diabetes estimates</b>                        |      |       |
| Prevalence   | 57   | -     |
| <b>Number of people affected (Million)</b>                   |      |       |
| Rank   | 43.9 | -     |
| <b>Total health care expenditure on diabetes</b>             |      |       |
| Mean expenditure per person with diabetes (USD)              | 92   | -     |
| Total deaths related to diabetes (Million)                   | 1.0  | -     |

**1.2 Antidiabetics with promising drugs targets:** In recent years, several medications have been developed for the management of type-II diabetes which is established as better therapeutics as compared to previous categories. Gastric

inhibitory polypeptide and glucagon-like peptide-1 are noticeable incretin hormones which show characterized secretion of insulin. Dipeptidyl peptidase-4 inhibitors are used to prevent the degradation of incretins. Therefore, Dipeptidyl peptidase-4 inhibitors are the widely used incretin-based therapy for the treatment of type -II diabetes.<sup>24</sup>

**1.2.1 Glucose dependent insulin release Peptides (GIP):** GIP's releases in a unique biologically active form through the K cells of the jejunum and duodenum with response to the administration of sugars or lipids. These drugs reported stimulating glucose-dependent insulin discharge in people. Likewise, GIP's consist of a crucial role in the metabolism of fat inside adipocytes and possess proliferatively action on the pancreatic  $\beta$  cells.<sup>25</sup>

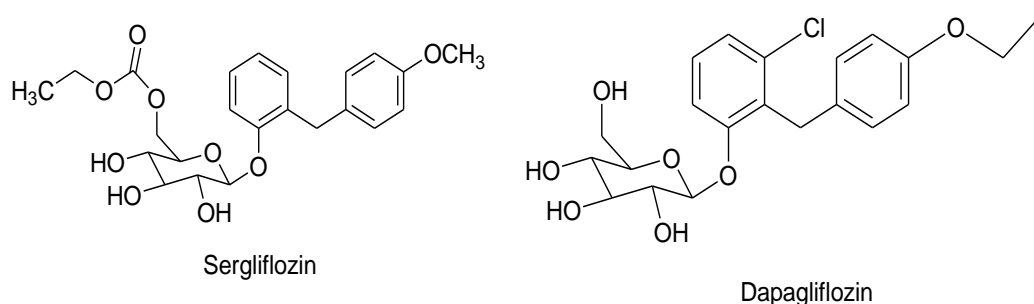
**1.2.2 Di-peptidyl peptidase-4 antagonists: DPP-4's** are capable into the complete destruction of glucagon like peptides (GLP-1) and gastric inhibitory polypeptides (GIP) which are the prominent incretin hormones. DPP-4's are majorly found in human tissues like kidneys, brain, pancreas, lungs, adrenals, lymphocytes and intestines. Insulin release can be upgraded by implication with DPP-4 inhibitor's which limit the destruction of incretin hormones that resulted to elevated concentration of the naturally present GIP and GLP-1 and in the blood circulation. Examples of these drugs are **Saxagliptin, Sitagliptin** and **Alogliptin** (Figure 2).<sup>26</sup>



**Figure 2:** Structures of Dipeptidyl Peptidase 4 inhibitors

**1.2.3 Sodium-subordinate glucose co-transporters (SGLT-2):** Sodium subordinate co-transporters of glucose are a group of transporters present into the mucus membrane of small intestine and some parts of proximal tubule of the nephron (SGLT-2 and SGLT-1). By inhibiting SGLT-2 in the proximal convoluted tubule improve the glucose discharge from the urine, yet essentially discussed medications

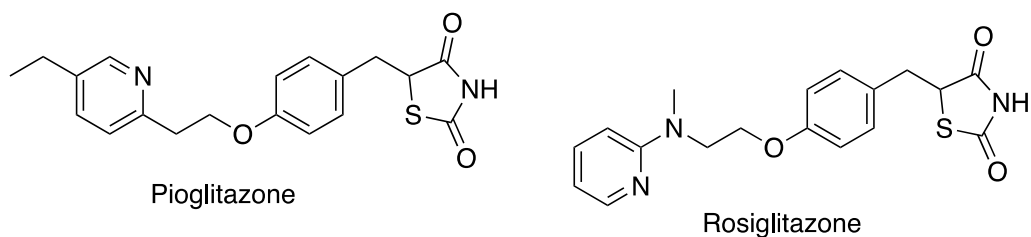
just seem to impact on hyperglycaemia by decreasing the glucose level in the plasma that could also indicate towards a novel therapeutic focus for the treatment hyperglycaemia. Hereby, SGLT drugs prove to be a good target for decreasing extreme hyperglycaemia and prevent the harmful effects of glucose related toxicity in the diabetic patients. Dapagliflozin and Sertgliflozin (Figure 3) and some of the Thio-glycosides are development from this class<sup>27</sup>



**Figure 3:** Structures of SGLT-2 inhibitors

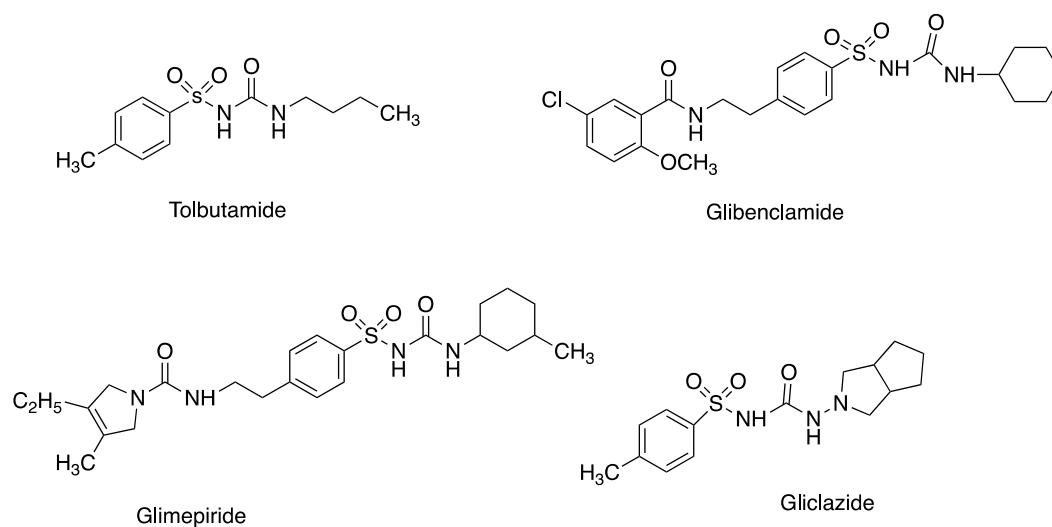
**1.2.4:Glucokinase agonists:** Glucokinase represents a category of glucose controlling enzyme present primarily in the pancreas and liver and known for the phosphorylation of d-glucose. Imperfection in the level of the enzyme result into several diseases, For example, maturity onset diabetes type-II specially in younger generation. Therefore, this category is also a perfect focus for the treatment of diabetes.<sup>28,29</sup>

**1.2.5:Peroxisome proliferator activated receptor:** PPAR $\gamma$  belongs to a group of 3 nuclear receptors that influence the interpretation and articulation level of various target qualities in adipocytes and other tissues or cells. PPARs are actuated by an extensive variety of normally processed FFA, eicosanoid-FFA subordinates and TZDs (Thiazolidinediones). By bringing down circulating lipids, PPARs restrict lipid-actuated insulin resistance. PPAR $\gamma$  is a widely examined individual from PPAR family since its agonist has been utilized clinically and financially for diabetes treatment for around 10 years. The main insulin sensitizer and PPAR $\gamma$  agonist utilized was troglitazone (Rezulin), which was taken off from the market in 2000 due to liver lethality. Recently, Rosiglitazone and Pioglitazone (Figure 4) are utilized for this reason and thought to be the most powerful and specific PPAR $\gamma$  agonists.<sup>30</sup>



**Figure 4:** Structures of PPAR agonist

**1.2.6: Sulphonyl urea's/insulin secretagogues:** Secretagogues are the drugs that stimulate the beta cells to secrete insulin. The anti-diabetic class of sulphonyl urea is commonly known as insulin secretagogues (Figure 5). Sulphonylureas are mainly effective in increasing plasma insulin concentrations. As a result, these drugs are efficacious only when the  $\beta$ -cells remain available in the pancreas. On the other side, Plasma insulin levels rise mainly for two different reasons. **a:** Due to the stimulation of insulin release by the  $\beta$ -cells of pancreas and **b.** Due to the lower the hepatic metabolism of insulin. Sulphonyl urea drugs act primarily via interacting to its specific membrane receptor present on pancreatic  $\beta$  cells. These drugs block the influx of potassium ions ( $K^+$ ) on the ATP-dependent potassium channels due to that the influx of  $K^+$  ions inside the  $\beta$ -cell stops which resulted to membrane depolarization. Depolarized membrane stops the penetration of  $Ca^{2+}$  ions inside cytosol which result to increase in the concentration of  $Ca^{2+}$  ions inside  $\beta$ -cells which further stimulates the contraction of actomyosin filaments of that are responsible to the release of insulin hormone.<sup>31</sup>



**Figure 5:** Structures of sulphonyl urea drug

**1.3 Recent targets for the management of diabetes:** The targets currently employed are based on selective inhibition or actuation of the onco-genes through lipids by the process of gene expression which is modulated by particular supplements through molecular level knowledge of the possibilities of specific kind of interactions that could lead the discovery of novel therapeutic agents for the management for diabetes. For example, targeting adipocytes or intestinal endocrine cells. Recent investigating targets are mentioned below:

**1.3.1 PTEN:** It represents a specific protein phosphatase which is engaged with transduction of signals and the suppression of tumours. PTEN act on phosphoinositide 3-phosphatase and is thus fit for smothering PI3K motioning by dephosphorylating. Since several metabolic actions of insulin is proceed via the stimulation of PI3-K which subsequently increase intracellular PIP-3 concentration.<sup>32</sup>

**1.3.2 Adipocytes:** Adipocytes considerably affect the homeostasis of glucose which is mediated through endocrine glands. The mechanism of adipocytes is primarily begins by the biosynthesis and release of adipokines which is a peptide hormones. In addition, it plays specific role in the balance of energy, leptins reverses hyperglycaemia via enhancing the response of insulin in liver and muscles.<sup>33</sup>

**1.3.3 Adipokines:** These are specific type of cytokines primarily proteins, peptides or polypeptides, which work as an important molecules that used widely in cells and discharged through fat tissues. For example: adiponectin, resistin and leptin are considered as primary focus for the management of diabetes.<sup>32</sup>

**1.3.4 Leptin:** It is a protein based hormone that have imperative impacts in maintaining body metabolism and weight. It is expressed mainly through adipocytes and the absence of practical leptin or its receptor result into excessive fat in people affected with diabetes. In addition, It essential in regulating food intake and energy utilization in the body. Moreover, it's a major Hormone that affect neuro-endocrine and metabolic functions.<sup>34</sup>

**1.3.5 Adiponectin:** These are hormones based on adipocyte, which are identified through four different categories. It suppresses hepatic glucose production and also

increases the fatty acid oxidation mainly in the liver and skeletal muscle. Accordingly, it also keeps the aggregation of lipids in insulin target tissues. A study based on mice model indicates that adiponectin injections induce weight loss and reduce obesity by decreasing serum adiponectin levels.<sup>35</sup>

**1.4 Drug designing:** designing of novel molecules is the essential approach for discovering new drugs in view of their respective targets. So, naturally a drug target can be expressed as an important molecule or basic nucleus of the drugs that is responsible for metabolism pathways which is also selective to an illness situation, Pathology or the infectivity or survival rate of a pathogenic microorganism responsible for that illness. A few methodologies have been introduced to control the functioning of these pathways in the diseased condition by featuring or focusing on the potent molecular nucleus or pharmacophore functioning. Nevertheless, these drugs would also have to be framed in such a manner that it should not affect the important side of the molecule that alongside serve as a prime role in framing the structure of particular drug molecule.<sup>36</sup>

**1.5 Discovery of novel therapeutic agents from natural origin:** discovery of novel the rapeutic compounds is a composite, tedious and exorbitant process. The amount of time required for this process (since designing of novel therapeutics to clinical studies and FDA approval) takes around twelve years. The entire process cost nearly 1 billion US\$ in the present context. Principally, the discovery of novel therapeutics requires signalling out of novel therapeutically active moieties (TAM<sub>s</sub>). These TAM<sub>s</sub> or active templates could be screened either via extraction and isolation from natural products or through organic synthesis. Considering the sources of novel therapeutics discovered since 1981 to 2020, revealed that almost half of the molecules approved during this period are based on natural products. In addition, throughout almost 39 year (1981- 2019) total 48 natural products based active templates have been approved.<sup>37</sup> There are different cases of advancement of new drugs through the plant sources. For example, Morphine was the first natural products based therapeutics segregated from opium poppy plant (*Papaver somniferous*). More than 100 natural product based derived drugs are already in the process of Clinical trials and approximately 150 small molecules are already in the phase of pre-clinical



development. Almost identified active templates currently in the development phase are either plant derived products or obtained through micro-organisms.<sup>38</sup>

### **1.6 Drug likeness of extracted plant based active templates:**

Difficulties observed in the search for new chemical entity is the most past experienced issue.<sup>39</sup> Naturally driven chemical entities usually contain the following unique features in their structures:

- Increased steric complexity
- Lower number of aromatic ring.
- Higher figure of oxygen atoms.
- large number of chiral centres
- More number of solvated H-bond donors or acceptors.
- Dealing out molecular level issues such as ring systems diversity, octanol-water partition co-efficient and high molecular mass.

These unique features present in naturally driven chemical entities represent a series of difficulties for researchers specifically while gain of development of new analogues or derivatives. Enhancement in the drug absorption, minimization of toxicity or to the improve effectiveness researches face issues due to their unique structural feature. However this issue could be resolved by addition/deletion of specific functional group's or bioisosteres.<sup>40</sup>

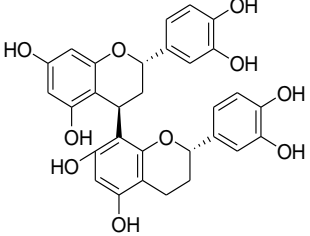
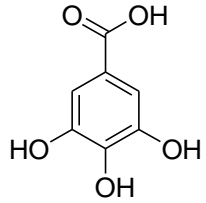
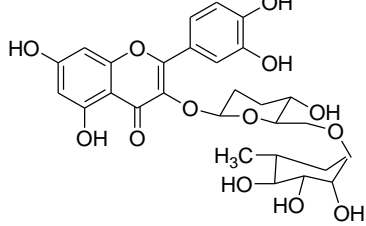
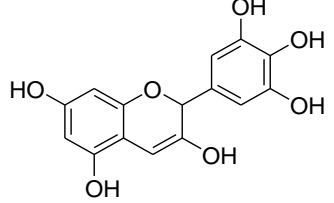
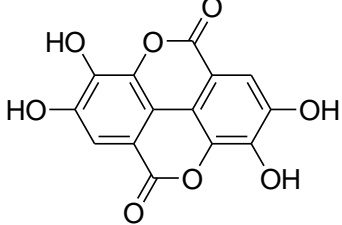
### **1.7: Anti-diabetics of plant origin:**

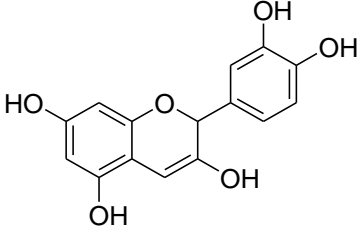
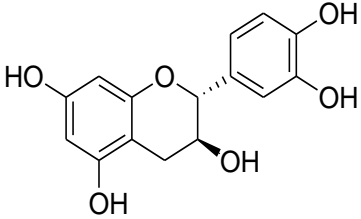
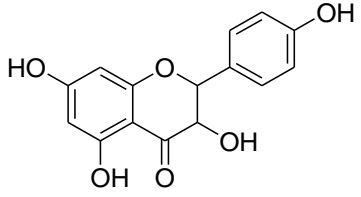
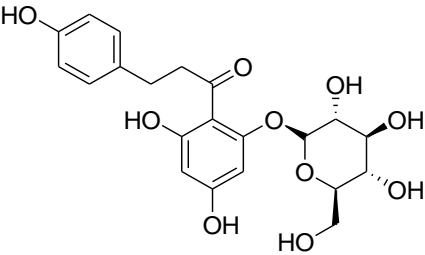
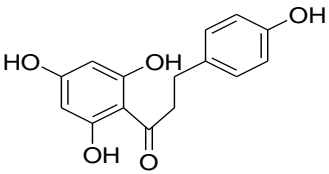
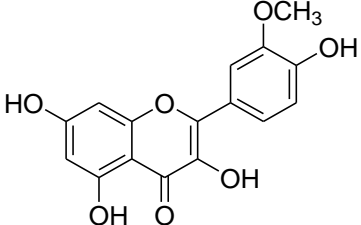
Throughout the history of anti-diabetic drugs, various treatment options and medications are in use to treat diabetes, this include insulin as well even before the understanding of its mode of action. Most of the compounds have been identified from natural sources such as most of phenolic compounds, galegine and pycnogenol derived from plant sources whereas miglitol, acarbose and voglibose are derived through microorganisms.

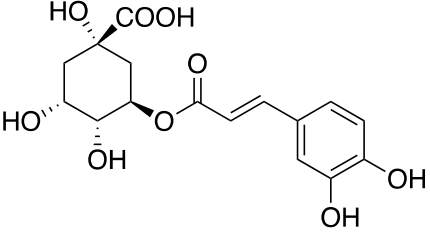
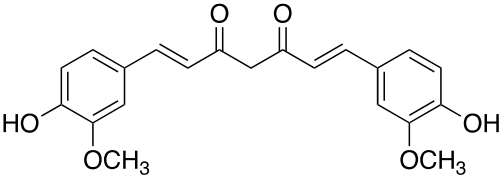
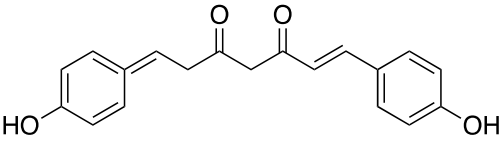
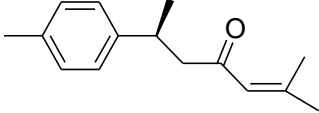
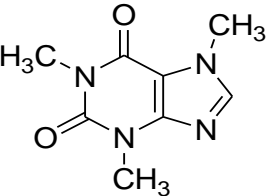
Variety of phenolic compounds such as alkaloidal flavonoids and anthocyanins shows therapeutic effects for the treatment of diabetes.<sup>41</sup> For example: divergent

anthocyanins obtained from *Poir* (*Convolvulaceae*), *Ipomoea batatas* (*L.*) Choisy (*Convolvulaceae*) and *Pharbitis nil* (*L.*) observed as potential antagonists of intestinal  $\alpha$ -glucosidase effect.

**Table 2** : list of active chemical moieties identified from natural sources reportedly showing Antidiabetic effect in the period of 2005-2019.<sup>42</sup>

| S. No. | Active compounds      | Chemical Structure   | Sources  |
|--------|-----------------------|--|--|
| 1.     | Procyanidin B2        |    | <ul style="list-style-type: none"> <li>• Grapes</li> </ul>   |
| 2.     | Gallic acid           |    | <ul style="list-style-type: none"> <li>• blackberry, strawberry, plums</li> </ul>                      |
| 3.     | Rutin                 |  | <ul style="list-style-type: none"> <li>• Sweet granadilla</li> </ul>                                   |
| 4.     | Delphinidin-Glucoside |   | <ul style="list-style-type: none"> <li>• Maqui-Berry</li> </ul>  |
| 5.     | Ellagic Acid          |  | <ul style="list-style-type: none"> <li>• Brazil plum</li> <li>• Camucamu</li> <li>• Cagaita</li> </ul> |

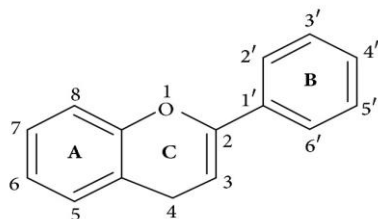
|     |              |  |  |
|-----|--------------|--|--|
| 6.  | Cyanidin     |     | <ul style="list-style-type: none"> <li>• Camucamu</li> </ul>                       |
| 7.  | Catechin     |    | <ul style="list-style-type: none"> <li>• Star fruit</li> <li>• Graviola</li> </ul> |
| 8.  | Kaempferol   |   | <ul style="list-style-type: none"> <li>• Araza</li> <li>• Açaçá</li> </ul>         |
| 9.  | Phlorizin    |  | <ul style="list-style-type: none"> <li>• Apple</li> </ul>                          |
| 10. | Phloretin    |   | <ul style="list-style-type: none"> <li>• Apple</li> </ul>                          |
| 11. | Isorhamnetin |  | <ul style="list-style-type: none"> <li>• Apple</li> </ul>                          |

|     |                        |   |   |
|-----|------------------------|---|---|
| 12. | Chlorogenic acid       |   | <ul style="list-style-type: none"> <li>• Apple</li> </ul>     |
| 13. | Curcumin               |   | <ul style="list-style-type: none"> <li>• Turmeric</li> </ul>  |
| 14. | Bis-demethoxy curcumin |  | <ul style="list-style-type: none"> <li>• Turmeric</li> </ul>  |
| 15. | ar-turmerone           |  | <ul style="list-style-type: none"> <li>• Turmeric</li> </ul>  |
| 16. | Caffeine               |  | <ul style="list-style-type: none"> <li>• Green tea</li> </ul> |

### 1.8 Flavonoids-The most potent poly-phenols as antidiabetic agents:

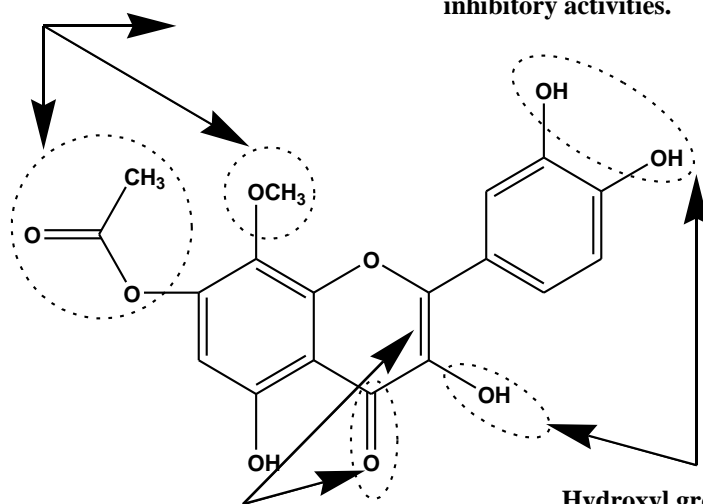
Flavonoids come from a category of phenolic compounds having hydroxyl groups. In recent years, flavonoids have achieved an enormous interest of researchers as a possible therapeutic agents against variety of medical illnesses, specifically in case of diabetes mellitus.<sup>43</sup> These are the derivatives of benzo-pyrones and mainly consist of oxygen containing functional groups as mentioned in Figure 6. The therapeutic effects of flavonoids in biologicals are generally attributed to its ability as hydrogen exchanger or producing free radicals,<sup>44</sup> To activate antioxidant enzymes present in the

plants,<sup>45</sup> chelating metal catalyst,<sup>46</sup> to reduce  $\alpha$ -tocopherol radicals<sup>47</sup> and to inhibit oxidase enzymes.<sup>48</sup>



**Methylated acetate groups decreased antidiabetic effects of Flavonoids**

**A pair of hydroxyl group at position C-3' and C-4' and C-5' (catechol) enhances the DPPH, FRAP and alpha glucosidase and DPP-4 inhibitory activities.**



**The absence of C-2,C-3 double bond and ketonic group at C-4 reduced the xanthine oxidase, alpha glucosidase and DPP-4 inhibitory activities**

**Hydroxyl group: the total number and the configuration of hydroxyl groups play an important role in regulating bioactivity of flavanoids**

**Figure 6:** Representation of flavonoids backbone and importance of functional groups to determine biological effects

Oxidization of nucleic acids, cell proteins, lipids and reactive oxygen-species (ROS's) is suitable. Multiple studies and clinical evaluations have investigated that the increments in reactive oxygen-species present in a molecular frame specifically in the case of diabetes is beneficial with the possible mechanism of oxidative degradation, oxidation and non-enzymatic glycation of protein.<sup>49,50</sup> Increment of reactive oxygen species like endothelial cell bound mitochondrial super oxides<sup>51</sup>, Endoplasmic reticulum, Stress induced lower antioxidant defence mechanism

produces lipid peroxides and impairment of enzymes, which further increase the chances of insulin secretion and ultimately lead to hyperglycaemia.<sup>52</sup>

From the timeline of mid 80s, researchers are continuously studying the therapeutic effects of flavonoids mainly for the treatment of type-II linked diabetes as compared to type-I.<sup>53</sup> Moreover alkaloidal flavonoids witnessing potent antioxidant effect are in lime light as a therapeutic lead for managing diabetes. Antioxidants usually limit the dangerous effect of hyperglycaemia in the body and help to enhance the metabolism of glucose. So, the consideration of antioxidants as a lead molecule is an alternative option for the management of serious complications associated with diabetes. In addition, due to the antioxidant effect of alkaloidal flavonoids these could be considered as biological targets for the designing of novel entity linked with the treatment of type-II diabetes-mellitus.

Observations so far suggests that there is still a need for more learnings required for the management of diabetes through the vigorous search for more better therapeutic agents with less adverse effects. As of now, the difficulties faced by researchers in the designing of potent antidiabetics is the profound aspect of mortalities and morbidities in this disease. Folk remedies used today have not undergone extensive experimental evaluations and scientific approval. Many of them could possibly produce dangerous toxicity and strong drug- drug interaction due to less experimental exposure. Therefore, there is a need for the development of novel potent antidiabetic agent from natural resources with least adverse effects for the management of serious diabetic complications. An orderly research and development required in the field of drug-discovery to represent the pharmacological activities of natural products for the management of diabetes.

**Hypothesis of research:** Diabetes mellitus has been considered as a serious metabolic disorder observed as an imperfection in insulin management in the body. The figure of diabetic patients are expected to reach nearly 642 millions in the next 25 years. Meanwhile, Natural chemical constituents have already established as important compounds in the management and treatment of several illnesses and in the discovery of modern medicines. Natural chemical entities have continue to exist as a source for

discovery of novel molecules with variety of possible structural modifications with biological activities to the treatment of several diseases. Medicines obtained from natural products are proven to be more safer, least costly, economically cheaper and most of the times more potent than the medications obtained from entirely synthetic routes. In the present years, researchers are in the search for safe and more potent drug molecules derived through natural resources, Specifically through medicinal plants or herbs. Traditional natural remedies used today have not undergone sufficient scientific trials so most of them could possibly produce serious effects and major drug- drug interactions. Therefore, it's high time for the search of novel therapeutics based from natural resources for the management of this serious illness like diabetes.

## CHAPTER 2

### REVIEW OF LITERATURE

#### Natural products and herbs as anti-diabetic agents:

**Grover, Yadav, et al. (2002)**, reviewed 45 different plants and their products (crude extracts and active & natural principles) that have been described in the traditional system of Indian medicine and have reported potent clinical antidiabetic effect.<sup>54</sup>

**Maiti, Jana, et al. (2004)**, studied anti-diabetic impact of *Tamarindus indica* (*Tamarind*). The aqueous extract of its seeds was used to treat streptozotocin diabetes induced rats in the time dependent manner. The treatment with aqueous seed extract of *Tamarindus indica* was continued for 7 days to the diabetes induced animals and it came out the huge lessening in fasting blood glucose level in the diabetic rats.<sup>55</sup>

**Hays, Galassetti, et al. (2008)**, reviewed the properties and pharmacological involvement of NP's for the management and cure of type-II diabetes mellitus. Similarly, **Nahas and Moher** studied a few important natural resources with therapeutic properties and possible potential as anti-diabetics, considering their clinical trials.<sup>56</sup>

**Sushma, Venkata, et al. (2013)**, evaluated the anti-diabetic, anti-hyperlipidaemic and antioxidant actions of *Buchanania lanzan* in streptozotocin prompted type-I and type-II diabetes. STZ was administered to rats to induce diabetes. Oral dosing of extract for the amount of 21 days brought about relative decrease Plasma glucose in fasting rats as compared to diabetic ones. The effect was claimed to produce due to the presence of potent flavonoids present in the plant extract. Result indicated the slight decrease in the level of blood glucose, total cholesterol, triglycerides in streptozotocin induced diabetic rats.<sup>57</sup>

**Liang, Guo, et al. (2013)**, Estimated anti-hyperglycaemic and anti-hyperlipidaemic properties of *Hericium erinaceus* in its extract. *In vivo* studies on diabetic rats estimated the relevant decrease in plasma glucose level and a noteworthy ascent in plasma insulin as well. Moreover, aqueous extract of *Hericium erinaceus* dosing



increased the level of enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase and protein antioxidant i.e. Glutathione diminished the level of lipid peroxidation product malondialdehyde in the liver tissue altogether.<sup>58</sup>

**Pàmies (2013)**, Identified natural products as promising anti-diabetic molecules with following objectives:<sup>59</sup>

1. 3D QSAR model development for PPAR $\gamma$  agonists.
2. Structure based designing of novel PPAR $\gamma$  partial agonists identified with high affinity score and less trans activation action.
3. Computational screening of natural products as promising PPAR $\gamma$  partial agonists.
4. Evaluation of natural extracts for potential PPAR $\gamma$  agonistic effect.
5. Evaluation of DPP-4 inhibitory effect of extracted natural products as potential anti-diabetic agents.

**Mishra, Pradhan, et al. (2013)**, evaluated antioxidant and hypoglycemic effect of *scoparia dulcis* in its methanolic extract. The isolated extract was tested on *in-vitro* and *in-vivo* both models. Study indicated that antioxidant effect was prominent in both the models that could be related to anti diabetic effect as well, as the intestinal enzymes amylase and glucosidase are responsible to increase glucose in the body. Therefore an *in-vitro* model was tested on these two enzymes which gave positive results in the favor of *S. dulcis* methanolic extract. For *in-vivo* studies, STZ-induced high fat diet model was chosen to check the hypoglycemic effect of methanolic extract of *S. dulcis*. Diabetes induced rats groups showed significant decrease in plasma glucose level as compared to negative control and the hypoglycemic effect of methanolic extract was observed as similar as standard drug Glibenclamide. Study proved the traditional value of *scoparia dulcis* plant for the treatment of diabetes mellitus.<sup>60</sup>

**Pan, Litscher, et al. (2014)**, Collectively studied on four different anti diabetes plants utilized as folk arab medicines that could also maintain a balance physiological blood glucose level termed as 'Glucolevel'. Clinical studies have also proved that these plant components start showing their effect during the period of 2 to 4 weeks of

therapy with no significant side effect. Results of the study shows safety, efficacy and tolerability of the herbal combinations of four plant components that shows synergistic effect to regulate glucose-homeostasis.<sup>61</sup>

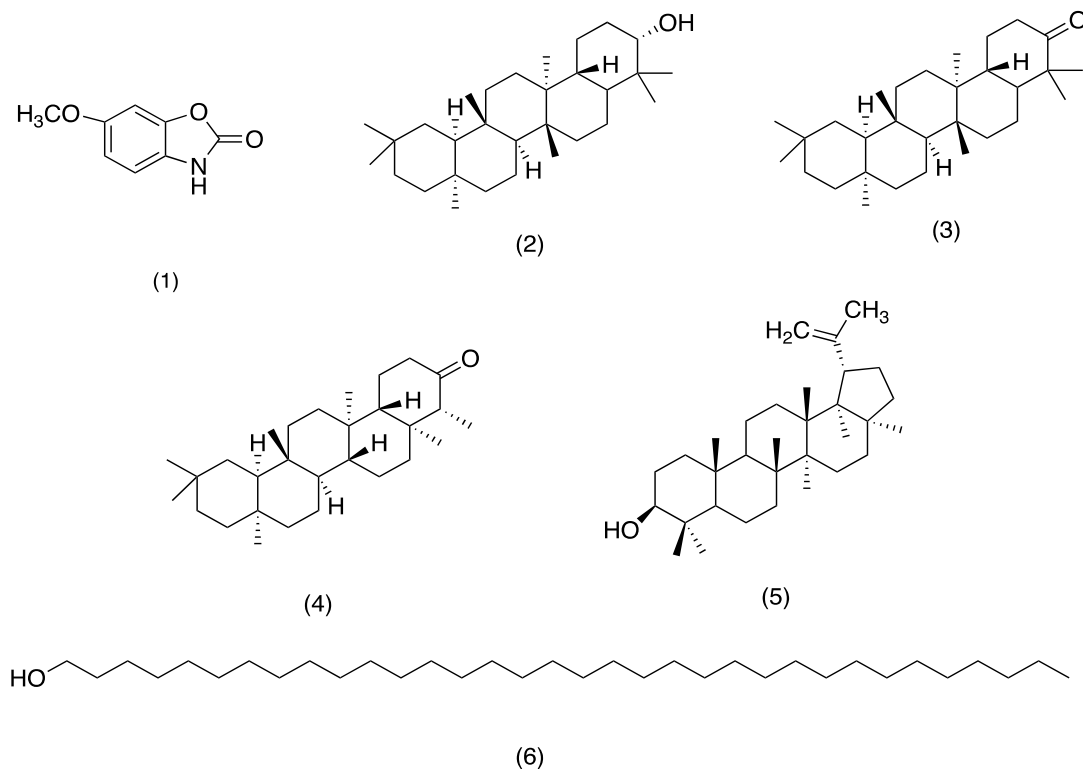
**Chikhi, Allali, et al. (2014)**, Observed antidiabetic effect of aqueous leaves extract of *Atriplex halimus* (*Chenopodiaceae*) in STZ induced diabetic-rats and results suggested that the *A. halimus* extract contain a therapeutic properties to lower the increased plasma glucose level in STZ induced diabetic rats.<sup>62</sup>

**Siddiqui, Chowdhury, et al. (2014)**, Investigated *Buchanania Lanza* plant that is commonly called as achar, char or chironji as a class with huge therapeutic potential. They found that rhizome of *Buchanania lanza* plays an important part in ancient medicine as diuretic, carminative and expectorant. They also reported some more biological effects i.e. antihypertensive, larvicidal, anticancer and anti-diabetics.<sup>63</sup>

**Rios et al. (2015)**, Suggested some natural moieties for the management of type-II diabetes and emphasized on several preclinical studies that are ongoing all over the world to check the efficacy of plants with medicinal properties specially in the cure of diabetes. They provided systematic reviews for recent and on-going clinical trials. In addition, they also explained an elaborative historical depth of traditional indigenous medical practices. In their study, they talked about several Indian, Chinese and Arabic medicines originate from herbal resources and emphasized on their relative contribution in the treatment of this illness mainly in the undeveloped and developing nations.<sup>15</sup>

**Sharma, Adhikari, et al. (2015)**, reported Potent insulin secretagogues from *Scoparia dulcis* linn. of Nepalese origin and yielded six compounds i.e. (1) Coixol, (2) Glutinol, (3) glutinone, (4) friedelin, (5) betulinic acid and (6) tetratriacontane - 1-ol (C<sub>34</sub>H<sub>70</sub>O) showed in Figure 7. All six compounds were analysed for insulin secretagogues effect on sacrificed mice pancreatic islets and  $\beta$ -cell line i.e. MIN-6. Compound 1(Coixol) and 2 (Glutinol) were shown significantly potent effect as compared to other compounds. Coixol was later on analysed for *in-vitro* cytotoxicity tests towards 3T3-cell lines, MIN-6, pancreatic islet and *In-vivo*

acute toxicity tests. *In-vivo* acute toxicity results in experimental mice reportedly shown nontoxic effect.<sup>64</sup>



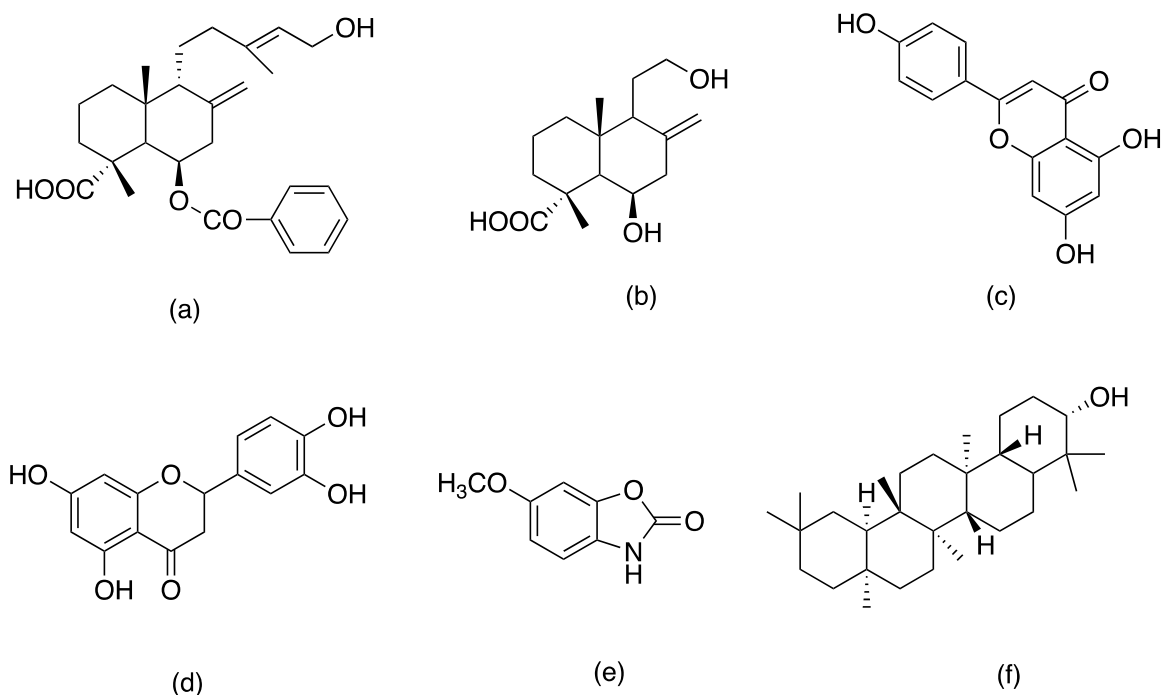
**Figure 7:** Structure of Chemical constituents isolated from *Scoparia dulcis* plant

**Moravej, Salehi, et al. (2016)**, Isolated chemical constituents from walnut leaves and evaluated the level of glycaemic control on patients with type-I diabetes. They found that flavonoids and phenolic acids are the two main category of phenolic moieties present in the leaves of walnut and concluded that walnut hydrosols are responsible for significant control in glycaemic level and release of insulin in patients affected with type-I diabetes with some minor adverse effects. Moreover, *in vitro* studies indicated that walnut oil contain seven compounds that are potent enough to determine the effectiveness and complications of walnut hydrosols in people affected with diabetes.<sup>65</sup>

**Mousavi, Leila, et al. (2016)**, reviewed some of the potent anti-diabetic substances isolated through folk medicinal plants and described them as original bio actives with anti-diabetic activities and investigated the pharmacological effects of these substances. For example: Oleanolic acid, ursolic acid, Tormentic acid and

Gymnemic acid in Terpenoids and Kola flavanone, Coixan, Leucocyanidin, Swerchirin, Bellidiflorin and (-)-Epicatechin in Flavonoids.<sup>66</sup>

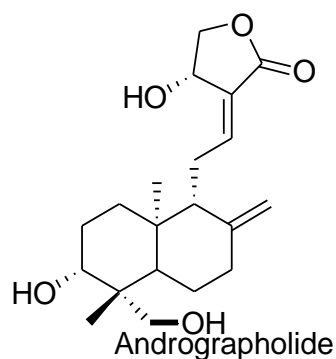
**Pamunuwa, Karunaratne, et al. (2016)**, verified anti-diabetic, anti-inflammatory and antioxidant potential of *Scoparia dulcis* considering the ancient uses of this plant for the treatment of several ailments like, jaundice, skin diseases, diabetes, fever, kidney stones and reproductive issues. They mainly focused to evaluate the anti-diabetic potential of *S. dulcis* and its bioactive constituents in the crude extracts and reported that its constituents basically show anti diabetic effect through the following mechanism through:  $\alpha$ -glucosidase antagonism, PPAR- $\gamma$  and release of Insulin. The potent chemical constituents identified as **(a)** Scoparic acid A, **(b)** scoparic acid-D, **(c)** apigenin, **(d)** luteolin, **(e)** Coixol and **(f)** Glutinol (Figure 8) are reportedly accountable for the mentioned effects.<sup>67</sup>



**Figure 8:** Structures of identified flavonoids as promising insulin secretory agents

**Brahmachari (2016)**, Identified a natural compound for its anti-diabetic properties, i.e. andrographolide. It's a diterpenoid lactone and a major bioactive chemical constituent present in *andrographispaniculata*. The key ingredient andrographolide is responsible for prime therapeutic effects henceforth it could be utilized to develop

it's semisynthetic derivatives which can be extensively evaluated as a lead molecule for drug discovery process of anti-diabetic molecules. <sup>68</sup>



**Figure 9:** Structure of Andrographolides

**Hayes (2016)**, proposed computational discovery of glycogen-phosphorylase inhibitors exploiting NP's by adopting different *in silico* methods and explored seven distinguished active sites of the receptor including, Catalytic, allosteric, novel allosteric inhibitor site, quercetin site, glycogen storage site and benzimidazole binding sites and concluded that imino sugars, glucose analogues, flavonoids, PTs and indirubins are the most efficacious and promising natural product inhibitors that has been discovered till date. <sup>69</sup>

**Dev, Ramakrishna, et al. (2016)**, screened natural products at a huge scale to evaluate their biological effect on glucose transporter-4 translocation receptor (GLUT-4) in the search for novel anti-diabetic molecules. The natural products of different classes were examined including alkaloids and amino acids, fatty acids, flavonoids, iso-flavonoids, chalcones, coumarins, anthraquinones, quinones and naphtha quinones, biphenyl and lignans, steroids, terpenes, phenols, saponins, tannins and iridoids and reportedly, most of the natural products were found to be efficacious for the activation of GLUT-4 receptors. They further concluded that these efficacious molecules could be used as a promising lead for the further discovery of novel molecules against diabetes. <sup>70</sup>

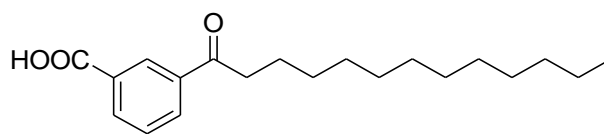
**Abbas, Al-Harrasi, et al. (2016)**, reviewed some natural products for  $\alpha$ -glucosidase enzyme inhibitory effect and examined new supportive natural product based inhibitors for  $\alpha$ -glucosidase enzyme which has been evaluated in *in vitro* and *in vivo*

both test. Study also indicated elaborated work on some of the natural products based  $\alpha$ -glucosidase enzyme inhibitors and claimed that these inhibitors might play the potential role for the treatment of diabetes mellitus in the future.<sup>71</sup>

**Khan, Chester, et al. (2017)**, Stated hypoglycaemic effect of aqueous extract of *Moringa oleifera* Leaves and evaluated *in-vivo* metabolic effect by using GC MS. They estimated total flavonoid and phenolic content present in the plant which resulted into its anti-diabetic effect. Basically, its seeds, leaves and pods have showed significant effect at low dose and proved to have anti diabetic potential. Thus this study is desirable to the establishment of natural therapy or novel Phyto-pharmaceuticals that could be used for the treatment of diabetes. Result of this study indicated that even minimum dose of extract was comparable to the specific dose given as per Ayurveda Pharmacopoeia's and have significantly high anti diabetic potential.<sup>72</sup>

**Bharti, Krishnan, et al. (2018)**, Extracted the seeds of *Cucurbita pepo* and isolated tocopherol which was further checked for its antidiabetic effect. The *in-vivo* experiment for induced diabetic rats demonstrated that tocopherol present in *C. pepo* seed extract is antioxidant and anti-diabetic in nature. Subsequently, this examination presents that these seeds extricate a supplement to the current oral anti-diabetic treatment that could limits the change of pre-diabetics into diabetic patients.<sup>42</sup>

**Prabha, Neethu, et al. (2018)**, Investigated phyto-chemical portions of *Myristicafatua Houtt bark*. This study lead to the identification of a new constituent named **1,3-tridecanoylbenzoic** (Figure 11). The molecular docking and dynamics investigation confirmed the prominent binding of the novel compound **1,3-tridecanoylbenzoic** acid with C-terminal of human maltase glucoamylase. In addition, the isolated compound was checked for promising antidiabetic effect against  $\alpha$ -amylase and  $\alpha$ -glucosidase which resulted into moderate antagonistic effect on  $\alpha$ -amylase and a significant effect on  $\alpha$ -glucosidase. Thus the entire investigation suggest that the identified novel constituent could be considered as a lead in future for promising anti diabetic effect.<sup>73</sup>



1,3-tridecanoylbenzoic acid

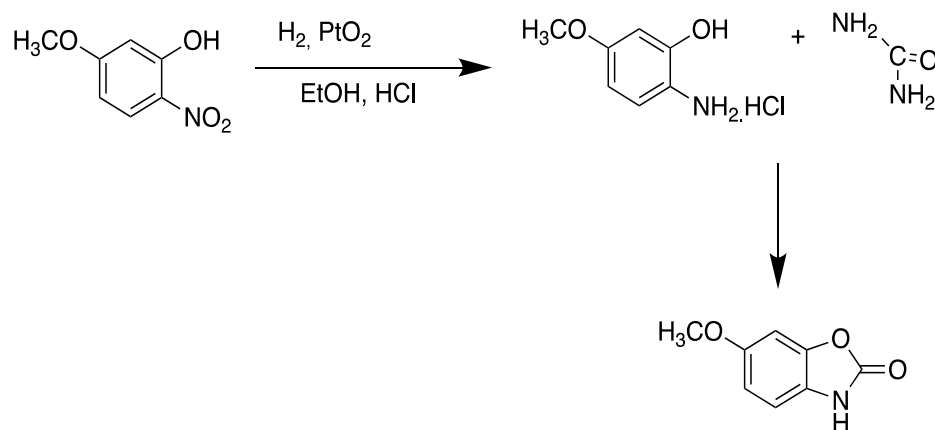
**Figure 10:** Chemical structure of new chemical constituent 1-(3-tridecanoylbenzoic acid) isolated from *Myristica fatua* Houtt.

**Jian, Halyang *et al.* (2018)**, Reviewed natural products as a crucial source for the invention of multi-target drugs and regulation of hepatic glucose-metabolism. The entire study emphasized on benefits and viability of discovering multiple target drugs (MTs) through natural resources. This review provided a new perspective on findings of drug specifically for drugs that could act on multiple targets.<sup>74</sup>

#### Literatures on synthetic approaches:

**Wright (1951)**, Introduced different synthetic strategies for the efficient synthesis of benzimidazolinone and its derivatives.<sup>75</sup>

**Smisman, Lapidus and Stanley (1957)**, worked on isolation and chemical synthesis of an insect resistance compound 6-methoxy benzoxazolinone present in Corn. The method involved the synthesis of 2-nitro-5-methoxyphenol which was further reduced to the 2-amino-4-methoxyphenol which was then converted to its stable hydrochloride salt. The final coupling with urea yielded 6-methoxy benzoxazolinone.<sup>76</sup>

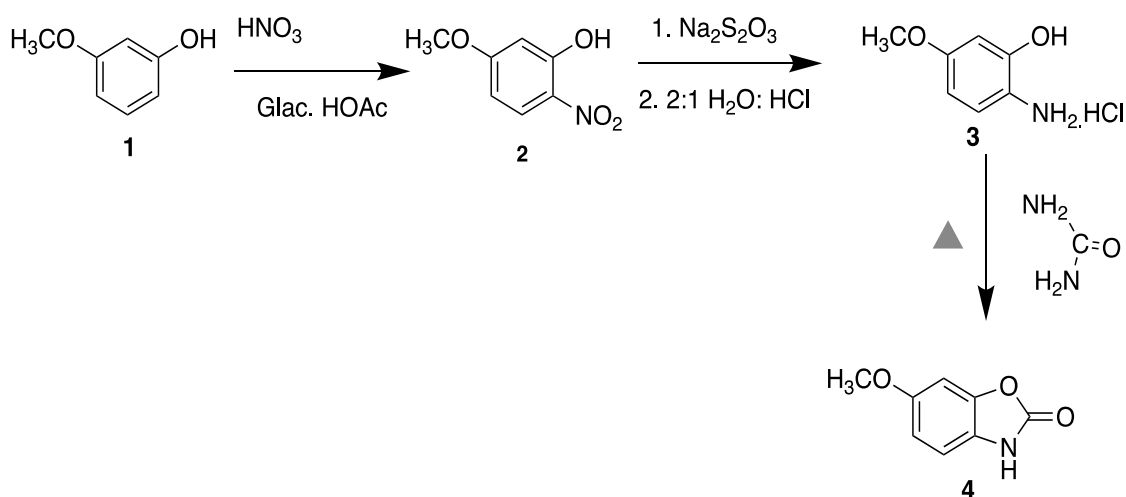


**Figure 11:** Synthesis of 6MBOA from 2-nitro-5-methoxyphenol

**Robert, Clark and Pessolano (1958)**, derived the synthetic route for some of the substituted benzimidazolones. They studied substitution in the aromatic ring and on the nitrogen atoms, and the necessary intermediates were synthesized. Some of them possessed anti-convulsant, antimitotic and anti-leukemic activity.<sup>77</sup>

**Chien-Pen, Philadelphia, Pa, asisgnor to Rohrn dz (1962)**, Patented the synthesis of 3-Thiocyanomethyl-2-benzothiazolinones and Benzoxazolinone.<sup>78</sup>

**Richey, Allen, Scism, (1975)**, Synthesized 6-Methoxy benzoxazolinone by a simplified and improved procedure. They illustrated the detailed procedure for the synthesis and thereafter reduction of 5-methoxy-2-nitrophenol and subsequent fusion of converted amine hydrochloride salt with urea in the absence of 1,3 butanediol.<sup>79</sup>



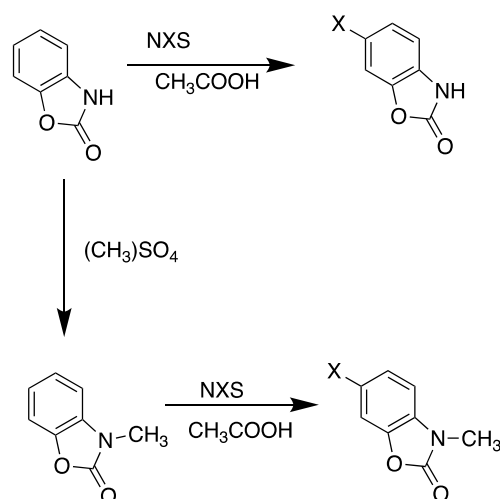
**Figure 12:** Synthetic scheme for 6MBOA using starting material 3 methoxy phenol

**Saxena, Khajuria, (1982)**, Studied the synthesis and spectral analysis of 2- mercapto benzimidazole derivatives. While preparing such compounds they noticed that 2-mercapto benzimidazole reacts predominantly as a thione under anhydrous reaction and in the presence of an alkali.<sup>80</sup>

**Pilli, Erdogan and Sunal (1993)**, worked on Some new benzoxazolinone derivatives with analgesic and anti-inflammatory activities. They synthesized Fourteen new 6-acyl-2-benzoxazolinone, ethyl-(6-acyl-2-benzoxazolinone) acetate and (6-acyl-2-benzoxazolinone-3-yl) acetic acid derivatives and their physical properties and UV absorption data were also examined.<sup>81</sup>

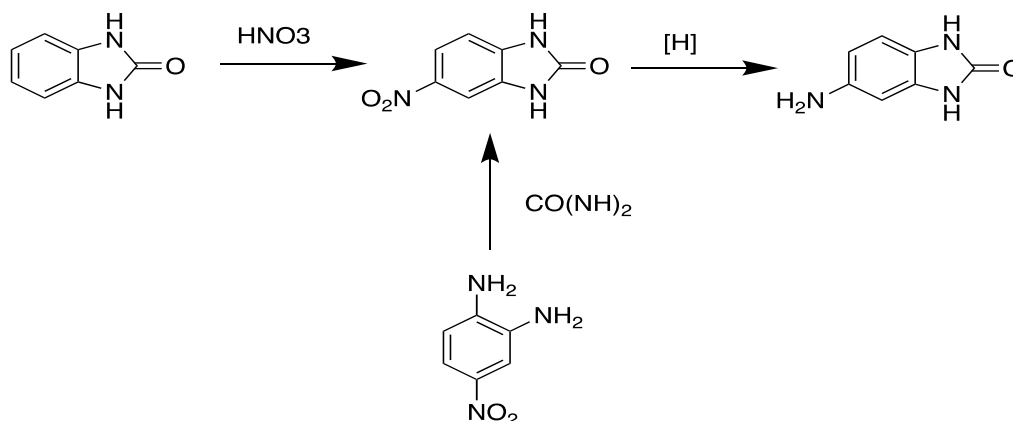


**Gershon, Clarke, et al. (1993)**, Synthesized substituted halogenated compounds of 2-aminophenol reacted with N-chloro succinimides and N-bromo succinimides at relatively ambient temperatures in the presence of glacial acetic acid. Study also indicates re-evaluation of thermo sensitive change of 2 halogenated phenyl azides to 2 aminophenols and other products which resulted into significantly different products as compared to earlier published reports.<sup>82</sup>



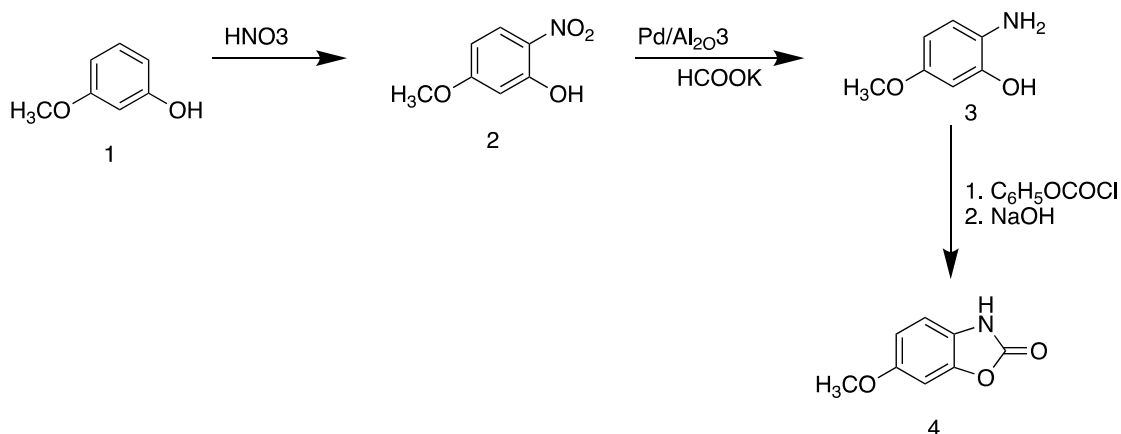
**Figure 13:** Synthetic route for benzoxazolinone substituted halogenated compounds

**Boruszczar, Kraska, (1998)**, Provided the synthetic route for 5-nitro and 5-amino 2-Benzimidazolinone. They mainly emphasized on the synthesis of pure mono nitro compounds of Benzimidazolinone that could be synthesized only when the nitration reaction is performed at mild and specific conditions by optimising specific DVS values.<sup>83</sup>



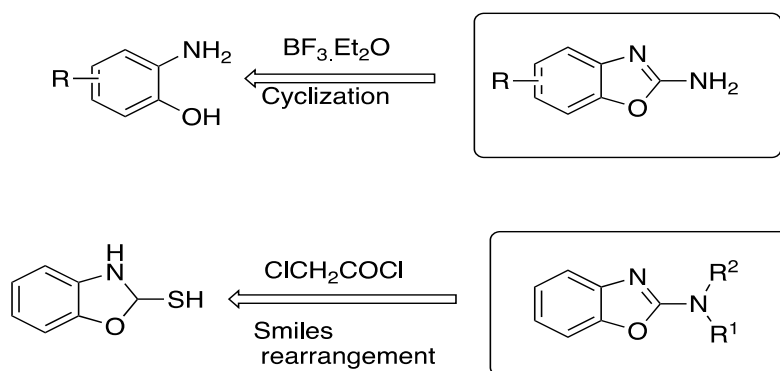
**Figure 14:** Synthetic scheme for 5-nitro and 5-amino 2-Benzimidazolinone.

**Maleski (2006)**, Introduced an improved method for the synthesis of 2-Nitro-5-methoxyphenol and 6-Methoxy Benzoxazolone by using starting material 3-methoxyphenol. They emphasized on selective nitration of 3-methoxyphenol in propionic acid, and the formed intermediate 2-nitroso-5-methoxyphenol was immediately oxidized to nitro product by using  $\text{HNO}_3$ .<sup>84</sup>



**Figure 15:** Reaction scheme for the synthesis of 6-methoxy Benzoxazolone using 3-methoxyphenol

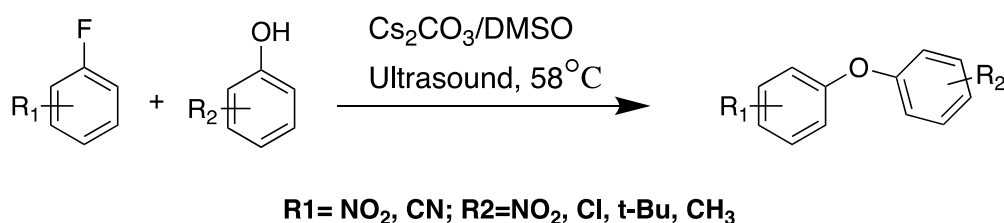
**Šlachtová, Chasák, et al. (2007)**, Reported synthetic scheme for 2-aminobenzoxazole derivatives via cyclization and Smiles rearrangement. The synthesized compounds formed by reaction between o-amino-phenols and N-cyanophenyl-p-toluene sulphonamide as a non-hazardous chemical. Whereas, the another synthetic pathway uses Smiles rearrangement method by activating benzoxazole-2-thiol with chloro acetyl chloride.<sup>85</sup>



**Figure 16:** Synthetic scheme for 2-Amino benzoxazoles via cyclization and Smiles rearrangement

**Köksal, Gökhan, et al. (2007)**, Introduced a novel series of mannich base of 5-nitro-3-substituted piperazino- methyl-2H -benzoxazolinones. Synthesized products were analysed for *in-vivo* anti-inflammatory and analgesic effect by using 2 bioassays, a: carrageenan-induced hind paw edema and p-aminobenzoquinone induced abdominal constriction effect in mice. The ulcer oriented effect of the compounds was also evaluated.<sup>86</sup>

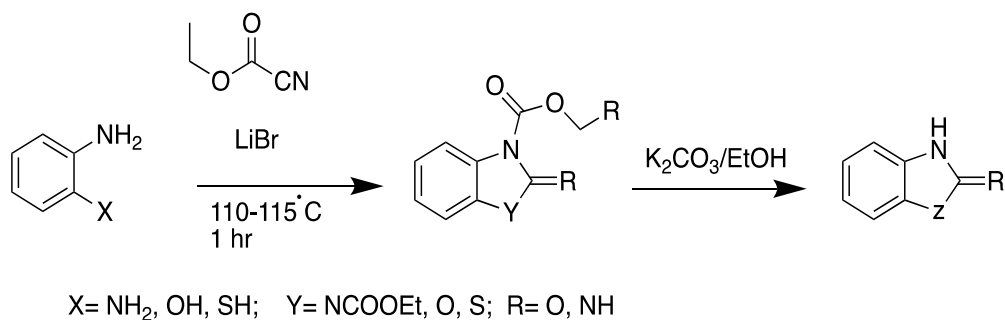
**Hui, Liao and Hong-Feng (2007)**, Developed a mild and efficient ultrasound-assisted synthesis of di aryl ethers without any catalyst by reacting different phenols specifically those having an electron withdrawing group with activated fluoro arenes via ultrasonication. The synthesized products afforded good yield even at the low temperature i.e. 60°C condition of the reaction and without adding any catalyst.<sup>87</sup>



**Figure 17:** Ultrasound-assisted synthetic approach for Diaryl ethers

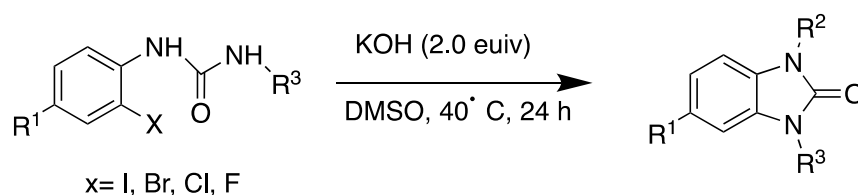
**Dinesh, Rudrawar, et al. (2008)**, Developed a suitable synthesis of 2-substituted benzoxazoles by reacting acid chloride (generated from carboxylic acid) 2-aminophenols by using methane sulphonic acid (MeSO<sub>3</sub>H) as a catalyst. The mentioned reaction was direct one pot synthesis carboxylic acid to 2 substituted benzoxazoles. All the Synthesized products afforded good yields. In addition, the reaction was found to be compatible with other substituent's such as Nitro, bromo, chloro, phenoxy and methoxy that also indicates the chemo-selectivity of the method as well.<sup>88</sup>

**Dekhane, Shivaji, et al. (2011)**, Emphasised on synthesis of benzimidazolones, benzoxazolones, 2-amino-benzothiazoles from ethyl cyanofornate and *o*-phenylene Diamines, *o*-aminophenol's, *o*- amino thiophenols promoted by lithium bromide.<sup>89</sup>



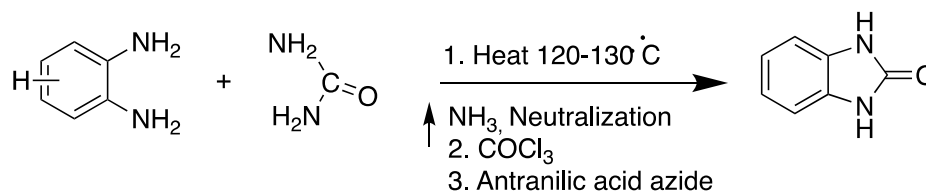
**Figure 18:** Synthetic scheme for Benzimidazolones, Benzooxazolones, 2-amino-benzothiazoles derivatives

**Beyer, Christine, Reucher *et al.* (2011)**, Set a novel method N-arylation of urea to form benzimidazolinone. The intramolecular cyclization scheme occurs in the presence of potassium hydroxide as base and di methyl sulphoxide (DMSO) as a solvent at relatively ambient temperature. As specified condition improves the yield of the product and stability of the functional groups.<sup>90</sup>



**Figure 19:** Intramolecular N-arylation of urea to form benzimidazole-2-ones

**Abbas, Hameed *et al.* (2013)**, Synthesized 2-benzimidazolines and its Derivative by applying time saving aqueous reaction conditions in the search for potential antidiabetic agents. They developed a novel and effective way for *in situ* synthesis of five member heterocyclic ring imidazolone by reaction of phenylene diamine with urea in a particular solvent phase THF:water.<sup>91</sup>

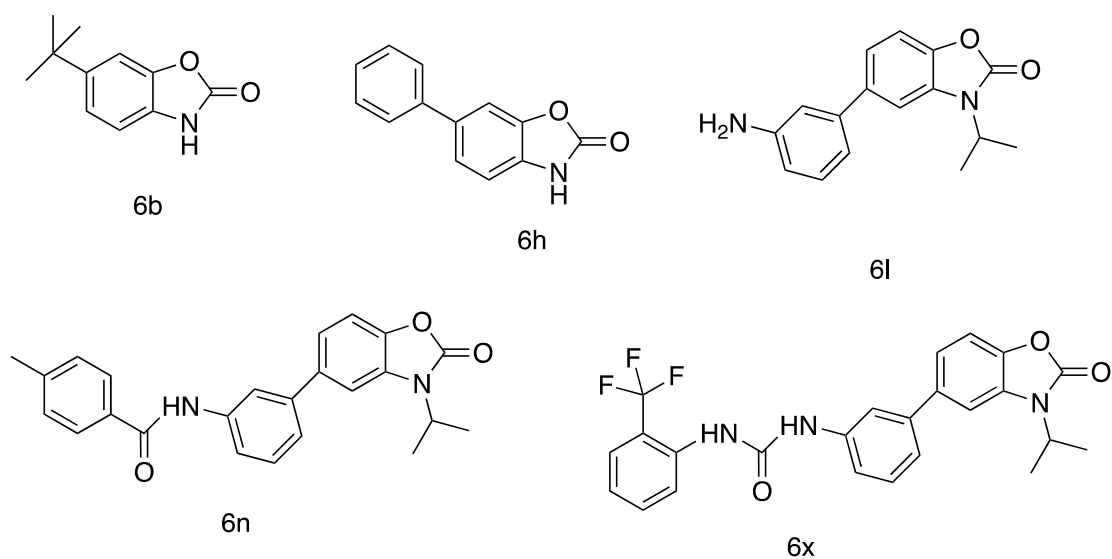


**Figure 20:** Proposed scheme for Benzimidazolinone preparation

**Jain, et al. (2014)**, Performed the synthesis of novel glycogen synthase kinase inhibitors to prevail over insulin resistance. Six compounds were synthesized as hydantoin analogs on the basis of docking results via two different approaches i.e. Stiglich Esterification reaction and Knoevenagel condensation. Considering ester substitution on benzylidene ring and hydantoin ring based pharmacophore afforded into potent and selective GSK-3 $\beta$  inhibitory effect for control of glycogen metabolism. The synthesized molecules were further checked for in vivo activity towards STZ induced diabetic rats which resulted into significant decrease in blood glucose level and increased glycogen content in liver of the diabetic rat's.<sup>92</sup>

**Wang et al. (2014)**, worked on synthesis and assessment of novel imidazolidine-diones compounds as potent anti-diabetics. Imidazolidine-diones scaffold based molecules were synthesized and tested on a mice model against hyperglycemia induced by alloxan. Accordingly, three of the compounds indicated anti-hyperglycaemic effect.<sup>93</sup>

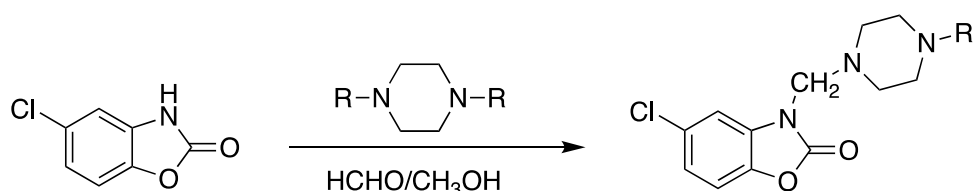
**Reddy, Sudhakar, et al. (2014)**, Introduced a basic and effective synthetic route for the synthesis of benzoxazolinone derivatives and evaluated for its anti-cancer and anti-mycobacterial properties. The in vitro cytotoxicity assay was performed against human non-carcinoma lung cells and pancreatic adenocarcinoma cell-lines. Result indicated the excellent to moderate performance of the synthesized compounds. The labelled compounds **6b**, **6l**, **6n** and **6x** performed well and considered as lead moieties. Specifically **6l** and **6n** shown potent effect against pancreatic adenocarcinoma cell line and **6x** shown potent effect against human lung cell carcinoma cell lines. On the other hand, synthesized compound **6l-6x** was found ineffective towards Mycobacterium tuberculosis. However, Among all synthesized molecules, only **6h** identified with promising anti-mycobacterial effect. Labelled molecules are mentioned in the figure 21.<sup>94</sup>



**Figure 21:** Structures of benzoxazolinone derivatives showed prominent effect as anti-cancer and anti-mycobacterial.

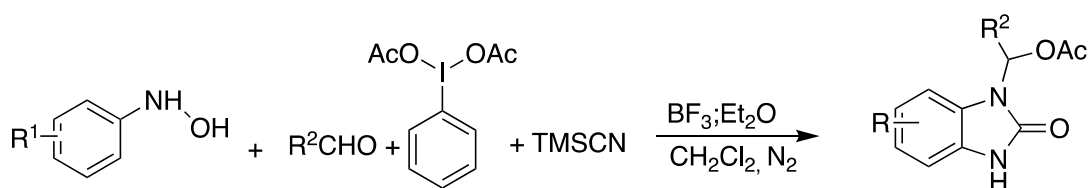
**Kazemi, Shiri and Heidari (2016),** Briefed a review on different microwave based ethers synthesis methods in the recent times as ethers place a unique space for the synthesis of biologicals, pharmaceuticals, industrials and natural products. Considering, this approach as the best towards the scope of green chemistry it was suggested to incorporate in organic synthesis due to speedy reactions, high reactivity, less intermediate products, high purity of products, excellent yields, wide range of temperature, sophisticated measurement and safety.<sup>95</sup>

**Mulazim, Berber, et al. (2017),** Studied microwave-assisted synthesis and analgesic activities of novel 5-chloro-2(3H)-Benzoxazolone derivatives. They utilized microwave-assisted synthesis to prepare piperazine substituted 5-chloro-2(3H)-Benzoxazolone derivatives also.<sup>96</sup>



**Figure 22:** Synthetic scheme for 5-chloro-2(3H)-Benzoxazolone derivatives

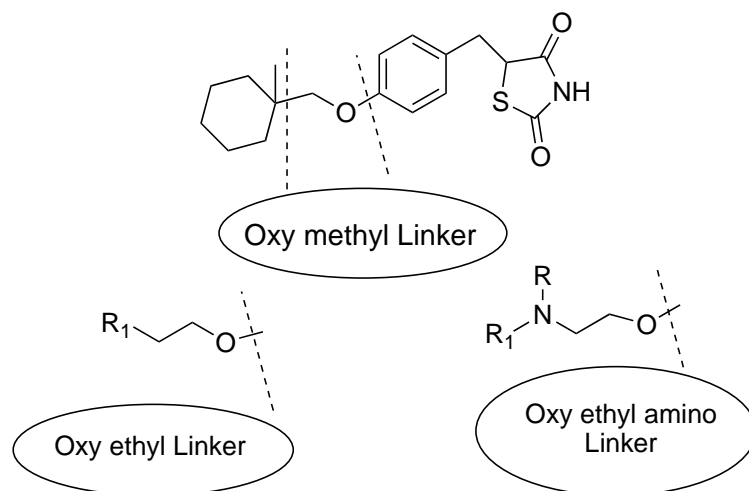
**Zhang, Huang, Wang, et al. (2017)**, Synthesized Benzimidazolones via one-pot reaction of hydroxyl amines, aldehydes, and trimethyl silyl cyanide promoted by diacetoxy iodo benzene. The resulting compounds obtained as N-substituted benzimidazolones with good yields under specific reaction environment. The proposed method was found to be robust to synthesize benzimidazolinone derivatives from feasible starting reactants.<sup>97</sup>



**Figure 23:** One pot synthetic scheme of Benzimidazole derivatives

**Safakish, Hajimahdi, et al. (2017)**, Designed and synthesized a novel category of benzoxazolinone effective as antiviral (HIV) drugs. *In vitro* evaluation of synthesized compounds shown prominent effect towards HIV at maximum concentration 100  $\mu$ M as no compound reported cytotoxicity at this concentration. The synthesized molecules which observed as potent effect against HIV had benzoxazolinone as pharmacophore and thiadiazol ring as a linker. Thus, the study suggests that benzoxazolinone based derivatives could provide a lead for further development of anti-human immunodeficiency virus (HIV-1) agents.<sup>98</sup>

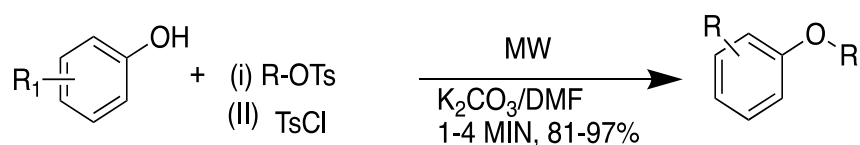
**Yasmin, Jayaprakash, et al. (2017)**, Summarized the structural modifications of the class thiazolidinediones from past two decades in the search for potential antidiabetic drugs. Thiazolidinediones were primarily named for their hypoglycaemic & hypolipidemic properties but later on also proved to possess PPAR $\gamma$  effect. This review mainly focused on the thiazolidine Dione pharmacophore specifically the linker region. The Optimal linker while the discovery of the cliglitazone was oxy methyl which was later changed to oxy-ethyl in the structure pioglitazone and oxy-ethyl amino in rosiglitazone. However, certain attempts were made to change alkyl linkers to cyclic one but the research is yet to be investigated for the same.<sup>99</sup>



**Figure 24:** Development in the linker region of thiazolidinedione Pharmacophore

**Alaqeel (2017),** Reviewed various synthetic approaches towards the synthesis of benzimidazoles from o-phenylenediamine.<sup>100</sup>

**Javaherian, Kazemi, et al. (2017),** Stabilised a microwave based scalable method by applying Williamson aryl alkyl ether reactions under Mild and solvent free conditions by using  $K_2CO_3$  as a mild base. This method was observed as rapid, easy, and potential and direct for wide applications to meet industrial requirements and synthesis of products.<sup>101</sup>



R= Alkyl groups,  $R_1 = CH_3, OH, NO_2$  etc.

**Figure 25:** Microwave assisted synthesis for alkyl aryl ethers

**Khatik, Datusalia, et al. (2018),** Performed a retrospect study on thiazole derivatives as the potential antidiabetic agents in the field of drug discovery and developments. Considering thiazole presence in the natural products such as vitamin B1 and penicillin's promote thiazole derivatives as an important lead for future research. Available antidiabetic drugs with thiazole moieties are pioglitazone and rosiglitazone that have overcome the serious side effects produced earlier by different categories.



This could have been possible due to the presence of thiazole moiety present the drugs. Thus, the review describe overall importance of thiazole nucleus and its derivatives as antidiabetic agents with a focus on the its history and current developments.<sup>102</sup>

**Gamba, Mori *et al.* (2018)**, Worked on identification of novel 2-benzoxazolinone compounds for HIV-1 inhibitory effect in nucleocapsid protein via optimizing virtual screening. The *in silico* evaluation on a large library of benzoxazolinone molecules shown putative inhibitory properties that could be further helpful for designing a series of benzoxazolinone analogues. The structure activity relationship study also revealed that the anti-HIV properties of these molecules could be achieved by adding specific substituents on the benzoxazolinone scaffold. Thus the entire investigation give a direction towards the development of novel class of target specific anti retro viral drugs against HIV-1 nucleocapsid protein.<sup>103</sup>

**Wu, Huang, *et al.* (2018)**, Introduced a novel series of pyrimidone derivatives via designing, synthesis and biological evaluations via linking the pathological processes of Alzheimer's disease and type-II diabetes mellitus altogether. Rosiglitazone act as both PDE-9 inhibitors and PPAR $\gamma$  agonists. Considering this, they designed a series of PDE9 inhibitors combining the pharmacophore of rosiglitazone and achieved optimum effects in some of the designed molecules. Result indicated that four of the compounds were reportedly less toxic towards SH-SY5Ycelllines whereas synthesized compound **11a** had the best efficacy to antagonize PDE-9 with I C50 value of 1.1 nmol/L.<sup>104</sup>

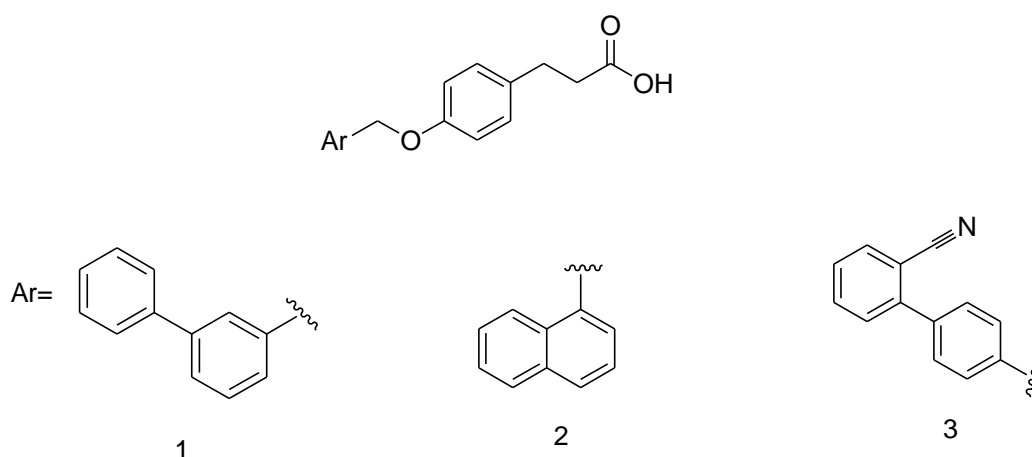
**Nejati, Ahmadi, *et al.* (2018)**, Synthesized di-aryl ethers by applying a new concept of nano catalyst based carbon- oxygen cross-coupling reactions. This method observed to be appropriate to Ullmann type C-O ether coupling reactions which are mainly dependent on catalysts.<sup>105</sup>

**Koothappan *et al.* (2018)**, worked on the synthesis of novel zinc incorporated complex of metformin (metformin-3-hydroxyflavone) and evaluated it's antidiabetic properties against high Fat Diet. + low dose of STZ (35 mg/kg) initiated type-II diabetes in rats. The metformin based zinc complex was estimated for hypoglycaemic

effect via oral glucose tolerance test. Insulin resistance was monitored via homeostasis model and performed several bio-chemical tests. Result indicated that the complex shows significant antidiabetic properties at less concentration (10mg/kg) than Metformin as a standard drug.<sup>106</sup>

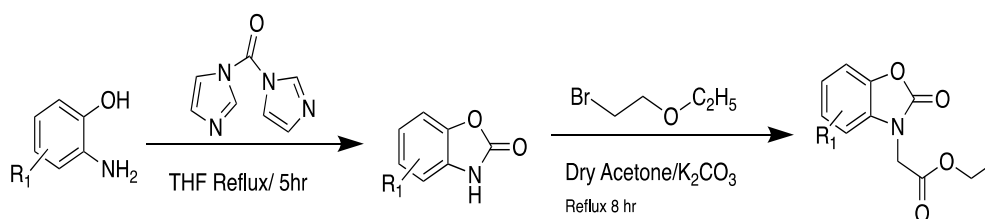
**Luthra, Lalitha, et al. (2018)**, studied separate class of Oxindoles as potent  $\alpha$ -glucosidase inhibitors and explained designing, chemical synthesis and *in vitro* evaluation of widely substituted oxindole derivatives.<sup>107</sup>

**Colín-Lozano, Estradasoto et al. (2018)**, introduced a novel series of Propanoic acid derivatives by applying a short and uncomplicated method. All the compounds were evaluated for *in vitro* analysis against 4 different targets. i.e. aldose reductase (AKR<sub>1</sub>B<sub>1</sub>), G-protein-coupled receptor (GPR-40), PPAR $\gamma$  and GLUT-4. The result of *in vitro* analysis indicated that compound 1 shows activity towards GPR40 receptor whereas Compounds 2 and 3 shown multiple effects as inhibitors of aldose reductase, potent agonist of GPR40 and showed an increased the mRNA expression of PPAR $\gamma$  protein 2 to 4 times as well as slight activity towards GLUT-4 receptor. So, as per the results of *in vitro* and *in vivo* analysis synthesized compounds 2 and 3 were found significantly potent as compared to compound 1 and could act as a lead for the promising antidiabetic effect.<sup>108</sup>



**Figure 26 :** Structures of 3-[4-arylmethoxy) phenyl] propanoic acid derivatives as potential anti diabetic agents

**Haider, Alam, et al. (2019)**, Designed and synthesized nineteen benzoxazolinone-based 1,3,4-thiadiazoles starting from different o-aminophenol derivatives. 2-aminophenol derivatives were refluxed with 1,1-carbonyl diimidazole in dry THF which was further reacted with ethyl bromo-acetate in dry acetone and  $K_2CO_3$ , leading to the formation of acetic acid ethyl ester derivatives which on further reaction with hydrazine hydrate in absolute ethanol yielded hydrazides.<sup>109</sup>



**Figure 27:** Synthetic scheme for benzoxazolinone derivatives

**Mostarda, Maz, Piccinno, et al. (2019)**, Optimized a novel experimental procedure of Benzimidazolinone Synthesis under specific flow Conditions. For this synthesis CDI (1,1-carbonyl diimidazole) was used for cyclo-carbonisation of o-phenylene diamine. The selection of co solvents was done as per their eco-friendly profile, i.e. polyethylene glycol-300 in the ratio 3:7 (v/v) with THF were used as co-solvents. the mixture was allowed reflux to at 160°C for the period of 16 hours to achieve the expected product with chromatographic purification. The flow condition was controlled by pumping a reservoir of THF which resulted to efficient synthesis of desired product. The mentioned method was found to be beneficial in case of multi scale synthesis of pure compound with high yield.<sup>110</sup>

**Proj, Sosic, et al. (2019)**, Worked on synthesis and characterization of chlorinated benzimidazole-thione derivatives with different positions of a substituted chloride on the benzene ring. Characterization of synthesized compounds was performed by NOESY and <sup>13</sup>C-NMR spectroscopy.<sup>111</sup>

**Cicco, Hernández, et al. (2021)**, Developed a time saving and efficient method for N-substituted amides by Goldberg C–N coupling reaction. This reaction uses CuI as catalyst and proceed between aryl halides and primary/secondary amines which run

either in deep eutectic solvents (DESs) or water as a reaction medium under mild reaction conditions<sup>112</sup>

#### **Literatures on Animal models:**

**Bell, Hye (1983)**, developed an animal models of diabetes mellitus by using streptozotocin.<sup>113</sup>

**Srinivasan, Viswanand, Asrat *et al.* (2005)**, worked on combination of high-fat diet-fed with lower dose of STZ administered model of rats for the induction of type-II diabetes and it's pharmacological evaluation. Study indicated that low dose of streptozotocin induced diabetes is the suitable method for testing novel promising anti diabetic molecules.<sup>114</sup>

**Gupta, Gaikwad, Tikko *et al.* (2010)**, Evaluated liver profiling that showed involvement of enzymes PKC-epsilon, DGK eta, Tnfaip and Rho kinase in type-II nephropathic diabetic rats.<sup>115</sup>

**Neto, de Vasconcelos, Thijan *et al.* (2013)**, Evaluated antihyperglycemic activity of *Calotropis procera* leaves extract on streptozotocin-induced diabetes in Wistar rats. *C. procera* leave extract were administered at the dose of 300 to 600 mg/kg/day, metformin at the dose of 500mg/kg/day and vehicle was given as per the specific groups of rats for the period of four weeks. Result were analysed for changes in body weight, biochemical parameters, fasting glucose levels and oral glucose tolerance tests.<sup>116</sup>

**Siddiqui, Hasan, Maiiraj *et al.* (2014)**, Identified two novel constituents present in the aerial parts of *Bergenia himalaica* and evaluated their anti-hyperglycaemic effect in diabetic rats induced by streptozotocin. Isolated compounds bergelin and bergenicin were determined thorough spectroscopic methods. The *in vivo* result indicated significant lower in the amount of plasma glucose observed after 2 hour of oral dosing (1.0 mg/kg) and 3 hours of dosing at concentration 0.5 mg/kg of bergenicin. Thus, the study reveal the insulin secretory property of active ingredient Bergenicin from the pancreatic tissue.<sup>117</sup>

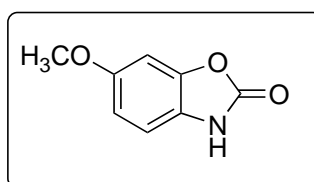
**Chakraborty, Mohammed, (2017),** Studied the effect of pomegranate -juice and tolbutamide in combination in the diabetes induced complications in rats by comparing the effect with of tolbutamide alone. Result indicated that pomegranate juice and tolbutamide combination exhibited complete protection against the disease as compared to tolbutamide alone. pharmacokinetic interaction findings also satisfied with the *in vivo* results.<sup>118</sup>

**Hameed, Hafizur, Khan, et al. (2019),** Reported the role of coixol (6-methoxy-2(3H Benzoxazolone) an alkaloid from the plant *Scoparia dulcis* as glucose dependent insulin secretory agent. They explored the insulinotropic property of coixol through *in vitro* studies. For the same purpose, Mice islets batches were incubated and perfused with coixol and insulin release was measured through ELISA assay. The intra-cellular cyclic AMP level was measured using enzyme immunoassay test. Coixol, like sulfonyl-urea's enhanced insulin in different batches of perfused and incubated and islets those are maintained at high glucose concentration. Hence, test data suggested that coixol increases insulin secretion glucose mediated by cAMP routes.<sup>119</sup>

## CHAPTER 3

### OBJECTIVES AND RATIONAL

**Coixol** is a natural product having potent insulin secretory action, present naturally in the crude extract of *Scoparia dulcis*<sup>60</sup> The seed of *Coix lacryma jobi*.<sup>120,121</sup> As of now no work has been reported on its derivatives. It has been reported as a potent insulin secretagogue and its mechanism of action is similar to sulfonyl urea therefore PDB file of 5YW7 (ATP-sensitive potassium channel bound with Glibenclamide) was chosen for preliminary docking study.



6-methoxybenzo[d]oxazol-2(3H)-one

**Figure 28:** Chemical structure of Coixol/ 6-methoxy Benzoxazolinone

**Work on Coixol:** Identified natural compound coixol known as an active constituent of *S. dulcis* and *Coix-Jobi*; responsible for producing insulin secretagogue and cytoprotective action. As of now, it has been assessed for insulinotropic effect on MIN 6 cell lines, *in-vitro* cytotoxicity test on 3T3cells, pancreatic islets and MIN-6 cells. Also, The *in-vivo* acute toxicity study suggested non-toxic effect of coixol. As a result, this investigation affirmed the insulinotropic effect of coixol which indicates the medicinal values of *S. dulcis* plant and *Coix lacryma jobi* with prominent insulin secretagogue properties.<sup>122</sup>

#### **Objectives of the Proposed work:**

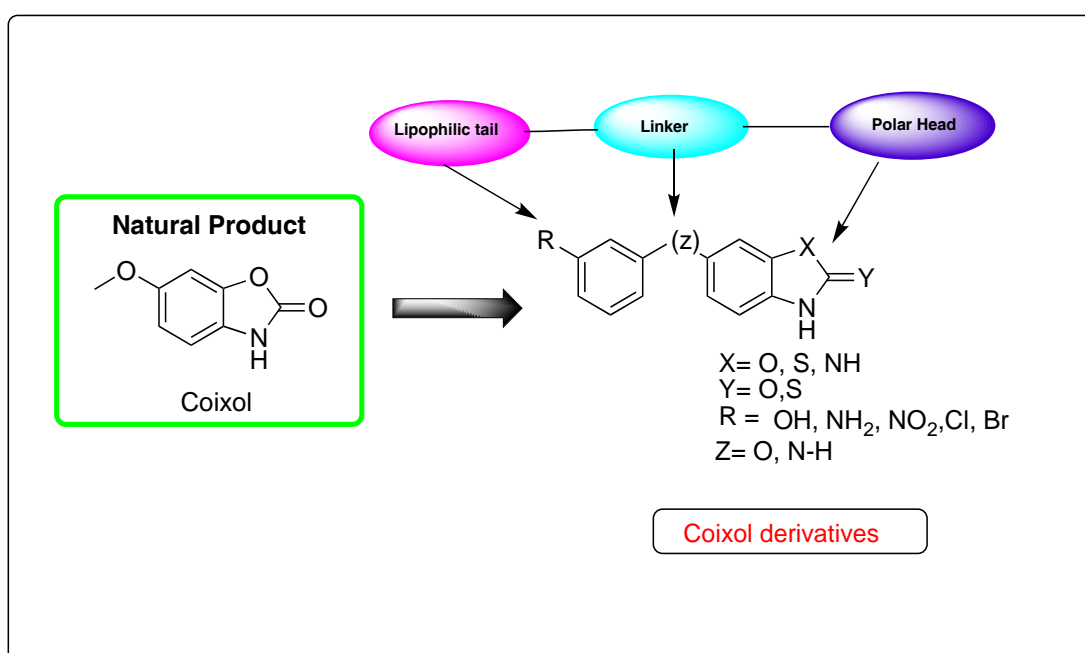
The research project aimed to identify modified coixol natural product derivatives that could potentially emerge as successful clinical candidate for the treatment of type-II diabetes.

### The specific objectives of the research project:

1. Design of novel coixol like derivatives using molecular docking tool of CADD.
2. Synthesis of designed novel coixol like derivatives.
3. Purification and characterization of coixol like derivatives by TLC, Column chromatography, IR, Mass and NMR techniques.
4. *In-vitro* antidiabetic evaluation of synthesized coixol like derivatives.
5. *In-vivo* antidiabetic evaluation of most potent coixol like derivative.

### Rational:

In this proposed research work we have optimized the skeleton by studying the SAR via varying electron donor, electron withdrawing and other possible substitution and found some more potent antidiabetic agents than coixol.



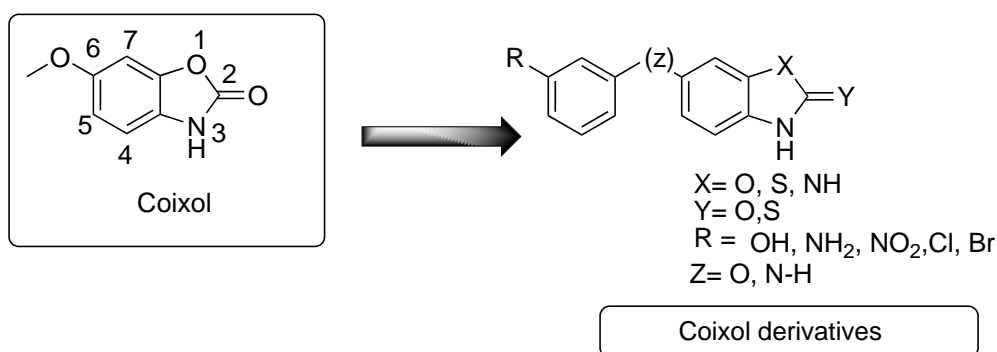
**Figure 29:** Designing of coixol based novel scaffold for potential anti diabetic effect

## CHAPTER 4

### WORK PLAN & HYPOTHESIS

#### 4.1 Structural modification:

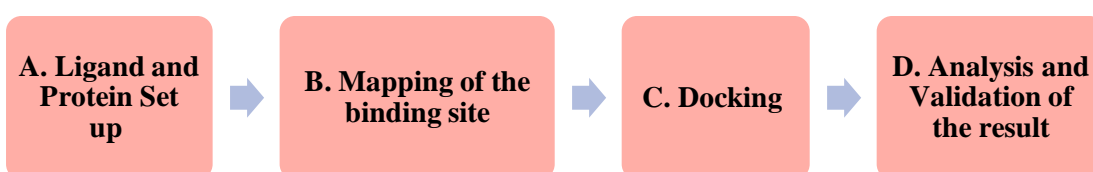
Detailed analysis of the literature indicates that coixol is known to be a potent insulin secretagogue obtained from *Scoparia dulcis*. It is proposed to be act on insulin receptor (ATP sensitive Potassium channels) and leading to increased insulin secretion. Based on this, A pharmacophore is proposed in which the structural modifications are done as showed in figure 18 and Scheme 1-4.



#### 4.2 Docking analysis of proposed series:

A PDB for insulin protein was downloaded from protein data bank that was isolated from organism *Mesocricetus auratus* with the resolution size 4.40 Å. Thereafter a protein-ligand docking with Auto dock/Auto dock-Vina was performed. Auto dock is a flexible ligand-protein docking programme which basically runs as a two-step procedure: the interactions with the binding sites will be mapped and calculated (performed with grid) and the posing of the other ligands after the interaction mapping (performed with docking).<sup>123,124</sup>

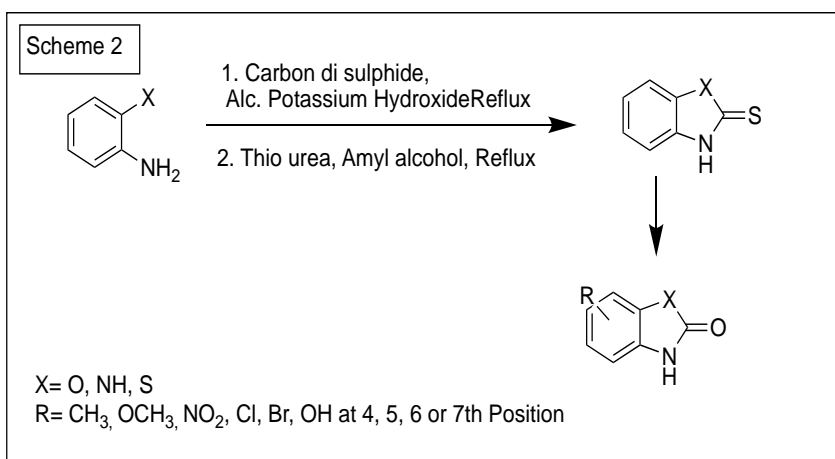
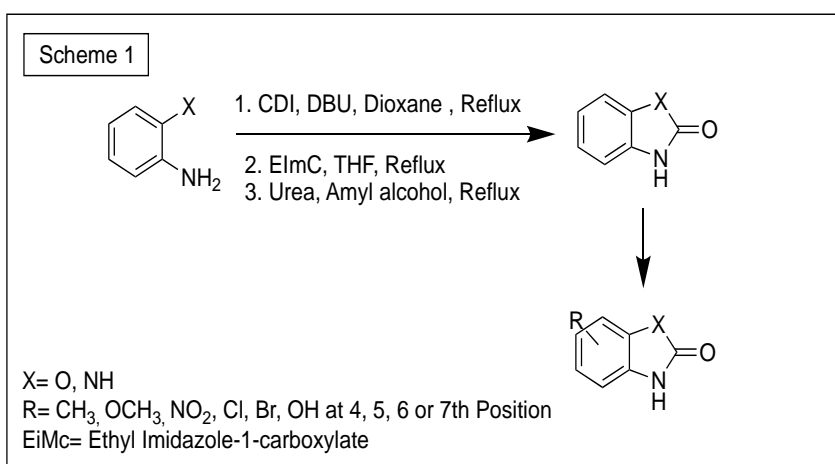
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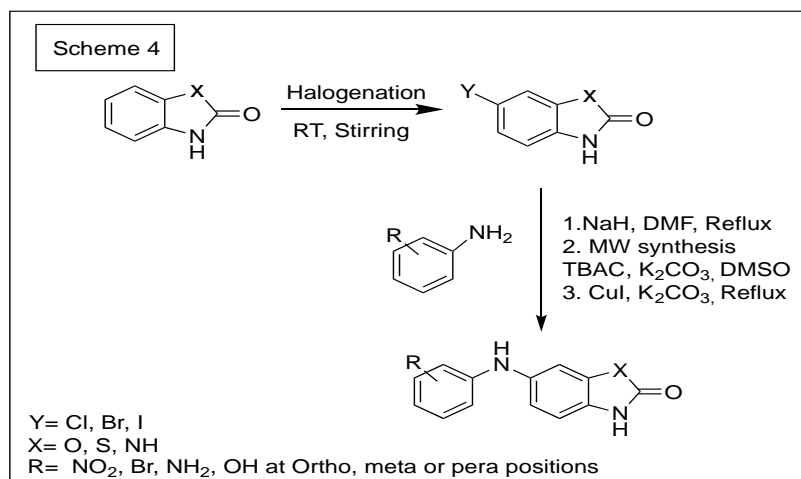
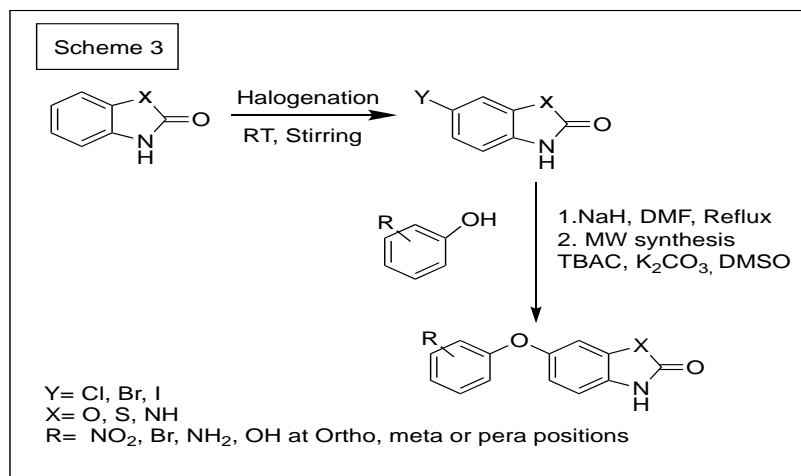




**4.3 Synthetic schemes of most potent and feasible compounds:** So, based on these observations we proposed scheme1-4 for series 1-4 by modifying the coixol natural product to further study the effects of other electron withdrawing or releasing substituents, aryl substituted ether/amine as a linker tail part and thione/ketone derivative at head part of pharmacophore. Synthesis of most potent and synthetically feasible compounds were carried out as shown in scheme 1, 2, 3 and 4 respectively.

**Proposed scheme for the synthesis of most potent compounds:**





**Figure 30:** Synthetic scheme for most potent molecules from series 1,2,3 and 4

**4.4 Characterization parameters of synthesized compounds:** The characterization will be done by TLC using R<sub>f</sub> value, IR, Mass and NMR techniques.

**TLC:** Thin layer chromatography is useful to check the progress of the reaction, compounds identification present in a reaction mixture and to check the purity of desired product . It consists of three steps - spotting, development and visualization.

**IR:** Infrared spectroscopy is a valuable tool in determination of functional groups within a compound and it has been widely applied to the characterization of molecules.

**Mass:** It is a useful spectroscopic technique for the determination of molecular weight of compounds through distinguishing different kinds of molecular ions on the basis of their  $m/z$  ratio.

**NMR:** It is one of the most important and powerful technique for the identification of particular compounds. NMR provides detail information about the structure by identifying proton ( $^1\text{H}$ NMR) or carbon atoms ( $^{13}\text{C}$ NMR) present in the structure of a compound.

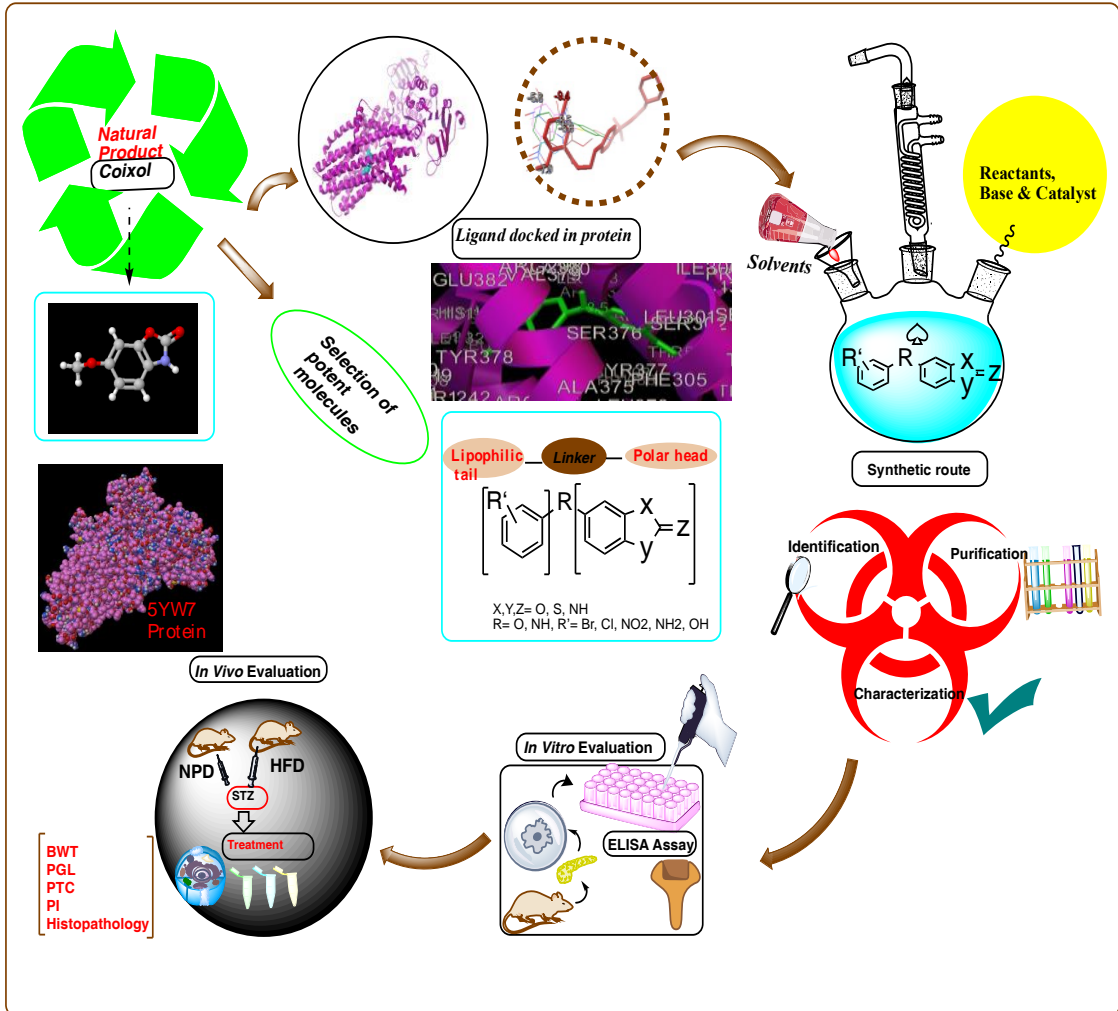
#### **4.5 *In-vitro* evaluation by insulin secretion assay:**

Islets isolation and Insulin secretion assay was performed as described previously by<sup>117</sup>.

#### **4.6 *In-vivo* evaluation of streptozotocin induced diabetes.<sup>114</sup>**

This research project is designed for the evaluation of synthesized coixol like derivatives against streptozotocin induced diabetic complication in high fat diet model.

## Research Hypothesis:



## CHAPTER 5

### MATERIAL, METHODS AND EXPERIMENTAL

#### 5.1: Molecular Docking studies:

Molecular docking is the well-known and established field of drug discovery. It minimizes the time and efforts of the researchers for identifying potent compounds. The initial steps are 3D visualization of molecules by performing computer simulations techniques on the protein. It's a computational technique for design and representation of molecular structure.<sup>125</sup> For the same purpose we used PyMOL and Auto Dock Vina 1.5.6 software's for visualizing active sites of protein and docking of designed ligands with the active sites of protein 5yw7 (K<sup>+</sup> ATPase Channel receptor). The promising ligands were designed in Chem draw professional 16.0 version and converted to 3D structure with the help of Chem Bio draw 3D. Th energy minimization was performed using MM2 interface method and converted to PDB file that is easily readable on ADT tool.<sup>126</sup> For the identification of potential anti diabetic compounds we choose protein 5yw7 (ATP sensitive potassium channel) which was downloaded from RCSB PDB: Homepage (<https://www.rcsb.org>). The protein analysis was performed on Auto Dock Vina software which reveal the appearance of hydrogen bonds, close contacts, hydrophilic and lipophilic interactions. The protein 5yw7 was prepared by Auto Dock Vina by removing water molecules, adding polar hydrogen atoms, repairing any missing atoms, adding Kollman charges and finally saved as a macromolecule by preparing its PDBQT file. The protein was validated by extracting the present ligand Glibenclamide in the B chain of the protein and docked it with the same ligand Glibenclamide which showed similar interaction as per the available reports.<sup>127</sup>

In the current work, we have designed all possible different novel Coixol like compounds by changing its substitution on different position and replacement of oxygen with other hetero atom with the help of Chem Draw and docked using Auto

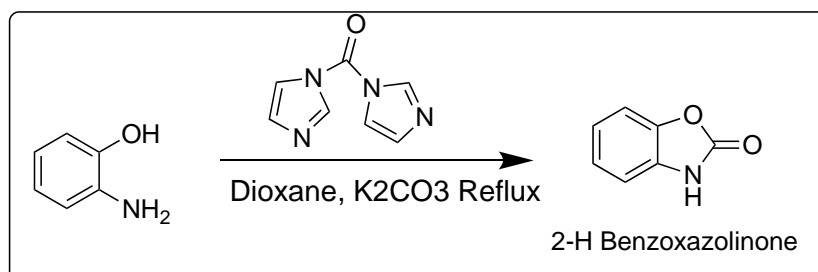
Dock vina. Among the all possible derivatives, we isolated and synthesized best ligands from each series on the basis of their affinity and binding scores.

## 5.2: Chemical synthesis and Characterization method:

All the synthetic, analytical grade chemicals & solvents were purchased from Sigma Aldrich, HI media, CDH and Loba chemicals supplied by a commercial supplier. Molecular docking was performed with the help of Auto Dock Vina 15.6 and PyMOL software's. For the determination of Melting point, Tempo capillary melting point apparatus was used after calibrating the Thermometer. The thermometer was calibrated in the range of -10°C- 250°C by using ice water and silicon oil. For the chromatographic analysis, precoated silica gel-G bound TLC plates were used (Merck). For the isolation and purification of synthetic compounds column chromatographic technique was used where silica gel with mesh size 100–200 was used as a stationary-phase. Evaporation of organic solvents from the product was carried by rotary evaporator under reduced pressure. The Fourier transform infrared spectrums were recorded ( $\nu$  max in  $\text{cm}^{-1}$ ) using PerkinElmer IR version 10.6.1 FT-IR spectrometer.  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR were recorded at 500 MHz on Bruker Advance NEO spectrometer using internal standard TMS.  $\text{CDCl}_3$  and DMSO utilized as a solvent to dissolve solid compounds for NMR spectroscopy. Mass spectra were recorded on Shimadzu 00018 mass spectrometer (GCMS-TQ8040 NX).

### 5.2.1. General synthetic procedure for Benzoxazolinone (DP100):

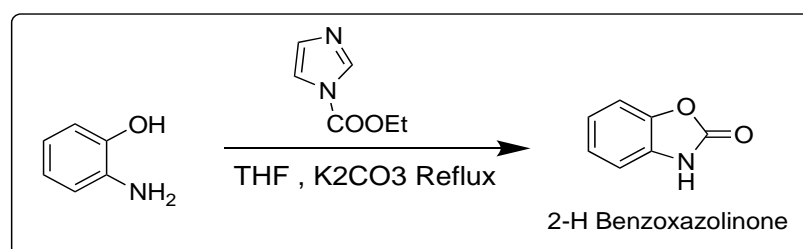
**Method-1:** Synthesis of Benzoxazolinone from o-amino phenol and CDI:



2- Amino phenol (1 mole) was refluxed with 1,1- carbonyl diimidazole (3 mole) in 25 ml dioxane for about 8 hours. Meanwhile added 3 equivalent potassium carbonate. The desired product benzoxazolinone was confirmed through TLC by taking Ethyl

acetate and Pet ether in the ration (3:7). The crude product was further purified via column chromatography by taking similar mobile phase *i.e.* Ethyl acetate and per ether at ratio (3:7). Evaporation of pure fraction lead to pure solid product with yield 65%.

**Method-2:** Synthesis of Benzoxazolinone from o-amino phenol and Ethyl imidazole-1- carboxylate

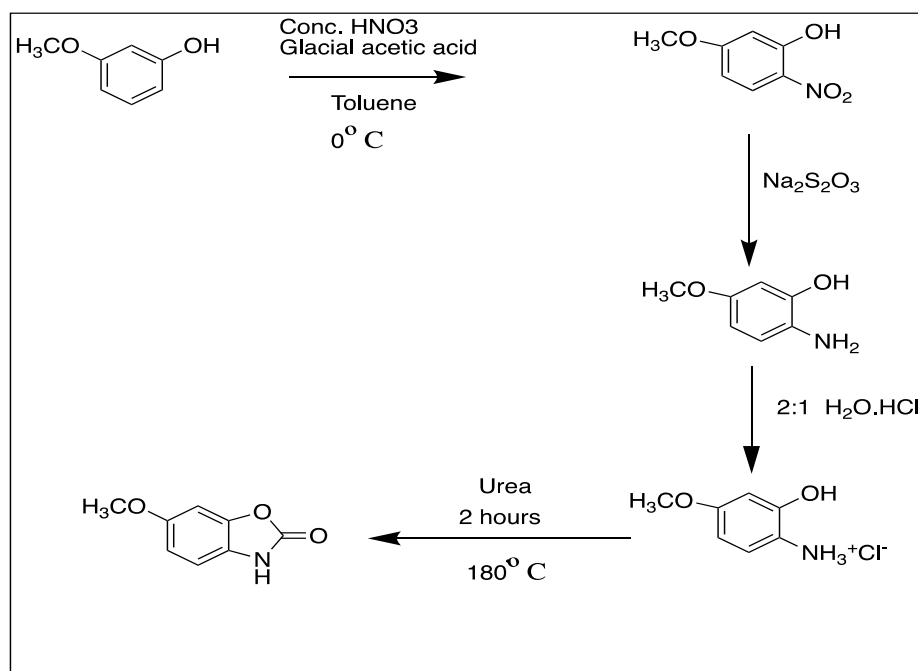


2-Aminophenol (1.2 g, 11.0 mmol) was dissolved in 20 mL of THF and reacted with ethyl-1H-imidazole-1-carboxylate (2.1 g, 15.0 mmol) with the gradual addition of potassium carbonate (8.0 mmol). The reaction was refluxed for 12-14 hours to produce 2-benzoxazolinone and monitored by TLC. Small fractions of diethyl ether were used for the extraction of the desired compound. The final product was purified by column-chromatography using mobile phase ethyl-acetate and hexane at a ratio (3:7) to obtain pure-2-benzoxazolinone in 85% yield.

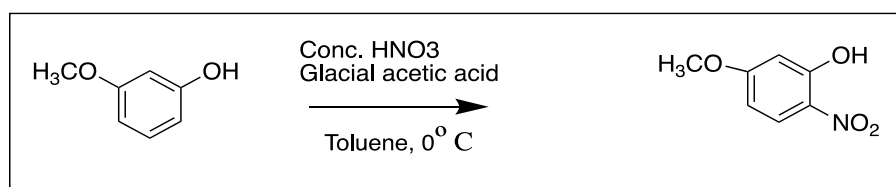
**2- Benzoxazolinone:** Physical properties and spectroscopic analysis were similar to earlier reports for this compound. Maximum yield achieved : 85%, cream colour amorphous solid, m p: 134-138°C, FTIR (KBr, cm<sup>-1</sup>): 1396 (C-O), 1622 (C=C), 1725 (C=O), 3201 (C-H), 3495 (N-H); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 7.10 (d, *J* = 10.0 Hz, 2H, ArH), 7.14 -7.19 (m, 1H, ArH), 7.22 (d, *J* = 10.0 Hz, 1H, ArH), 9.48 (s, 1H, N-H); <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>): δ 110.1, 110.2, 122.7, 124.2, 129.4, 143.9, 156.3; GC-MS (m/z) at 135 [M<sup>+</sup>]

**5.2.2: Synthesis of 6-methoxy benzoxazolinone (Coixol):** We Optimised the chemical synthesis of Coixol in total 4 steps.

### Synthetic scheme for Coixol (DP102):



### Step -1: Synthesis of 5- methoxy 2-nitro phenol:

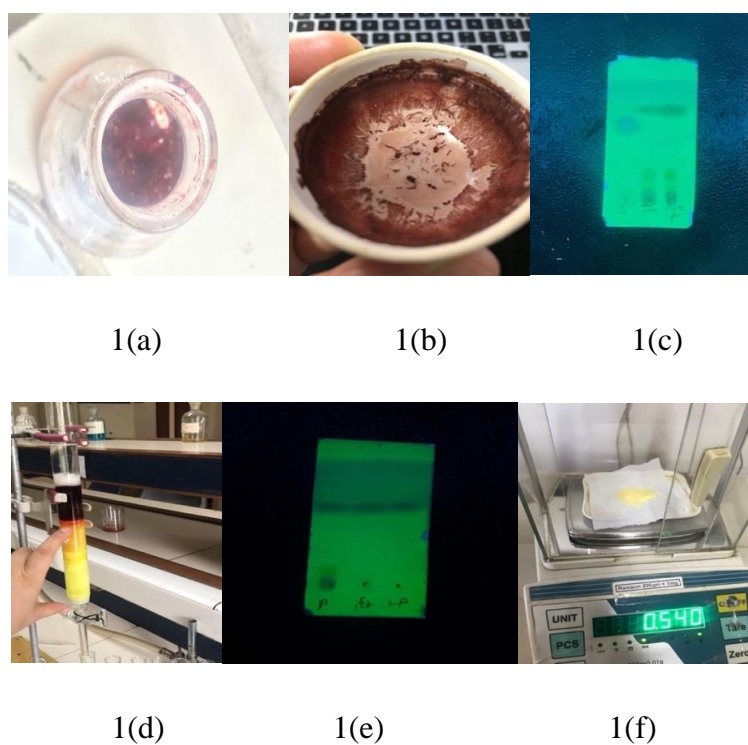


3- methoxy phenol (5gm, 0.396 mole) was slowly dissolved in Glacial acetic acid (5.2 gm) and toluene (0.4ml) was nitrated at 0°C by the gradual addition of conc. HNO<sub>3</sub> (4ml, 0.567 mole) in the glacial acetic acid (15.5ml) which resulted into brown, greenish mixture which was later kept at room temperature for 10-15 minutes which was later on followed by removal vigorous fumes of nitric oxide and the solution turned reddish quickly. The reddish solution was allowed to stand overnight in a refrigerator. After keeping it for overnight in a refrigerator the compound got precipitated on the bottom of the container, which was further washed several times with ice cold water and dried under a vacuum pump. For further purification,



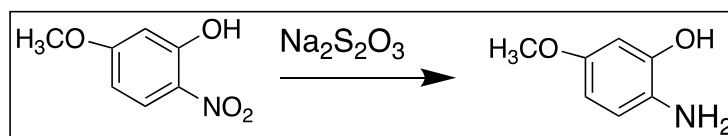
it was recrystallized by using hot ethanol but even after the recrystallization the purpose did not solve. Compound had not shown the single spot on TLC which was later isolated through Column Chromatography (Figure 31).

**Purification of crude mixture (5-methoxy2-nitrophenol) through column chromatography:** Column chromatography was performed using by silica gel (60-120 mesh) and the desired Non polar compound was eluted by using Mobile phase Ethyl acetate and Hexane at ratio 1:9. The pure compound was obtained as crystalline yellow solid.

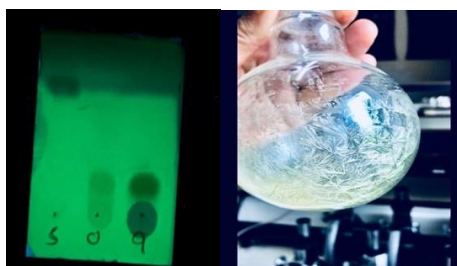


**Figure 31:** **1(a)** Shows the impure nitro compound obtained, **1(b)** represents the recrystallised nitro compound by using hot ethanol, **1(c)** shows the TLC of starting material, co-spot and desired product on the top, **1(d)** represents the preparation of silica gel column and loading of compounds, **1 (e)** represents the top spot (Non-polar) of the product on TLC, **1(f)** represents the pure dried compound isolated from the column.

### Step -2: Synthesis of 2-Amino-5-methoxyphenol:



Synthesized compound 5-methoxyphenol (1.16 gm, 0.016 mole) was placed into 45 ml distilled water and allowed to add solid anhydrous sodium dithionite (2.52gm, 0.0034 mole) into small proportions with continuous heating of the mixture. When the solution became a transparent yellow solution, the reaction components were cooled to room temperature. After that, extracted several times with small portions of di ethyl ether and evaporation of organic layer resulted into a white crystalline solid (Figure 32). The product was confirmed as reduced amine which was confirmed via TLC and FTIR through stretching ranges  $3320\text{ cm}^{-1}$  and  $3270\text{ cm}^{-1}$  (N-H stretch).

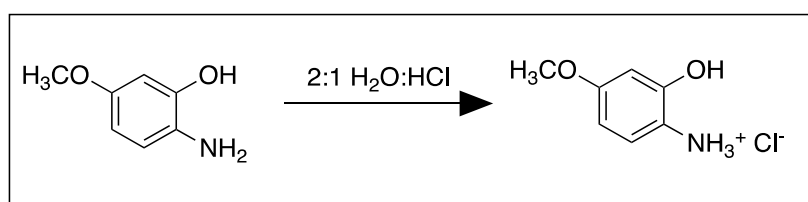


1 (a)

1(b)

**Figure 32:** 1(a) represents the spot of amine after the reduction of nitro compound which shows the presence on aqueous and organic both layers. Compound was further extracted from the aqueous layer. 1(b) represents the crystalline compound which obtained after evaporation of organic layer.

### Step-3: Synthesis of 2-Amino -5 methoxyphenol hydrochloride:



The white crystalline solid of 840mg amine was treated with 25 ml of 4M Hydrochloric acid solution. Upon adding 4M HCl solution quickly turned dark-purple and the amine hydrochloride crystals were precipitated at the bottom of the container which resulted to the formation of Wurster's salt (Figure 33). The blue-greyish solid was used without purification.

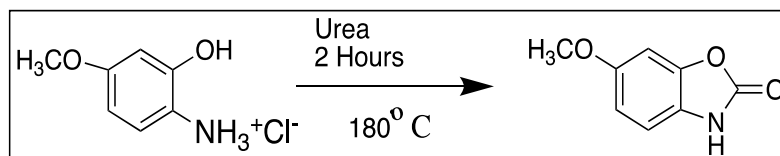


1 (a)

1(b)

**Figure 33:** 1(a) Represents the formation of purple solution by the addition of 4M HCl which leads to precipitation of crystal at the bottom. 1(b) shows the filtered product which can be used without purification for the next step.


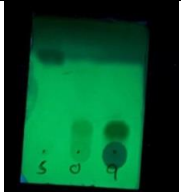
**Step-4: Synthesis of 6-methoxy 2- benzoxazolinone/ Coixol/ MBOA:**

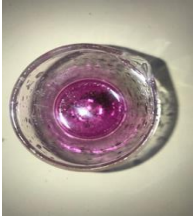


6-methoxy 2-benzoxazolinone/Coixol was synthesized by the fusion of amine hydrochloride (0.016 mole) with urea using an air condenser at 180°C without adding any solvent for the period of 30 minutes. The fusion residue/product was extracted several times with 4M HCl which turned to purple colour mixture. The solution was then extracted multiple times with small fractions of di ethyl ether. Evaporation of organic layer resulted into a residue which was further extracted with small fractions of hot water (95°C). water extract was filtered to remove any insoluble impurities and allowed to cool. After few minutes product precipitating in the filtrate. The dried product was then recrystallized from water and methylene chloride subsequently which resulted into yellow colour needle shaped crystals.

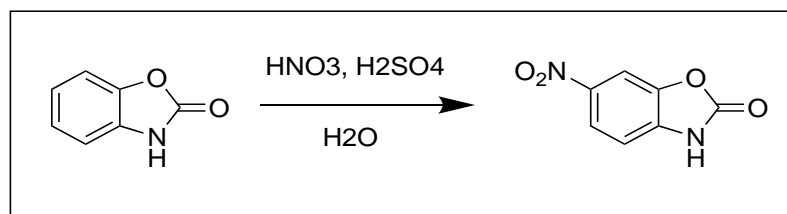
**Coixol/6MBOA (DP102):** Physical properties and spectroscopic analysis were similar to earlier reports for this compound Yield : 87%, Yellow crystalline solid; mp. 161-165°C; FTIR (KBr  $\text{cm}^{-1}$ ): 1485 (C-O), 1621 (C=C), 1783 (C=O), 3120 (C-H), 3296 (N-H);  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.81(s, 3H,  $\text{OCH}_3$ ), 6.44-6.46 (m, 2H, ArH),  $\delta$  7.96 (d, 1H,  $J = 10.0$  Hz, ArH), 10.96 (s, 1H, N-H);  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  56.1, 101.4, 109.5, 126.9, 158.0, 167.0; MS ES+  $m/z$  at 165.05 [M+].

**Table No. 3:** Steps followed for the Synthesis of 6-methoxy benzoxazolinone/Coixol

| S. No. | Reactants   | Reaction conditions             | Time         | Temperature  | Observation   |
|--------|---|---------------------------------|--------------|--|---|
| 1      | <b>Synthesis of 5-methoxy 2-nitro phenol:</b><br><br>3methoxy Phenol+ Glacial acetic acid+ $\text{HNO}_3$ + Toluene | Continuous stirring at (-2°C)   | 30 Minutes   | Addition of reactants at -2 °C which was further placed at room temperature. |   |
| 2      | <b>Synthesis of 5-methoxy 2-amino phenol:</b><br><br>5-methoxy 2-nitro phenol+ Sodium dithionite                    | Heating and continuous stirring | 20 minutes   | 60-70 °C   |  |
| 3      | <b>Synthesis of 2-Amino -5-methoxyphenol hydrochloride:</b><br><br>5-methoxy2-                                      | Stirring at room temperature    | 5-10 minutes | Room temperature   | Solution turned to deep purple with precipitates of hydrochloride                     |

|    |  |                 |         |            |  |
|----|--|-----------------|---------|------------|--|
|    | amino phenol+<br>4M HCl solution   |                 |         |            | crystals.<br> |
| 4. | <b>6-methoxy2-benzoxazolinone:</b><br><br>2-Amino-5-methoxyphenol hydrochloride + Urea | Fusion at 180°C | 2 hours | 150-180 °C | Yellow crystals<br><br>Melting point-161-165°C   |

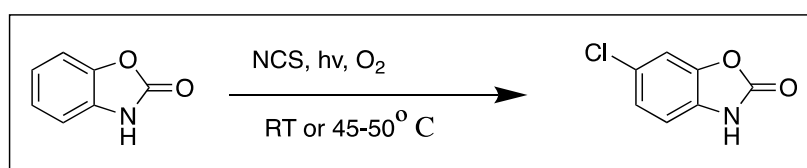
### 5.2.3: Synthesis of 6-nitro benzoxazolinone (DP104):



Powdered 2-benzoxazolinone (1.3 g, 10.0 mmol) was gradually added to the nitrating mixture containing 3mL of water, 23 mL of 96% H<sub>2</sub>SO<sub>4</sub> and 0.6 mL of 63% HNO<sub>3</sub>. The reaction was allowed to stir continuously at 10°C for about 30 min. After the required period, the reaction mixture was transferred to ice-cold water, and the precipitated product was filtered. The filtered product was given multiple times washing with water and NaHCO<sub>3</sub> to make the compound neutral. Finally filtered and dried, the final product was dried at 50-60°C for about one hour. Though, Benzoxazoles tend to react mainly at C-6 position in electrophilic substitutions and to a lesser extent at C-5, The resulting product appeared as 6-Nitro benzoxazolinone which was further confirmed through characterization techniques.

**6-nitro benzoxazolinone** : Yield 96%; light-yellow-solid; mp. 304-308°C; FTIR (KBr  $\text{cm}^{-1}$ ): 1520 ( $\text{NO}_2$ , asymmetrical), 1318  $\text{NO}_2$ , symmetrical), 1618 ( $\text{C}=\text{C}$ ), 1708 ( $\text{C}=\text{O}$ ), 3203 ( $\text{C}-\text{H}$ ), 3342 ( $\text{N}-\text{H}$ );  $^1\text{H-NMR}$  (500 MHz, d-DMSO):  $\delta$  7.28 (d,  $J = 10.0$  Hz, 1H, ArH), 8.12 (d,  $J = 10.0$  Hz, 1H, ArH),  $\delta$  8.19 (s, 1H, ArH), 12.41 (s, 1H, NH),  $^{13}\text{C}$  NMR (125 MHz, d- DMSO):  $\delta$  105.3, 109.2, 120.6, 136.6, 141.9, 142.6, 154.1; Ms ESI+  $m/z$  at 181.02 [ $\text{M}^+$ ].

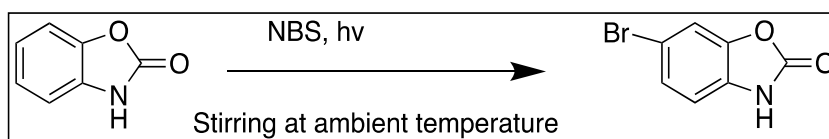
#### 5.2.4: Synthesis of 6-Chloro benzoxazolinone (DP105):



2-benzoxazolinone (0.54 g, 4mmol) and *N*-chlorosuccinimide (0.5 g, 3mmol) were added to 10 mL of glacial acetic acid, and the reaction was allowed to stir at 45-50°C for about 48 hours in the presence of light. The reaction progress was monitored through TLC by using an iodine chamber. Upon completion of the reaction, the crude product was transferred into 250 mL of distilled water with continuous stirring for 15 min. The precipitated product was recovered via filtration. The filtered product was washed multiple times with water and dried at 90°C for 1-2 hours. The final product appeared as the result of electrophilic substitution reaction at C-6 position.

**6-Chloro benzoxazolinone: (14b):** Yield 70%; white powder; mp. 191-193°C; FT-IR (KBr  $\text{cm}^{-1}$ ): 690 ( $\text{C}-\text{Cl}$ ), 1642 ( $\text{C}=\text{C}$ ), 1708 ( $\text{C}=\text{O}$ ), 3016 ( $\text{C}-\text{H}$ ), 3316 ( $\text{N}-\text{H}$ ),  $^1\text{H-NMR}$  (500 MHz, d-DMSO):  $\delta$  7.09 (d,  $J = 8.3$  Hz, 1H, ArH), 7.19-7.21 (m, , 1H, ArH), 7.48 (d,  $J = 2$  Hz, 1H, ArH), 11.79 (s, 1 H, N-H);  $^{13}\text{C}$ -NMR (125 MHz, d-DMSO):  $\delta$  110.1, 110.5, 123.5, 125.6, 129.3, 143.6, 154.0; GC MS ( $m/z$ ) at 170.99 [ $\text{M}^+$ ].

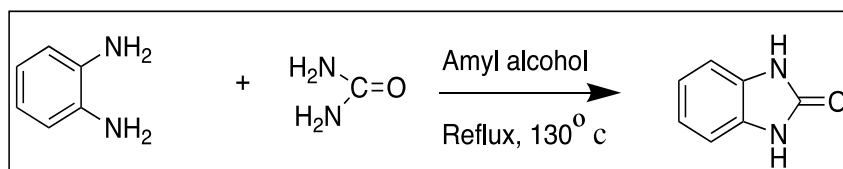
#### 5.2.5: Synthesis of 6- bromo benzoxazolinone (DP106):



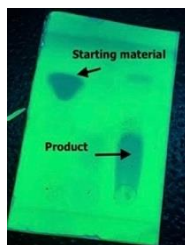
Benzoxazolione (0.67 g, 5.0 mmol) and *N*-Bromo succinimide (0.64 g, 3.0 mmol) were reacted at ambient temperature in the presence of 10 mL of glacial acetic acid. The reaction was allowed to stir at ambient temperature in the presence of light.

**6- bromo benzoxazolinone:** Yield 65%; cream colour amorphous solid; mp. 190-191°C; FT-IR (KBr  $\text{cm}^{-1}$ ): 690 (C-Br), 1642 (C=C), 1708 (C=O), 3016 (C-H), 3208 (N-H);  $^1\text{H-NMR}$  (500 MHz, d-DMSO):  $\delta$  7.04 (d,  $J = 5.0$  Hz, 1H, ArH), 7.31-7.33 (m, 1H, ArH), 7.58 (d,  $J = 5$ Hz, 1H, ArH), 11.79 (s, 1H, NH);  $^{13}\text{C}$  NMR (125 MHz, d- DMSO):  $\delta$  111.1, 112.6, 112.9, 126.3, 129.8, 143.9, 153.9, MS ESI+ (m/z) at 216.95 [M+]

### 5.2.6: Synthesis of Benzimidazolinone:



For the synthesis of 2-benzimidazolinone, *o*-phenylenediamine (1g, 10.0 mmol) and urea (1.2 g, 20.0 mmol) were fused in the presence of 25 mL of amyl alcohol. The reaction was refluxed for 4-5 h at 130°C. The progress of the reaction was checked through TLC. After completion of the reaction, the reaction mixture was filtered and washed multiple times with hexane to remove non-polar impurities. The Final product was crystallized using hot ethanol. Finally, the resulting compound was dried at 90-100°C for 2 hours (Figure 34).

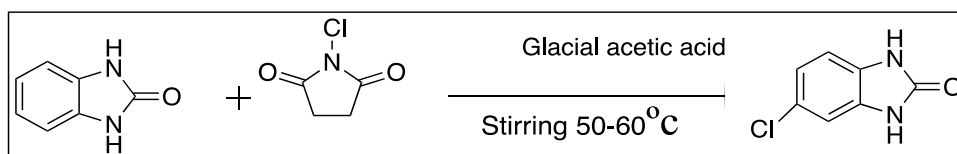


**Figure 34:** TLC represent the formation of Benzimidazolinone

**Benzimidazolinone:** Physical properties and spectroscopic analysis were similar to earlier reports for this compound. Yield: 88%; pale white amorphous powder; mp.

300-305°C; FTIR: (KBr  $\text{cm}^{-1}$ ): 1630 (C=C), 1710 (C=O), 3017 (C-H), 3397 (N-H);  $^1\text{H-NMR}$  (500 MHz, d-DMSO):  $\delta$  7.25 (m, 2H, ArH),  $\delta$  7.90 (m, 2H, ArH), 10.51 (s, 2H, NH);  $^{13}\text{C}$  NMR (125 MHz, d- DMSO):  $\delta$  110.1, 108.9, 124.5, 129.8, 155.1; MS ESI+ m/z at 134.05.

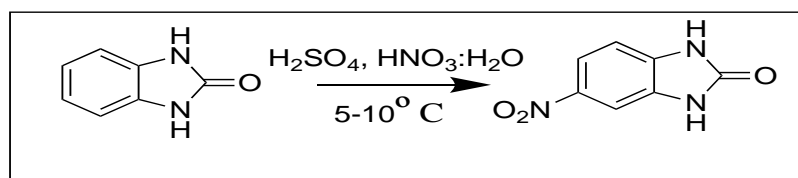
### 5.2.7: Synthesis of 5-Chloro benzimidazolone-2 (DP151):



2-benzimidazolone (0.53 g, 4.0 mmol) and *N*-chloro succinimide (0.5 g, 4.0 mmol) were gradually added into 15 mL of glacial acetic acid. The reaction was allowed to stir for about 48 h at 50-60°C. The reaction progress was checked through TLC by using an iodine chamber for detection. After the completion, the reaction mixture was transferred into ice-cold water with continuous stirring for 10-15 min. The resulting product was precipitated, filtered, and washed several times with water. Finally, it was dried at 90°C for 1-2 h.

**5-Chloro 2-benzimidazolone:** Yield: 70%; pale white amorphous powder; mp. 240-250°C; FT- IR (KBr,  $\text{cm}^{-1}$ ): 694 (C-Cl), 1728 (C=O), 2913 (C-H), 3303 (N-H),  $^1\text{H-NMR}$  (500 MHz, d-DMSO):  $\delta$  6.90-6.96 (m, 2H, ArH), 7.10-7.16 (m, 1H, ArH), 10.74 (s, 1H, N-H), 10.91 (s, 1 H, N-H);  $^{13}\text{C-NMR}$  (125 MHz, d-DMSO):  $\delta$  120.0, 122.2, 124.4, 129.7, 130.7, 155.1; MS ES + (m/z) at 169.01.

### 5.2.8: Synthesis of 5- Nitro 2-benzimidazolone (DP154):



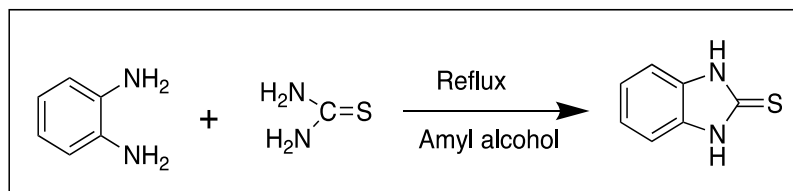
Powdered 2-benzimidazolone (1.0 g, 8.0 mmol) was added gradually to the nitrating mixture consisting of 3 mL of water, 23 mL of 96%  $\text{H}_2\text{SO}_4$ , and 0.7 mL of 63%



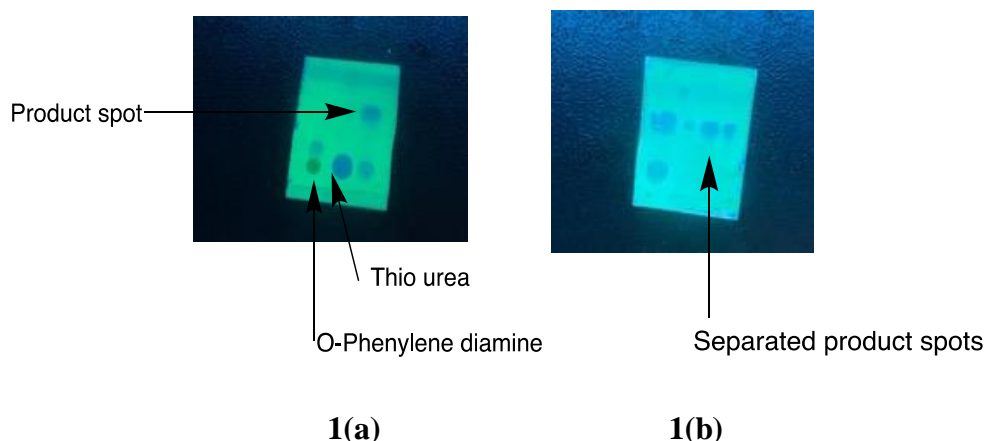
HNO<sub>3</sub>. The reaction was stirred at 10-15°C for a period of 0.5 h. After the required period, the reaction mixture was poured into crushed ice, resulting in fine product precipitates. The product was filtered under a vacuum and washed with a mixture of water and 5% aqueous NaHCO<sub>3</sub> solution. The washing was continued several times with water until the pH appeared neutral. The filtered product was then dried at 50-60°C to afford the desired product.

**5-Nitro 2-benzimidazolone:** Yield: 95%; yellow powder; m.p. 305-307°C; FT-IR (KBr, cm<sup>-1</sup>): 1475 (NO<sub>2</sub>, asymmetrical), 1333(NO<sub>2</sub>, symmetrical), 1698 (C=O), 3013 (C-H), 3403 (N-H); <sup>1</sup>H-NMR (500 MHz, d-DMSO): δ 7.10 (d, *J* = 10.0 Hz, 1H, ArH), 7.71 (s, 1H, ArH), 7.94 (d, *J* = 10.0 Hz, 1H, ArH), 11.17 (s, 1H, N-H), 11.40 (s, 1H, N-H); <sup>13</sup>C NMR (125 MHz, d-DMSO): δ 107.97, 117.67, 120.31, 129.59, 135.61, 141.18, 155.22; MS ES + (m/z) at 180.04 [M+].

**5.2.9: Synthesis of 2- Benzimidazole-thione (1,3-dihydro-2H-benzo[d]imidazole-2-thione) DP200:**



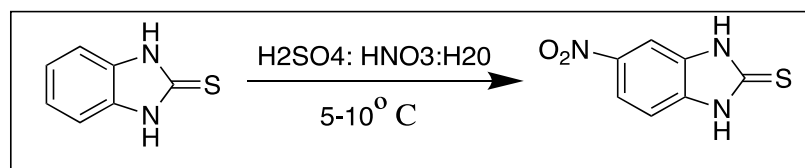
Benzimidazole-2-thione was synthesized by reacting a mixture of o-phenylenediamine (1.2 g, 11.2 mmol) and thiourea (1.2 g, 15.5 mmol) in 25 mL of isoamyl alcohol. The reaction was refluxed at 170-180°C for nearly 8h. The progress of product formation was checked through TLC. Later the reaction mixture was kept overnight at room temperature to afford solid precipitate, which was filtered and washed 2-3 times with water. The pure product was obtained through column chromatographic with mobile phase ethyl acetate and hexane (1:9). (Figure 35). Evaporation of column fractions lead to final product.



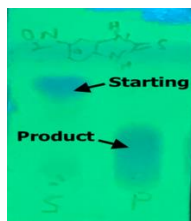
**Figure 35:** 1(a) TLC represents the formation of product & 1 (b) represents the separation of product via Column chromatography.

**1,3-dihydro-2H-benzo[d]imidazole-2-thione:** Physical properties and spectroscopic analysis were similar to earlier reports for this compound. Yield: 75%; white to slight pink colour crystalline solid; mp. 302-310°C; FT-IR (KBr  $\text{cm}^{-1}$ ): 1620 (C=C), 1732 (C=S), 2875 (C-H), 3112 (N-H);  $^1\text{H-NMR}$  (500 MHz, DMSO):  $\delta$  7.11-7.12 (m, 2H, ArH), 7.13-7.15 (m, 2H, ArH), 12.51 (s, 2H, NH);  $^{13}\text{C NMR}$  (125 MHz,  $\text{CDCl}_3$ ),  $\delta$  110.1, 125.6, 130.3, 132.4, 165.6, MS ES+ (m/z) at 151.04.

#### 5.2.10: Synthesis of 6-nitro-1,3-dihydro-2H-benzo[d]imidazole-2-thione (DP260):



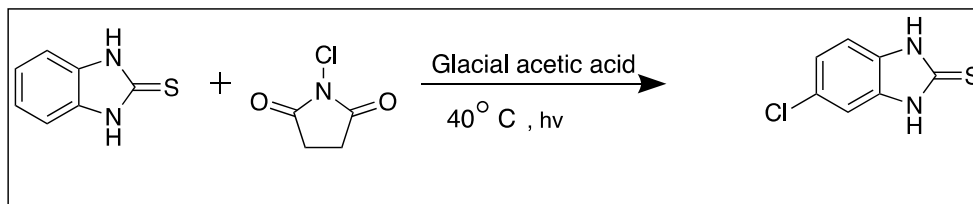
The compound benzimidazole-2-thione (1.1 g, 7.5 mmol) was gradually added in the nitrating mixture consist of 3 mL of  $\text{H}_2\text{O}$ , 23 mL of 96%  $\text{H}_2\text{SO}_4$  and 0.7 mL of 63%  $\text{HNO}_3$  with continuous stirring at  $10-15^\circ\text{C}$ , over about 30 min (Figure 36). After the completion of the reaction, the crude product was transferred into ice-crushed water, leading to precipitating. The precipitate was filtered under a vacuum and washed multiple times with water and a 3% solution of  $\text{NaHCO}_3$  to make its pH neutral. Finally, the filtered product was dried at  $80-90^\circ\text{C}$  to remove the moisture from the product.



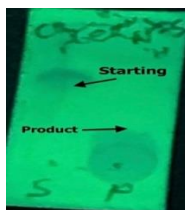
**Figure 36:** TLC represent the complete conversion of Benzimidazole thione into 6-Nitro benzimidazole thione.

**6-nitro-1,3-dihydro-2H-benzo[d]imidazole-2-thione:** Yield: 95%; mustard colour solid; mp; 305-310 °C; FT-IR (KBr  $\text{cm}^{-1}$ ): 1587 ( $\text{NO}_2$ , asymmetrical), 1340( $\text{NO}_2$ , symmetrical), 1628 (C=C), 1732 (C=S), 2952 (C-H), 3262 (N-H);  $^1\text{H-NMR}$  (500 MHz, d-DMSO):  $\delta$  7.16-7.18 (m, 1H, ArH), 7.27-7.32 (m, 1H, ArH), 7.60- 7.61 (m, 1H, ArH), 7.87 -7.88 (m, 1H, NH), 9.57 (s, 1H, NH);  $^{13}\text{C-NMR}$  (125 MHz, d-DMSO):  $\delta$  114.3, 119.1, 122.5, 126.1, 135.8, 140.5, 162.7; MS ES+ (m/z) at 197.58 [M + 1].

#### 5.2.11: Synthesis of 5-chloro-1,3-dihydro-2H-benzo[d]imidazole-2-thione (DP263):



Synthesized compound benzimidazole thione (1 mmol) was stirred well with N-Chloro succinimide (1mmol) in the presence of Glacial acetic acid 30ml for nearly 48 hours. For the first 2 hours the reaction mixture was allowed to stir at 60°C and then the residue was kept at room temperature by using a  $\text{CaCl}_2$  guard tube. The formation of product was confirmed through TLC which was detected through UV cabinet and iodine chamber (Figure 37). After the completion of reaction the reaction mixture was poured into distilled water which lead to the precipitation of product. The final product was then filtered and washed 2-3 times with water and dried well.



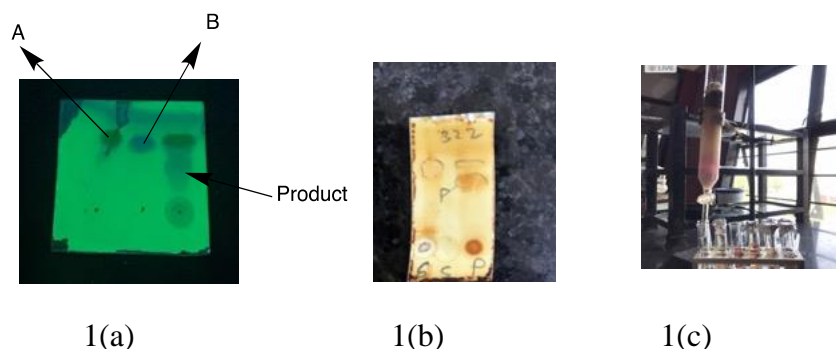
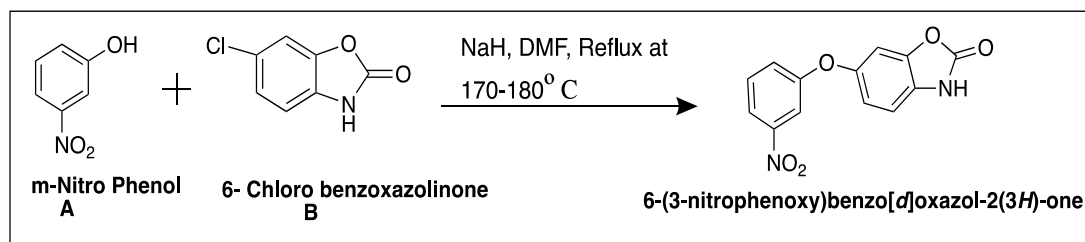
**Figure 37:** TLC represent the complete conversion of Benzimidazole thione into 5-Chloro benzimidazole thione

**5-chloro-1,3-dihydro-2H-benzo[d]imidazole-2-thione:** Yield 68%; pale yellow colour solid; mp. 296-298°C; FT-IR (KBr  $\text{cm}^{-1}$ ): 740 (C-Cl), 1619 (C=C), 1733 (C=S), 2923 (C-H), 3060 (N-H);  $^1\text{H-NMR}$  (500 MHz, d-DMSO):  $\delta$  7.27-7.28 (m, 2H, ArH), 7.60 (s, 1H, ArH), 11.05 (s, 1 H, N-H), 13.05 (s, 1 H, N-H);  $^{13}\text{C-NMR}$  (125 MHz, d- DMSO):  $\delta$  93.8, 105.8, 113.8, 130.4, 137.5, 143.1, 164.0; MS ES+ (m/z) at 183.01 [M+].

#### 5.2.12: Synthesis of 6-(3-nitrophenoxy) benzo-[d] oxazol-2 (3H) one (DP322)

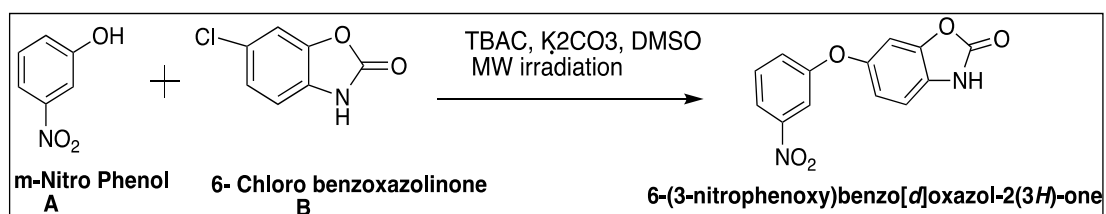
We tried to optimize the product using different methods, including Williamson's ether and microwave-assisted synthesis. The formation of desired product through different methods is shown in Figure 27 and 28.

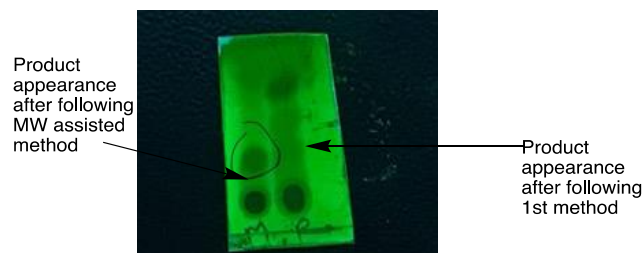
**Method -1:** Based on the principle of Williamson's synthesis, 3-nitro phenol (1.5 g, 11.0 mmol) was first reacted with a base NaH (0.1 g, 5.0 mmol) in the presence of 10 mL dimethyl furan. After gradually adding 6-chloro-2-benzoxazolinone (1.3 g, 8.0 mmol), the reaction refluxed at 160-170°C for 24 hours. The reaction mixture was poured into ice-cold water and extracted several times with diethyl ether. The organic layer's TLC ensured the formation of the product along with the remaining traces of starting material (figure 38). The formed product was isolated through column chromatography by applying silica gel as adsorbing medium, hexane, and ethyl acetate (6:4) as the mobile phase. The pure compound was separated as a semisolid reddish-brown product with a 40-50% yield.



**Figure 38** : 1(a) & 1(b) represents the formation of product 1(c) represents the isolation technique which was followed.

**Method 2:** Microwave-assisted coupling was followed by mixing m-nitro phenol (0.69 g, 5.0 mmol) and 6-chloro-2-benzoxazolinone (0.67 g, 4.0 mmol) with a mild base  $\text{K}_2\text{CO}_3$  (0.96 g, 7.0 mmol) and tetrabutylammonium chloride (0.2 g, 2.0 mmol) in the presence of 5 mL dimethyl sulphoxide and exposed to microwave radiation for about 4-5 min. The work-up and isolation procedure was performed in the same manner as per the above-mentioned method 1. This method was found to be more effective in terms of yield and not lead to the formation of other intermediates (Figure 39).





**Figure 39:** TLC represents the product spot in Microwave assisted and Williamson's coupling method.

**6-(3-nitrophenoxy) benzo-[d] oxazol-2 (3H) -one** : Yield; 65%, Reddish semi solid, FT-IR ( $\text{cm}^{-1}$ ): 1481 ( $\text{NO}_2$ , asymmetrical), 1347( $\text{NO}_2$ , symmetrical), 1606 (C=C), 1760 (C=O), 2923 (C-H), 3073 (N-H);  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  6.80-6.86 (m, 2H, ArH), 6.98- 7.00 (m, 1H, ArH) 7.18- 7.21 (m, 1H, ArH), 7.23- 7.26 (m, 1H, ArH), 7.69-7.76 (m, 1H, ArH), 8.25 (s, 1H, ArH), 9.77 (s, 1H, N-H);  $^{13}\text{C}$  NMR (125 MHz, d-DMSO):  $\delta$  111.1, 112.9, 114.3, 120.6, 122.6, 126.3, 129.8, 140.5, 143.9, 153.9, 154.0, 158.01; GC MS (m/z) 273 [M+]

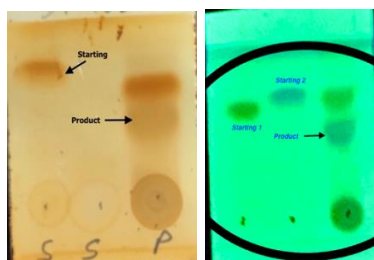
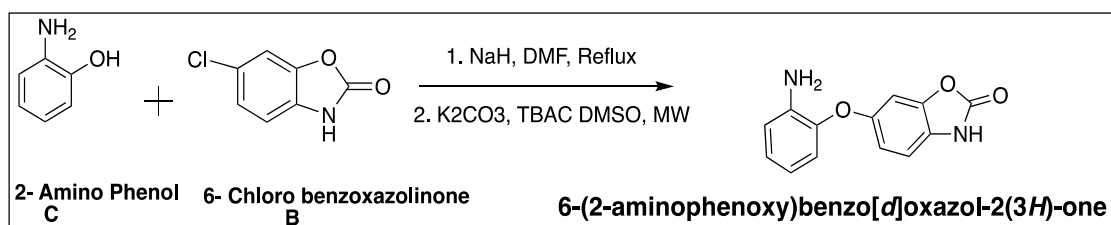
### 5.2.13: Synthesis of 6 - (2-aminophenoxy) benzo-[d] oxazole-2 (3H) -one (DP330):

For the synthesis of 6-(2-aminophenoxy) benzo-[d] oxazole -2 (3H)-one; following methods were applied. The formation of desired product is mentioned in Figure 40.

**Method-1:** 2- Amino Phenol (1.1 g, 11.0 mmol) was refluxed with 6-chloro Benzimidazolinone (1.2 g, 7.5 mmol) with slight addition of base NaH in the presence of DMF. The strong base was used to produce a Nucleophile which could further displace the chloride ion from 6-chloro benzoxazolinone to get the desired ether product. The reaction was allowed to reflux for about 24-hours. The progress of the reaction was monitored through TLC and after the completion of reaction slight amount of water was added in the reaction mixture which was then extracted multiple times with Di-ethyl-ether. After the extraction the product appeared in the organic layer (Di ethyl ether) which was later evaporated but the residue wasn't pure which was further purified by column-chromatography using

mobile phase Ethyl-acetate and Hexane at ratio 4:6. The purified produce was appeared as pinkish-reddish oil. The reported yield was 45%

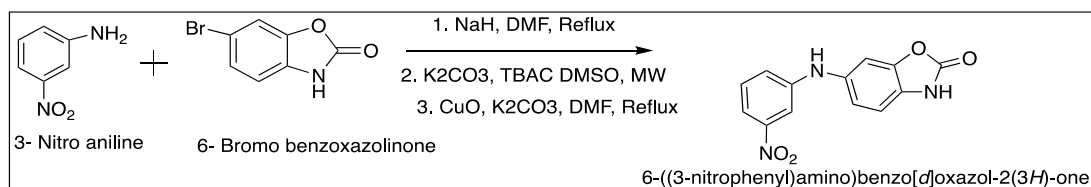
**Method 2:** 2- Amino Phenol (1.1 g, 11.0 mmol) and 6-chloro benzoxazolinone (1.2 g, 7.5 mmol) was mixed with 3 ml of DMSO with slight addition of mild base  $K_2CO_3$  and TBAC (2mmol) to facilitate the reaction. The reaction mixture was exposed to microwave radiation for about 3-4 minute. The work up and isolation procedure was performed as per method-1. The final purified product appeared as pinkish-reddish oil. This method was found to be more effective in terms of yield and no formation of intermediates.



**Figure 40:** TLC's representing the formation of desired product 6-(2-aminophenoxy) benzo[d]oxazol-2(3H)-one.

**6-(2-aminophenoxy)benzo[d]oxazol-2(3H)-one:** Maximum obtained yield: 75%, Pinkish-reddish oil, FTIR ( $cm^{-1}$ ): 1348 (C-O), 1618 (C=C), 1731 (C=O), 2924 (C-H), 3272 ( $2^\circ$  N-H), 3403 ( $1^\circ$  N-H);  $^1H$ -NMR (500 MHz,  $CDCl_3$ ):  $\delta$  5.37 (s, 1H,  $1^\circ$ NH), 7.25 (d,  $J = 10.0$  Hz, 2H, ArH), 7.40 (s, 1H, ArH), 7.43-7.45 (m, 2H, ArH), 7.54 (d,  $J = 10.0$  Hz, 1H, ArH), 8.08 (d,  $J = 10.0$  Hz, 1H, ArH), 9.71 (s, 1H,  $2^\circ$  NH);  $^{13}C$ -NMR (125 MHz, d-DMSO):  $\delta$  109.3, 113.8, 117.6, 119.5, 122.1, 132.1, 141.9, 142.6, 158.7, 168.0; GC MS (m/z) 242 [M+].

### 5.2.14: Synthesis of 6-((3-nitrophenyl)amino) benzo-[d] oxazol-2-(3H)-one (DP422):



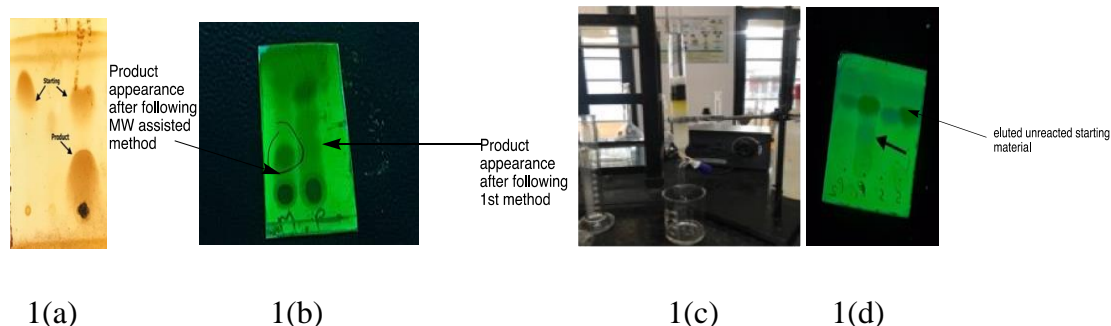
For the synthesis series 4 Compounds we tried 3 different methods to produce good yield. Goldberg-type catalyst based C–N coupling reaction, Williamson’s coupling, and Microwave assisted methods were employed to yield desired product. The appearance of product from three different methods is mentioned in figure 41.

**Method-1:** Based on the principle of Williamson’s coupling; 3-nitroaniline (1 g, 7.8 mmol) was first reacted with a base NaH in the presence of solvent DMF in order to produce deprotonated amine (Nucleophile) which was further reacted with 6-Bromo benzoxazolinone (1.3 g, 7.0 mmol). After the gradual addition of 6-bromo benzoxazolinone, the reaction was refluxed at 180°C for the duration of 24-hours. After that the reaction mixture was poured on ice cold water and extracted several times with di-ethyl-ether. The TLC ensured the formation of product along with the remaining traces of starting material. The formed product was isolated through Column-chromatography by using Silica gel as adsorbing medium and Hexane and Ethyl-acetate as mobile phase at ratio 6:4. The pure compound was separated as semisolid reddish- yellow semisolid product with 50% yield.

**Method 2:** 3-nitroaniline (0.5 g, 3.6 mmol) was reacted with 6-Bromo-2-benzimidazolinone (0.51 g, 2.6 mmol) in DMF along with slight addition of base K<sub>2</sub>CO<sub>3</sub> (0.2 g, 2.0 mmol) and a catalyst CuI (0.07 g, 0.4 mmol). The reaction was refluxed for 24 h at 180°C. After the completion, the reaction mixture was extracted multiple times with diethyl ether and the organic layer was allowed to evaporate. The evaporated residue was further loaded into the column for purification using mobile phase ethyl acetate and hexane (1:1). The purified product appeared as reddish-yellow oil with 65% yield.



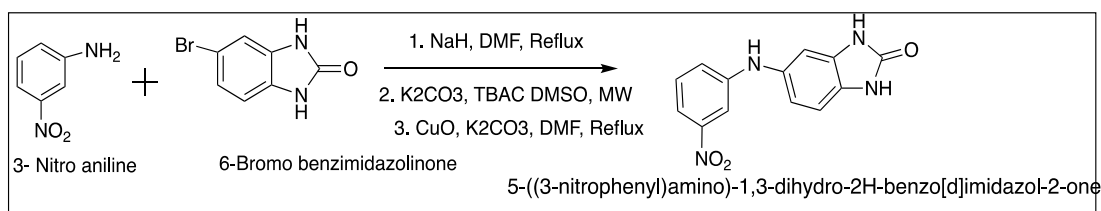
**Method 3:** 3-nitroaniline (0.6 g, 5.0 mmol) was reacted with 6-Bromo-2-benzimidazolinone (0.79 g, 4.0 mmol) in DMSO along with slight addition of mild base  $K_2CO_3$  (0.5 g, 4.0 mmol) and tetrabutylammonium chloride (TBAC) (0.34 g, 3.0 mmol). All the reactants were exposed to microwave radiation for about 4 min. The final pure product was isolated through column chromatography with a yield of 70%.



**Figure 41:** **1(a)** Represents the formation of product through Goldberg method, **1(b)** Showing the comparison of product appearance from Microwave and Williamsons method **1(c)** represents the isolation technique followed. **1(d)** Shows the elution of untreated starting material from the column.

**6-((3-nitrophenyl)amino) benzo-[d] oxazol-2 (3H) -one:** Yield: 70%; reddish brown gel; FT-IR (KBr  $cm^{-1}$ ): 1521( $NO_2$ , asymmetrical), 1345 ( $NO_2$ , symmetrical), 1617 (C=C), 1766 (C=O), 3089 (C-H), 3260 (N-H);  $^1H$ -NMR (500 MHz,  $CDCl_3$ ):  $\delta$  7.36 – 7.38 (m, 2H, ArH), 7.43 -7.46 (m, 1 H, ArH), 7.50 – 7.53 (m, 1 H, ArH), 7.96 – 8.01 (m, 2H, ArH), 8.38- 8.39 (m, 1 H, ArH), 8.45 (s, 1 H, N-H), 9.89 (s, 1-H, N-H);  $^{13}C$  NMR (125 MHz,  $CDCl_3$ ):  $\delta$  109.6, 111.9, 114.6, 117.5, 119.8, 125.6, 127.0, 128.6, 130.1, 138.5, 143.0, 158.7; GC MS (m/z) at 270.

### 5.2.15: Synthesis of 5-((3-nitrophenyl)amino)-1,3-dihydro-2H-benzo-imidazol-2-one (DP442):



The desired product was obtained by reacting 3-nitro aniline with 6-Bromo Benzimidazolinone by applying 3 different methods. The appearance of the product is mentioned in Figure 42.

**Method-1:** 3-nitroaniline (1.0g, 7.7mmol) was reacted with 6 bromo Benzimidazolinone (1.3 g, 7.0 mmol). in the presence of DMF. The reaction was allowed to reflux for about 22-24 hours. The progress of the reaction was monitored through TLC and after the completion of reaction, slight amount of water was added in the reaction mixture which was then extracted multiple times with di-ethyl-ether. Thereafter, the product appeared in the organic layer which was later evaporated but the residue was not pure which was further purified through column chromatography by using mobile phase ethyl-acetate and hexane. The pure spot of product obtained at mobile phase ratio (5:5, Ethyl acetate: Hexane). The purified product was appeared as reddish yellow oil with yield 40%.

**Method 2:** 3-nitro aniline (1.0g, 7.7mmol) was reacted with 6 bromo Benzimidazolinone (1.3 g, 7.0 mmol). in the presence of solvent DMSO along with slight addition of mild base  $K_2CO_3$  and TBAC to facilitate the rate of reaction. All the reactants were exposed to microwave radiation for about 4 minutes. The progress of the reaction was checked through TLC in between. The work up and purification process was followed as per Method-1. This method was observed as effective, time saving and high yield of the product was achieved. i.e. 72%. Although it has not changed the appearance of the product.

**Method-3:** 3-nitro aniline (1.0 g, 7.7 mmol) was reacted with 6-bromo Benzimidazolinone (1.3 g, 7.0 mmol) in the presence of Solvent DMF along with slight addition of base  $K_2CO_3$  and a catalyst CuI was added (0.4 mmol). The reaction was refluxed for 24 hours at 180°C. The progress of the reaction was monitored through TLC which was detected through UV cabinet. After the completion of reaction, the reaction mixture was extracted multiple times with di ethyl ether and the organic layer was allowed to evaporate. The evaporated residue was further loaded into the column for purification using mobile phase Ethyl acetate and Hexane at ratio 5:5. The purified product was appeared as reddish-yellow oil with 65% yield.



**Figure 42:** TLC's representing the formation of desired product 5 ((3-nitrophenyl) amino) -1,3- di-hydro-2H- benzo- imidazole -2- one which was detected through Iodine chamber.

**5-((3-nitrophenyl)amino)-1,3-dihydro-2H-benzoimidazol-2-one:** Yield: 72%; reddish yellow oil; FT-IR (KBr  $\text{cm}^{-1}$ ): 1526 ( $\text{NO}_2$ , asymmetrical), 1348 ( $\text{NO}_2$ , symmetrical), 1598 (C=C), 1665 (C=O), 2925 (C-H), 3325 (N-H),  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  6.78 (d,  $J = 10.0$  Hz, 1 H, ArH), 7.02 (d,  $J = 10.0$  Hz, 1H, ArH), 7.10 (d,  $J = 10.0$  Hz, 1H, ArH), 7.14 (d,  $J = 10.0$  Hz, 1H, ArH), 7.16-7.18 (m, 2H, ArH), 7.20 (d, 1H,  $J = 10.0$  Hz, ArH), 7.37 (s, 1H, ArH), 9.61 (s, 2H, NH);  $^{13}\text{C NMR}$  (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  101.4, 110.0, 112.1, 116.6, 126.3, 129.8, 132.1, 143.9, 153.9, 155.1; MS  $m/z$  at 271 [ $\text{M}^+$ ].

**5.3: Description of *In-silico* toxicity prediction:** The synthesized molecules were analysed to check if there is any toxicity associated issue in terms of carcinogenicity and mutagenicity. For the same, particular compound SMILES was copied from Chem Draw Professional 16.0 version and used as an input to check their toxicity using online available software Lazar toxicity predictor <https://lazar.in-silico.ch/predict>.<sup>128</sup>

**5.4 Description of *In-silico* ADME Prediction:** For the estimation of ADME properties of the synthesized compounds SWISS ADME software was used which is available online at <http://www.swissadme.ch/index.php>. The software provided the complete profile of the synthesized molecules as a calculated value for Physico-chemical parameters like: lipophilicity, water solubility, drug likeness of the molecules following Lipinski rule of five and Pharmacokinetic profile.<sup>129</sup>

## 5.5 Description of *In Vitro* evaluation:

**5.5.1: *In vitro* assay protocol:** For the *In vitro* analysis of twelve synthesized potential antidiabetic compounds, A rat insulin ELISA kit from Abbkines was chosen.<sup>130</sup> For this study, pancreatic islets of the Sprague-Dawley rats were isolated using collagenase IV *Clostridium histolyticum* enzyme purchased from Hi Media. In brief, rats were anaesthetized with chloroform and the pancreas was expanded in 4-5 ml collagenase solution at concentration expanded in collagenase solution 1 mg/ml at 37°C for the duration of 15 minutes. The separated islets were kept in Hanks balanced salt solution (HBSS) without calcium, magnesium and phenol-red and purified through cold centrifugation at 4°C using rpm 1000 for about 1 min which was further filtered through a 70 mm thickness cell-strainer. Finally, the islets were seen under microscope. Immediately after purification, the islets were incubated at 37 °C for 30 min in Krebs Ringer bicarbonate (KRB) buffer solution composed of 3 mM glucose and 0.1% BSA. Thereafter, different batches were prepared for similar sized islets which were later incubated in KRB medium with 3 mM (basal) and 16.7 mM (stimulatory) glucose for 1 hour in the absence and presence of test compounds. At the end of incubation 150-200µL samples from each tubes were immediately frozen until insulin assay. To check the insulin release concentration, Rat Insulin ELISA kit was used. For *in -vitro* insulin secretion assay glibenclamide was utilised as a standard at concentration 100-200 µM.

## 5.5.2: Pre-assay reagents preparations:

**Table 4: Preparation of HBBS buffer without magnesium, calcium and phenol red.**<sup>131</sup>

| S. No. | Components                         | Amount (gm/L) |
|--------|------------------------------------|---------------|
| 1      | Sodium Chloride (NaCl)             | 8 gm          |
| 2      | Potassium chloride (K Cl)          | 0.4 gm        |
| 3      | Sodium phosphate dibasic dihydrate | 0.06 gm       |
| 4      | Potassium phosphate monobasic      | 0.06 gm       |
| 5      | D-Glucose                          | 1 gm          |
| 6      | Sodium bicarbonate                 | 0.35 gm       |
| 7      | Distilled water                    | 1000ml        |

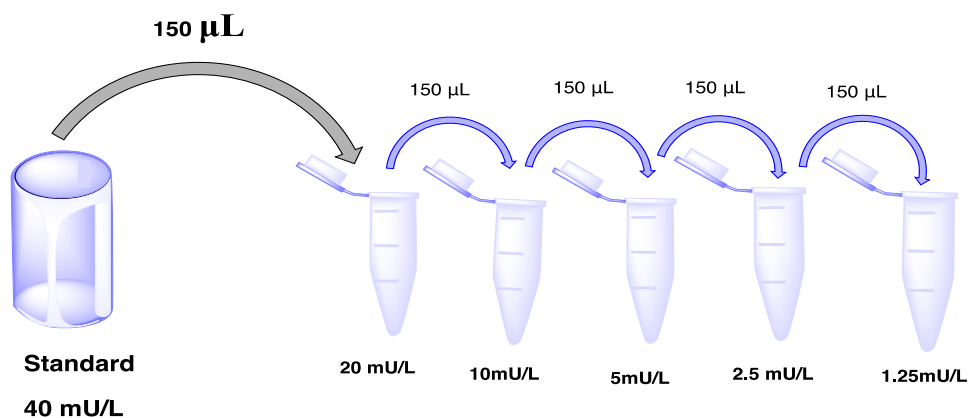
**Table 5: Preparation of KRB buffer containing BSA and HEPES.**<sup>132</sup>

| S. No. | Ingredients (INORGANIC SALTS)         | Amount (mg/L) |
|--------|---------------------------------------|---------------|
| 1      | Disodium hydrogen phosphate anhydrous | 100 mg        |
| 2      | Magnesium chloride hexahydrate        | 223           |
| 3      | Potassium chloride                    | 372 mg        |
| 4      | Sodium chloride                       | 7010 mg       |
| 5      | Sodium dihydrogen phosphate anhydrous | 180 mg        |
| 6      | Bovine serum albumin                  | 2000 mg       |
| 7      | Calcium chloride                      | 367 mg        |
| 8      | HEPES buffer                          | 2380 mg       |

**Reagents preparation:** All the reagents were maintained at room temperature prior to use. In case of crystals formation the buffer concentrates were allowed to warm until the crystals dissolved completely.

**Wash buffer:** For the preparation of wash buffer, distilled water was diluted in the ratio 0.5:15 fold for the preparation of 46 tests.

**Preparation of Standard solutions at different concentrations :** 150  $\mu$ L of standard diluent was added into each tubes. (used this standard solution for the preparation of 2 times dilution series at various concentrations). Each tubes was mixed properly before the next addition whereas the standard remains undiluted and labelled as standard (40 mU/L) as mentioned in figure 43.



**Figure 43:** Preparation of standard solutions at various conc. 20,10,5,2.5 and 1.25 mU/L

### **5.5.3: Rat insulin ELISA assay procedure:**

The ELISA assay was initiated with the addition of diluted standards maintained at different concentrations i.e. 1.25, 2.5, 5, 10 and 20 mU/L into separate wells. Each well was filled with 50  $\mu$ L of standard diluents of different concentrations. Similarly, remaining wells were filled with 40  $\mu$ L of sample diluents and named as sample wells. Thereafter, 10  $\mu$ L amount of 12 different test samples were added at triplicate concentration in different sample wells except the blank well. All the wells were covered with a plate cover provided with the kit and thereafter incubated at 37°C for about 45 minutes. Post incubation, each well was aspirated and washings were given four times for 1-3 minutes each time. The washings were given by the wash buffer (250  $\mu$ L each time) provided with the kit. After the washing, each well was again aspirated to remove any traces of wash buffer. Following the addition of 50  $\mu$ L HRP conjugated detection antibody into each well except the blank. All the wells were covered again and incubated at 37°C for 30 minutes. Later on, each plate was again aspirated and washed for minimum five times with wash buffer. Thereafter, added 50  $\mu$ L Chromogen solution-B into each well which was gently mixed allowed for re-incubating the wells at 37°C for the duration of 15 minutes. At last, added 50  $\mu$ L of stop solution into each well and observed a colour change from blue to yellow. Once the colour turned to yellow, immediately measured the optical absorbance at 450-nm wavelength using a micro-plate ELISA reader and finally recorded the results.

**5.6: Description of *In-vivo* evaluation:** The *in vivo* estimation of synthesised compounds was performed under the IAEC approval number: LPU/IAEC/2021/89.

**5.6.1: Experimental animals:** For this study experimental animals were female Sprague dawley rats of weight 150–250 grams were placed in poly-propylene cages which are maintained at standard conditions (12-hour light/ dark cycles, 25  $\pm$  5°C) in paddy husk beds. A high fat diet model along with single low dose of streptozotocin (35mg/kg of body weight) induces type-II diabetes which causes pancreatic insulinitis as well as resistance to insulin producing diabetes in rats. It is believed that the mechanism behind this is pathogenic involvement of pancreas leading to low insulin

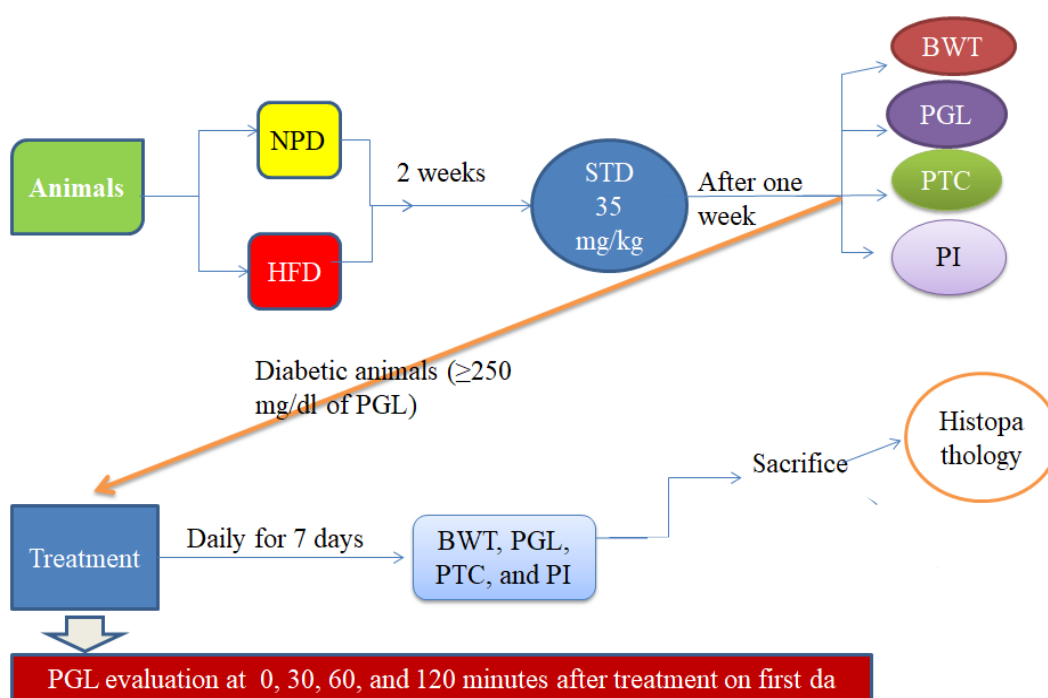
production. This method is very much useful in case of IIDDM type of diabetes or type-II diabetes.

**Steps for Induction of diabetes and treatment:**

1. Diabetes was induced via administering a fresh aqueous medium of Streptozotocin (35 mg/ kg body weight) in phosphate buffer PH 7.4 through intraperitoneal (i.p.) route.
2. Seven days after STZ administration, serum was collected from fasting rats via the vein from the tail for analysis of plasma glucose. fasting glucose level in rats ranged from 301 to 318 mg/d which indicated the clear signs of polyphagia, polyuria, and polydipsia that is the consideration of diabetes.
3. After the induction of diabetes, rats were given the treatment for 7 days through the oral route. Thereafter, Plasma glucose level and Total cholesterol level was checked using commercial available kits. Plasma insulin level was monitored using rat insulin enzyme linked immunosorbent assay kit (ELISA).

**5.6.2: Experimental Procedure:** For this study, insulin resistance type-II diabetes was induced in female Sprague dawley rats fed with High-Fat-Diet (HFD) with low dose of STZ (35mg/kg). The rats were divided into eleven different groups and assigned them two different dietary regimens. Group 1-4 was fed with Normal Pellet diet (NPD) whereas group 5-11 were fed with High Fat diet (HFD) composed of 17% carbohydrate, 25% protein and 58% fat at total kcal percentage respectively, for the initial period of 2 weeks or 15 days.<sup>115</sup> The NPD and HFD rats were separated into following groups i.e. NPD (Vehicle control) , NPD + Synthesized compound-1, DP104 (5mg/kg) , NPD + Synthesized compound-2 DP422 (5mg/kg), NPD + synthesized Coixol (5mg/kg), HFD + Streptozotocin (Negative control), HFD + Streptozotocin + Glibenclamide (10mg/kg) , HFD + STZ + coixol (5mg/kg). HFD + STZ + synthesized compound-1 (low and high dose: 2.5 and 5mg/kg ), HFD + STZ + synthesized compound-2 (low and high dose: 2.5 and 5mg/kg). After day 15<sup>th</sup> (2 weeks of dietary manipulation), the HFD fed rats were administered the minimum

dose of Streptozotocin (35 mg /kg), via intraperitoneal route and NPD fed groups were administered with 0.5% Carboxy Methyl Cellulose as a vehicle. Thereafter 7 days of streptozotocin administration, the rats were analysed for their change in body weight and blood glucose levels using tail pricking method to ensure the induction of disease. On day 22<sup>nd</sup>, The disease induced animals were fed orally as per the treatment protocol mentioned below in table no. 6. Immediately after the treatment started, Blood glucose level and body weight index were again evaluated at 30, 60, 90, and 120 minutes interval after the administration of test compounds to see the acute effect of test compounds. Treatment was continued for the 7 days and thereafter the body weight and biochemical estimations (i.e. blood glucose, insulin and cholesterol) were evaluated on Day 28<sup>th</sup> to check the effect of treatment on diseased rats. Rats were continued on HFD diet till the end of protocol. At the end, rats were sacrificed for the histopathological evaluations.



**Figure 44:** *In Vivo* study plan



**Table 6:** Approved treatment protocol for type-II diabetes model:

| S. No.    | Name of the Groups                   | Diet + Dose and route of drug-treatment                                      | Animals in each group |
|-----------|--------------------------------------|--|-----------------------|
| Group- 1  | Control group (Vehicle)              | NPD* + CMC at 0.5% (Vehicle) (p.o.)  | 6                     |
| Group- 2  | Synthesized molecule-1 <i>per se</i> | NPD + 5 mg/kg  | 6                     |
| Group- 3  | Synthesized molecule-2 <i>per se</i> | NPD + 5 mg/kg  | 6                     |
| Group- 4  | Synthesized Coixol <i>per se</i>     | NPD + 5 mg/kg  | 6                     |
| Group- 5  | Negative control                     | HFD** + 35 mg/kg of STZ (single dose, i. p.)                                 | 7                     |
| Group- 6  | Positive control-1 (Standard drug)   | HFD + 35 mg/kg of STZ (single dose, i. p.) +10 mg/kg of glibenclamide (p.o.) | 7                     |
| Group- 7  | Positive control-2 (Natural Product) | HFD + 35 mg/kg of STZ (single dose, i. p.) + 5 mg/kg of coixol (p.o.)        | 7                     |
| Group- 8  | Synthesized molecule-1 low dose      | HFD + 35 mg/kg of STZ (single dose, i. p.) + 2.5 mg/kg (p.o.)                | 7                     |
| Group- 9  | Synthesized molecule-1 high dose     | HFD + 35 mg/kg of STZ (single dose, i. p.) + 5 mg/kg (p.o.)                  | 7                     |
| Group- 10 | Synthesized molecule-2 low dose      | HFD + 35 mg/kg of STZ (single dose, i. p.) + 2.5 mg/kg (p.o.)                | 7                     |
| Group- 11 | Synthesized molecule-2 high dose     | HFD + 35 mg/kg of STZ (single dose, i. p.) + 5 mg/kg (p.o.)                  | 7                     |
|           |                                      | Total  | 73                    |

\*NPD = Normal Pellet Diet

\*\*HFD = High Fat Diet

**5.6.3 Parameters of evaluation:** Following parameters were evaluated during the entire protocol to monitor the effect of the test compounds and response of rats in insulin resistance type-II diabetes.

**5.6.3.1: Description of Body weight:** Animals were measured for their respective body weights on 1<sup>st</sup>, 15<sup>th</sup>, 22<sup>nd</sup> and 28<sup>th</sup> day of protocol respectively.

**5.6.3.2: Description of Biochemical Estimation:**

**A. Plasma glucose level:** This test is used to evaluate the level of glucose after its oxidative conversion to gluconic acid and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The density of pink colour indicates the concentration of glucose present in the blood. Plasma glucose level was evaluated using enzymatic method called GOD-POD by using a commercial available kit.<sup>133</sup> The level of available glucose in the blood was evaluated on Day 22<sup>nd</sup> and Day 28<sup>th</sup> of the study as per the mentioned procedure in the kit. In detail, 10µL of plasma was collected from each rats which was mixed with the glucose oxidase peroxidase reagent (GOD-POD).The blank, standard and tests solutions were taken separately in three different test tubes following the addition of Glucose oxidase peroxidase reagent into all the test tubes for the estimation of glucose level as mentioned in Table no. 7. All the mixtures were finally incubated for nearly 30 minutes at 37°C that allowed the change in colour in standard and test solutions which optical density was further measured by calorimetry at 510nm wavelength for the estimation of glucose level present in Test solutions. The absorbances of the test and standard samples were observed in comparison to the reagent blank.

**Each sample was performed in triplicate concentration.**

**Table 7:** Preparation of sample for GOD/POD method of glucose estimation:

|                                 | <b>Blank</b> | <b>Standard</b> | <b>Test</b> |
|---------------------------------|--------------|-----------------|-------------|
| GOD POD test reagent            | 1000 µL      | 1000 µL         | 1000 µL     |
| Distilled water (Blank)         | 10 µL        | --              | --          |
| Glucose (Standard) at 200 mg/dl | --           | 10 µL           | --          |
| Test solution                   | --           | --              | 10 µL       |

Calculation of glucose concentration:  $\text{Glucose (mg/dl)} = (\text{Absorbance of test} / \text{Absorbance of Standard}) \times 200 \text{ mg/dL (Concentration of standard solution)}$

**B. Plasma cholesterol:** This test is used to evaluate the amount of total cholesterol in the blood after the conversion of cholesterol ester to cholesterol and fatty acids in the presence of cholesterol esterase enzyme. The intensity of red colour indicates the concentration of available cholesterol in the blood. For the estimation of total cholesterol, Erba cholesterol kit was utilized.<sup>134</sup> The kit component contains cholesterol reagent and Cholesterol standard at concentration 200 mg/L.

**Assay procedure:** Collected 20µL plasma of each fasted rat which was mixed with working cholesterol reagent for the estimation of cholesterol level in test samples. Similarly, Standard and blank i.e. distilled water was also mixed separately with working cholesterol reagent. All the reagents were mixed appropriately and incubated at room temperature for 10 minutes. Aspirated blank, standard and test samples separately into the flow cell and recorded the absorbance at 505 nm. The absorbances of the test and standard samples were recorded in comparison to the blank.

All values were carried in triplicates:

**Table 8:** Preparation of sample for Cholesterol estimation:

|                         | <b>Blank</b> | <b>Standard</b> | <b>Test</b> |
|-------------------------|--------------|-----------------|-------------|
| Cholesterol reagent     | 1000 µL      | 1000 µL         | 1000 µL     |
| Distilled water (Blank) | 20 µL        | --              | --          |
| Standard                | --           | 20 µL           | --          |
| Test solution           | --           | --              | 20 µL       |

Calculation for plasma cholesterol:  $\text{Cholesterol (mg/dL)} = (\text{Absorbance of test} / \text{Absorbance of standard}) \times 200; \text{mg/dL (Standard Concentration)}$

**C. Plasma insulin concentration:** To evaluate plasma insulin level, rats plasma was collected on Day 22<sup>nd</sup> and 28<sup>th</sup> respectively to check the level of insulin in diabetic rats before and after the treatment.

**Assay Procedure:** Plasma was collected from each rat using EDTA as an anticoagulant. Within 30 minutes of plasma collection, All the samples were allowed to cold centrifugation for 15 minutes at 4°C with approximately 1000rpm. The supernatant plasma layer was recovered and performed the ELISA assay immediately by following the similar procedure discussed earlier in *in vitro* assay protocol.

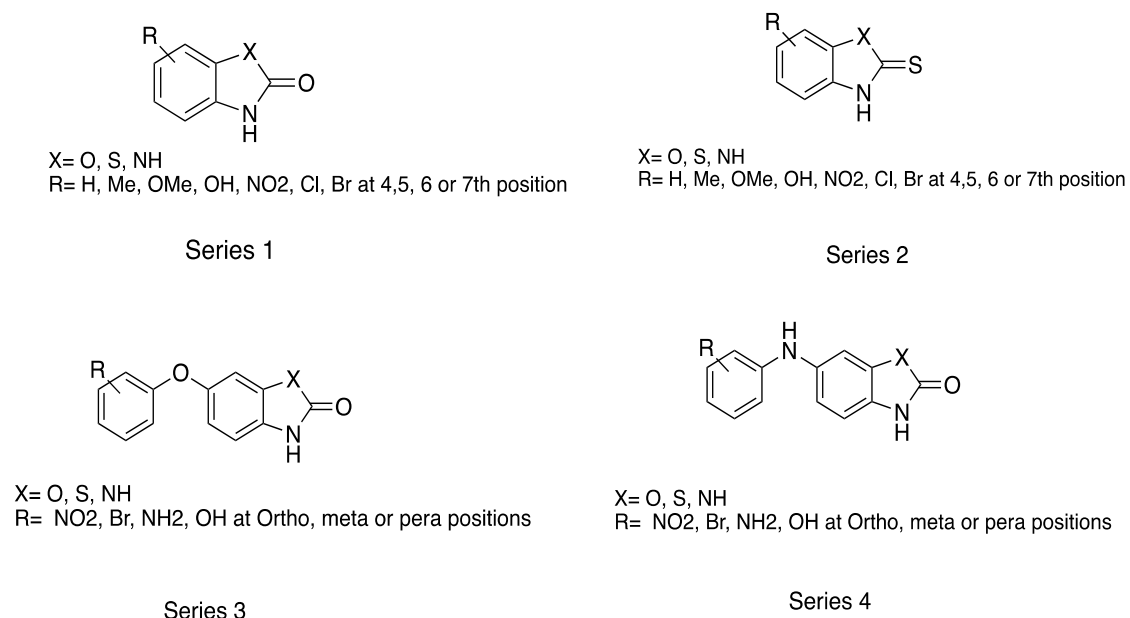
**5..6.3.3. Description of Histopathological evaluation:** The pancreases of S.D. rats were collected after the sacrifice of rats from each groups and stored in 10 % formalin solution immediately after the dissection to avoid tissue drying or damage. Slides were prepared and stained with haematoxylin and eosin.<sup>135</sup> Histopathological examination was by a performed by a pathologist at Gargi Diagnostic Laboratory, Jalandhar.

**5.6.4 Description of Statistical Calculations :** The results of *in vitro* and *in vivo* evaluation were analysed as mean±SD. The statistics data analysis was performed for behavioural studies and Biochemical data by applying One-way ANOVA method via Tukey-test ( Sigma-Stat-Software, 4.0). The significant difference were found at 5% and 1% and 0.1 % level ( $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ ).

## CHAPTER 6

### RESULT AND DISCUSSION

**6.1 Molecular docking:** After the exclusive molecular docking studies, we selected specific heterocyclic nucleus on the basis of their potency and synthesized their derivatives. As follows: Series 1 having benzoxazolinone and Benzimidazolinone derivatives, Series 2 having Benzimidazole-thiones derivatives, Series-3 having substituted aromatic phenols with the benzoxazolinone nucleus and Series-4 having substituted aromatic amines with benzoxazolinone and Benzimidazolinone nucleus as mentioned in Figure 34.



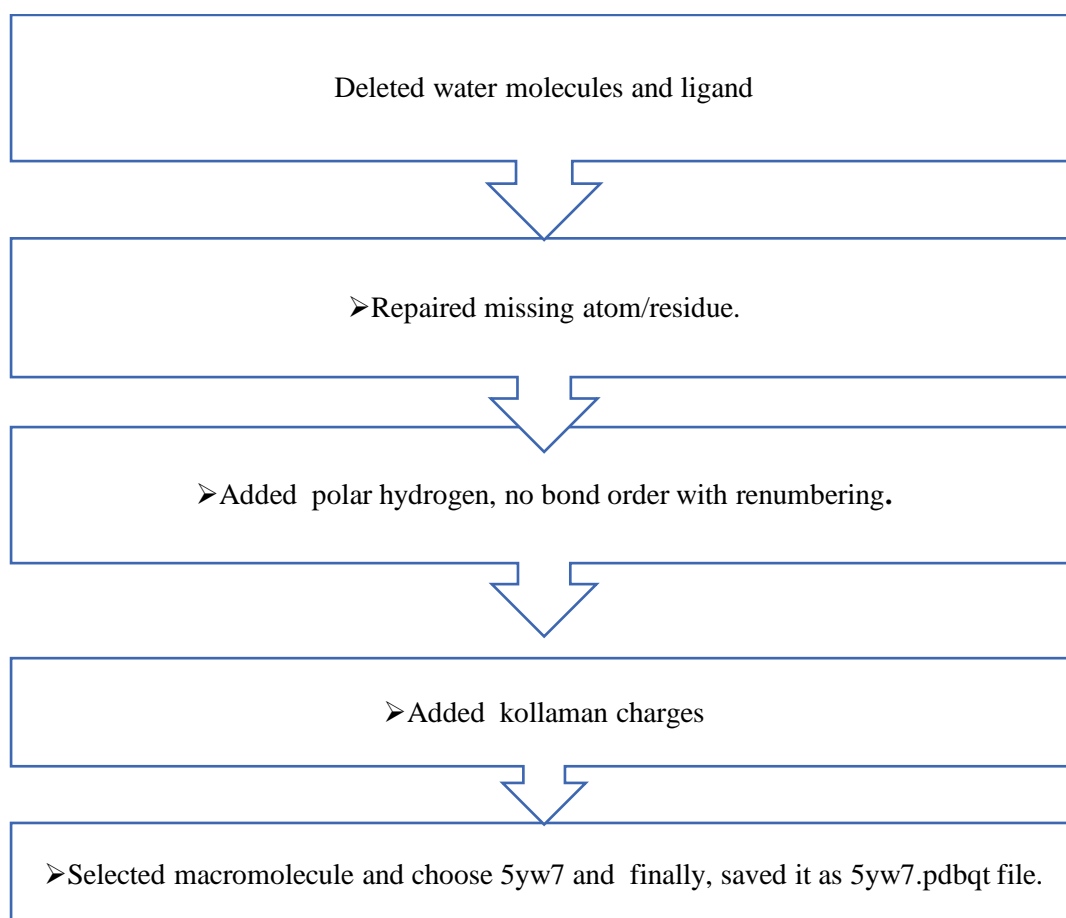
**Figure 45: Designed Coixol like molecules in series 1,2,3 and 4 as Insulin secretagogues.**

To determine most potential molecules towards K<sup>+</sup> ATPase channel receptors as Insulin secretagogues Auto dock vina software was used. For molecular docking studies, A pancreatic ATP-sensitive potassium channel receptor was downloaded from protein data bank. PDB file of 5yw7 receptor was used to evaluate all the designed compounds with Coixol and Glibenclamide as standard drug. All the designed molecular structure were drawn using Chem Bio draw software. A step wise discussion on molecular docking is given below:

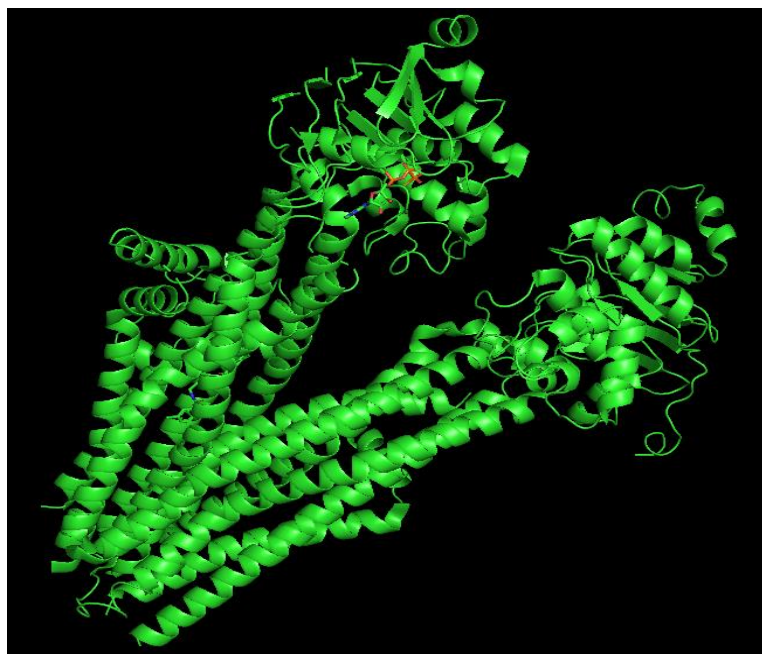
### 6.1.1 Preparation of Protein 5yw7 for Molecular docking studies:

The Auto Dock Vina Tool-1.5.6 version and PyMOL were used for the Molecular docking studies and visualization of protein (figure 47). The structures of molecules were designed using Chem Draw 16.0 and Chem Bio draw 3D for the conversion of the structures into 3D and energy minimization purpose using MM2 interface. All the files were saved in PDB extension format that was readable at Auto dock vina interface to check any issue with the ligand or protein PDB file, such as missing atoms or bonds and to remove any irrelevant structure such as water molecules. Careful examination of protein PDB file is done before the molecular docking studies to remove any irrelevant molecule or structure present in it and kept only the protein and its cofactors bounded to it naturally.

Following steps for preparing the Protein 5yw7 for molecular docking studies:



**Figure 46:** Flow chart for Protein preparation and validation in Auto dock vina

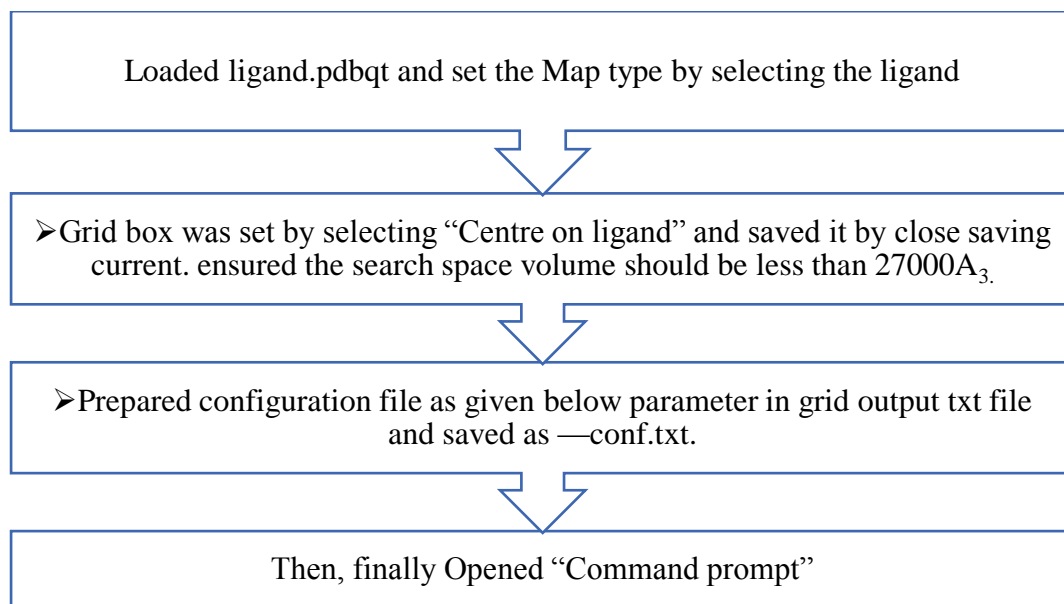


**Figure 47:** Visualization of Ribbon Structure of pancreatic ATP-sensitive potassium channel bounded receptor with glibenclamide (5yw7) using PyMOL

### 6.1.2: Docking and Validation of selected protein 5yw7:

Validation was performed by docking the internal ligand Glibenclamide with 5yw7 protein itself (A pancreatic ATP-sensitive potassium channel receptor) as showed in Figure no. 50 with good overlay and least rmsd value. 5yw7 is a hetero-octameric membrane protein complexes distribute on pancreatic beta cells and responsible for regulation of insulin release. Upon binding of glibenclamide or glyburide, it inhibit  $K^+$ ATPase channel and release insulin<sup>136</sup>. The same model was used for further evaluation of newly designed coixol based molecules from series 1-4 and compared with GBM and coixol as shown in **Table 9-12**. Configuration file “conf.txt” was prepared for Auto dock Vina molecular docking, command prompt was used by giving command ‘C:\users\intel>intel>cd “downloads

C:\users\intel\docking “\program files (x86)\the Scripps research institute\vina\vina.exe” –help C:\users\intel\dock2 “\program files (x86)\the Scripps research institute\vina\vina.exe” config ;conf. txt – log log. txt”and thus, generated the output file including the binding-score or interaction-affinity (Kcal/mol).



**Figure 48:** Flow chart of configuration file preparation procedure in Auto dock vina

conf - Notepad

```

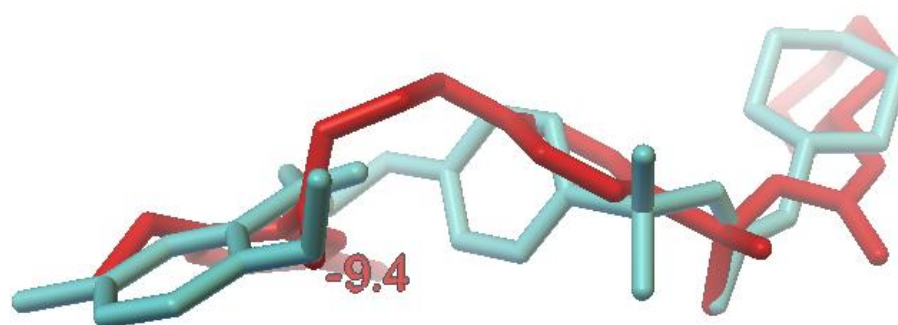
File Edit Format View Help
receptor = 5yw7.pdbqt
ligand = ligand.pdbqt
center_x = 199.552
center_y = 220.077
center_z = 179.357

size_x = 30
size_y = 30
size_z = 30
  
```

```

Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -474695376
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
done.
Refining results ... done.
mode | affinity | dist from best mode
      | <kcal/mol> | rmsd l.b. | rmsd u.b.
-----|-----|-----|-----
1     | -9.5      | 0.000    | 0.000
2     | -9.4      | 6.669    | 10.898
3     | -9.1      | 3.733    | 4.461
4     | -9.0      | 4.851    | 8.514
5     | -9.0      | 5.725    | 10.315
6     | -8.9      | 7.137    | 11.302
7     | -8.9      | 3.389    | 8.279
8     | -8.7      | 5.785    | 10.466
9     | -8.6      | 7.184    | 11.394
Writing output ... done.
C:\Users\intel\My Documents\dock>
  
```

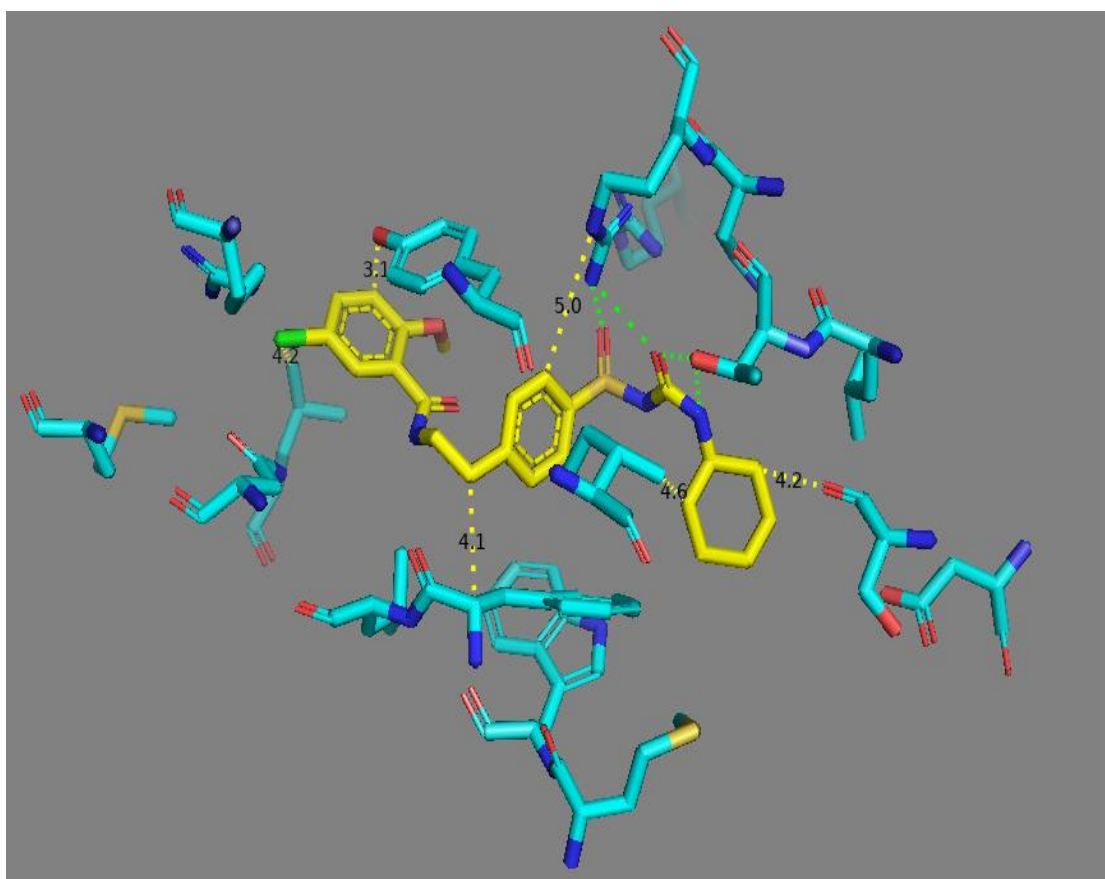
**Figure 49:** Configuration file and a Command Prompt used in Auto Dock Vina



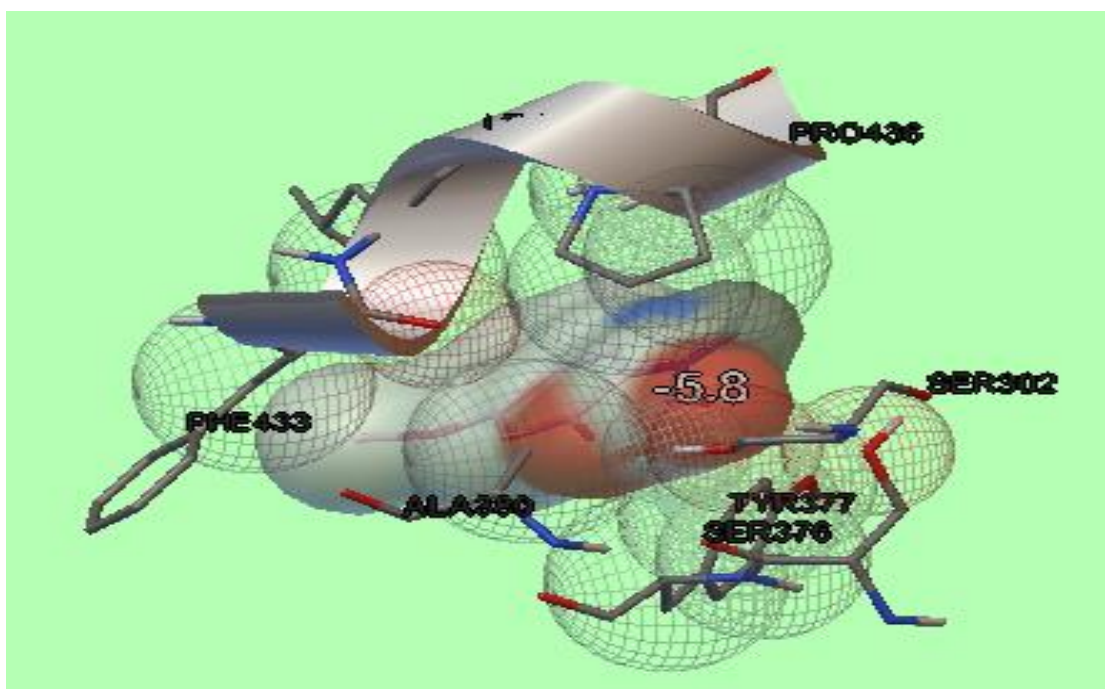
**Figure 50:** Validation of model by overlay of GBM internal ligand (Magenta colour), with docked GBM (Red colour).



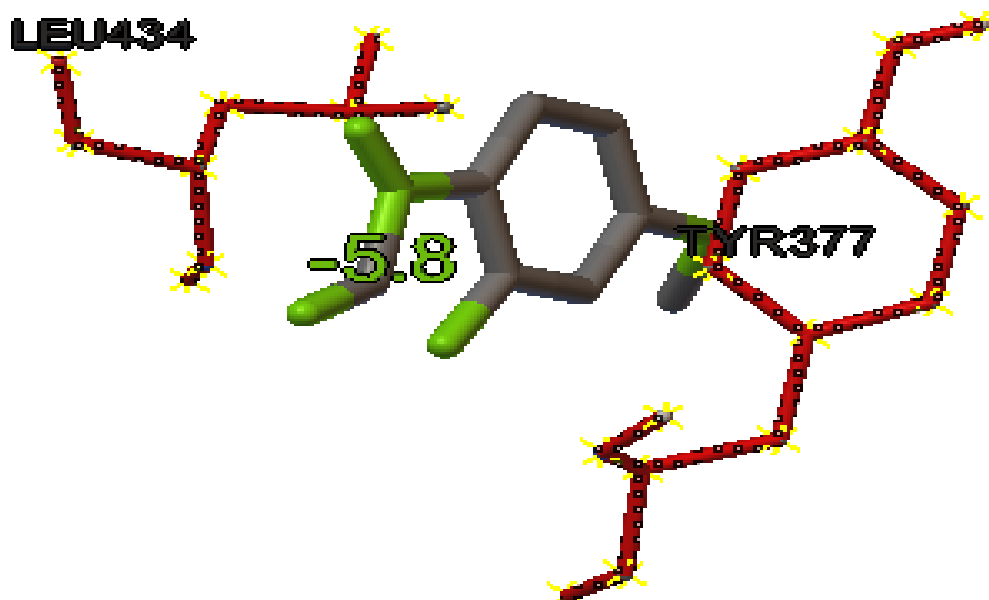
**6.1.3: Observation of potent most molecules via Molecular docking studies:** We examined the interaction of best molecules along with the naturally existing potent molecule Coixol with the selected protein. The Figure 51 Shows the interactions of standard drug Glibenclamide with the membrane receptor 5y7. Figure 52 and 53 represents the interaction of Coixol with the proteins and its neighbouring amino acids . Figure 54, 55, 56 depicted the in depth interaction of DP422 with 5y7 protein. There are four H- bond observed between DP422 and 5y7 as shown in Figure 56. One Hydrogen bond observed in DP442 as shown in Figure 57 and similarly, One hydrogen bond observed in DP322 as shown in Figure 58. Whereas, Figure 59 represents the interaction of ligand DP330 with neighbouring amino acids of the protein 5y7.



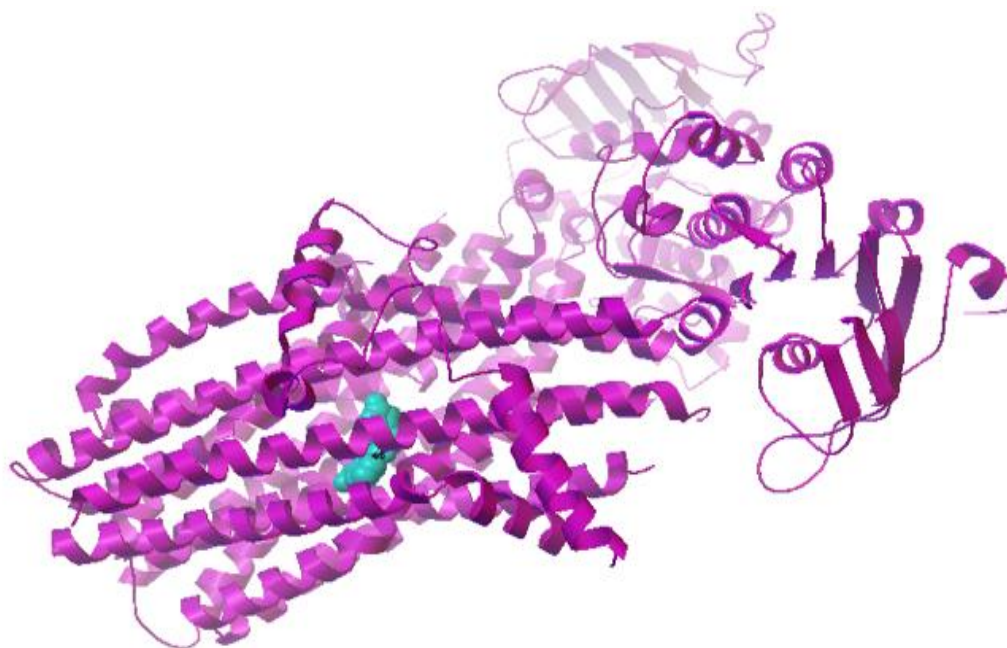
**Figure 51:** Binding orientation of internal ligand GBM showing possible interaction in the limit 5 Å with the amino acid residues of the protein 5y7



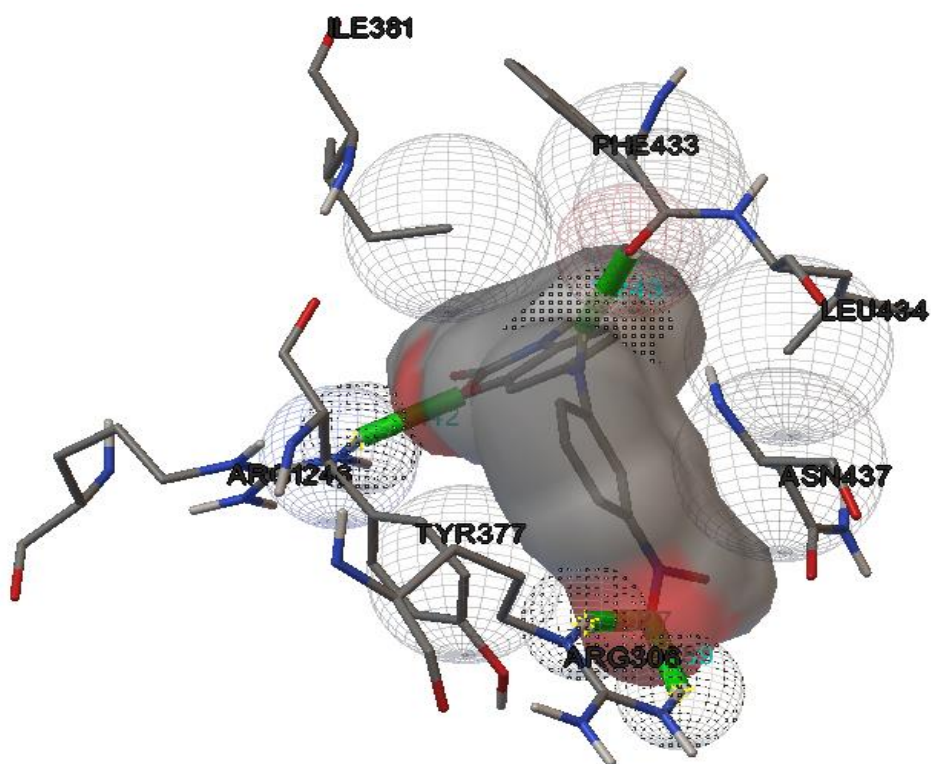
**Figure 52:** Interaction of Coixol with neighbouring amino acid residues of the protein 5yw7.



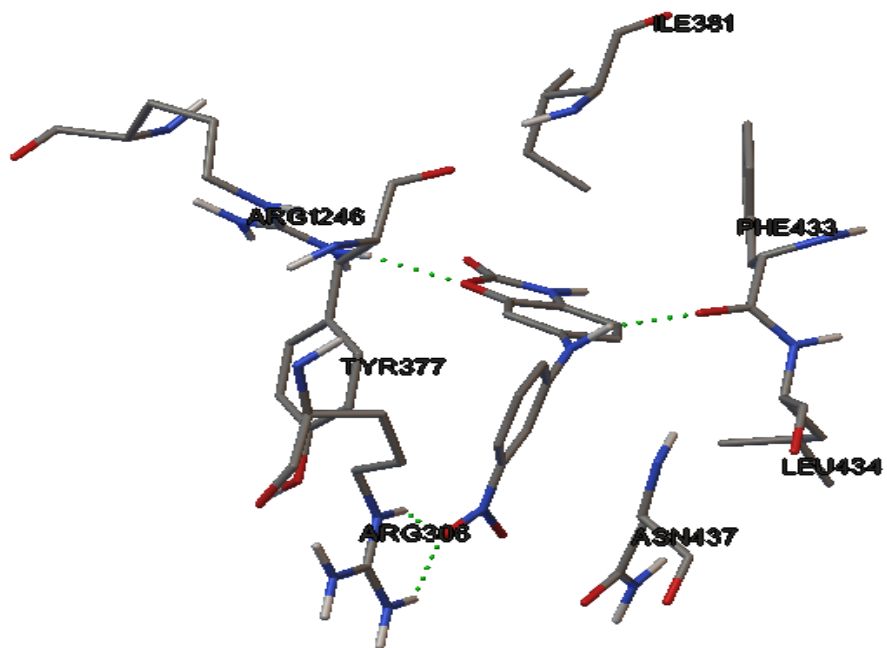
**Figure 53:** Overlay of close contact of Coixol with 5yw7 amino acids residues LEU434 and TYR377.



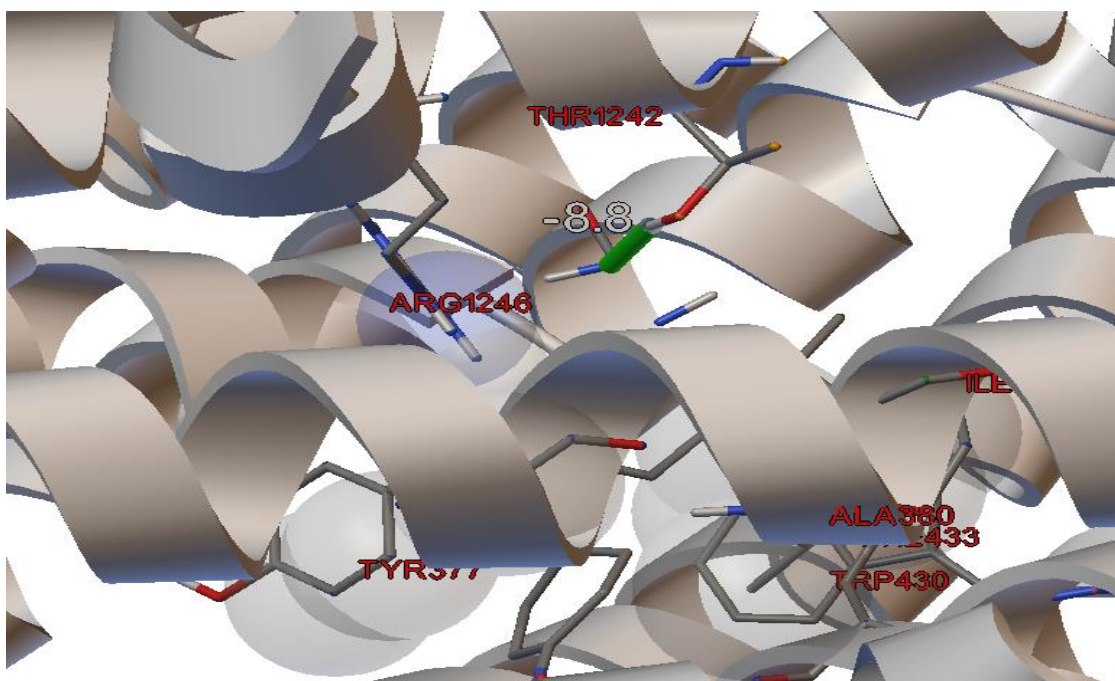
**Figure 54.** Interaction of DP422 (Green colour), with 5y7 (Ribbon structure, pink colour).



**Figure 55:** Interaction of ligand DP422 with protein's neighbouring amino acid residues.



**Figure 56:** Close overlay of nearby contacts and Hydrogen bonds (4-H) of DP422 with neighbouring amino acid residues of 5y7 protein.

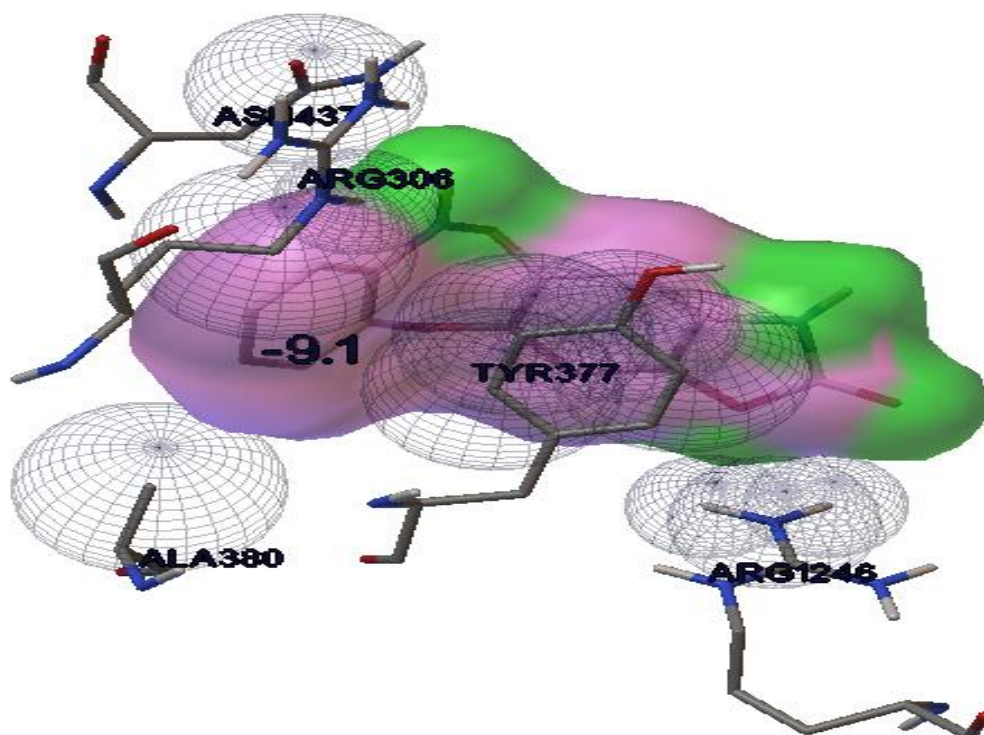


**Figure 57:** Overview of close contacts and Hydrogen bonding (Green colour rod) Of ligand DP442 with neighbouring amino acid residues of 5y7 protein in Ribbon structure.



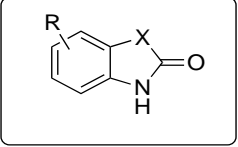


**Figure 58:** Overview of ligand DP322 close contacts with neighbouring amino acid residues and Hydrogen bonding interaction with ARG306 amino acid.



**Figure 59:** Visualization of active sites of protein 5yw7 and close contacts of ligand DP330 with its amino acid residues.

**Table 9** : Binding affinity scores of series 1 designed molecules Benzoxazolinone and Benzimidazolinone with the protein 5yw7.

|  |              |   |                  |                 |                            |
|---|--------------|---|------------------|-----------------|----------------------------|
| S. No   | Code         | X | R                | Position of R   | Affinity score (Kcal/Mole) |
| 1   | DP101        | O | CH <sub>3</sub>  | 6 <sup>th</sup> | -5.8                       |
| 2   | DP102/Coixol | O | OCH <sub>3</sub> |                 | -5.8                       |
| 3   | DP103        | O | OH               |                 | -6.2                       |
| 4   | DP104        | O | NO <sub>2</sub>  |                 | -7.3                       |
| 5   | DP105        | O | Cl               |                 | -6.5                       |
| 6   | DP106        | O | Br               |                 | -6.6                       |
| 7   | DP107        | O | OH               | 4 <sup>th</sup> | -6.1                       |
| 8   | DP108        | O | NO <sub>2</sub>  |                 | -6.8                       |
| 9   | DP109        | O | Cl               |                 | -6.0                       |
| 10  | DP110        | O | Br               |                 | -6.1                       |
| 11  | DP111        | O | CH <sub>3</sub>  |                 | -6.1                       |
| 12  | DP112        | O | OCH <sub>3</sub> |                 | -6.2                       |
| 13  | DP113        | O | OH               | 5 <sup>th</sup> | -6.1                       |
| 14  | DP114        | O | NO <sub>2</sub>  |                 | -5.8                       |
| 15  | DP115        | O | Cl               |                 | -6.3                       |
| 16  | DP116        | O | Br               |                 | -5.8                       |
| 17  | DP117        | O | CH <sub>3</sub>  |                 | -5.9                       |
| 18  | DP118        | O | OCH <sub>3</sub> |                 | -5.9                       |
| 19  | DP119        | O | OH               | 7 <sup>th</sup> | -6.0                       |
| 20  | DP120        | O | NO <sub>2</sub>  |                 | -7.0                       |
| 21  | DP121        | O | Cl               |                 | -6.1                       |
| 22  | DP122        | O | Br               |                 | -6.0                       |
| 23  | DP123        | O | CH <sub>3</sub>  |                 | -6.3                       |

|    |       |     |                  |                 |                 |
|----|-------|-----|------------------|-----------------|-----------------|
| 24 | DP124 | O   | OCH <sub>3</sub> |                 | -6.2            |
| 25 | DP125 | S   | CH <sub>3</sub>  | 6 <sup>th</sup> | -6.0            |
| 26 | DP126 | S   | OCH <sub>3</sub> |                 | -5.8            |
| 27 | DP127 | S   | Cl               |                 | -5.9            |
| 28 | DP128 | S   | Br               |                 | -6.0            |
| 29 | DP129 | S   | OH               |                 | -6.1            |
| 30 | DP130 | S   | NO <sub>2</sub>  |                 | -6.7            |
| 31 | DP131 | S   | CH <sub>3</sub>  |                 | 7 <sup>th</sup> |
| 32 | DP132 | S   | OCH <sub>3</sub> | -6.0            |                 |
| 33 | DP133 | S   | Cl               | -5.9            |                 |
| 34 | DP134 | S   | Br               | -5.8            |                 |
| 35 | DP135 | S   | OH               | -5.9            |                 |
| 36 | DP136 | S   | NO <sub>2</sub>  | -6.1            |                 |
| 37 | DP137 | S   | CH <sub>3</sub>  | 5 <sup>th</sup> |                 |
| 38 | DP138 | S   | OCH <sub>3</sub> |                 | -5.9            |
| 39 | DP139 | S   | Cl               |                 | -5.9            |
| 40 | DP140 | S   | Br               |                 | -6.0            |
| 41 | DP141 | S   | OH               |                 | -6.2            |
| 42 | DP142 | S   | NO <sub>2</sub>  |                 | -6.6            |
| 43 | DP143 | S   | CH <sub>3</sub>  |                 | 4 <sup>th</sup> |
| 44 | DP144 | S   | OCH <sub>3</sub> | -6.0            |                 |
| 45 | DP145 | S   | Cl               | -5.9            |                 |
| 46 | DP146 | S   | Br               | -5.8            |                 |
| 47 | DP147 | S   | OH               | -5.7            |                 |
| 48 | DP148 | S   | NO <sub>2</sub>  | -6.3            |                 |
| 49 | DP149 | -NH | CH <sub>3</sub>  | 6 <sup>th</sup> |                 |
| 50 | DP150 | -NH | OCH <sub>3</sub> |                 | -6.6            |
| 51 | DP151 | -NH | Cl               |                 | -6.8            |
| 52 | DP152 | -NH | Br               |                 | -6.7            |
| 53 | DP153 | -NH | OH               |                 | -6.1            |
| 54 | DP154 | -NH | NO <sub>2</sub>  |                 | -7.1            |

|    |       |     |                  |                 |      |
|----|-------|-----|------------------|-----------------|------|
| 55 | DP155 | -NH | CH <sub>3</sub>  | 7 <sup>th</sup> | -6.3 |
| 56 | DP156 | -NH | OCH <sub>3</sub> |                 | -6.3 |
| 57 | DP157 | -NH | Cl               |                 | -6.1 |
| 58 | DP158 | -NH | Br               |                 | -6.1 |
| 59 | DP159 | -NH | OH               |                 | -6.2 |
| 60 | DP160 | -NH | NO <sub>2</sub>  |                 | -6.7 |
| 61 | DP161 | -NH | CH <sub>3</sub>  | 5 <sup>th</sup> | -5.9 |
| 62 | DP162 | -NH | OCH <sub>3</sub> |                 | -6.1 |
| 63 | DP163 | -NH | Cl               |                 | -5.8 |
| 64 | DP164 | -NH | Br               |                 | -5.8 |
| 65 | DP165 | -NH | OH               |                 | -6.1 |
| 66 | DP166 | -NH | NO <sub>2</sub>  |                 | -6.7 |
| 67 | DP167 | -NH | CH <sub>3</sub>  | 4 <sup>th</sup> | -6.3 |
| 68 | DP168 | -NH | OCH <sub>3</sub> |                 | -6.3 |
| 69 | DP169 | -NH | Cl               |                 | -6.1 |
| 70 | DP170 | -NH | Br               |                 | -6.0 |
| 71 | DP171 | -NH | OH               |                 | -6.2 |
| 72 | DP172 | -NH | NO <sub>2</sub>  |                 | -6.5 |

**Table 10:** Binding affinity scores of series 2 designed molecules Benzimidazole-thiones with the protein 5yw7.

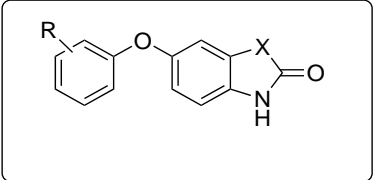
| S. No | Code  | X | R                | Position of R   | Affinity scores (Kcal/mol) |
|-------|-------|---|------------------|-----------------|----------------------------|
| 1     | DP201 | O | CH <sub>3</sub>  | 4 <sup>th</sup> | -6.8                       |
| 2     | DP202 | O | OCH <sub>3</sub> |                 | -5.8                       |
| 3     | DP203 | O | Cl               |                 | -5.8                       |
| 4     | DP204 | O | Br               |                 | -5.8                       |
| 5     | DP205 | O | OH               |                 | -5.7                       |
| 6     | DP206 | O | NO <sub>2</sub>  |                 | -6.4                       |
| 7     | DP207 | O | CH <sub>3</sub>  | 5 <sup>th</sup> | -5.6                       |
| 8     | DP208 | O | OCH <sub>3</sub> |                 | -5.5                       |



|    |       |   |                  |                 |      |
|----|-------|---|------------------|-----------------|------|
| 9  | DP209 | O | Cl               | 6 <sup>th</sup> | -5.4 |
| 10 | DP210 | O | Br               |                 | -5.3 |
| 11 | DP211 | O | OH               |                 | -6.0 |
| 12 | DP212 | O | NO <sub>2</sub>  |                 | -5.7 |
| 13 | DP213 | O | CH <sub>3</sub>  |                 | -5.9 |
| 14 | DP214 | O | OCH <sub>3</sub> |                 | -5.5 |
| 15 | DP215 | O | Cl               |                 | -5.5 |
| 16 | DP216 | O | Br               |                 | -5.9 |
| 17 | DP217 | O | OH               |                 | -6.4 |
| 18 | DP218 | O | NO <sub>2</sub>  |                 | -5.7 |
| 19 | DP219 | O | CH <sub>3</sub>  | 7 <sup>th</sup> | -6.6 |
| 20 | DP220 | O | OCH <sub>3</sub> |                 | -5.8 |
| 21 | DP221 | O | Cl               |                 | -5.6 |
| 22 | DP222 | O | Br               |                 | -6.0 |
| 23 | DP223 | O | OH               |                 | -5.8 |
| 24 | DP224 | O | NO <sub>2</sub>  |                 | -5.7 |
| 25 | DP225 | S | CH <sub>3</sub>  | 4 <sup>th</sup> | -5.7 |
| 26 | DP226 | S | OCH <sub>3</sub> |                 | -5.7 |
| 27 | DP227 | S | Cl               |                 | -5.5 |
| 28 | DP228 | S | Br               |                 | -5.4 |
| 29 | DP229 | S | OH               |                 | -5.5 |
| 30 | DP230 | S | NO <sub>2</sub>  |                 | -6.2 |
| 31 | DP231 | S | CH <sub>3</sub>  | 5 <sup>th</sup> | -5.3 |
| 32 | DP232 | S | OCH <sub>3</sub> |                 | -5.2 |
| 33 | DP233 | S | Cl               |                 | -5.3 |
| 34 | DP234 | S | Br               |                 | -5.3 |
| 35 | DP235 | S | OH               |                 | -6.2 |
| 36 | DP236 | S | NO <sub>2</sub>  |                 | -6.2 |
| 37 | DP237 | S | CH <sub>3</sub>  | 6 <sup>th</sup> | -5.5 |
| 38 | DP238 | S | OCH <sub>3</sub> |                 | -5.5 |
| 39 | DP239 | S | Cl               |                 | -5.3 |
| 40 | DP240 | S | Br               |                 | -5.2 |

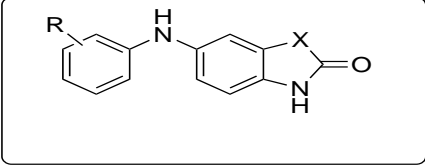
|    |       |     |                  |                 |                 |      |
|----|-------|-----|------------------|-----------------|-----------------|------|
| 41 | DP241 | S   | OH               | 7 <sup>th</sup> | -6.0            |      |
| 42 | DP242 | S   | NO <sub>2</sub>  |                 | -6.2            |      |
| 43 | DP243 | S   | CH <sub>3</sub>  |                 | -6.0            |      |
| 44 | DP244 | S   | OCH <sub>3</sub> |                 | -5.5            |      |
| 45 | DP245 | S   | Cl               |                 | -5.7            |      |
| 46 | DP246 | S   | Br               |                 | -5.5            |      |
| 47 | DP247 | S   | OH               |                 | -6.0            |      |
| 48 | DP248 | S   | NO <sub>2</sub>  |                 | -6.3            |      |
| 49 | DP249 | -NH | CH <sub>3</sub>  | 4 <sup>th</sup> | -6.0            |      |
| 50 | DP250 | -NH | OCH <sub>3</sub> |                 | -5.9            |      |
| 51 | DP251 | -NH | Cl               |                 | -5.9            |      |
| 52 | DP252 | -NH | Br               |                 | -5.6            |      |
| 53 | DP253 | -NH | OH               |                 | -5.8            |      |
| 54 | DP254 | -NH | NO <sub>2</sub>  |                 | -6.8            |      |
| 55 | DP255 | -NH | CH <sub>3</sub>  |                 | 5 <sup>th</sup> | -5.7 |
| 56 | DP256 | -NH | OCH <sub>3</sub> |                 |                 | -5.8 |
| 57 | DP257 | -NH | Cl               | -5.5            |                 |      |
| 58 | DP258 | -NH | Br               | -5.4            |                 |      |
| 59 | DP259 | -NH | OH               | -6.0            |                 |      |
| 60 | DP260 | -NH | NO <sub>2</sub>  | -6.8            |                 |      |
| 61 | DP261 | -NH | CH <sub>3</sub>  | 6 <sup>th</sup> |                 | -5.7 |
| 62 | DP262 | -NH | OCH <sub>3</sub> |                 |                 | -6.1 |
| 63 | DP263 | -NH | Cl               |                 | -6.6            |      |
| 64 | DP264 | -NH | Br               |                 | -5.3            |      |
| 65 | DP265 | -NH | OH               |                 | -6.0            |      |
| 66 | DP266 | -NH | NO <sub>2</sub>  |                 | -6.7            |      |
| 67 | DP267 | -NH | CH <sub>3</sub>  |                 | 7 <sup>th</sup> | -6.0 |
| 68 | DP268 | -NH | OCH <sub>3</sub> |                 |                 | -5.9 |
| 69 | DP269 | -NH | Cl               | -5.9            |                 |      |
| 70 | DP270 | -NH | Br               | -5.0            |                 |      |
| 71 | DP271 | -NH | OH               | -5.8            |                 |      |
| 72 | DP272 | -NH | NO <sub>2</sub>  | -6.7            |                 |      |

**Table 11:** Binding affinity scores of series 3 designed Aryl substituted ether derivatives with the protein 5yw7.

|  |       |   |                  |               |                |
|--|-------|---|------------------|---------------|----------------|
| S. No  | Code  | X | R                | Position of R | Affinity score |
| 1  | DP301 | S | CH <sub>3</sub>  | <b>Ortho</b>  | -7.7           |
| 2  | DP302 | S | OCH <sub>3</sub> |               | -7.6           |
| 3  | DP303 | S | OH               |               | -7.6           |
| 4  | DP304 | S | NO <sub>2</sub>  |               | -7.4           |
| 5  | DP305 | S | Cl               |               | -7.6           |
| 6  | DP306 | S | Br               |               | -7.8           |
| 7  | DP307 | S | NH <sub>2</sub>  |               | -7.9           |
| 8  | DP308 | S | CH <sub>3</sub>  | <b>meta</b>   | -7.4           |
| 9  | DP309 | S | OCH <sub>3</sub> |               | -7.6           |
| 10   | DP310 | S | OH               |               | -8.3           |
| 11   | DP311 | S | NO <sub>2</sub>  |               | -7.6           |
| 12   | DP312 | S | Cl               |               | -7.4           |
| 13   | DP313 | S | Br               |               | -7.2           |
| 14   | DP314 | S | NH <sub>2</sub>  |               | -8.1           |
| 15   | DP315 | S | CH <sub>3</sub>  | <b>para</b>   | -7.5           |
| 16   | DP316 | S | OCH <sub>3</sub> |               | -7.5           |
| 17   | DP317 | S | OH               |               | -7.6           |
| 18   | DP318 | S | NO <sub>2</sub>  |               | -7.8           |
| 19   | DP319 | S | Cl               |               | -7.4           |
| 20   | DP320 | S | Br               |               | -7.5           |
| 21   | DP321 | S | NH <sub>2</sub>  |               | -8.3           |
| 22   | DP322 | O | NO <sub>2</sub>  | <b>meta</b>   | <b>-8.3</b>    |
| 23   | DP323 | O | OH               |               | -8.1           |
| 24   | DP324 | O | Br               |               | -8.2           |
| 25   | DP325 | O | Cl               |               | -8.0           |
| 26   | DP326 | O | NH <sub>2</sub>  |               | -8.2           |

|    |       |          |                 |              |             |
|----|-------|----------|-----------------|--------------|-------------|
| 27 | DP327 | O        | CH <sub>3</sub> |              | -7.9        |
| 28 | DP328 | O        | OH              | <b>ortho</b> | -8.2        |
| 29 | DP329 | <b>O</b> | Br              |              | -8.6        |
| 30 | DP330 | O        | NH <sub>2</sub> |              | <b>-9.1</b> |
| 31 | DP331 | O        | NO <sub>2</sub> |              | -8.4        |
| 32 | DP332 | O        | CH <sub>3</sub> |              | -7.9        |
| 33 | DP333 | O        | Cl              |              | -8.4        |
| 34 | DP334 | O        | OH              |              | <b>para</b> |
| 35 | DP335 | O        | Br              | -8.1         |             |
| 36 | DP336 | <b>O</b> | NH <sub>2</sub> | -8.3         |             |
| 37 | DP337 | O        | NO <sub>2</sub> | -8.4         |             |
| 38 | DP338 | O        | CH <sub>3</sub> | -7.8         |             |
| 39 | DP339 | O        | Cl              | -8.3         |             |
| 40 | DP340 | -NH      | NH <sub>2</sub> | <b>Ortho</b> |             |
| 41 | DP341 | -NH      | CH <sub>3</sub> |              | -7.9        |
| 42 | DP342 | -NH      | OH              |              | -8.0        |
| 43 | DP343 | -NH      | NO <sub>2</sub> |              | -7.9        |
| 44 | DP344 | -NH      | Cl              |              | -7.7        |
| 45 | DP345 | -NH      | Br              |              | -7.9        |
| 46 | DP346 | -NH      | NH <sub>2</sub> | <b>Meta</b>  | -8.0        |
| 47 | DP347 | -NH      | CH <sub>3</sub> |              | -7.7        |
| 48 | DP348 | -NH      | OH              |              | -8.3        |
| 49 | DP349 | -NH      | NO <sub>2</sub> |              | -8.1        |
| 50 | DP350 | -NH      | Cl              |              | -7.8        |
| 51 | DP351 | -NH      | Br              |              | -7.8        |
| 52 | DP352 | -NH      | NH <sub>2</sub> | <b>para</b>  | -7.9        |
| 53 | DP353 | -NH      | CH <sub>3</sub> |              | -7.5        |
| 54 | DP354 | -NH      | OH              |              | -8.0        |
| 55 | DP355 | -NH      | NO <sub>2</sub> |              | -8.0        |
| 56 | DP356 | -NH      | Cl              |              | -7.6        |
| 57 | DP357 | -NH      | Br              |              | -7.6        |

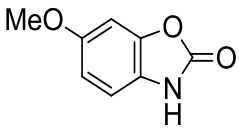
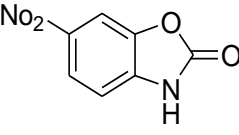
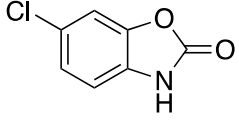
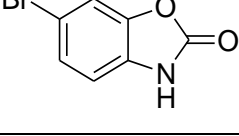
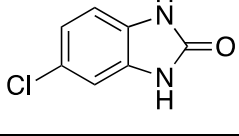
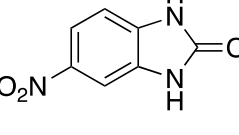
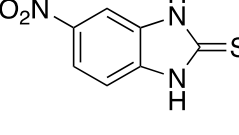
**Table 12:** Binding affinity scores of series 4 designed Aryl substituted amine derivatives with the protein 5yw7.

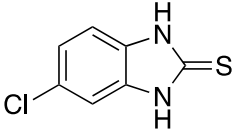
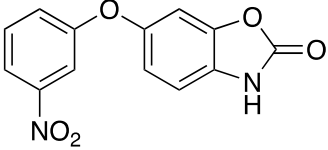
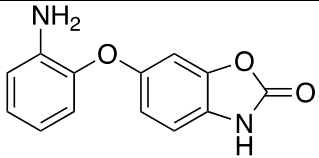
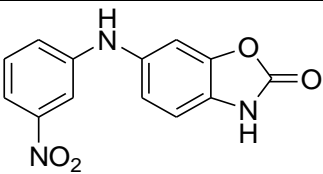
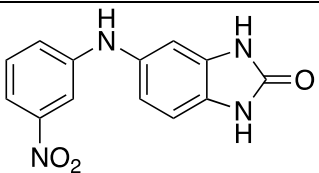
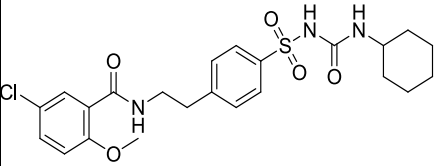
|  |       |   |                  |               |                |
|--|-------|---|------------------|---------------|----------------|
| S. No  | Code  | X | R                | Position of R | Affinity score |
| 1  | DP401 | S | CH <sub>3</sub>  | <b>meta</b>   | -7.9           |
| 2  | DP402 | S | OCH <sub>3</sub> |               | -7.7           |
| 3  | DP403 | S | OH               |               | -7.5           |
| 4  | DP404 | S | NO <sub>2</sub>  |               | -8.8           |
| 5  | DP405 | S | Cl               |               | -7.8           |
| 6  | DP406 | S | Br               |               | -7.7           |
| 7  | DP407 | S | CH <sub>3</sub>  | <b>ortho</b>  | -7.5           |
| 8  | DP408 | S | OCH <sub>3</sub> |               | -7.7           |
| 9  | DP409 | S | OH               |               | -8.1           |
| 10   | DP410 | S | NO <sub>2</sub>  |               | -8.2           |
| 11   | DP411 | S | Cl               |               | -7.3           |
| 12   | DP412 | S | Br               |               | -7.4           |
| 13   | DP413 | S | CH <sub>3</sub>  | <b>para</b>   | -7.6           |
| 14   | DP414 | S | OCH <sub>3</sub> |               | -7.3           |
| 15   | DP415 | S | OH               |               | -7.2           |
| 16   | DP416 | S | NO <sub>2</sub>  |               | -8.5           |
| 17   | DP417 | S | Cl               |               | -7.3           |
| 18   | DP418 | O | CH <sub>3</sub>  |               | <b>meta</b>    |
| 19   | DP419 | O | OCH <sub>3</sub> | -8.2          |                |
| 20   | DP420 | O | OH               | -8.4          |                |
| <b>21</b>  | DP421 | O | Cl               | -7.7          |                |
| 22   | DP422 | O | NO <sub>2</sub>  | <b>-9.5</b>   |                |
| 23   | DP423 | O | Br               | -7.7          |                |

|    |       |     |                  |              |             |
|----|-------|-----|------------------|--------------|-------------|
| 24 | DP424 | O   | CH <sub>3</sub>  | <b>ortho</b> | -7.8        |
| 25 | DP425 | O   | OCH <sub>3</sub> |              | -8.1        |
| 26 | DP426 | O   | OH               |              | -8.5        |
| 27 | DP427 | O   | Cl               |              | -7.7        |
| 28 | DP428 | O   | NO <sub>2</sub>  |              | -8.6        |
| 29 | DP429 | O   | Br               |              | -7.7        |
| 30 | DP430 | O   | CH <sub>3</sub>  |              | <b>Para</b> |
| 31 | DP431 | O   | OCH <sub>3</sub> | -7.6         |             |
| 32 | DP432 | O   | OH               | -7.6         |             |
| 33 | DP433 | O   | NO <sub>2</sub>  | -8.7         |             |
| 34 | DP434 | O   | Cl               | -7.7         |             |
| 35 | DP435 | O   | Br               | -7.7         |             |
| 36 | DP436 | -NH | NO <sub>2</sub>  | <b>ortho</b> |             |
| 37 | DP437 | -NH | CH <sub>3</sub>  |              | -7.9        |
| 38 | DP438 | -NH | OCH <sub>3</sub> |              | -7.8        |
| 39 | DP439 | -NH | OH               |              | -8.0        |
| 40 | DP440 | -NH | Cl               |              | -7.9        |
| 41 | DP441 | -NH | Br               |              | -7.9        |
| 42 | DP442 | -NH | NO <sub>2</sub>  |              | <b>meta</b> |
| 43 | DP443 | -NH | CH <sub>3</sub>  | -8.5         |             |
| 44 | DP444 | -NH | OCH <sub>3</sub> | -8.5         |             |
| 45 | DP445 | -NH | OH               | -7.6         |             |
| 46 | DP446 | -NH | Cl               | -7.4         |             |
| 47 | DP447 | -NH | Br               | -8.0         |             |
| 48 | DP448 | -NH | NO <sub>2</sub>  | <b>para</b>  |             |
| 49 | DP449 | -NH | CH <sub>3</sub>  |              | -7.6        |
| 50 | DP450 | -NH | OCH <sub>3</sub> |              | -8.8        |
| 51 | DP451 | -NH | OH               |              | -7.6        |
| 52 | DP452 | -NH | Cl               |              | -7.7        |
| 53 | DP453 | -NH | Br               |              | -7.6        |

After having consideration from Molecular docking studies of total 254 designed molecules towards pancreatic ATP-sensitive potassium channel receptor (5yw7). Best potent molecules were selected as per the feasibility of the reactions and availability of the reagents for the chemical synthesis and biological evaluations, as mentioned in Table no. 13.

**Table 13:** Synthesized molecules on the basis of best binding affinity from each series 1-4.

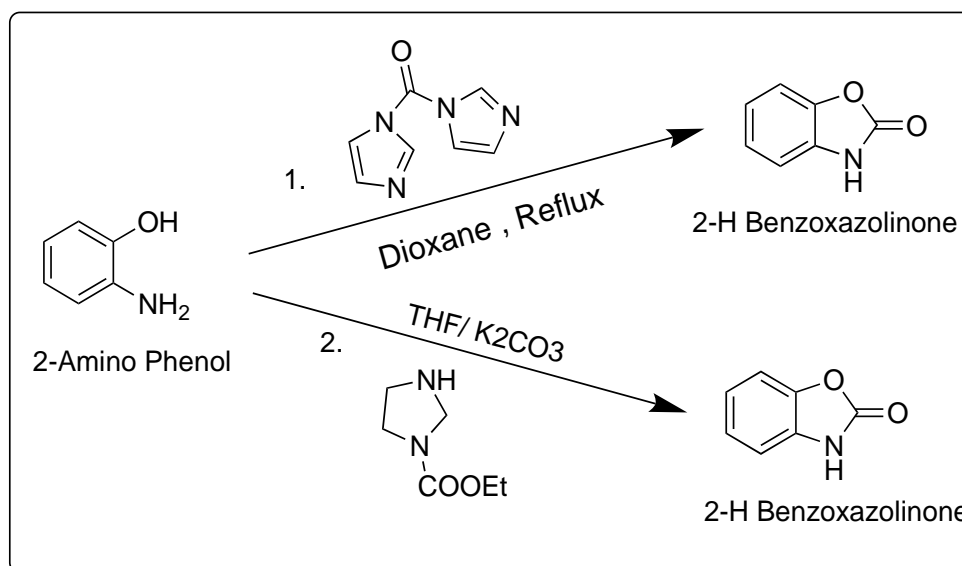
| Sr. | Code                              | Structure   | Binding Affinity (kcal/mol) | Yield |
|-----|-----------------------------------|---|-----------------------------|-------|
| 1   | Coixol/ 6-methoxy benzoxazolinone |   | -5.8                        | 85%   |
| 2   | DP104                             |  | -7.3                        | 88%   |
| 3   | DP105                             |  | -6.5                        | 90%   |
| 4   | DP106                             |  | -6.6                        | 89%   |
| 5   | DP150                             |  | -6.8                        | 92%   |
| 6   | DP154                             |  | -7.1                        | 90%   |
| 7   | DP260                             |  | -6.8                        | 85%   |

|    |       |  |      |     |
|----|-------|--|------|-----|
| 7  | DP263 |     | -6.6 | 70% |
| 9  | DP322 |    | -8.3 | 77% |
| 10 | DP330 |    | -9.1 | 75% |
| 11 | DP422 |   | -9.5 | 75% |
| 12 | DP442 |  | -8.8 | 60% |
| 13 | GBM   |  | -9.4 |     |



## 6.2: Synthesis of series 1 compounds:

### 6.2.1: Synthetic description of 2-Benzoxazolinone (DP100) and its derivatives:

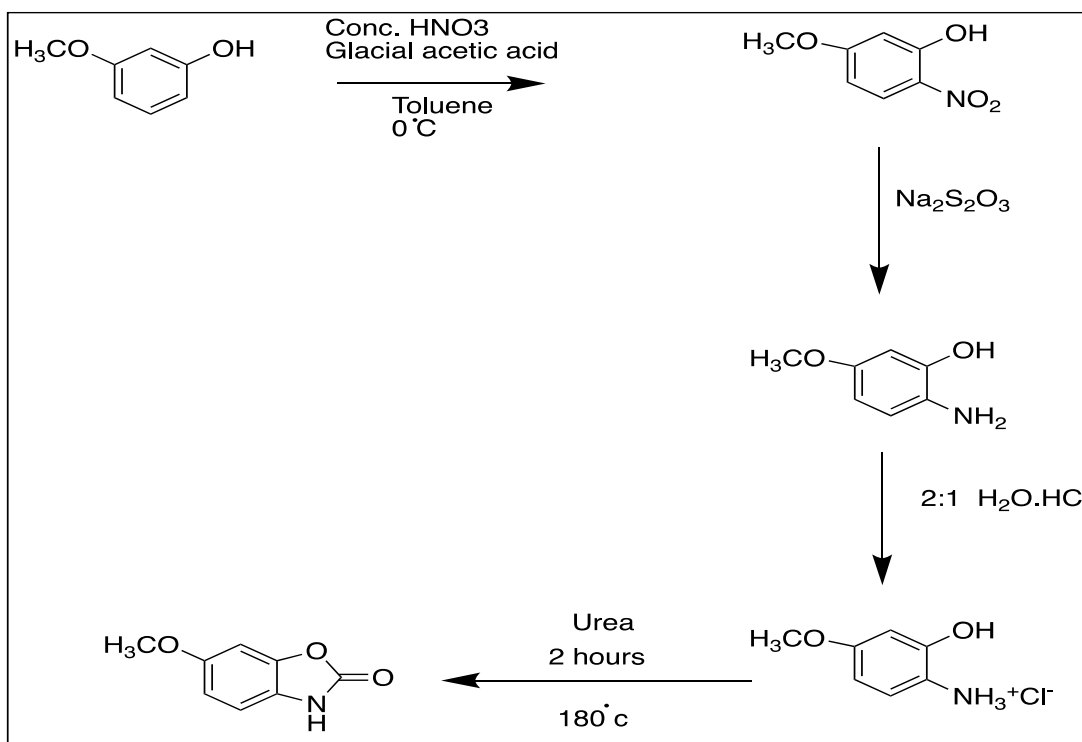


For the synthesis of 2- Benzoxazolinone we followed two methods.

**Method-1 :** 2- Amino phenol (1 mole) was refluxed with 1,1- carbonyl diimidazole (3 mole) in 25 ml Dioxane for 8 hours. The formation of the product was confirmed by TLC. The resulted product was further purified using gravity column with mobile phase Ethyl-acetate and Hexane at ;ratio (3:7) with yield 65%.

**Method-2:** 2- Amino phenol (1 mole) was reacted with Ethyl -1-H-imidazole-carboxylate (1.2 mole) with mild base potassium carbonate (3 mole) in 20 ml THF under reflux condition for about 12 hours to produce 2-benzoxazolinone. The progress was monitored through TLC and after the formation of product, the crude product was further purified via Column chromatography which appeared as cream colour amorphous powder with 85% yield.

### Synthesis of Coixol/6-Methoxy Benzoxazolinone:



**For the Chemical synthesis of Coixol;** 3- methoxy phenol (0.396 mole) was dissolved in glacial acetic acid (5.2 gm) mixed with toluene (0.4ml) and nitration was performed at 0°C with the slight addition of conc. HNO<sub>3</sub> (4ml, 0.567 mole) in the remaining amount of glacial acetic acid (15.5ml). The brown, greenish mixture was further kept at room temperature for 15 minutes which was later on followed by removal by vigorous fumes of nitric oxide and the solution turned reddish quickly. The reddish solution was allowed to sit overnight in a refrigerator. After keeping it for overnight in a refrigerator the compound got precipitated on the bottom of the container which was further washed with ice cold water and dried under a vacuum-pump. The compound was further purified through Column-chromatographic technique using mobile phase Ethyl acetate and Hexane. The formed Nitro compound was further undergone reduction in the presence of sodium Thio sulphite (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>) which yielded into white crystalline compound which was further converted to hydrochloride salt and finally fused with urea at 180°C for 2 hours which yielded into pure yellow needle shaped crystals of coixol.

### **Synthesis of Benzoxazolone derivatives (DP104,105,106):**

Synthesized compound 2-H benzoxazolinone **DP100** was further used for various substitution reactions. To synthesize 6-nitro benzoxazolinone **DP104**, synthesized compound benzoxazolinone was added in small portions to a nitrating mixture (DVS ratio) containing 3ml of water, 23 ml of 96% H<sub>2</sub>SO<sub>4</sub> and 0.6 ml of 63% HNO<sub>3</sub>. Reaction was stirred continuously at 10°C for 30 minutes. After the completion reaction components were transferred in ice-water and the formed product was vacuum filtered followed by multiple washings with water and then with NaHCO<sub>3</sub> in order to make compound neutral. Dried at 50-60°C for about 1 hour which resulted into light yellow amorphous powder.

Compound coded as **DP105 & DP106** synthesized by reacting **DP100** (2-H benzoxazolinone) (0.01 mol) and N-Chloro succinimide and **DP100** & N-Bromo succinimide separately in 10 ml of Glacial-acetic-acid and reaction was stirred at 45-50°C for 48 hours. The reaction course was checked by TLC by using Iodine chamber. Upon completion of reaction the reaction mixture was poured into 250 ml of water with continuous stirring for 15 minutes and finally the product was filtered. After washing with water and drying at 90°C for 1-2 hours **DP104** obtained as white powder and **DP105** obtained as slight yellow amorphous powder.

### **6.2.2: Synthetic description of Benzimidazolone-2 Derivatives:**

For the synthesis of Benzimidazole 2-one; O-Phenylene diamine (1 mol) and Urea (1.2 mol) were fused together by using amyl alcohol as a solvent (25 ml) and the reaction was refluxed for 4-5 hours at 130°C. After the purification of the desired product, further substitution reactions were successfully performed as like benzoxazolinone derivatives and final compounds coded as **DP151** and **DP154**.

### **6.3: Synthetic description of Series 2 compounds Benzimidazole thiones and its derivatives:**

For the synthesis of 2-Benzimidazole thiones; we used O-Phenylene diamine and fused with Thio-urea in the presence of amyl alcohol at 170°C. After the completion of reaction, the resulted compound (**DP200**) was further purified by Column

chromatography and confirmed through spectroscopic data. We also successfully achieved the Nitro and Chloro derivatives of 2- Benzimidazole thiones by following substitution on 6<sup>th</sup> position **DP260** and **DP263**.

#### **6.4: Brief discussion on the synthesis of series 3 compounds coded as DP322 and DP330:**

##### **1. Synthesis of 6-(3-nitrophenoxy) benzo-[d]-oxazol-2 (3H)-one (DP322)**

To achieve the synthesis of desired product we tried different methods in order to improve to yield i.e. Williamson's ether synthesis and Microwave assisted synthesis.

Based on the principle of Williamson's synthesis m-nitro phenol was first reacted with a base NaH in the presence of solvent DMF, 6-Chloro Benzoxazolinone was added gradually in the reaction. The reaction mixture was allowed to heat at 180°C for 24-hours. The desired compound (C-O coupling) was purified by performing column chromatography. The pure compound was isolated from the column at ration (4:6 ) Ethyl acetate: Hexane with yield 40%. In order to achieve high yield we tried to synthesize the compound by Microwave assisted coupling where NaH was replaced by Base K<sub>2</sub>CO<sub>3</sub> due to its explosive nature and added TBAC (0.2 mmol). The reaction mixture was given the exposer of microwave radiation for 1-2 min for the complete conversion of product. Purification was done as per the previous method. This method was found to be more effective than above mentioned method in terms of final compounds yield (65%).

**2. Synthesis of 6-(2-aminophenoxy) benzo-[d]-oxazol-2(3H)-one (DP330):** For synthesizing 6-(2-aminophenoxy) benzo-[d] oxazol-2 (3H)-one, 2-Amino phenol was allowed to react with 6-chloro benzoxazolinone as per the same procedure mentioned above for the synthesis of DP322. In order to achieve microwave assisted synthesis the reactants were dissolved in DMSO with minute addition of base K<sub>2</sub>CO<sub>3</sub> and TBAC as a phase transfer catalyst. K<sub>2</sub>CO<sub>3</sub> was grounded to a fine powder using a mortar and pestle prior to use.

### **6.5: Brief discussion on the synthesis of series 4 compounds (DP422 and DP442)**

**Synthesis of 6-((3-nitrophenyl)amino) benzoxazol-2(3H)-one (DP422):** C-N coupling based compounds DP422 and DP442 were synthesized by trying different methods i.e. Goldberg-type catalyst based C–N coupling reaction, Williamson's coupling, and Microwave assisted method.

**Method -1:** Based on the principle of Williamson's coupling; m-nitro aniline was first reacted with a base NaH in DMF in to produce deprotonated amine (Nucleophile) which was further reacted with 6-Bromo benzoxazolinone (As bromo are better leaving groups than Chloro) . The reaction was allowed to reflux at 180°C for 24-hours. The product formation was confirmed through TLC. The crude product was subjected to work-up procedure and thereafter purified the final product by using gravity columns with mobile phase Ethyl acetate and Hexane (1:1). The yield was significantly less as compare to other methods (40%).

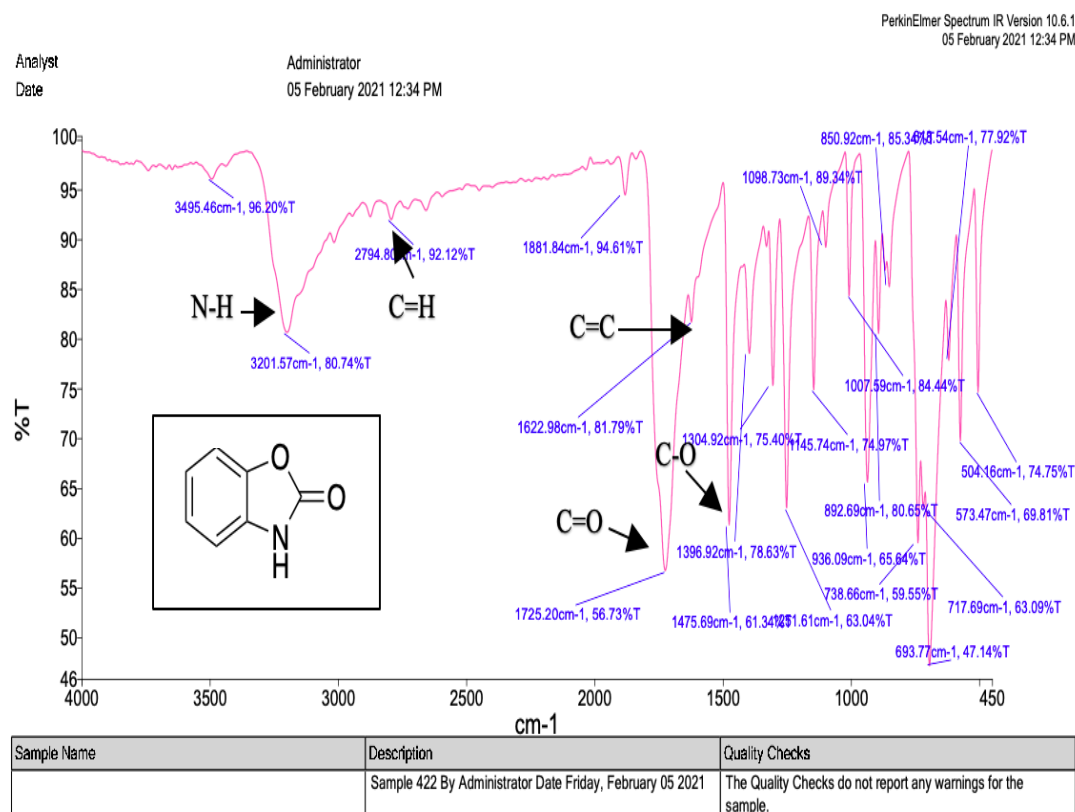
**Method-2 : For the** Goldberg-type catalyst based C–N coupling, m-nitro aniline (1.1 mmol) and 6 bromo benzoxazolinone (1 mmol) placed together in a RBF using DMF as a solvent and K<sub>2</sub>CO<sub>3</sub> as base (2 mmol) and CuI as a catalyst (0.4 mmol). The reaction was refluxed at 180 °C for about 24 hours. The desired product was isolated from column with 65% yield.

**Method-3:** Microwave assisted coupling was followed by adding compound 3-nitro aniline and 6 bromo benzoxazolinone (1 mmol each), K<sub>2</sub>CO<sub>3</sub> as base (2 mmol) and TBAC (0.2 mmol) in the presence of 5ml DMSO solvent and exposed to microwave radiation for about 2-3 minute. The work up and isolation procedure was performed in the same manner as above mentioned method. This method was found to be time saving with the final product yield.(55%).

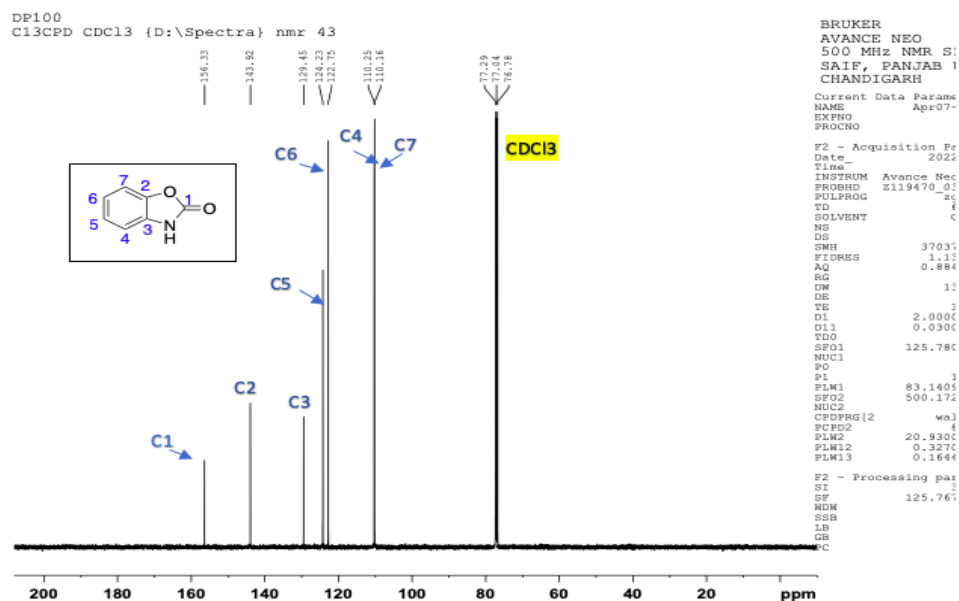
**Synthesis of 5-((3-nitrophenyl)amino)-1,3 dihydro-2H-benzo-[d]imidazol-2-one (DP442):** The synthesis of DP442 was achieved by reacting 3- nitro aniline with 6-Bromo Benzimidazolinone as per the same procedure we applied for the synthesis of DP422.

## 6.6: Characterization of Synthesized compounds from series 1-4:

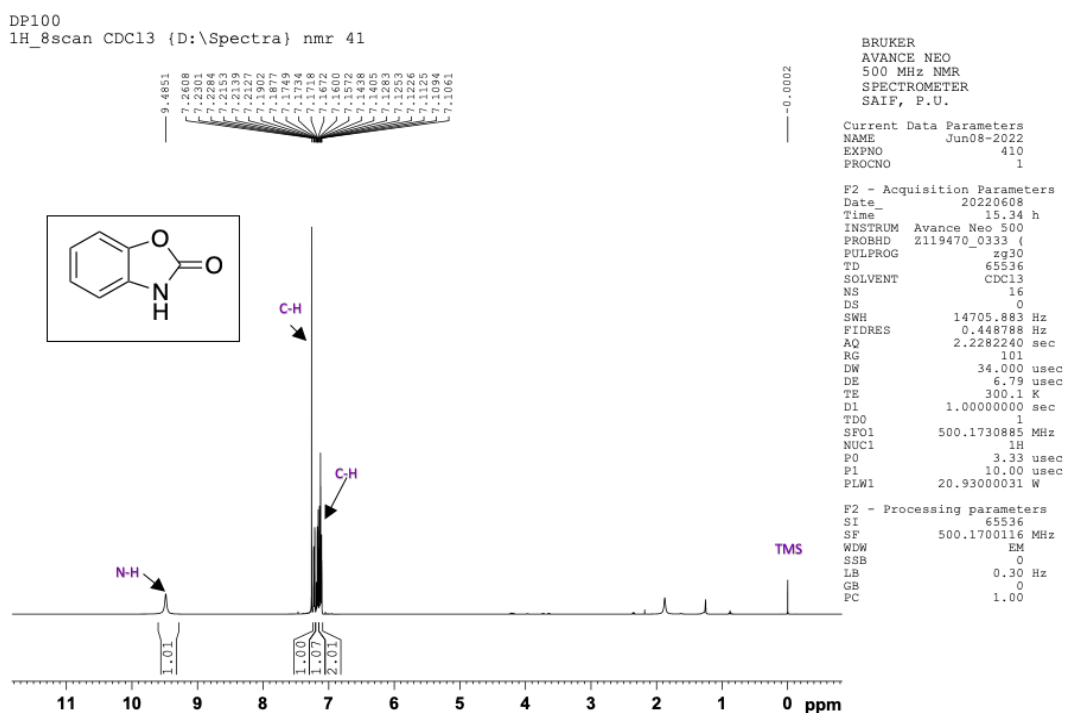
### 1. Spectral Characterization of Benzoxazolinone (DP100):



**Figure 60:** FTIR Spectra of Benzoxazolinone Coded as **DP100** IR (cm<sup>-1</sup>): FTIR (KBr, cm<sup>-1</sup>): 1396 (C-O), 1622 (C=C), 1725 (C=O), 3201 (C-H), 3495 (N-H)



**Figure 61:**  $^{13}\text{C}$  NMR Spectra of Benzoxazolinone coded as **DP100**.  $^{13}\text{C}$  NMR (500MHz, d-  $\text{CDCl}_3$ ):  $\delta$  110.16, 110.25, 122.75, 124.23, 129.45, 143.92 and 156.33.



**Figure 62:**  $^1\text{H}$ -NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.10 (d,  $J = 10.0$  Hz, 2H, ArH), 7.14 -7.19 (m, 1H, ArH), 7.22 (d,  $J = 10.0$  Hz, 1H, ArH), 9.48 (s, 1H, N-H).

### Sample Information

Analyzed by : Admin  
Analyzed : 11/12/2021 12:21:40  
Sample Type : Unknown  
Level # : 1  
Sample Name : DP100\_MS-091221-82\_111221  
Sample ID : DP100\_MS-091221-82\_DI\_111221  
\$EndIf\$Data File : E:\Mass\_DI\_Analysis\GCMS-MS Data 2021\Mass Analysis 2021\Dec 2021\DP100\_MS-(  
Org Data File : E:\Mass\_DI\_Analysis\GCMS-MS Data 2021\Mass Analysis 2021\Dec 2021\DP100\_MS-(  
Method File : E:\Mass\_DI\_Analysis\GCMS-MS Data 2021\Mass Analysis 2021\Dec 2021\DP100\_MS-(  
Org Method File : E:\Mass\_DI\_Analysis\GCMS-MS Data 2021\Mass Analysis 2021\Dec 2021\DP100\_MS-(  
Report File :  
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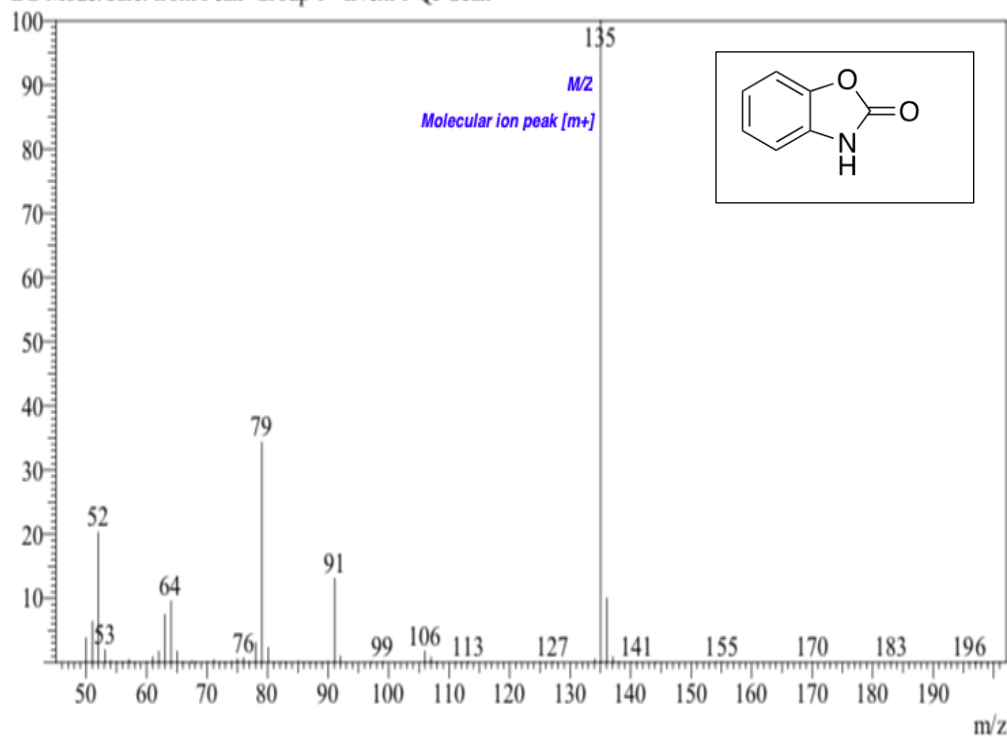
### Spectrum

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MassPeaks:64

RawMode:Averaged 4.210-4.220(841-843) BasePeak:135(584652)

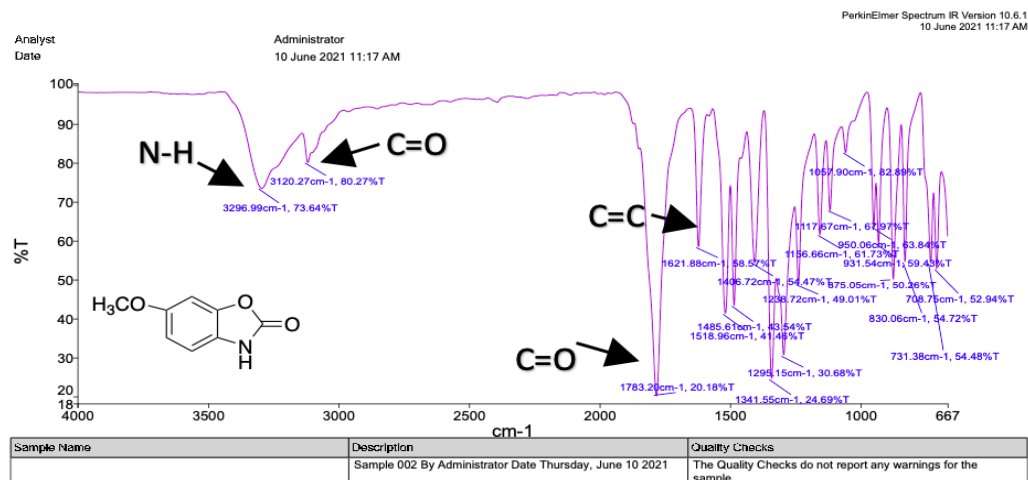
BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan



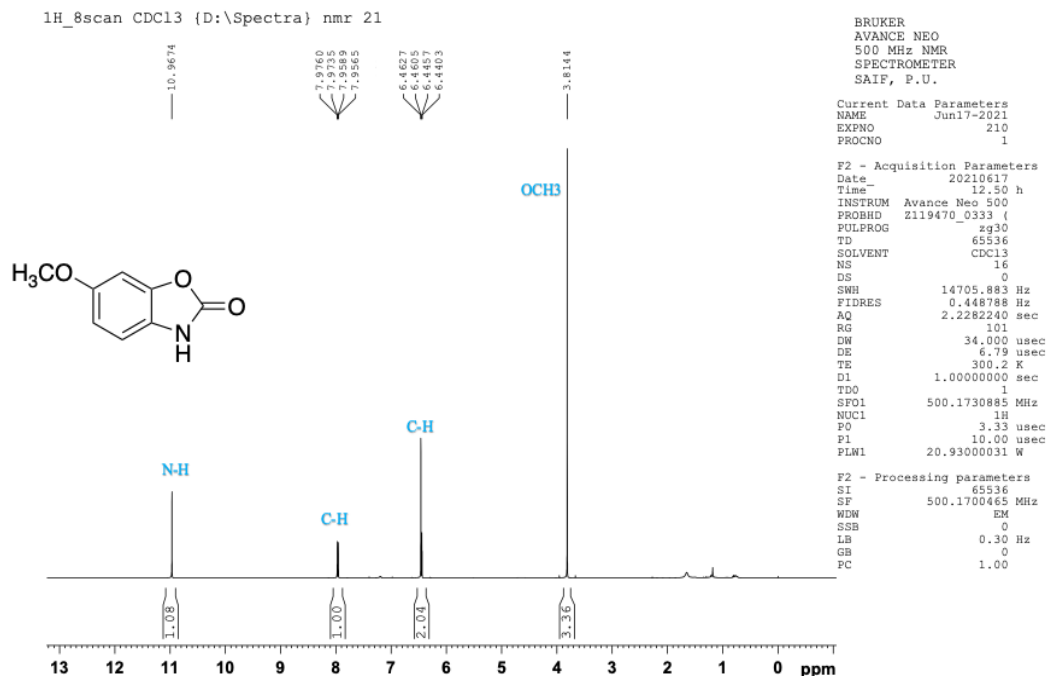
**Figure 63:** Mass spectra of Benzoxazolinone coded as **DP100** [ $M^+$ ] 135, m/z 135.



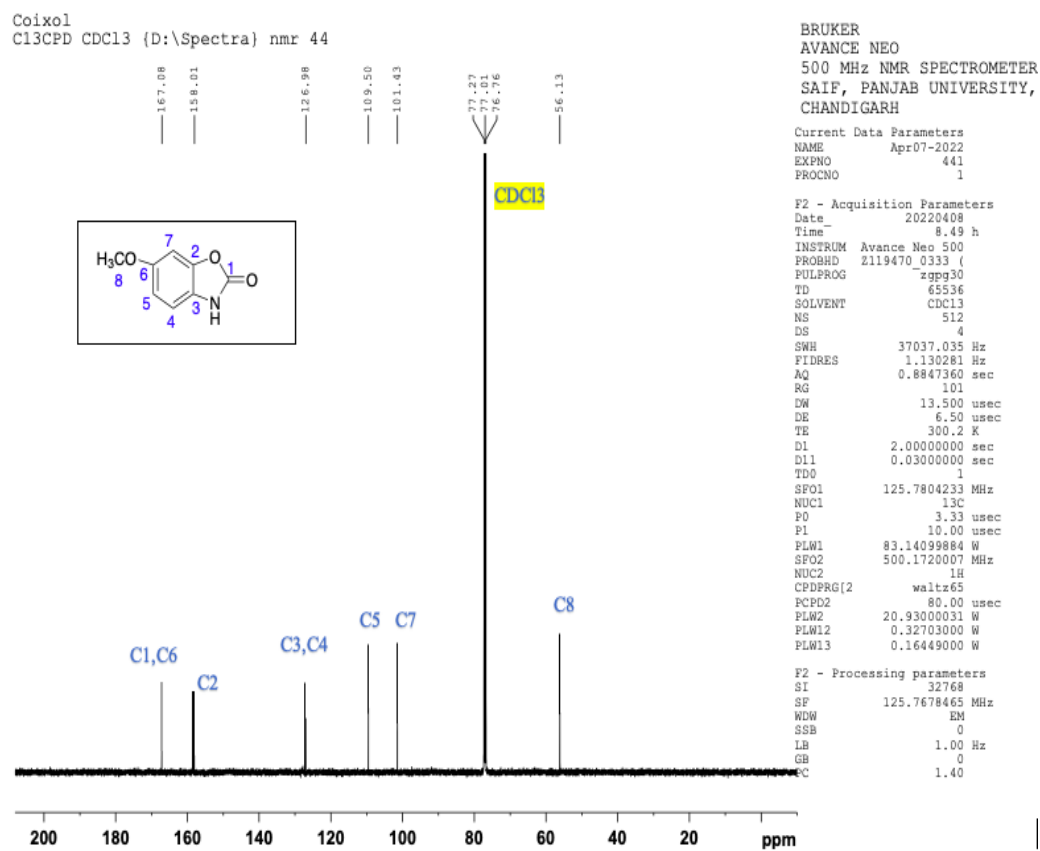
## 2. Spectral Characterization of Coixol/6MBOA (DP102):



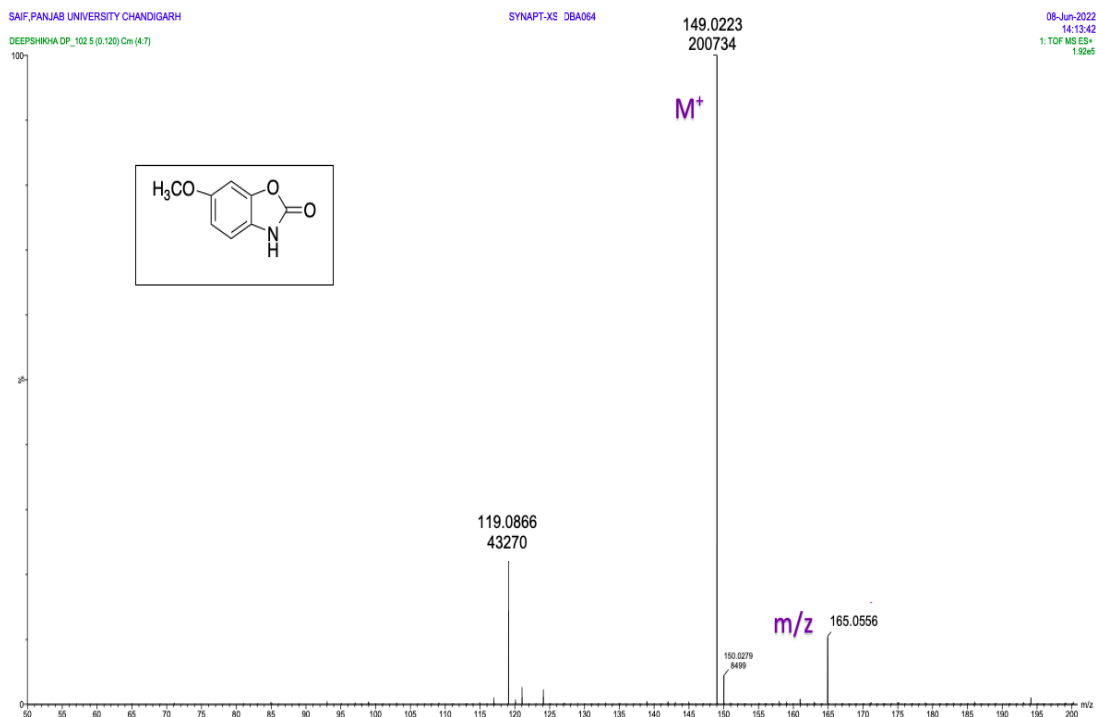
**Figure 64:** FTIR Spectra of Coixol/6MBOA coded as DP102. FTIR ( $\text{KBr cm}^{-1}$ ): 1485 (C-O), 1621 (C=C), 1783 (C=O), 3120 (C-H), 3296 (N-H)



**Figure 65:**  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.81(s, 3H, OCH<sub>3</sub>), 6.44-6.46 (m, 2H, ArH),  $\delta$  7.96 (d, 1H,  $J = 10.0$  Hz, ArH), 10.96 (s, 1H, N-H)

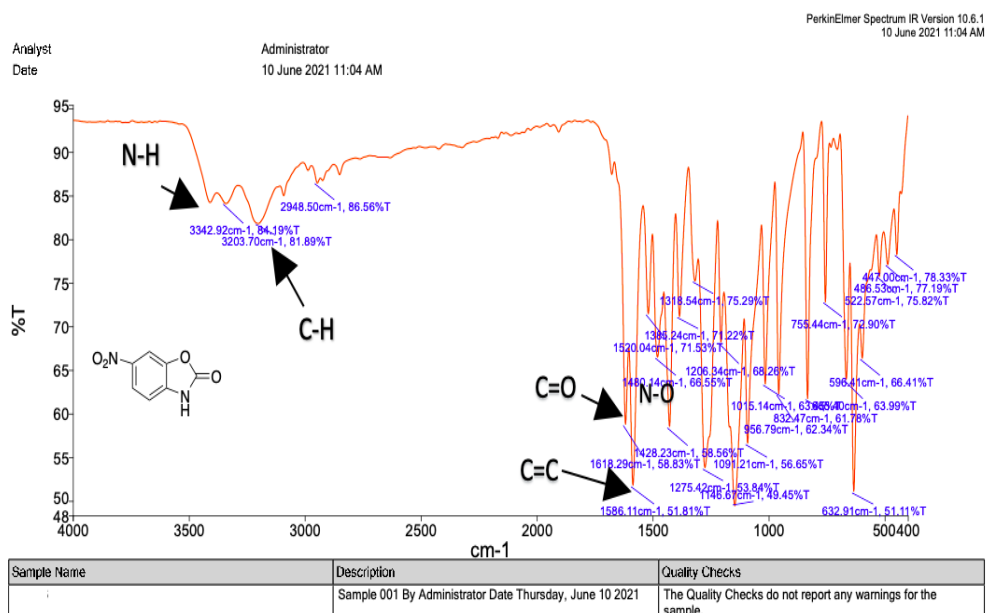


**Figure 66:** <sup>13</sup>C NMR Spectra of Coixol coded as DP102. <sup>13</sup>C NMR (500MHz, d-CDCl<sub>3</sub>): δ 56.13, 101.43, 109.50, 126.98, 158.01, 167.08



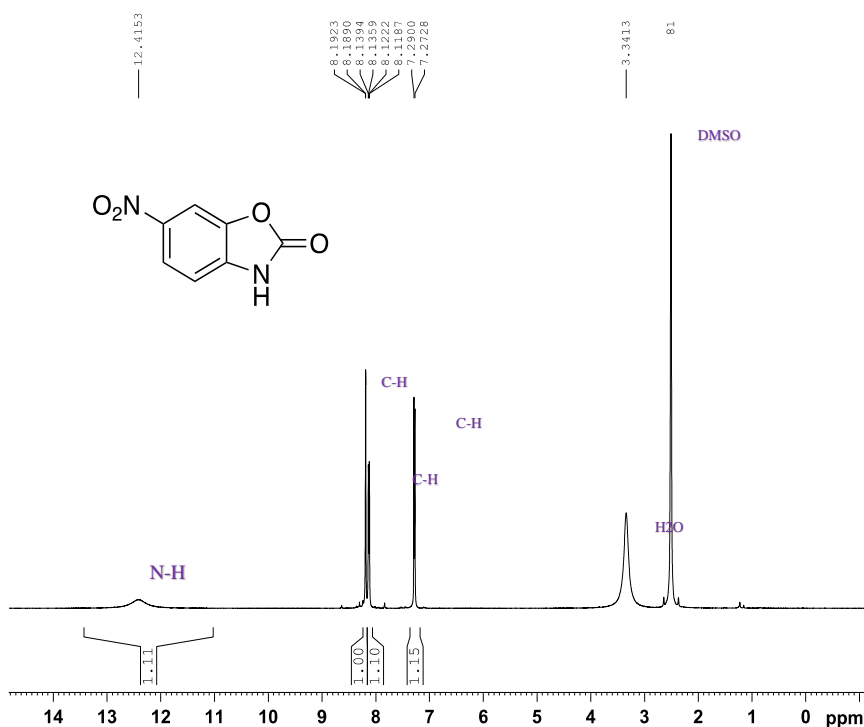
**Figure 67:** Mass spectra of Coixol coded as DP102 MS ES+  $m/z$  at 165.05 [ $M^+$ ].

### 3. Spectral Characterization of 6-Nitro Benzoxazinone (DP104):



**Figure 68:** FT-IR Spectra for Compound coded as DP104. IR (cm<sup>-1</sup>): FTIR (KBr cm<sup>-1</sup>): 1520 (NO<sub>2</sub>, asymmetrical), 1318 NO<sub>2</sub>, symmetrical), 1618 (C=C), 1708 (C=O), 3203 (C-H), 3342 (N-H)

DP-906  
 1H\_8scan DMSO {D:\Spectra} nmr 22



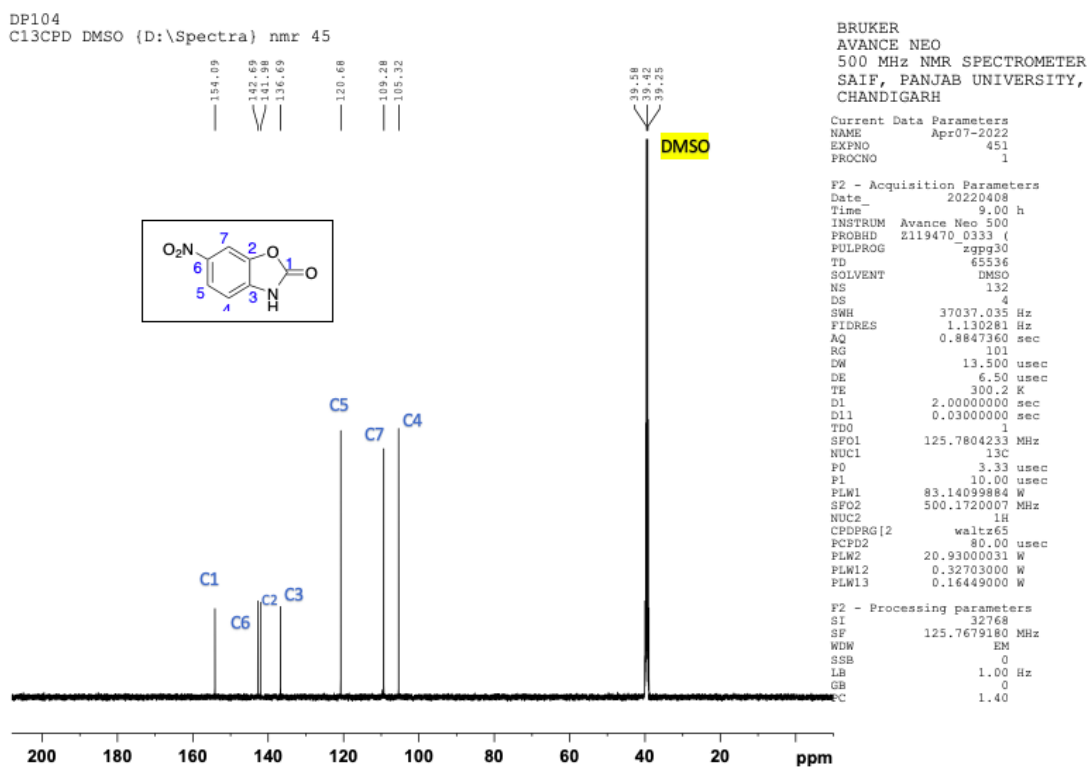
BRUKER  
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 500 MHz NMR  
 SPECTROMETER  
 SAIF, P.U.

Current Data Parameters  
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 EXPNO 220  
 PROCNO 1

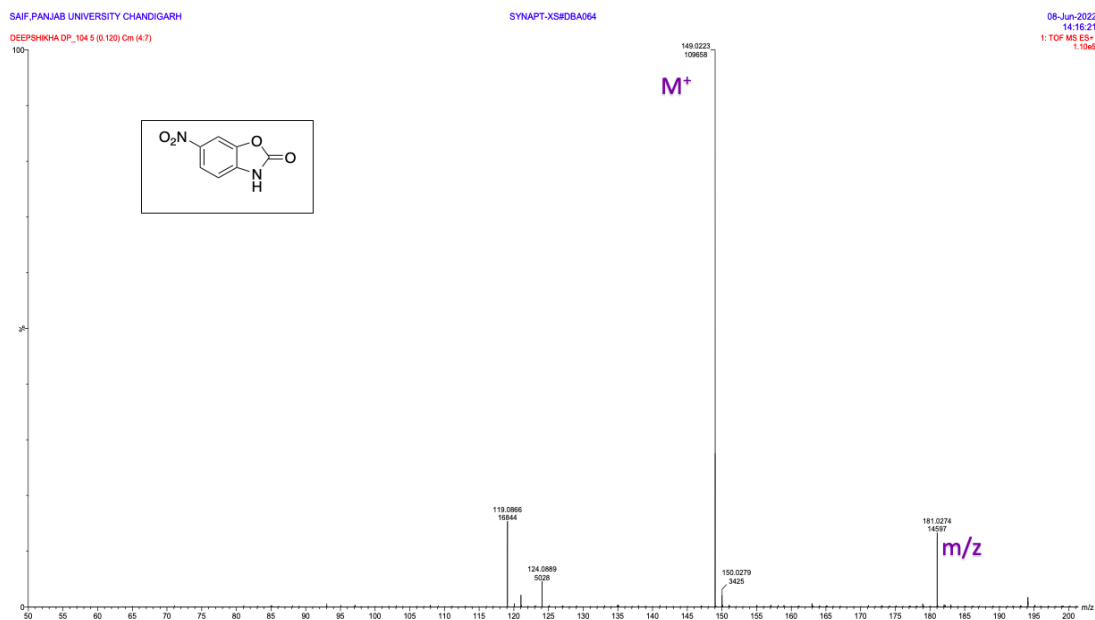
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 RG 95.7854  
 DW 34.000 usec  
 DE 6.79 usec  
 TE 300.2 K  
 D1 1.00000000 sec  
 TD0 1  
 SF01 500.1730885 MHz  
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 P0 3.33 usec  
 P1 10.00 usec  
 PLW1 20.93000031 W

F2 - Processing parameters  
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 WDW EM  
 SSB 0  
 LB 0.30 Hz  
 GB 0  
 PC 1.00

**Figure 69:**  $^1\text{H-NMR}$  spectra of compound coded as DP104.  $^1\text{H-NMR}$  (500 MHz, d-DMSO):  $\delta$  7.28 (d,  $J = 10.0$  Hz, 1H, ArH), 8.12 (d,  $J = 10.0$  Hz, 1H, ArH),  $\delta$  8.19 (s, 1H, ArH), 12.41 (s, 1H, NH),

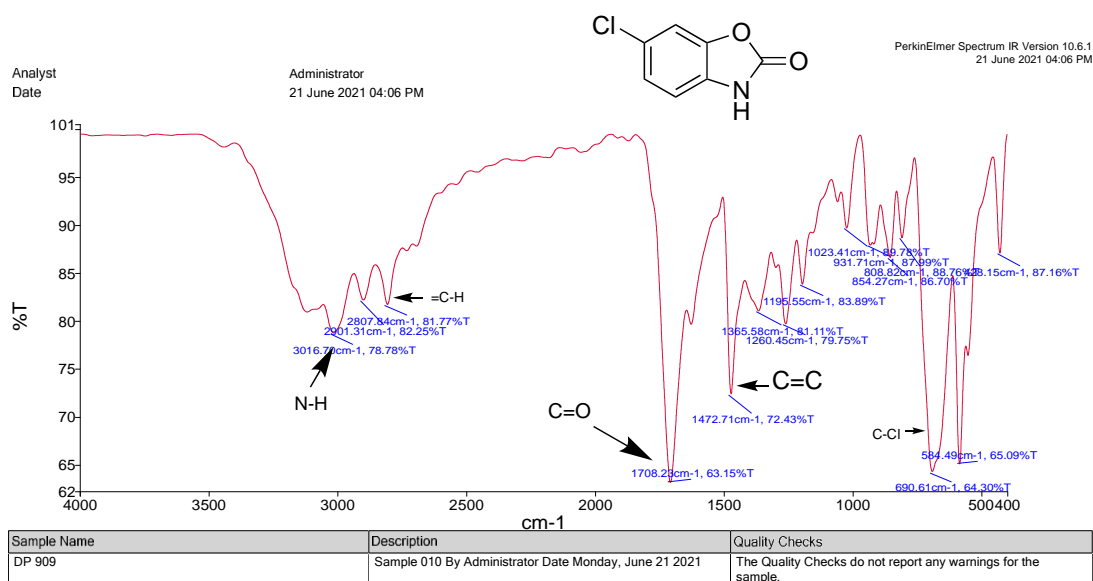


**Figure 70:**  $^{13}\text{C}$  NMR Spectra of Compound coded as DP104.  $^{13}\text{C}$  NMR (500MHz, d-DMSO):  $\delta$  105.32, 109.28, 120.68, 136.69, 141.98, 142.69, 154.09

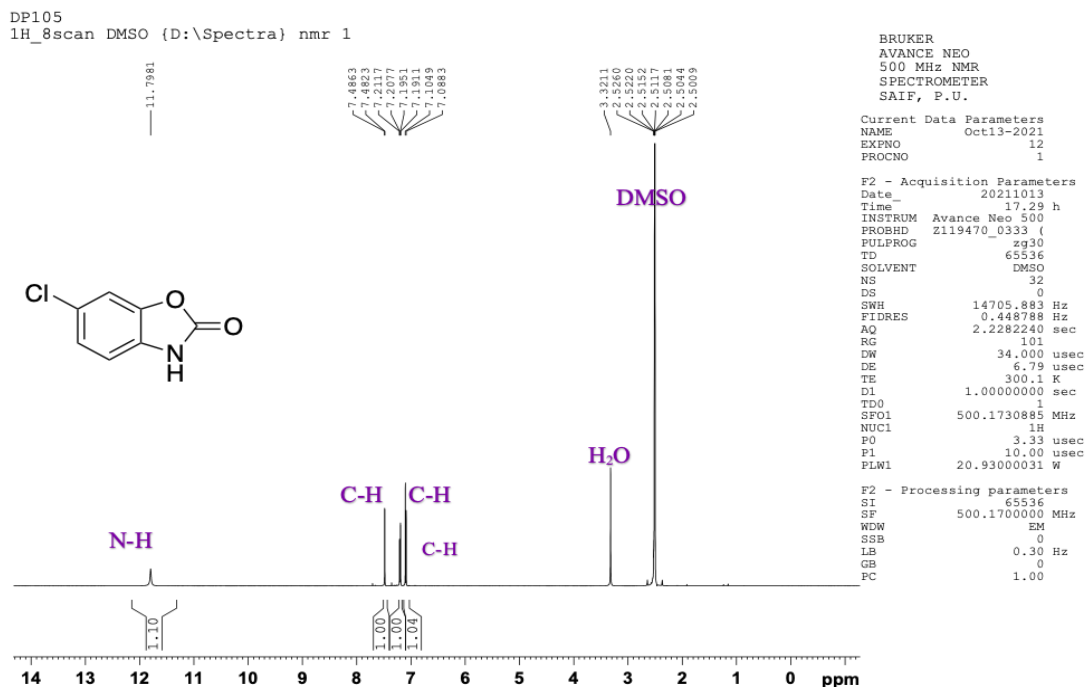


**Figure 71:** Mass spectra of 6-Nitro Benzoxazinone coded as DP104 Ms ESI+ m/z at 181.02 [M+].

#### 4. Spectral Characterization of 6-Chloro Benzoxazolinone (DP105):

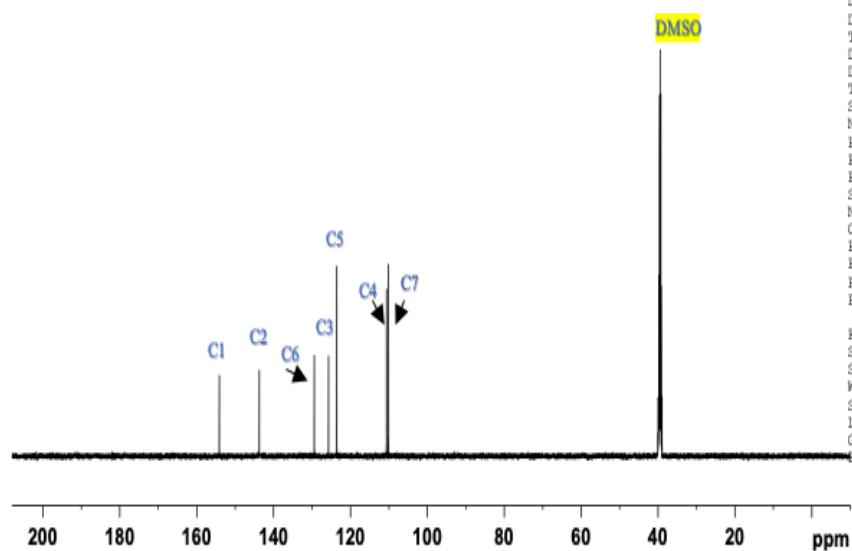
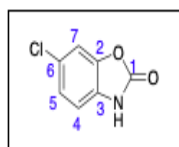


**Figure 72:** FT-IR Spectra for Compound coded as DP105. FT-IR (KBr cm<sup>-1</sup>): 690 (C-Cl), 1642 (C=C), 1708 (C=O), 3016 (C-H), 3316 (N-H)



**Figure 73:** <sup>1</sup>H-NMR Spectra of Compound coded as DP105<sup>1</sup>H-NMR (500 MHz, d-DMSO): δ 7.09 (d, *J* = 8.3 Hz, 1H, ArH), 7.19-7.21 (m, , 1H, ArH), 7.48 (d, *J* = 2 Hz, 1H, ArH), 11.79 (s, 1 H, N-H)

DP105  
C13CPD DMSO (D:\Spectra) nmr 46



BRUKER  
AVANCE NEO  
500 MHz NMR SPECTROMETER  
SAIF, PANJAB UNIVERSITY,  
CHANDIGARH

Current Data Parameters  
NAME Apr07-2022  
EXPNO 461  
PROCNO 1

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SOLVENT DMSO  
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DS 4  
SWH 37037.035 Hz  
FIDRES 1.130281 Hz  
AQ 0.8847360 sec  
RG 101  
DW 13.500 usec  
DE 6.50 usec  
TE 300.1 K  
D1 2.0000000 sec  
D11 0.03000000 sec  
TD0 1  
SFO1 125.7804233 MHz  
NUC1 13C  
PO 3.33 usec  
P1 10.00 usec  
PLW1 83.14099884 W  
SFO2 500.1720007 MHz  
NUC2 1H  
CPDPRG12 waltz65  
PCPD2 80.00 usec  
PLW2 20.93000031 W  
PLW12 0.32703000 W  
PLW13 0.16449000 W

F2 - Processing parameters  
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WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
EC 1.40

**Figure 74:**  $^{13}\text{C}$  NMR Spectra of Compound coded as DP105.  $^{13}\text{C}$  NMR (500MHz, d-DMSO):  $\delta$  110.06, 110.57, 123.50, 125.66, 129.34, 143.67,154.04

### Sample Information

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 Analyzed : 21/06/2021 15:36:40  
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 Level # : 1  
 Sample Name : Spl A\_MS-210621-67\_210621  
 Sample ID : Spl A\_MS-210621-67\_210621\_DI  
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 Method File : E:\Mass\_DI\_Analysis\GCMS-MS Data 2021\Mass Analysis 2021\June 2021\Spl A\_MS\_2  
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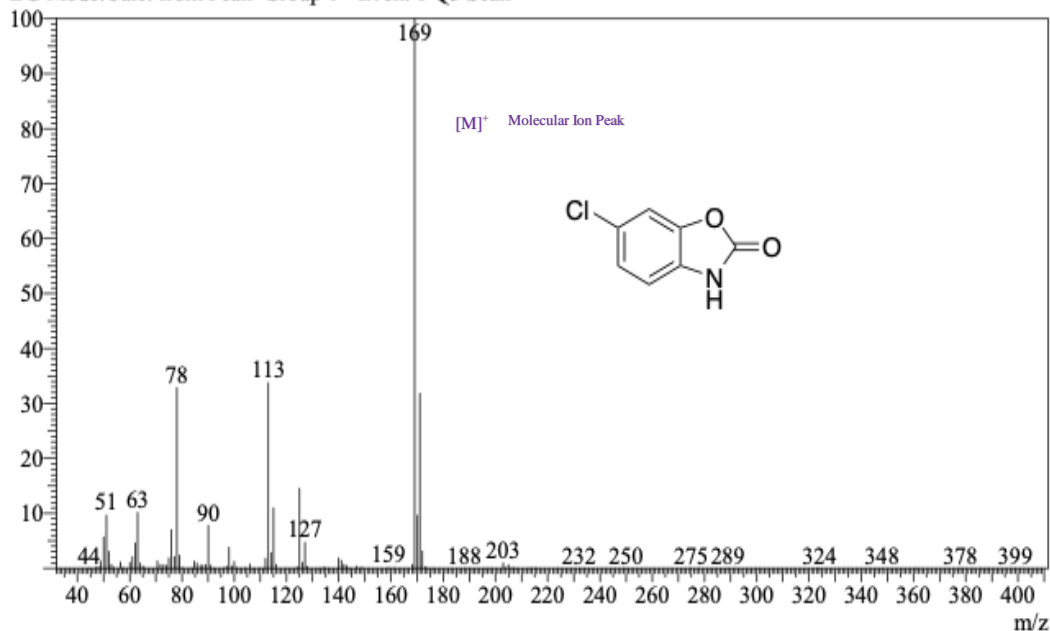
### Spectrum

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MassPeaks:115

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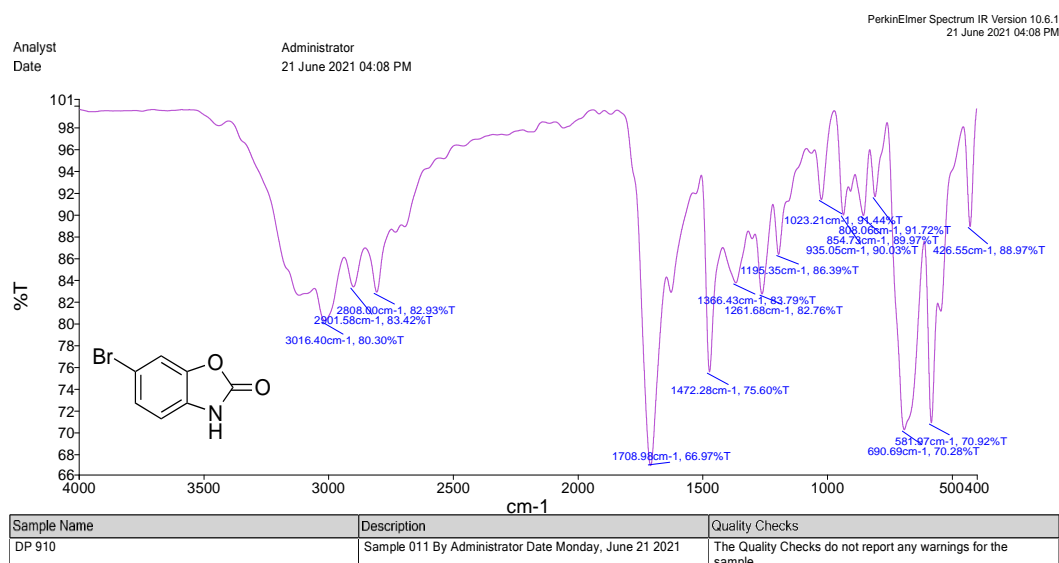
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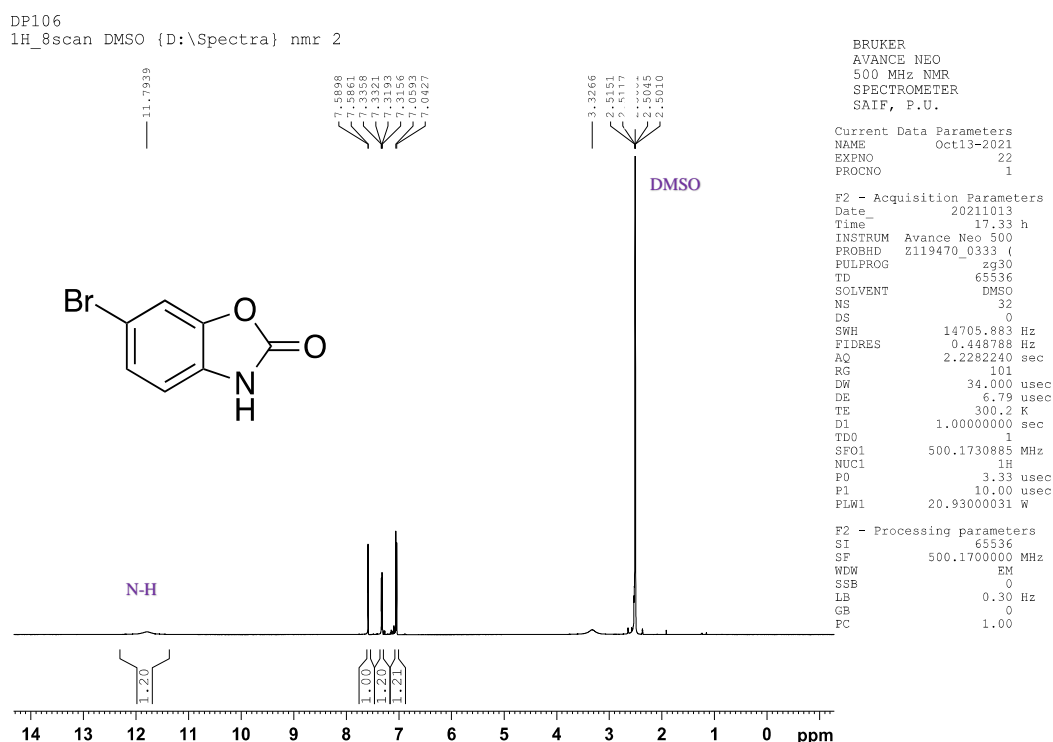
**Figure 75:** Mass spectra for compound coded as DP105. GC MS (m/z) at 170.99 [M+].



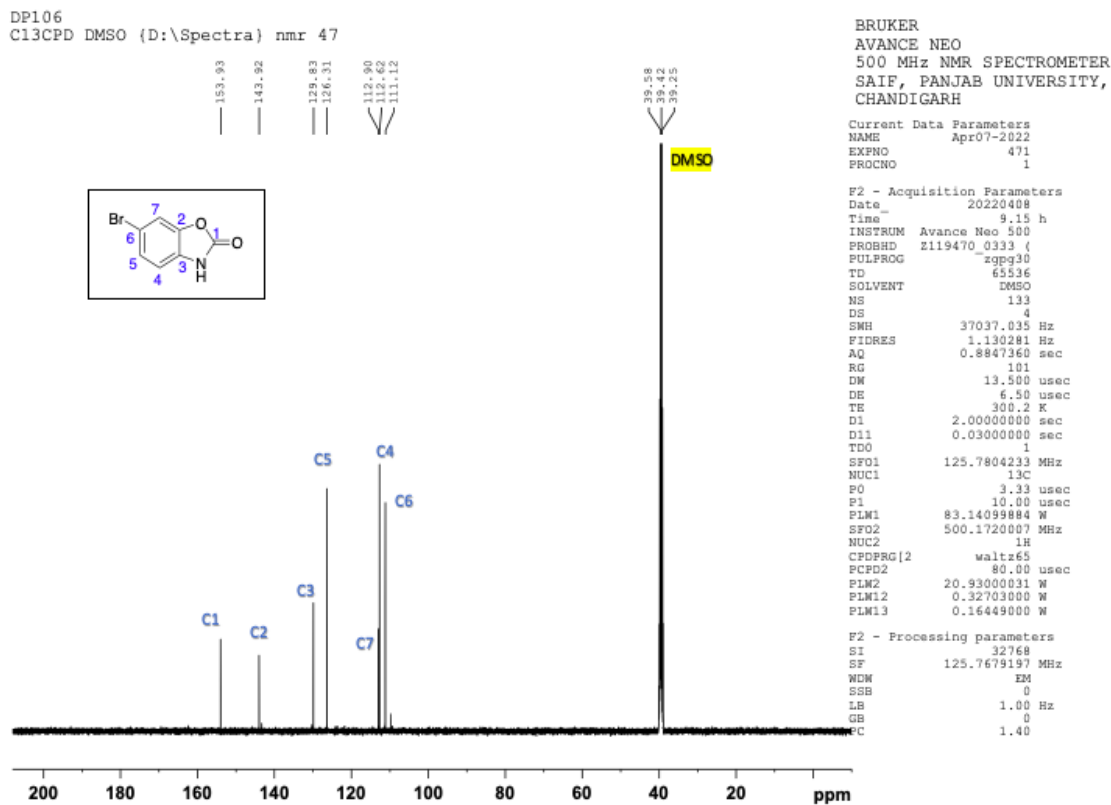
## 5. Spectral Characterization of 6-Bromo Benzoxazinone (DP106):



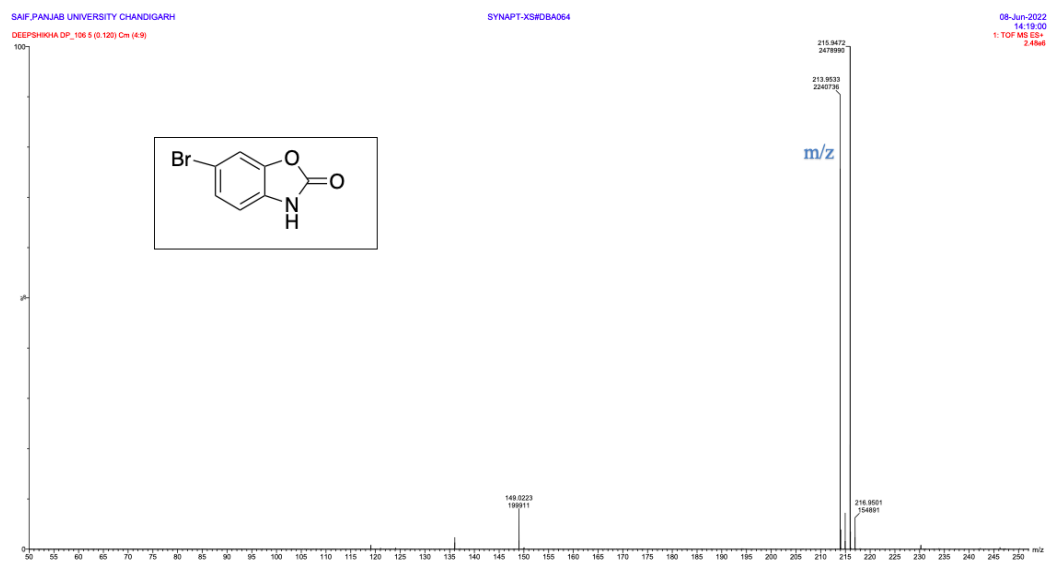
**Figure 76:** FT-IR Spectra for Compound coded as DP106. ; FT-IR (KBr  $\text{cm}^{-1}$ ): 690 (C-Br), 1642 (C=C), 1708 (C=O), 3016 (C-H), 3208 (N-H)



**Figure 77:**  $^1\text{H-NMR}$  spectra for compound coded as DP106.  $^1\text{H-NMR}$  (500 MHz, d-DMSO):  $\delta$  7.04 (d,  $J = 5.0$  Hz, 1H, ArH), 7.31-7.33 (m, 1H, ArH), 7.58 (d,  $J = 5$ Hz, 1H, ArH), 11.79 (s, 1H, NH)

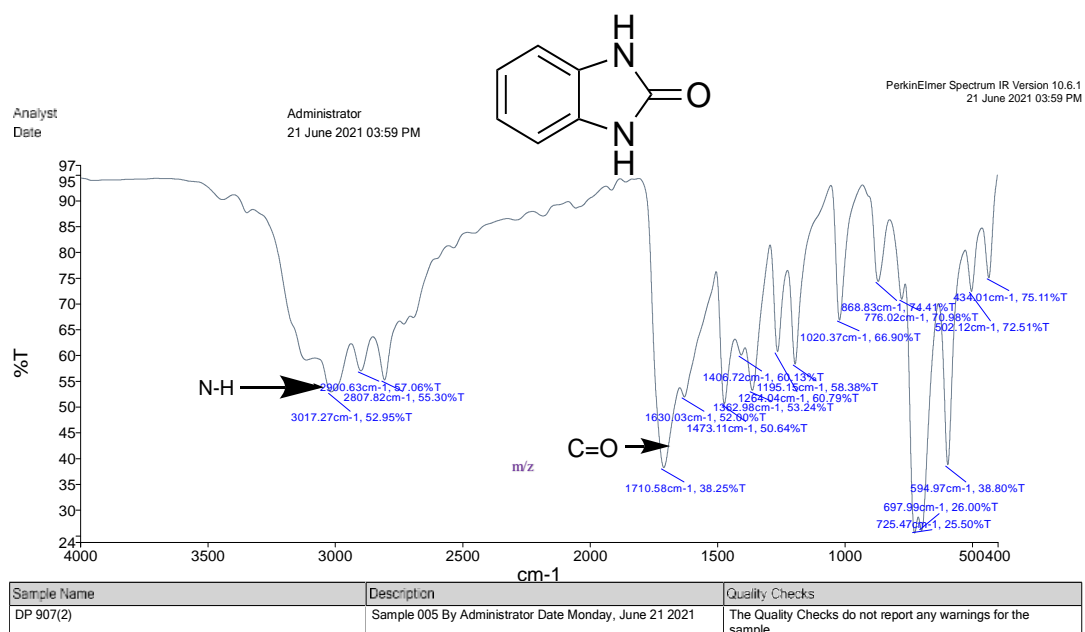


**Figure 78:**  $^{13}\text{C}$  NMR Spectra of compound coded as DP106.  $^{13}\text{C}$  NMR (500MHz, d-DMSO):  $\delta$  111.12, 112.62, 112.90, 126.31, 129.83, 143.92, 153.93.



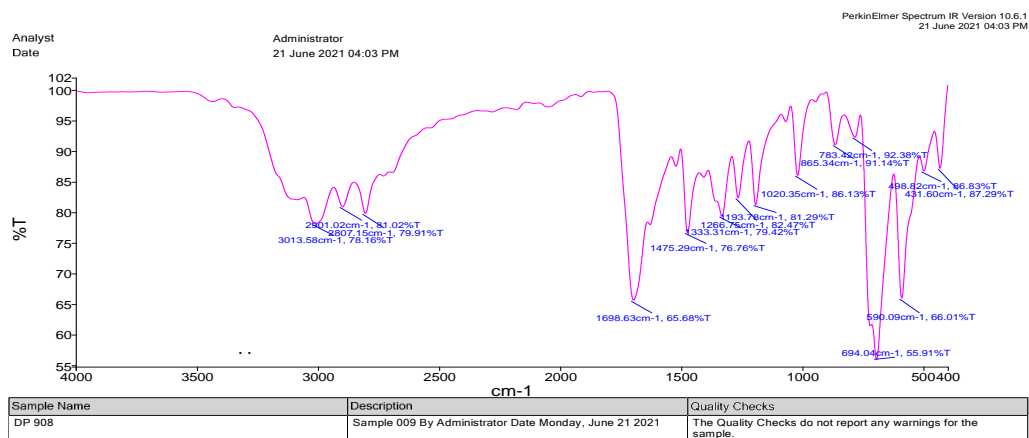
**Figure 79:** Mass spectra for compound coded as DP106 MS ESI+ (m/z) at 216.95 [M+]

## Spectral Characterization of Benzimidazolinone:

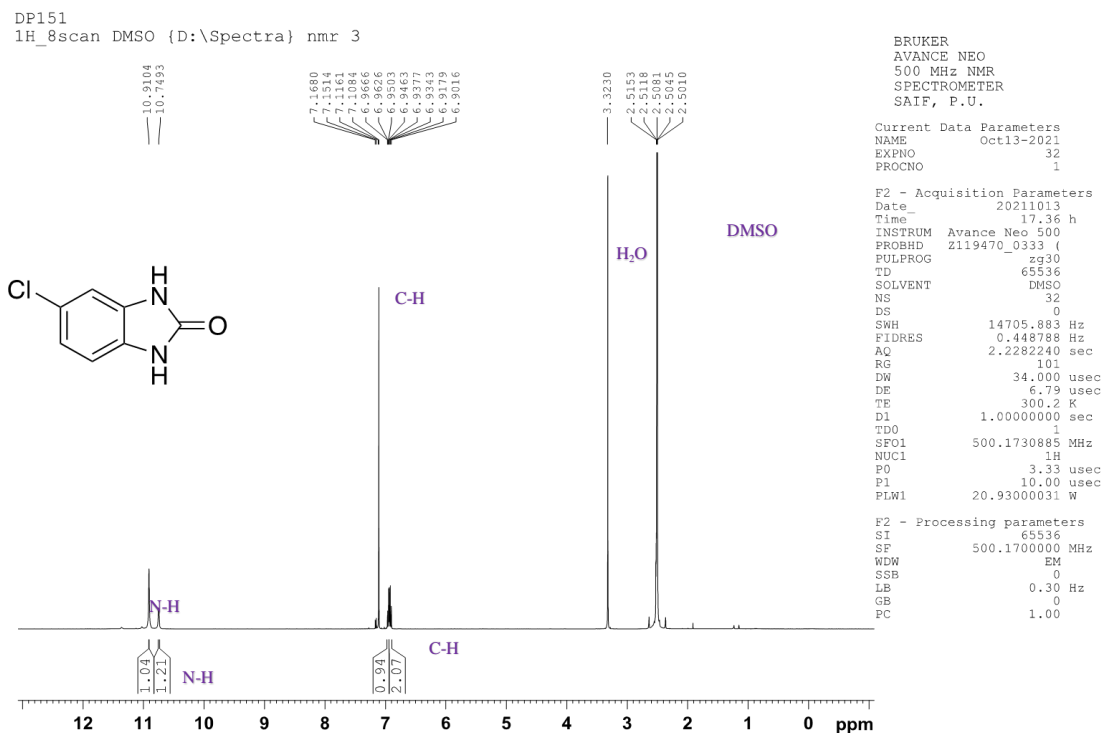


**Figure 80:** FT-IR Spectra for compound Benzimidazolinone. FTIR (cm<sup>-1</sup>): 1630 (C=C), 1710(C=O), 2900 (C-H), 3017(N-H)

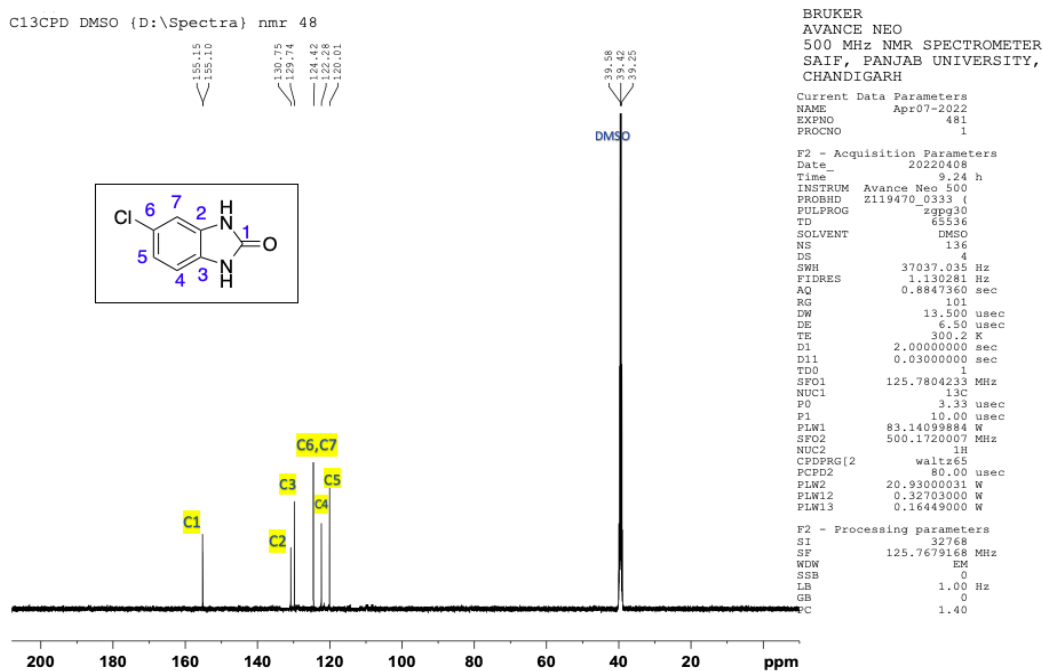
## 6. Spectral Characterization of 5-Chloro Benzimidazolinone (DP151):



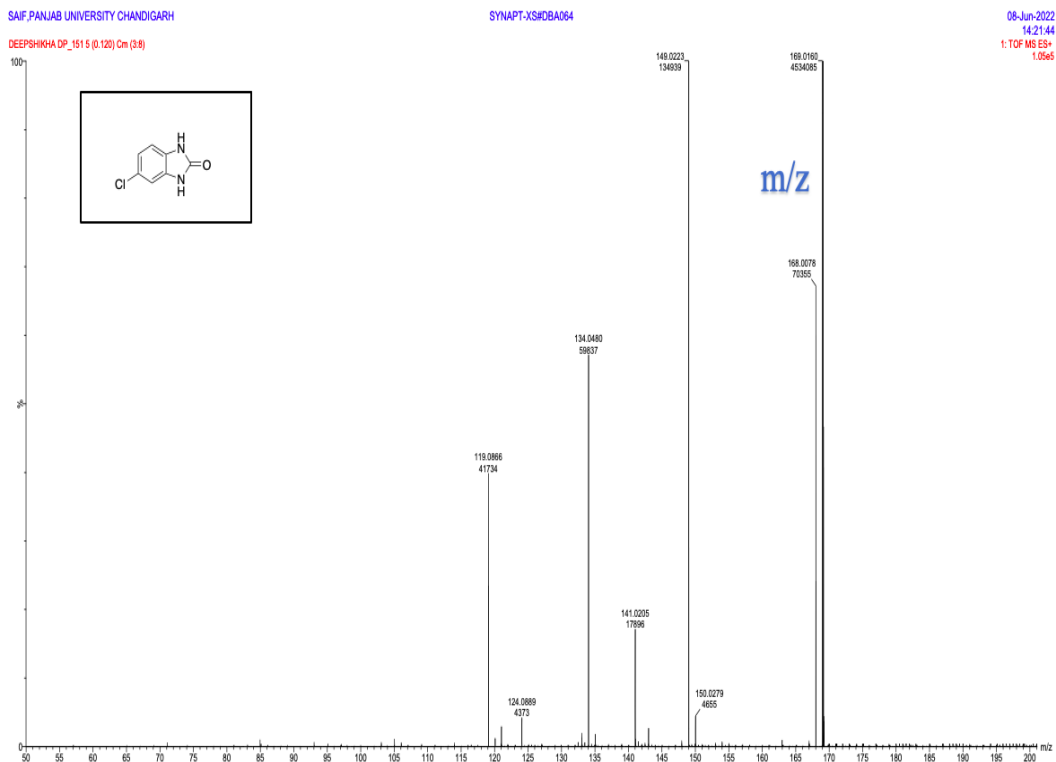
**Figure 81:** FT-IR Spectra for Compound coded as DP151. FT- IR (KBr, cm<sup>-1</sup>): 694 (C-Cl), 1728 (C=O), 2913 (C-H), 3303 (N-H)



**Figure 82:**  $^1\text{H}$ -NMR spectra for compound coded as DP151.  $^1\text{H}$ -NMR (500 MHz, d-DMSO):  $\delta$  6.90-6.96 (m, 2H, ArH), 7.10-7.16 (m, 1H, ArH), 10.74 (s, 1H, N-H), 10.91 (s, 1 H, N-H)

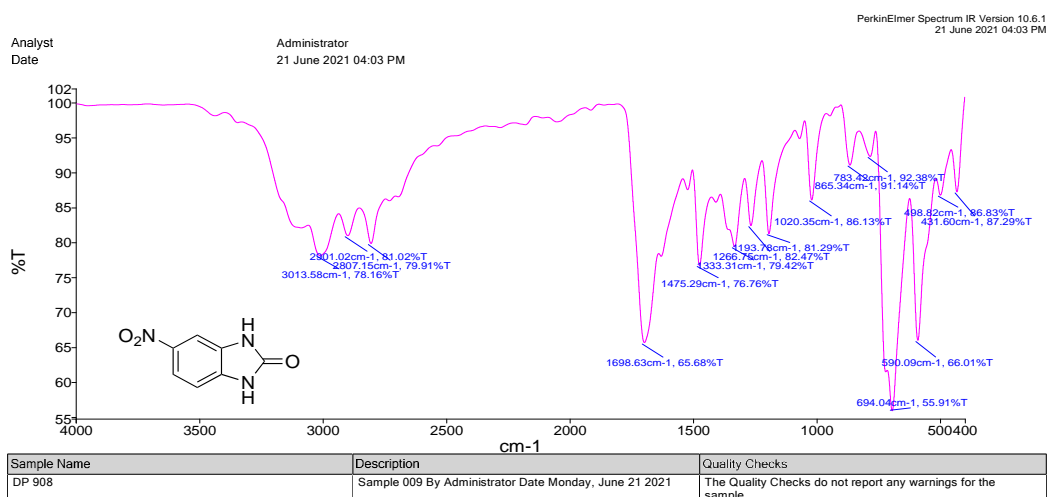


**Figure 83 :**  $^{13}\text{C}$  NMR spectra of compound coded as DP151.  $^{13}\text{C}$  NMR (500MHz, d-DMSO):  $\delta$  120.01, 122.28, 124.42 (2C), 129.74, 130.75, 155.10.

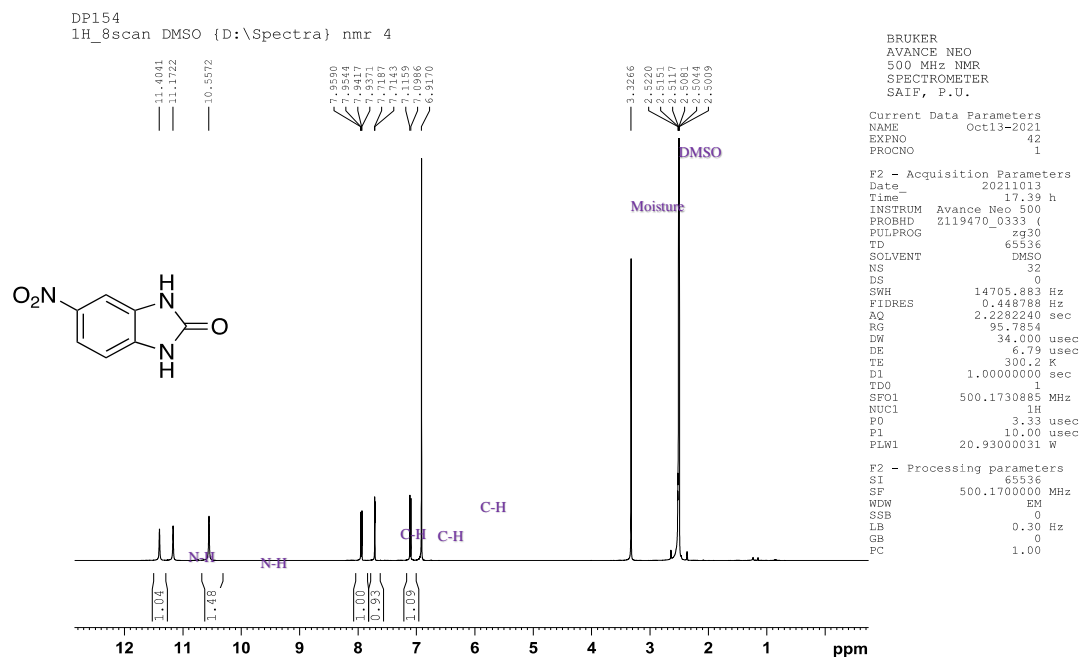


**Figure 84:** Mass spectra for compound coded as DP151 MS ES + (m/z) at 169.01.

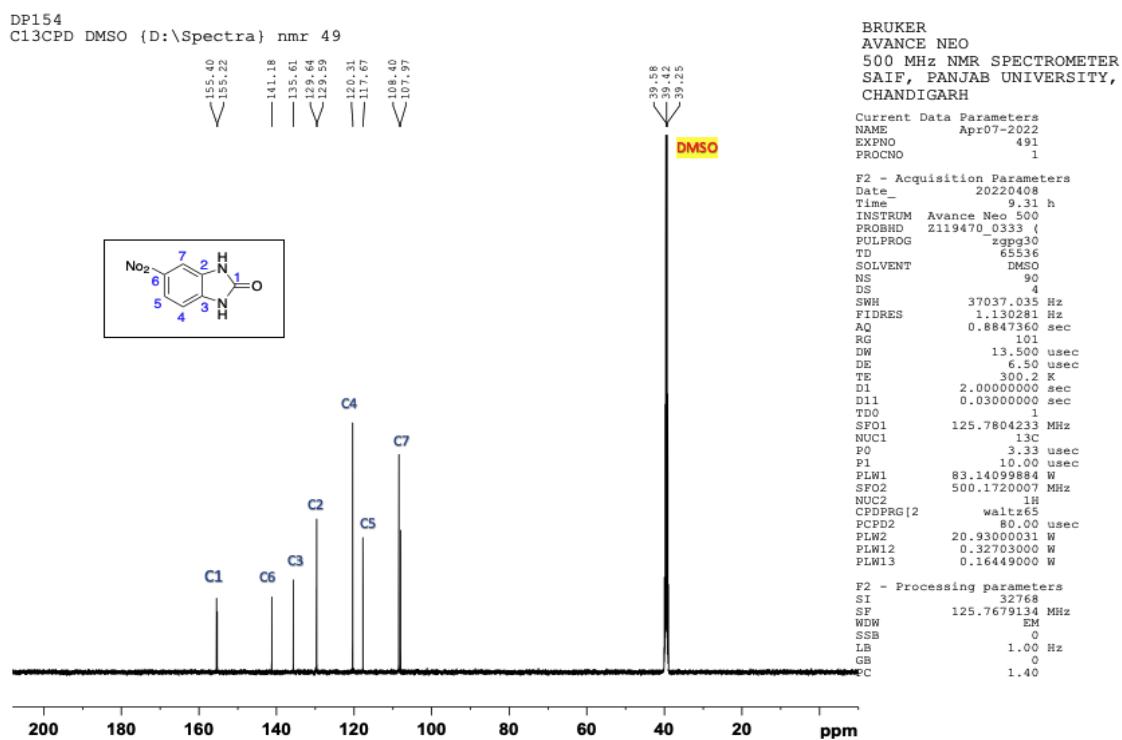
## 7. Spectral Characterization of compound coded as DP154:



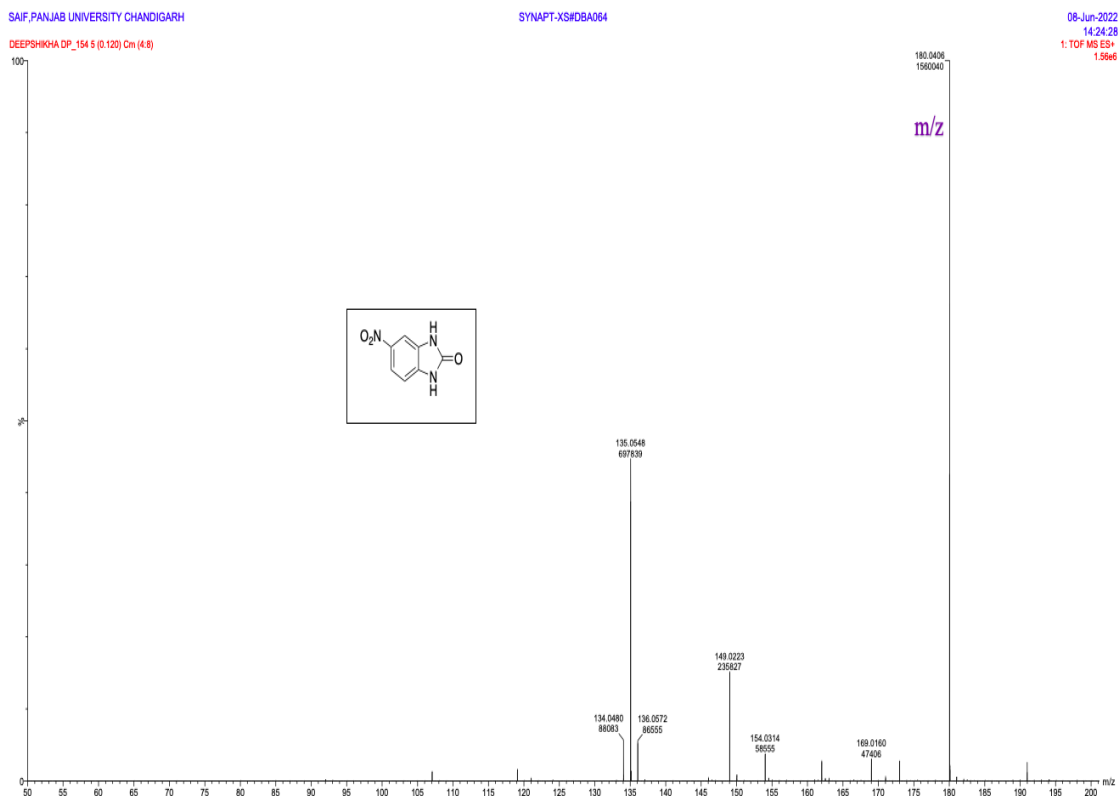
**Figure 85:** FT-IR spectra for compound coded as DP154. FT-IR (KBr,  $\text{cm}^{-1}$ ): 1475 ( $\text{NO}_2$ , asymmetrical), 1333( $\text{NO}_2$ , symmetrical), 1698 (C=O), 3013 (C-H), 3403 (N-H)



**Figure 86:**  $^1\text{H}$ -NMR spectra for compound coded as DP154.  $^1\text{H}$ -NMR (500 MHz, d-DMSO):  $\delta$  7.10 (d,  $J = 10.0$  Hz, 1H, ArH), 7.71 (s, 1H, ArH), 7.94 (d,  $J = 10.0$  Hz, 1H, ArH), 11.17 (s, 1H, N-H), 11.40 (s, 1H, N-H)

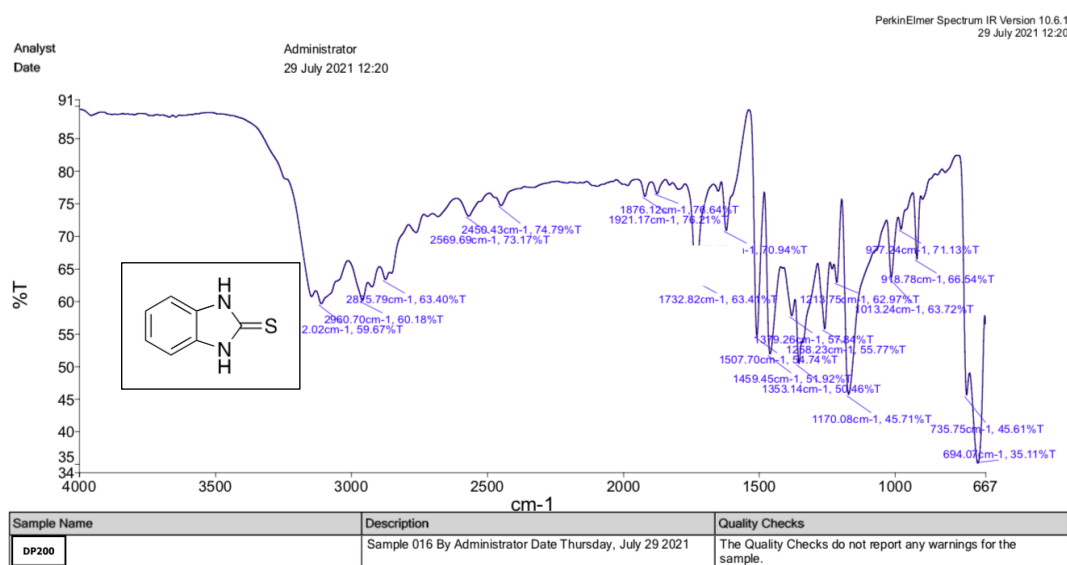


**Figure 87:**  $^{13}\text{C}$  NMR spectra of compound coded as DP154.  $^{13}\text{C}$  NMR (500MHz, d-DMSO):  $\delta$  107.97, 108.40, 117.67, 120.31, 129.59, 135.61, 141.18, 155.40.

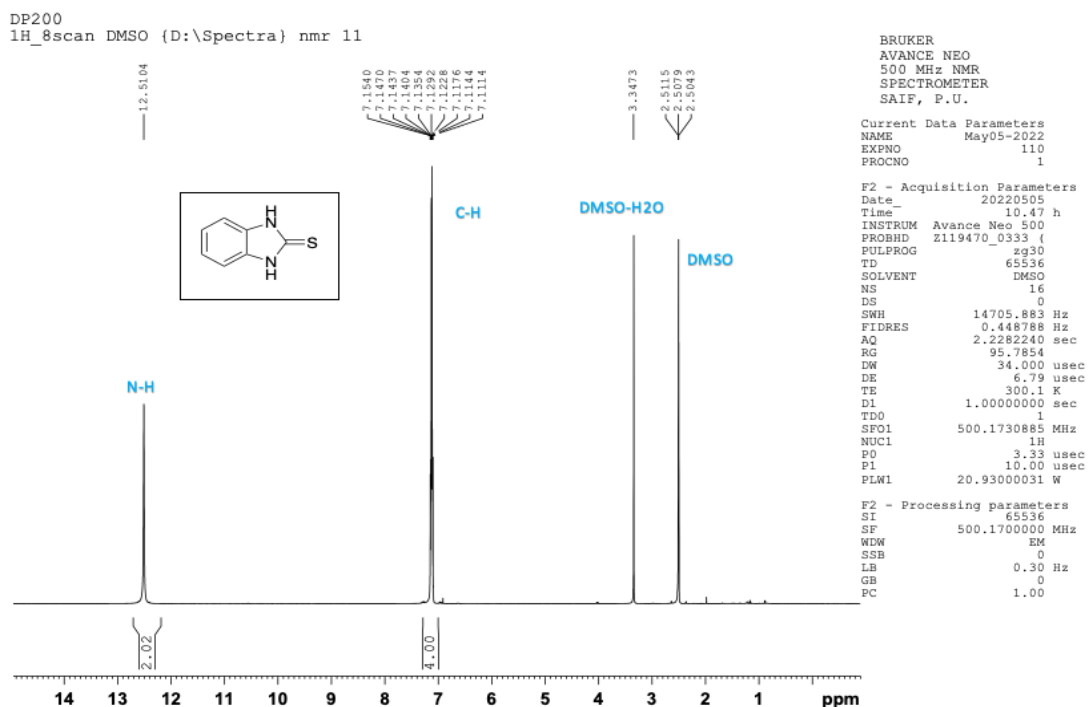


**Figure 88:** Mass spectra for compound coded as DP154. MS ES + (m/z) at 180.04 [M +].

### 8. Spectral characterization of Benzimidazole thiol (DP200):

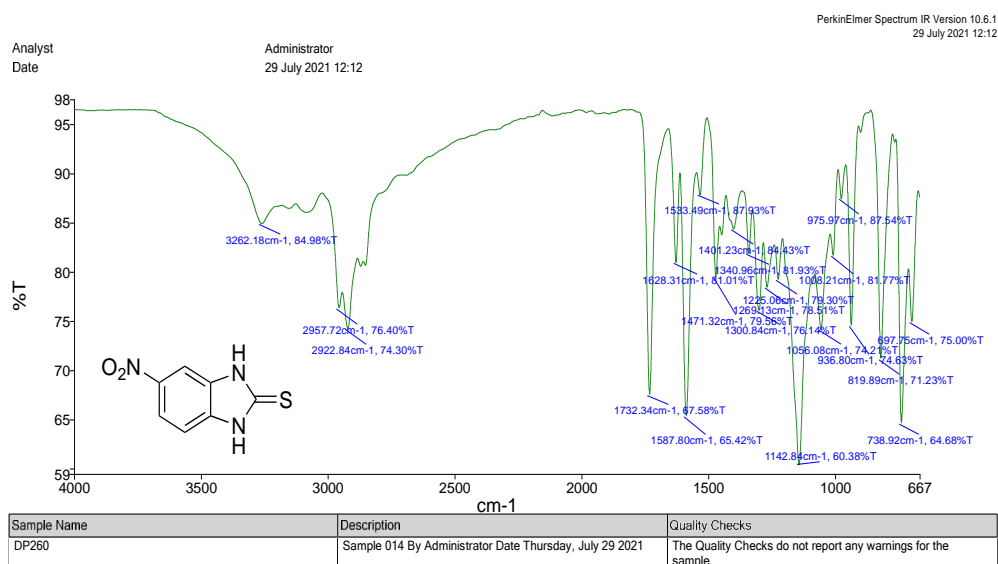


**Figure 89:** FT-IR spectra for compound coded as DP200. FT-IR (KBr cm<sup>-1</sup>): 1620 (C=C), 1732 (C=S), 2875 (C-H), 3112 (N-H)



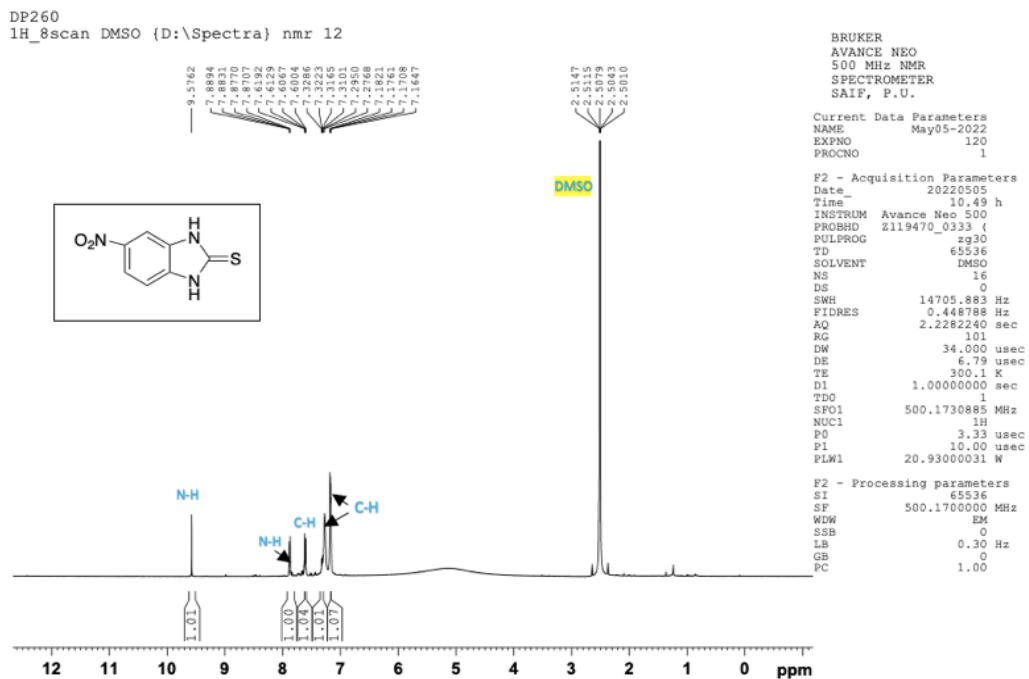
**Figure 90:**  $^1\text{H-NMR}$  spectra for compound coded as DP200.  $^1\text{H-NMR}$  (500 MHz, DMSO):  $\delta$  7.11-7.12 (m, 2H, ArH), 7.13-7.15 (m, 2H, ArH), 12.51 (s, 2H, NH).

### 9. Spectral Characterization of 6-Nitro Benzimidazole thione (DP260):

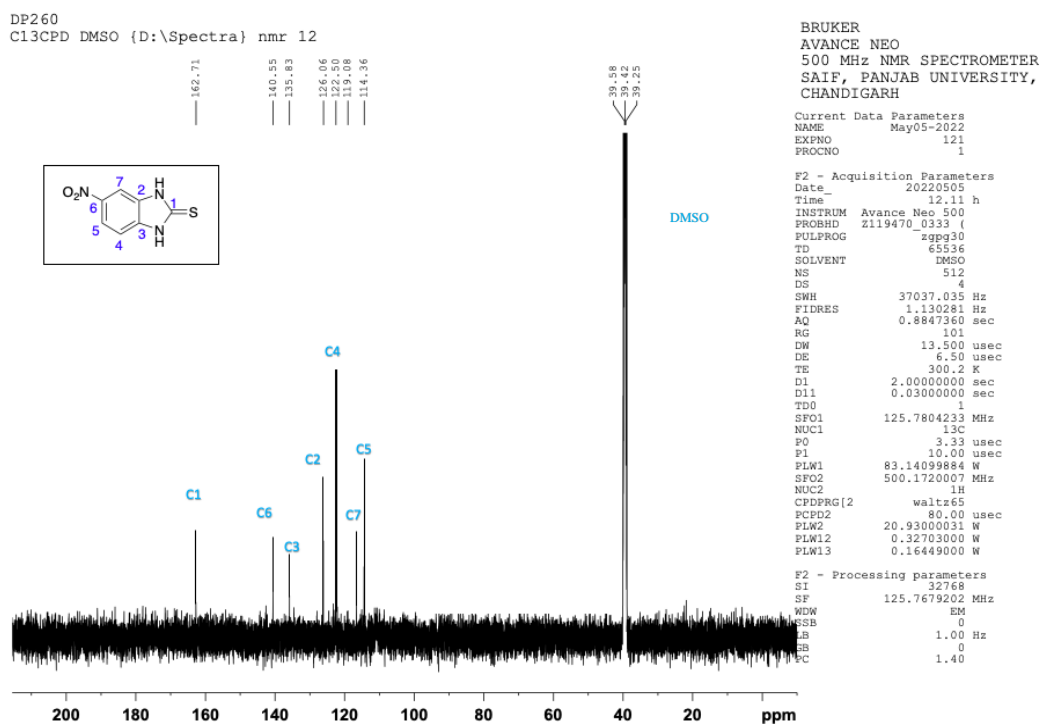


**Figure 91 :** FT-IR spectra for compound coded as DP260. FT-IR (KBr  $\text{cm}^{-1}$ ): 1587 ( $\text{NO}_2$ , asymmetrical), 1340( $\text{NO}_2$ , symmetrical), 1628 (C=C), 1732 (C=S), 2952 (C-H), 3262 (N-H)

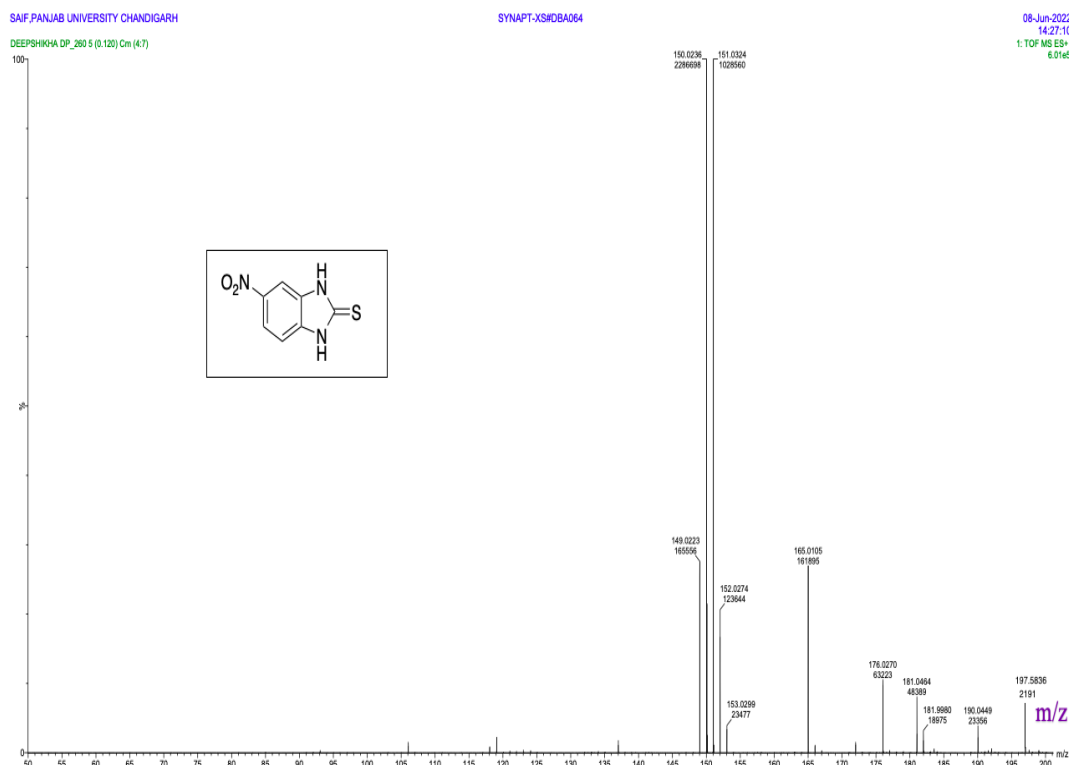




**Figure 92:**  $^1\text{H}$ -NMR spectra for compound coded as 260.  $^1\text{H}$ -NMR (500 MHz, d-DMSO):  $\delta$  7.16-7.18 (m, 1H, ArH), 7.27-7.32 (m, 1H, ArH), 7.60- 7.61 (m, 1H, ArH), 7.87 -7.88 (m, 1H, NH), 9.57 (s, 1H, NH)

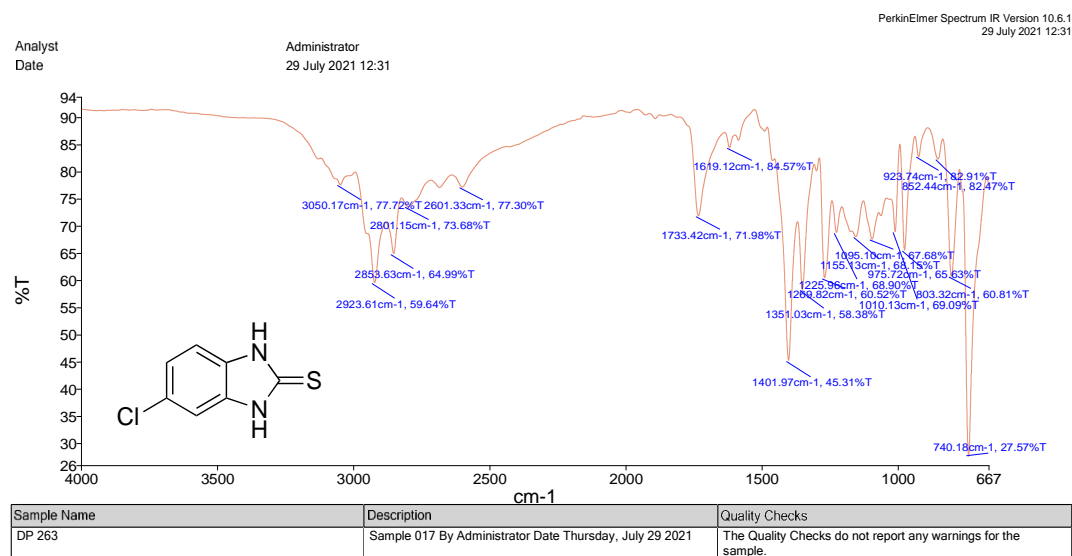


**Figure 93:**  $^{13}\text{C}$  NMR spectra of compound coded as DP260.  $^{13}\text{C}$  NMR (500MHz, d-DMSO):  $\delta$  114.36, 119.08, 122.50, 126.06, 135.83, 140.55, 162.71.



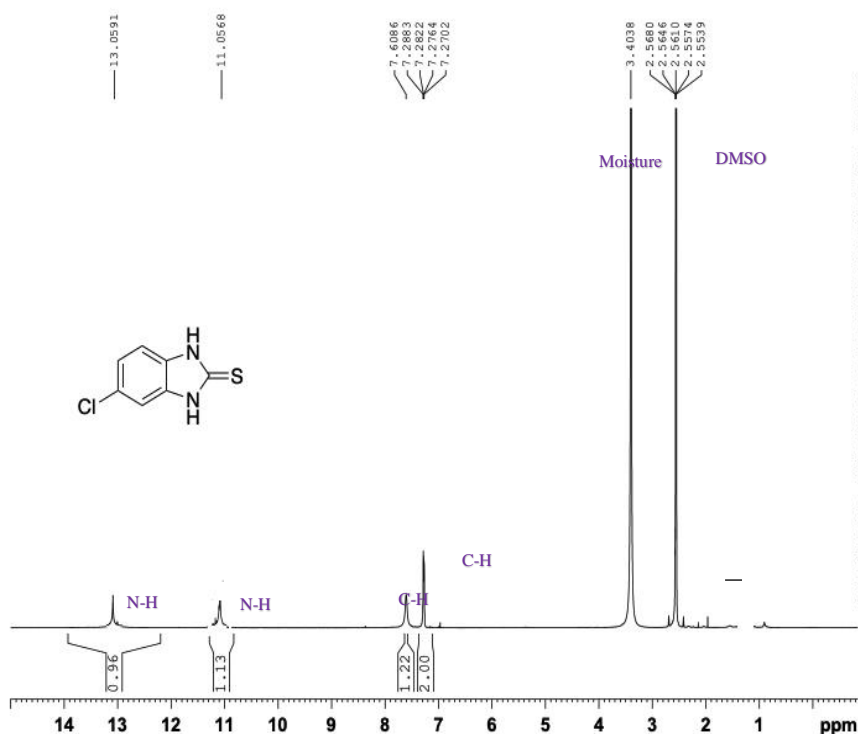
**Figure 94:** Mass spectra for compound coded as DP260. MS ES+ (m/z) at 197.58 [M + 1].

### 10. Spectral Characterization of compound coded as DP263:



**Figure 95:** FT-IR spectra for compound coded as DP263. FT-IR (KBr cm<sup>-1</sup>): 740 (C-Cl), 1619 (C=C), 1733 (C=S), 2923 (C-H), 3060 (N-H)

DP263  
1H\_8scan DMSO {D:\Spectra} nmr 6



BRUKER  
AVANCE NEO  
500 MHz NMR  
SPECTROMETER  
SAIF, P.U.

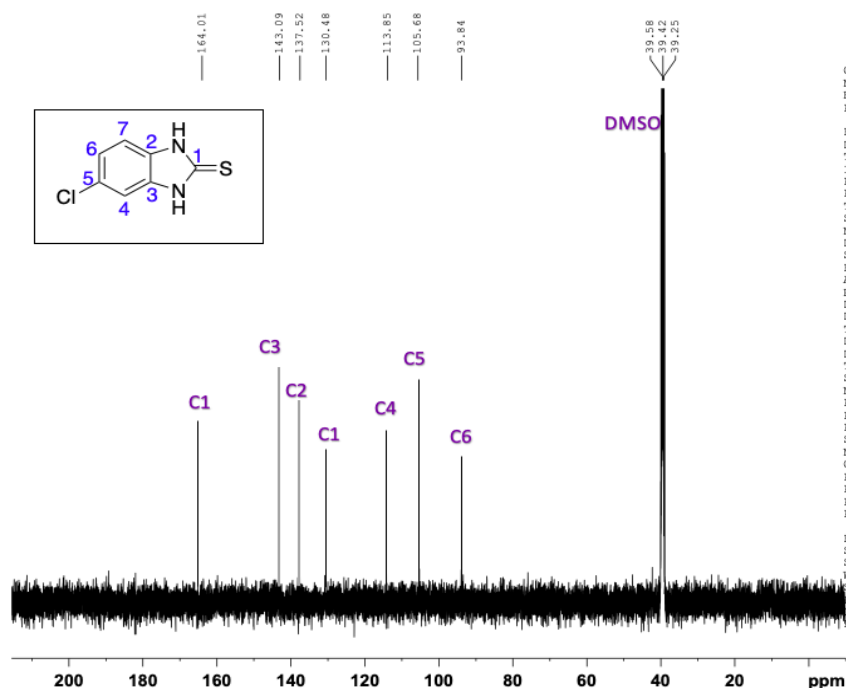
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DS 0  
SWH 14705.883 Hz  
FIDRES 0.448789 Hz  
AQ 2.2282240 sec  
RG 95.7854  
DW 34.000 usec  
DE 6.79 usec  
TE 300.1 K  
D1 1.00000000 sec  
TD0 1  
SFO1 500.1730885 MHz  
NUC1 1H  
P0 3.33 usec  
P1 10.00 usec  
PLW1 20.93000031 W

F2 - Processing parameters  
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SF 500.1699736 MHz  
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SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

**Figure 96:**  $^1\text{H}$ -NMR spectra of compound coded as 263.  $^1\text{H}$ -NMR (500 MHz, d-DMSO):  $\delta$  7.27-7.28 (m, 2H, ArH), 7.60 (s, 1H, ArH), 11.05 (s, 1 H, N-H), 13.05 (s, 1 H, N-H).

DP263  
C13CPD DMSO {D:\Spectra} nmr 13



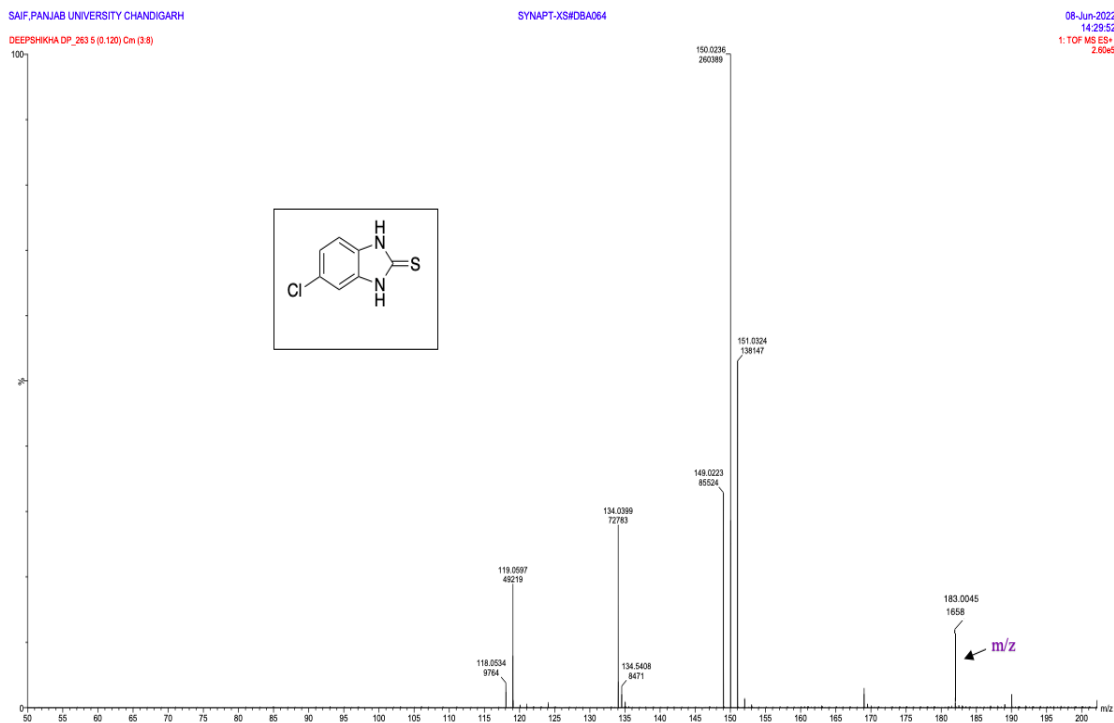
BRUKER  
AVANCE NEO  
500 MHz NMR SPECTROMETER  
SAIF, PANJAB UNIVERSITY,  
CHANDIGARH

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PROCNO 1

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SOLVENT DMSO  
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DS 4  
SWH 37037.035 Hz  
FIDRES 1.130281 Hz  
AQ 0.8847360 sec  
RG 101  
DW 13.500 usec  
DE 6.50 usec  
TE 300.1 K  
D1 2.00000000 sec  
D11 0.03000000 sec  
TD0 1  
SFO1 125.7804233 MHz  
NUC1 13C  
P0 3.33 usec  
P1 10.00 usec  
PLW1 83.14099884 W  
SFO2 500.1720007 MHz  
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PLW2 20.93000031 W  
PLW12 0.32703000 W  
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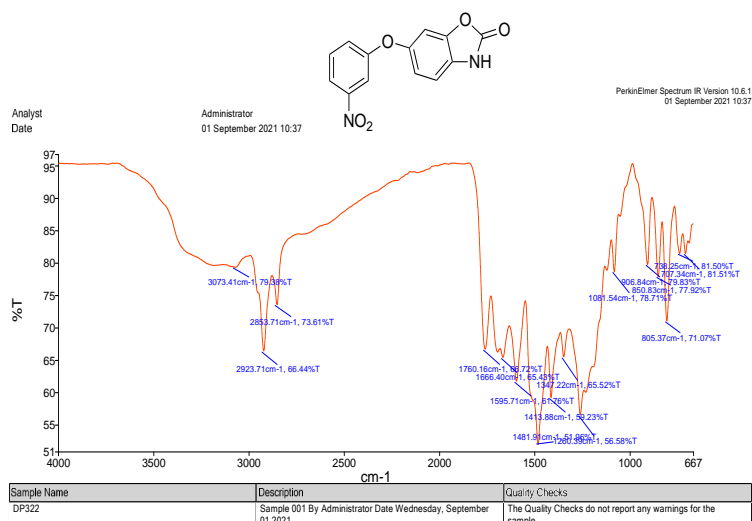
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PC 1.40

**Figure 97:**  $^{13}\text{C}$  NMR spectra of compound coded as DP263.  $^{13}\text{C}$  NMR (500MHz, d-DMSO):  $\delta$  93.84, 105.88, 113.85, 130.48, 137.52, 143.09, 164.01.

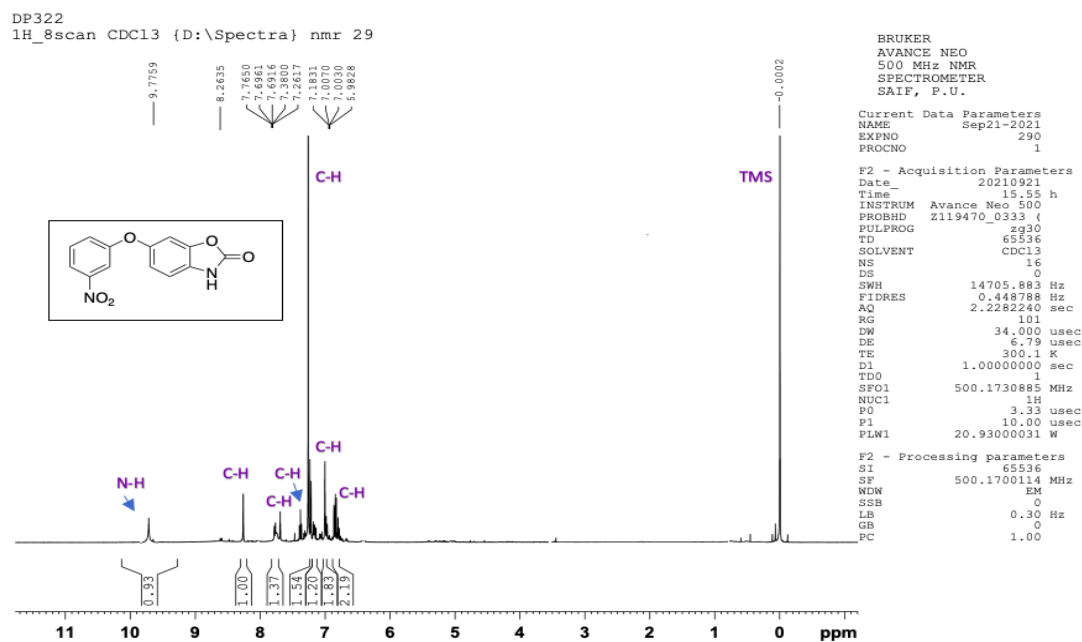


**Figure 98:** Mass spectra for compound coded as DP263. MS ES+ (m/z) at 183.01 [M+].

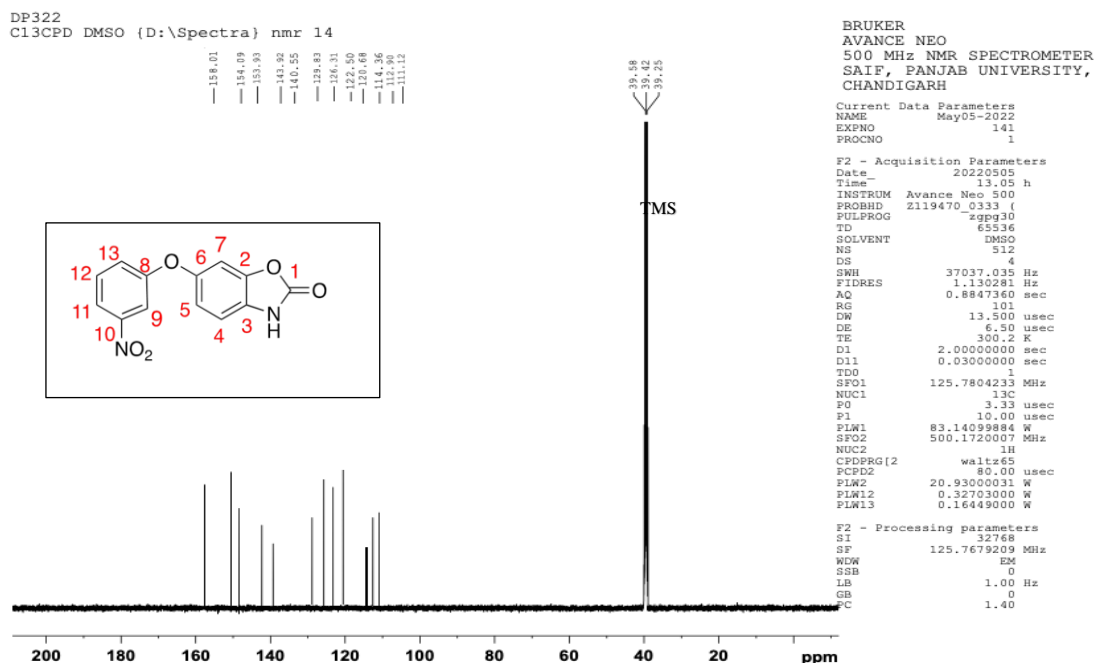
### 11. Spectral Characterization of 6-(3-nitrophenoxy)benzo[d]oxazol-2(3H)-one DP322:



**Figure 99:** FT-IR spectra for compound coded as DP322. FT-IR (cm<sup>-1</sup>): 1481 (NO<sub>2</sub>, asymmetrical), 1347(NO<sub>2</sub>, symmetrical), 1606 (C=C), 1760 (C=O), 2923 (C-H), 3073 (N-H)



**Figure 100:**  $^1\text{H}$ -NMR spectra for compound coded as DP322.  $^1\text{H}$ -NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  6.80-6.86 (m, 2H, ArH), 6.98- 7.00 (m, 1H, ArH) 7.18- 7.21 (m, 1H, ArH), 7.23- 7.26 (m, 1H, ArH), 7.69-7.76 (m, 1H, ArH), 8.25 (s, 1H, ArH), 9.77 (s, 1H, N-H)



**Figure 101:**  $^{13}\text{C}$  NMR Spectra of Compound coded as DP322.  $^{13}\text{C}$  NMR (500MHz, d- DMSO):  $\delta$  112.12, 112.90, 114.36, 120.68, 122.50, 126.31, 129.83, 140.55, 143.92, 153.93, 154.09, 158.

### Sample Information

Analyzed by : Admin  
Analyzed : 24/09/2021 11:40:15  
Sample Type : Unknown  
Level # : 1  
Sample Name : DP322\_MS\_230921-209\_240921-2  
Sample ID : DP322\_MS\_230921-209\_240921-2  
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Report File :  
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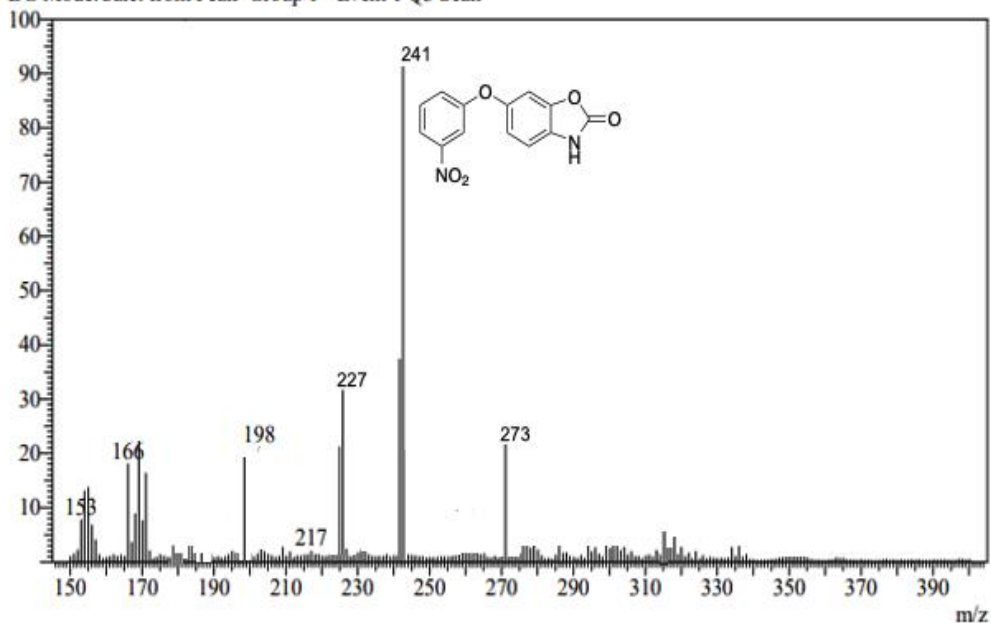
### Spectrum

Line#:1 R.Time:16.530(Scan#:3305)

MassPeaks:251

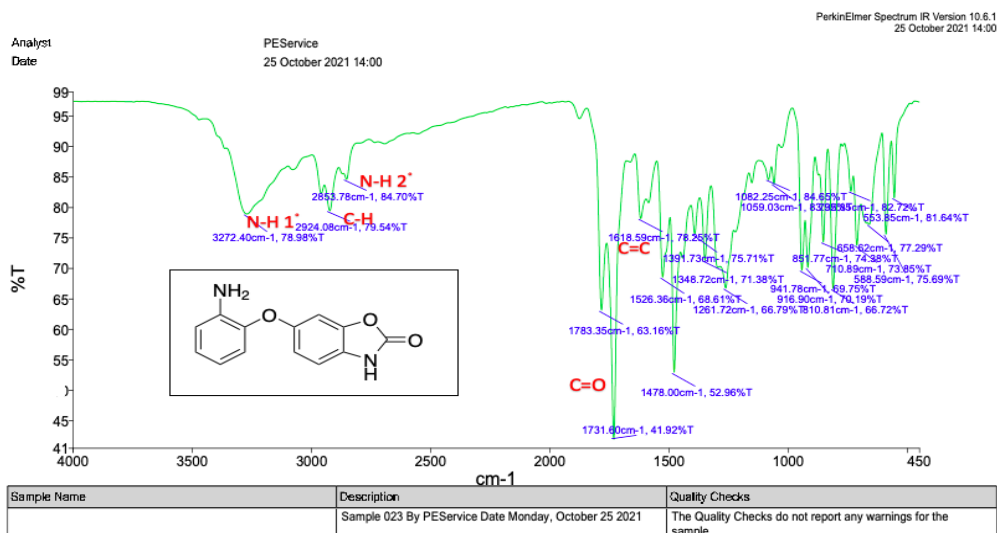
RawMode:Averaged 16.525-16.535(3304-3306) BasePeak:182(114777)

BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

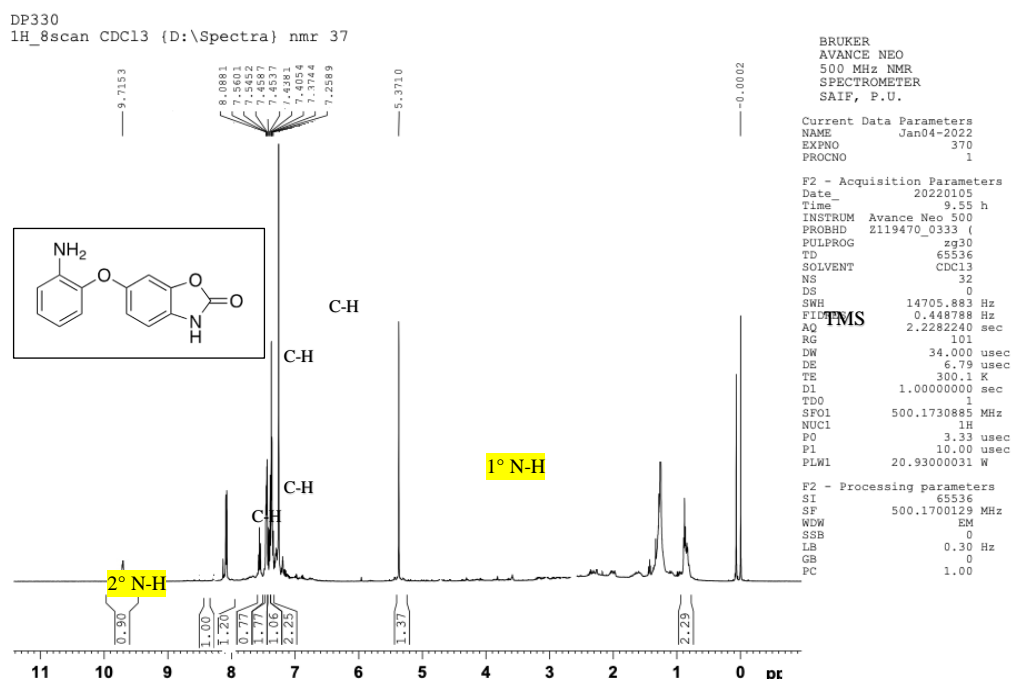


**Figure 102:** Mass spectra for compound coded as DP322. GC MS (m/z) 273 [M+]

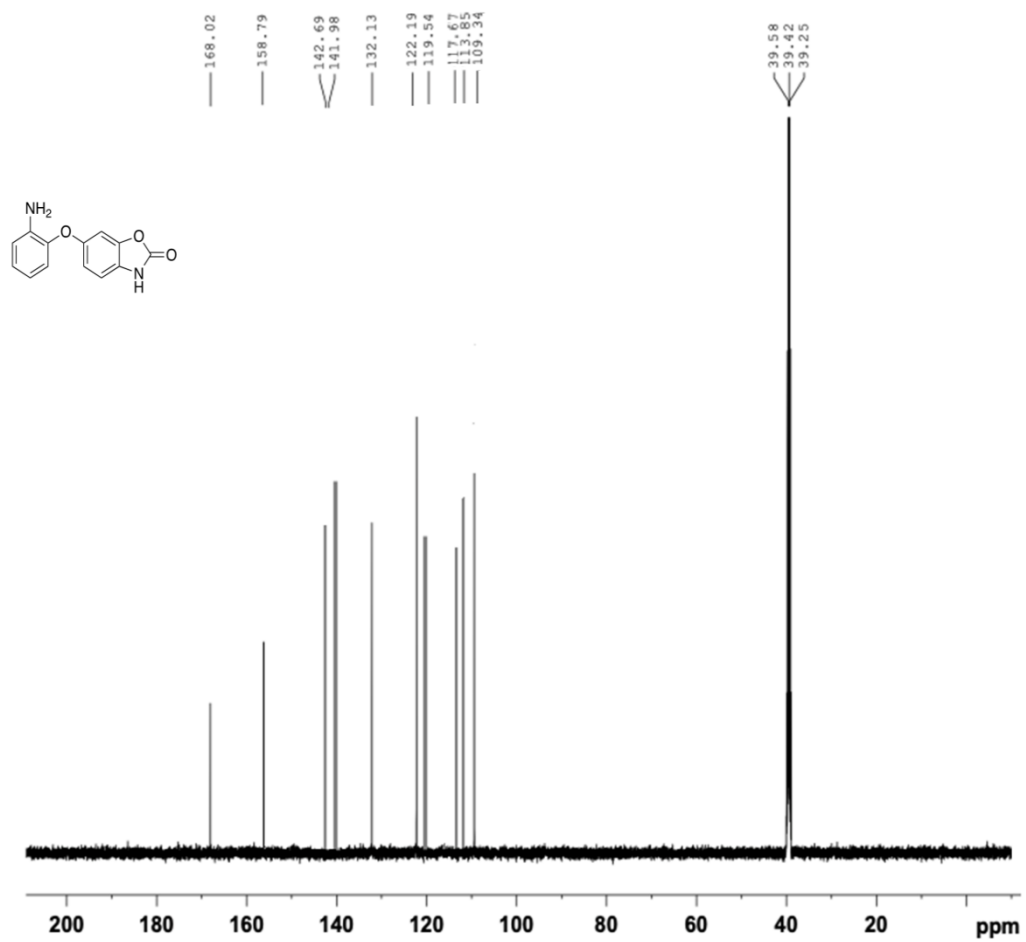
## 12. Spectral Characterization of compound coded as DP330:



**Figure 103:** FT-IR Spectra for Compound coded as DP330. FTIR ( $\text{cm}^{-1}$ ): 1348 (C-O), 1618 (C=C), 1731 (C=O), 2924 (C-H), 3272 ( $2^\circ$  N-H), 3403 ( $1^\circ$  N-H)



**Figure 104:**  $^1\text{H-NMR}$  for compound coded as DP330.  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  5.37 (s, 1H,  $1^\circ\text{NH}$ ), 7.25 (d,  $J = 10.0$  Hz, 2H, ArH), 7.40 (s, 1H, ArH), 7.43-7.45 (m, 2H, ArH), 7.54 (d,  $J = 10.0$  Hz, 1H, ArH), 8.08 (d,  $J = 10.0$  Hz, 1H, ArH), 9.71 (s, 1H,  $2^\circ$  NH)



**Figure 105:** <sup>13</sup>C NMR Spectra of Compound coded as DP330. <sup>13</sup>C-NMR (125 MHz, d-DMSO): δ 109.3, 113.8, 117.6, 119.5, 122.1, 132.1, 141.9, 142.6, 158.7, 168.0



### Sample Information

Analyzed by : Admin  
Analyzed : 31/12/2021 15:21:25  
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Level # : 1  
Sample Name : DP330\_MS-311221-268\_311221  
Sample ID : DP330\_MS-311221-268\_DI\_311221  
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Org Method File : E:\Mass\_DI\_Analysis\GCMS-MS Data 2021\Mass Analysis 2021\Dec 2021\DP330\_MS-3  
Report File :  
Tuning File : C:\GCMSsolution\System\Tune1\Dec 2021\131221\_Normal\_FL\_01\_CID\_ON\_Pass.qgt

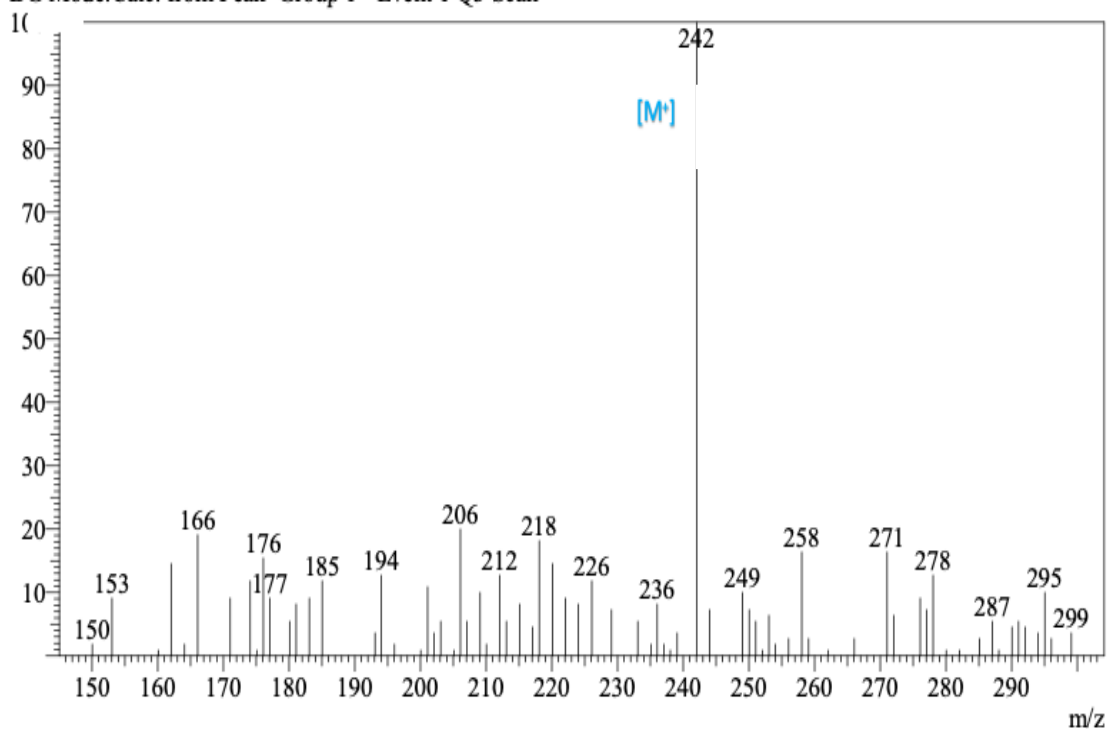
### Spectrum

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MassPeaks:7

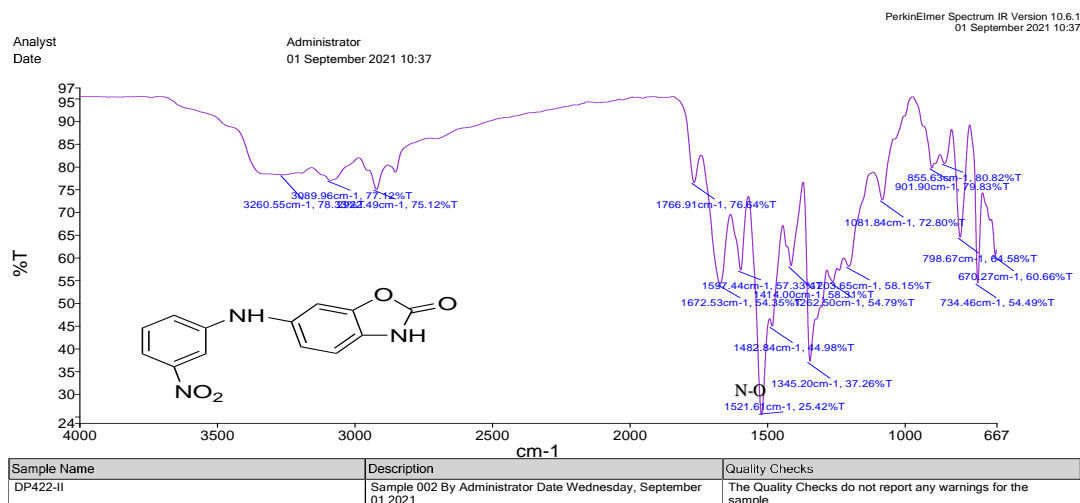
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BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

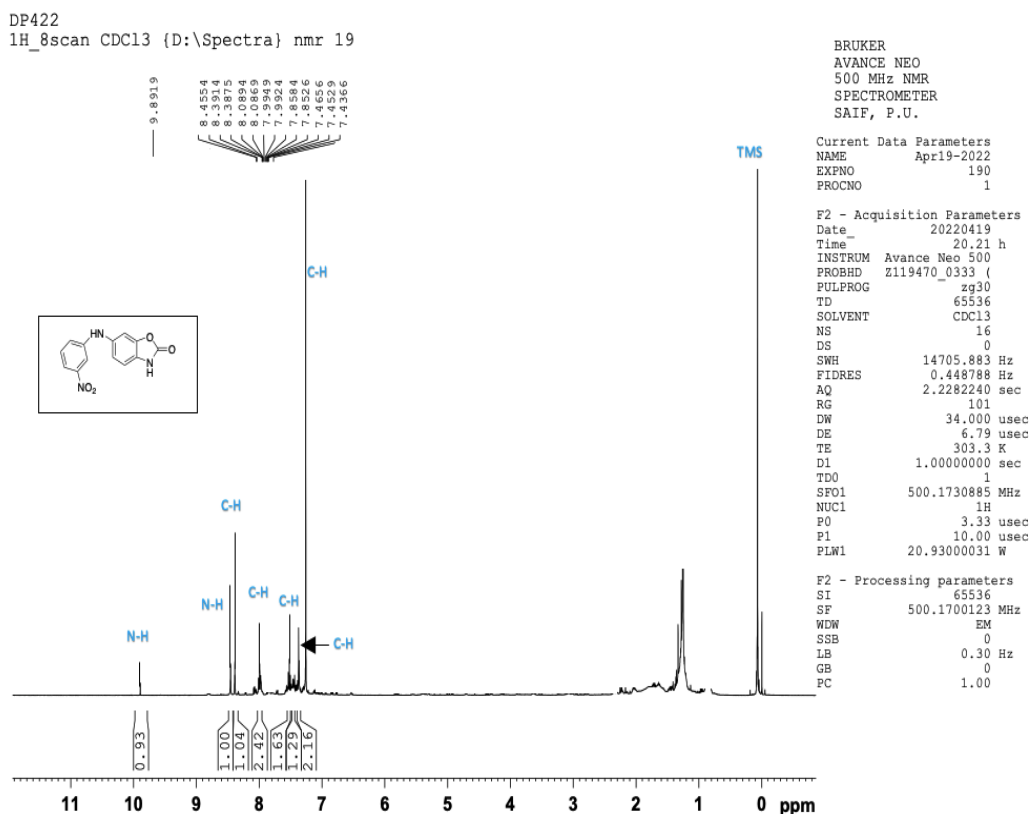


**Figure 106:** Mass spectra for compound coded as DP330. GC MS (m/z) 242 [M+].

### 13. Spectral Characterization of compound coded as DP422:



**Figure 107:** FT-IR Spectra for Compound coded as DP422. FT-IR (KBr  $\text{cm}^{-1}$ ): 1521( $\text{NO}_2$ , asymmetrical), 1345 ( $\text{NO}_2$ , symmetrical), 1617 ( $\text{C}=\text{C}$ ), 1766 ( $\text{C}=\text{O}$ ), 3089 ( $\text{C}-\text{H}$ ), 3260 ( $\text{N}-\text{H}$ ).



**Figure 108:**  $^1\text{H}$ -NMR of compound coded as DP422.  $^1\text{H}$ -NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.36 – 7.38 (m, 2H, ArH), 7.43 -7.46 (m, 1 H, ArH), 7.50 – 7.53 (m, 1 H, ArH), 7.96 – 8.01 (m, 2H, ArH), 8.38- 8.39 (m, 1 H, ArH), 8.45 (s, 1 H, N-H), 9.89 (s, 1-H, N-H);

DP422  
 C13CPD CDC13 {D:\Spectra} nmr 19

BRUKER  
 AVANCE NEO  
 500 MHz NMR SPECTROMETER  
 SAIF, PANJAB UNIVERSITY,  
 CHANDIGARH

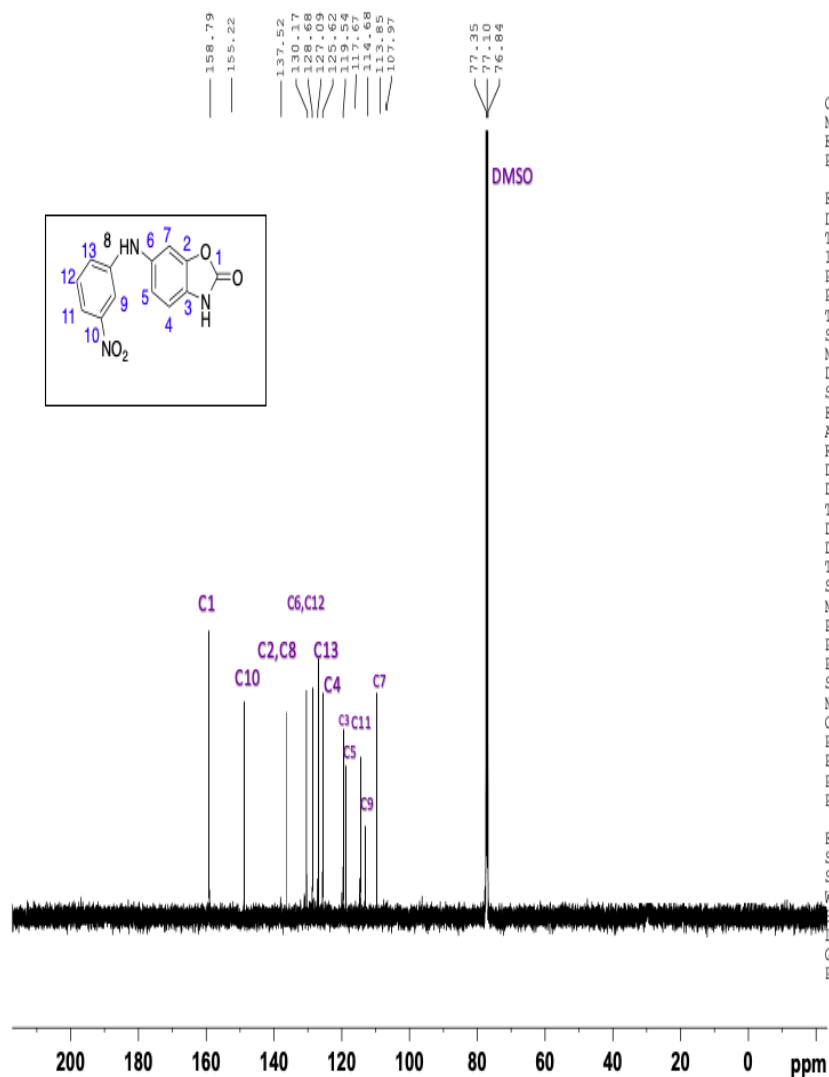
Current Data Parameters  
 NAME Apr19-2022  
 EXPNO 191  
 PROCNO 1

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 NS 1024  
 DS 4  
 SWH 37037.035 Hz  
 FIDRES 1.130281 Hz  
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 DE 6.50 usec  
 TE 304.5 K  
 D1 2.00000000 sec  
 D11 0.03000000 sec  
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 NUC1 13C  
 PO 3.33 usec  
 P1 10.00 usec  
 PLW1 83.14099884 W  
 SF02 500.1720007 MHz  
 NUC2 1H  
 CPDPRG2 waltz65  
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F2 - Processing parameters

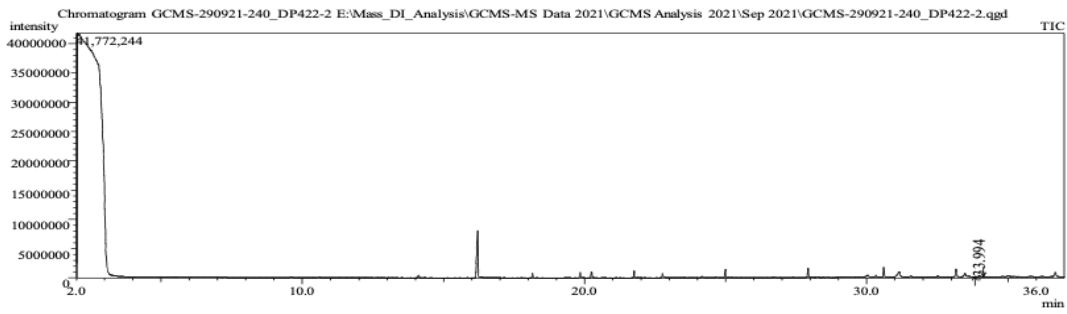
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 WDW EM  
 SSB 0  
 LB 1.00 Hz  
 GB 0  
 PC 1.40



**Figure 109:**  $^{13}\text{C}$  NMR Spectra of Compound coded as DP422.  $^{13}\text{C}$  NMR (500MHz, d-  $\text{CDCl}_3$ ):  $\delta$ 107.97, 113.85, 114.68, 117.67, 119.54, 125.62, 127.09, 128.68, 130.17, 137.52, 155.22, 158.79.

Sample Information

Analyzed : 29/09/2021 13:50:24  
 Sample Type : Unknown  
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 Sample ID : GCMS-290921-240\_DP422-2  
 Vial # : 2  
 Injection Volume : 1.00  
 Data File : E:\Mass\_DI\_Analysis\GCMS-MS Data 2021\GCMS Analysis 2021\Sep 2021\GCMS-290  
 Method File : E:\Mass\_DI\_Analysis\GCMS-MS Data 2021\GCMS Analysis 2021\Sep 2021\GCMS-290  
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 (!=)[Comment]



Peak Report TIC

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|--------|--------|--------|--------|---------|
| 33.994 | 876022 | 100.00 | 197469 | 100.00  |
|        | 876022 | 100.00 | 197469 | 100.00  |

Line#:1 R.Time:33.995(Scan#:6400)  
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 BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

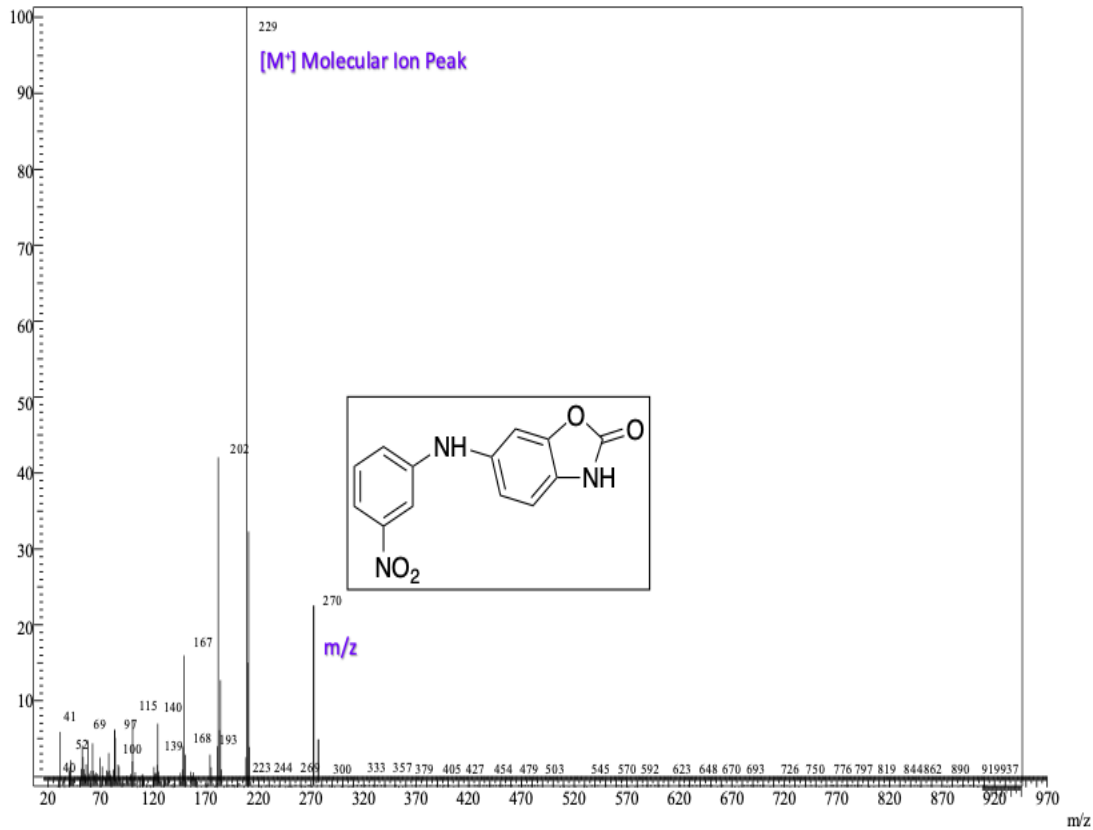
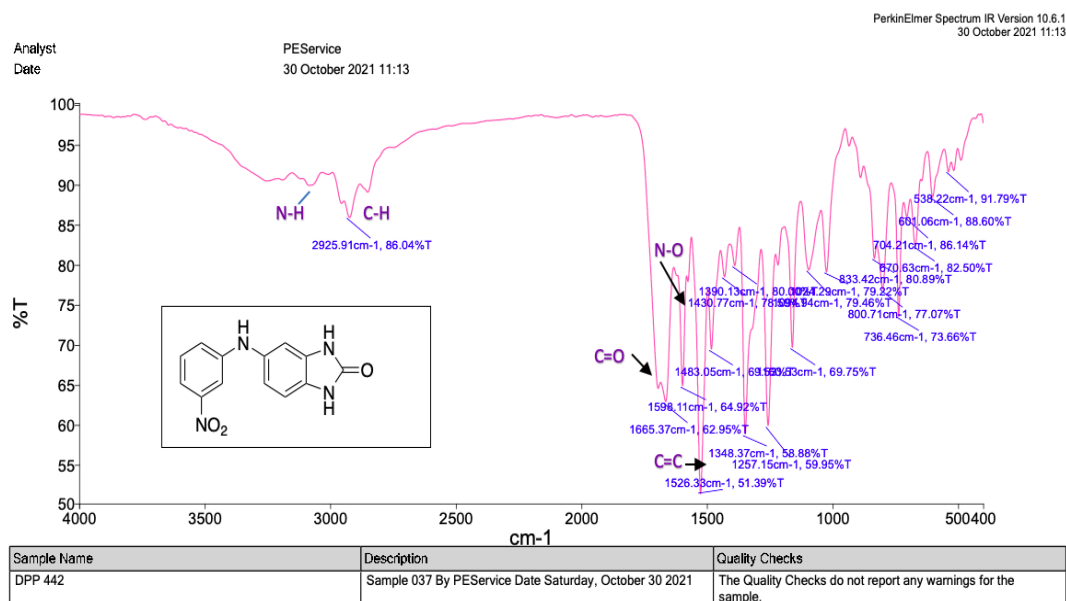
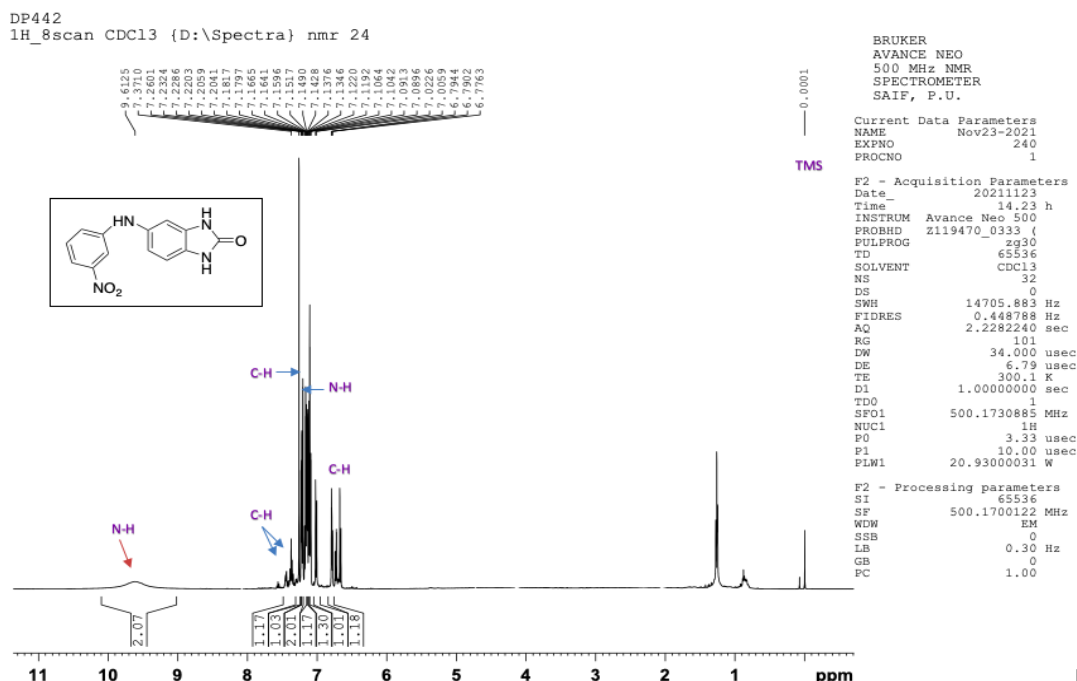


Figure 110:GC-MS spectra for compound coded as DP422. GC MS (m/z) at 270.

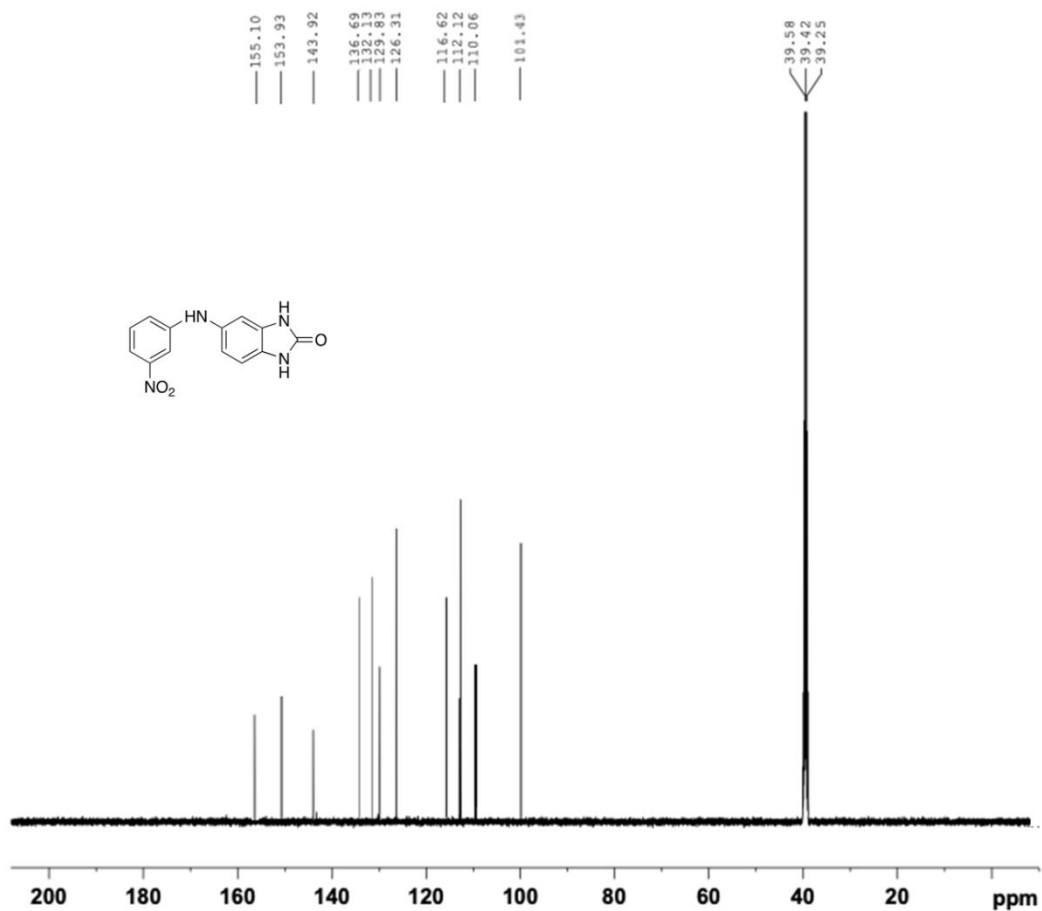
## 14. Spectral Characterization of compound coded as DP442:



**Figure 111:** FT-IR Spectra for Compound coded as DP442. FT-IR (KBr  $\text{cm}^{-1}$ ): 1526 (NO<sub>2</sub>, asymmetrical), 1348 (NO<sub>2</sub>, symmetrical), 1598 (C=C), 1665 (C=O), 2925 (C-H), 3325 (N-H).



**Figure 112:** <sup>1</sup>H-NMR Spectra for compound coded as DP442. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  6.78 (d,  $J$  = 10.0 Hz, 1 H, ArH), 7.02 (d,  $J$  = 10.0 Hz, 1H, ArH), 7.10 (d,  $J$  = 10.0 Hz, 1H, ArH), 7.14 (d,  $J$  = 10.0 Hz, 1H, ArH), 7.16-7.18 (m, 2H, ArH), 7.20 (d, 1H,  $J$  = 10.0 Hz, ArH), 7.37 (s, 1H, ArH), 9.61 (s, 2H, NH); (2H,C-H), 7.26 (1H,N-H), 6.93 (1H,C-H), 6.79 (1H, C-H)



**Figure 113:** <sup>13</sup>C NMR Spectra of Compound coded as DP442. <sup>13</sup>C NMR (500MHz, d- CDCl<sub>3</sub>): <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ 101.4, 110.0, 112.1, 116.6, 126.3, 129.8, 132.1, 143.9, 153.9, 155.1.

Sample Information

Analyzed by : Admin  
Analyzed : 31/12/2021 15:21:25  
Sample Type : Unknown  
Level # : 1  
Sample Name : |DP442\_MS-311221-268\_311221  
Sample ID : DP442\_MS-311221-268\_DI\_311221  
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Org Data File : E:\Mass\_DI\_Analysis\GCMS-MS Data 2021\Mass Analysis 2021\Dec 2021\MS-3  
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Report File :  
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Spectrum

Line#: 1 R.Time:16.125(Scan#:3224)

MassPeaks: 73

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BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

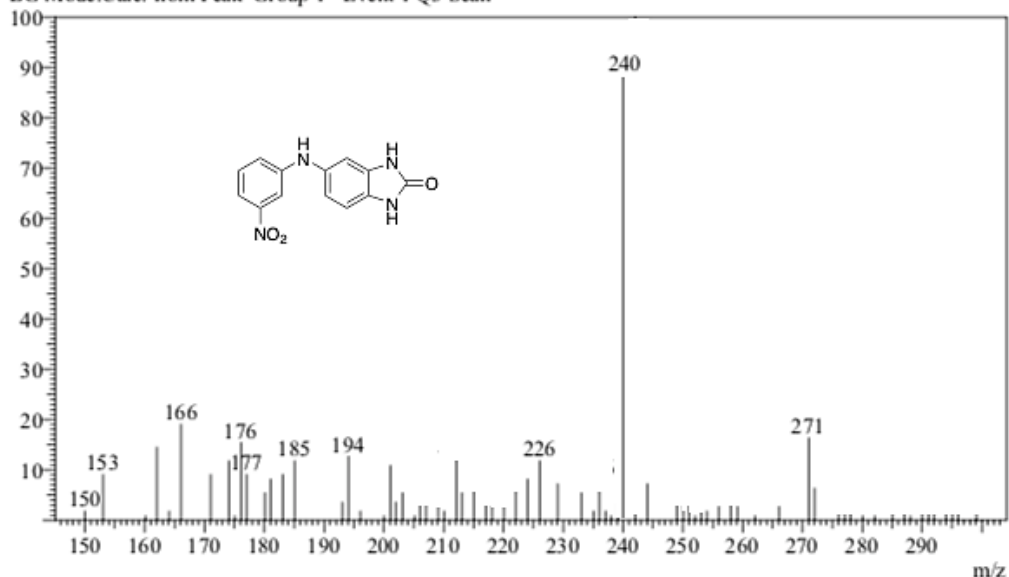


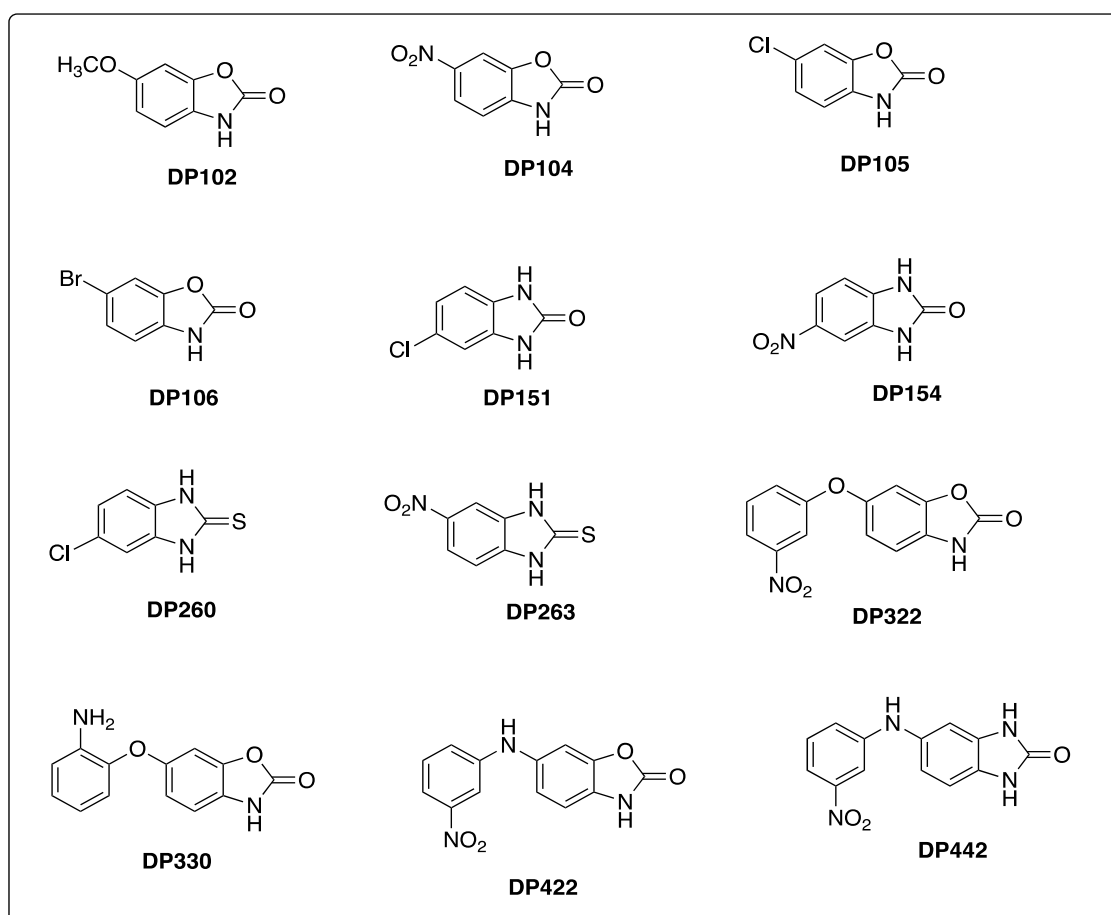
Figure 114: Mass spectra for compound coded as DP442. MS m/z at 271 [M+].

### 6.7: *In-silico* toxicity studies:

Presently, Toxicity of synthesized compounds is one of a major concern. Therefore all the synthesized molecules were checked if there is any toxicity issue associated with for their further development. We studied total twelve synthesized compounds from series 1 to 4. The *In silico* toxicity prediction was performed using software available online at <https://lazar.in-silicoboratory.ch/predict>.

Following steps are mentioned to predict the toxicity of designed molecules:

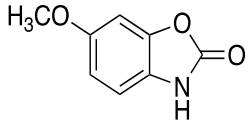
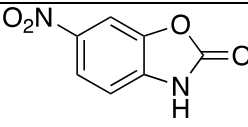
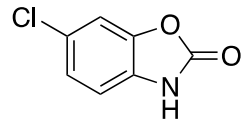
- Open or draw a 2D structure of designed compound in Chem Bio draw software.
- Copy the smileys for each structure from Chem Bio draw - Paste the smileys into the webpage.
- Thereafter predict all type of toxicity.

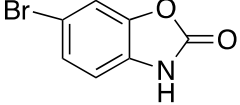
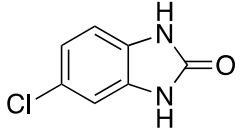
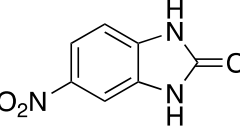
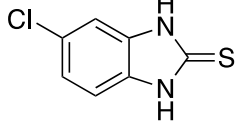
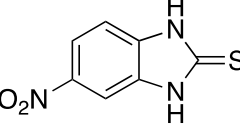


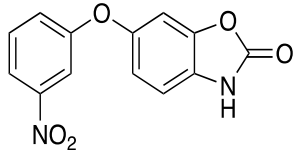
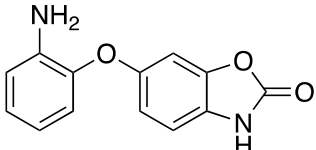
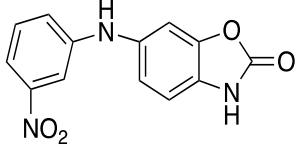
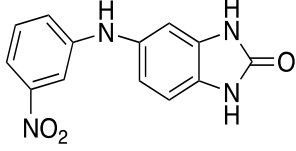
**Figure 115:** Designed and Synthesized molecules



**Table 14:** *In-silico* toxicity prediction by Lazar toxicity interface (<https://lazar.in-silico.de/predict>) NA = not available

| Compound structure   | Carcinogenicity (mouse)  | Carcinogenicity (Rat)   | Maximum Recommended Daily Dose (human)                | Mutagenicity (Salmonella-Typhimurium)   |
|--|--|---|---|---|
|  <p><b>DP102</b></p>  | <p><b>Non-carcinogenic</b><br/>(carcinogenic- 0.0932 vs non-carcinogenic-0.157)</p>                      | <p><b>Non-carcinogenic</b><br/>(carcinogenic: 0.111 vs non-carcinogenic: 0.174)</p>                       | <p>0.011 (mmol/kg-bw/day)<br/>1.82 (mg/kg bw/day)</p> | <p><b>Mutagenic</b><br/>Probability:<br/>non-mutagenic: 0.206<br/>mutagenic: 0.294</p>    |
|  <p><b>DP104</b></p>  | <p><b>Non-carcinogenic</b><br/>(non-carcinogenic-0.191 vs carcinogenic- 0.166)</p>                       | <p><b>Non-carcinogenic</b><br/>carcinogenic- 0.138<br/>Non- carcinogenic- 0.22</p>                        | <p>0.233(mmol/kg-bw/day)<br/>42.0 (mg/kg bw/day)</p>  | <p><b>Mutagenic</b><br/>Probability:<br/>mutagenic: 0.394<br/>non-mutagenic: 0.0729</p>   |
|  <p><b>DP105</b></p> | <p><b>Non-carcinogenic</b><br/><b>Probability:</b><br/>carcinogenic-0.119<br/>non-carcinogenic-0.153</p> | <p><b>Non-carcinogenic</b><br/><b>Probability:</b><br/>carcinogenic: 0.13<br/>non-carcinogenic: 0.143</p> | <p>0.13 (mmol/kg-bw/day)<br/>22.1 (mg/kg-bw/day)</p>  | <p><b>Non-mutagenic</b><br/>Probability:<br/>mutagenic-0.163<br/>non-mutagenic: 0.376</p> |

|   |  |   |   |  |
|---|--|---|---|--|
| <br><b>DP106</b>   | NA   | NA  | NA  | <b>Non-mutagenic</b><br>Probability<br>non-mutagenic: 0.496<br>mutagenic: 0.0427     |
| <br><b>DP151</b>   | <b>Non-carcinogenic</b><br>carcinogenic: 0.117<br>non-carcinogenic: 0.216                      | <b>Non-carcinogenic</b><br>carcinogenic: 0.166<br>non-carcinogenic:<br>0.167                        | 0.0477 (mmol/kg-bw/day)<br>8.04 (mg/kg-bw/day)    | <b>Non-mutagenic</b><br>Probability:<br>Non mutagenic- 0.25<br>non-mutagenic: 0.25   |
| <br><b>DP154</b>   | <b>Non-carcinogenic</b><br><b>Probability:</b><br>non-carcinogenic-0.32<br>carcinogenic: 0.295 | <b>Non-carcinogenic</b><br><b>Probability:</b><br>carcinogenic: 0.245<br>carcinogenic: 0.371        | 0.00822 (mmol/kg-bw/day)<br>1.47 (mg/kg-bw/day)   | <b>Mutagenic</b><br><b>Probability:</b><br>mutagenic: 0.585<br>non-mutagenic: 0.0814 |
| <br><b>DP260</b>  | <b>Carcinogenic</b><br><b>Probability:</b><br>non-carcinogenic: 0.117<br>carcinogenic: 0.216   | <b>Non-carcinogenic</b><br><b>Probability:</b><br>non-carcinogenic-<br>0.167<br>carcinogenic: 0.166 | 0.0484  | <b>Non-mutagenic</b><br><b>Probability:</b><br>non-mutagenic-0.25<br>mutagenic: 0.25 |
| <br><b>DP263</b> | <b>Non-carcinogenic</b><br><b>Probability:</b><br>non-carcinogenic-0.32<br>carcinogenic: 0.295 | <b>Non-carcinogenic</b><br><b>Probability:</b><br>carcinogenic-0.245<br>Non carcinogenic-<br>0.371  | 0.0064 (mmol/kg-bw/day)<br>1.25<br>(mg/kg-bw/day) | <b>mutagenic</b><br><b>Probability:</b><br>non-mutagenic: 0.0814<br>mutagenic: 0.585 |

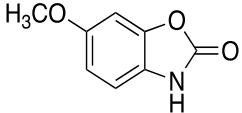
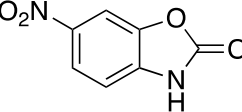
|   |  |  |  |   |
|---|--|--|--|---|
|  <p><b>DP322</b></p>   | <p><b>Non-carcinogenic</b><br/><b>Probability:</b><br/>noncarcinogenic: 0.168<br/>carcinogenic: 0.165</p>  | <p><b>Non-carcinogenic</b><br/><b>Probability:</b><br/>carcinogenic: 0.163<br/>Non carcinogenic:<br/>0.171</p> | <p>0.0214<br/>(mmol/kg-bw/day)<br/>5.85<br/>(mg/kg-bw/day)</p> | <p><b>Non mutagenic</b><br/><b>Probability:</b><br/>non-mutagenic: 0.409<br/>mutagenic: 0.0912</p>      |
|  <p><b>DP330</b></p>   | <p><b>Carcinogenic</b><br/><b>Probability:</b><br/>carcinogenic: 0.18<br/>non carcinogenic- 0.13</p>       | <p><b>Non-carcinogenic</b><br/><b>Probability:</b><br/>carcinogenic: 0.178<br/>non-carcinogenic:<br/>0.294</p> | <p>NA</p>  | <p><b>Mutagenic</b><br/><b>Probability:</b><br/>mutagenic: 0.352<br/>non-mutagenic: 0.119</p>           |
|  <p><b>DP422</b></p>  | <p><b>Non-carcinogenic</b><br/>non-carcinogenic- 0.21<br/>carcinogenic- 0.143</p>                          | <p><b>Non-carcinogenic</b><br/><b>Probability:</b><br/>non-carcinogenic-<br/>0.214<br/>carcinogenic: 0.139</p> | <p>0.0068 (mmol/kg-bw/day)<br/>1.85(mg/kg_bw/day)</p>          | <p><b>Non- mutagenic</b><br/><b>Probability:</b><br/>mutagenic:<br/>0.0795<br/>non-mutagenic: 0.391</p> |
|  <p><b>DP442</b></p> | <p><b>Non-carcinogenic</b><br/><b>Probability:</b><br/>non-carcinogenic- 0.271<br/>carcinogenic: 0.199</p> | <p><b>Non-carcinogenic</b><br/><b>Probability:</b><br/>carcinogenic- 0.193<br/>non carcinogenic:<br/>0.277</p> | <p>0.0011(mmol/kg-bw/day)<br/>0.299(mg/kg-bw/day)</p>          | <p><b>Non- mutagenic</b><br/><b>Probability:</b><br/>non-mutagenic: 0.447<br/>mutagenic: 0.116</p>      |

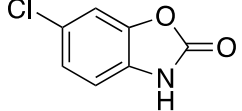
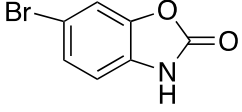
The Table 6 showed *in-silico* toxicity profile of the synthesized molecules, the result showed that synthetic compounds **Coixol (DP102), DP104, DP105, DP151, DP154, DP263, DP322, DP422** and **DP442** are found to be Non carcinogenic in Mouse and rats whereas compound **DP260** and **DP330** found to be carcinogenic in Mouse but Non carcinogenic in Rats.

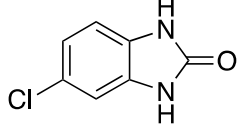
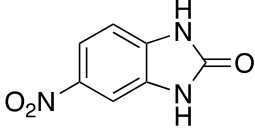
#### **6.8: *In-silico* ADME studies:**

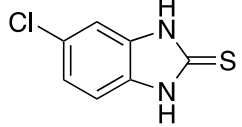
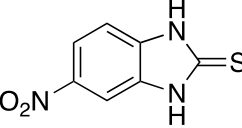
Since, toxicity is associated with the Pharmacokinetic profile of the compounds i.e. Adsorption, distribution, metabolism and excretion. Thus we analysed *in-silico* ADME properties of all the synthesized compounds to estimate their water solubility and lipophilicity with their toxicity (Table 7). The *in-silico* ADME properties of the compounds were estimated as a numeric value for the prediction of physicochemical properties, lipophilicity, water solubility, pharmacokinetic file and drug likeness by following Lipinski rule of five. <http://www.swissadme.ch/index.php>

**Table 15:** *In silico* ADME prediction:

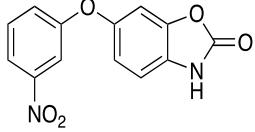
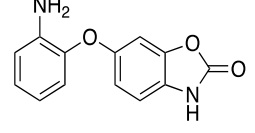
| Compounds Structure  | Physicochemical properties  | Water solubility  | Lipophilicity                               | Drug Likeness    | Pharmacokinetics  |
|--|---|---|---|------------------|---|
|  <p><b>DP102</b></p>  | Formula: C <sub>8</sub> H <sub>7</sub> NO <sub>3</sub><br>Molecular weight: 165.15 g/mol<br>Molar refractivity: 43.33               | <i>Log S (Ali):</i> - 1.88<br>Solubility- 2.16e+00 mg/ml; 1.31e-02 mol/l<br>Class- very soluble | <i>Log P<sub>o/w</sub> (i LOG P)</i> - 1.65 | Yes; 0 violation | GI absorption- High<br>BBB permeant- Yes<br>P-gp substrate- No<br>CYP1A2 inhibitor- Yes<br>CYP2C19 inhibitor-NO<br>CYP2C9 inhibitor-NO<br>CYP2D6 inhibitor-NO<br>CYP3A4 inhibitor- No<br>Log K <sub>p</sub> (Skin permeation) - 6.51 cm/s |
|  <p><b>DP104</b></p> | Formula: C <sub>7</sub> H <sub>4</sub> N <sub>2</sub> O <sub>4</sub><br>Molecular weight: 180.12 g/mol<br>Molar refractivity: 45.66 | <i>Log S (Ali):</i> - 2.51<br>Solubility- 5.61e+00 mg/ml; 3.11 e-02 mol/l<br>Class- soluble     | <i>Log P<sub>o/w</sub> (i LOG P)</i> - 0.92 | Yes; 0 violation | GI absorption- High<br>BBB permeant- NO<br>P-gp substrate- NO<br>CYP1A2 inhibitor- Yes<br>CYP2C19 inhibitor-NO<br>CYP2C9 inhibitor-NO<br>CYP2D6 inhibitor-NO<br>CYP3A4 inhibitor- No<br>Log K <sub>p</sub> (Skin permeation) - 6.70 cm/s  |

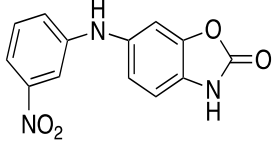
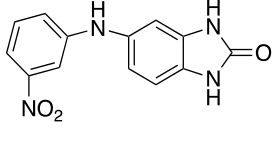
|   |   |  |  |                         |   |
|---|---|--|--|-------------------------|---|
| <p><b>DP105</b></p>  | <p>Formula: C<sub>7</sub>H<sub>4</sub>ClNO<sub>2</sub><br/> Molecular weight: 169.57 g/mol<br/> Molar refractivity: 41.84</p> | <p><i>Log S (Ali):</i> - 2.37<br/> Solubility- 7.16e-01 mg/ml;<br/> 4.22e-03 mol/l<br/> Class- soluble</p> | <p>Log P<sub>o/w</sub> (iLOGP)- 1.54</p> | <p>Yes; 0 violation</p> | <p>GI absorption- High<br/> BBB permeant- Yes<br/> P-gp substrate- NO<br/> CYP1A2 inhibitor- Yes<br/> CYP2C19 inhibitor-NO<br/> CYP2C9 inhibitor-NO<br/> CYP2D6 inhibitor-NO<br/> CYP3A4 inhibitor- NO<br/> Log K<sub>p</sub> (Skin permeation) - 6.06 cm/s</p> |
| <p><b>DP106</b></p>  | <p>Formula: C<sub>7</sub>H<sub>4</sub>BrNO<sub>2</sub><br/> Molecular weight: 214.02 g/mol<br/> Molar refractivity: 44.53</p> | <p><i>Log S (Ali):</i> - 2.44<br/> Solubility- 7.83-01 mg/ml;<br/> 3.66e-03 mol/l<br/> Class- Soluble</p>  | <p>Log P<sub>o/w</sub> (ILOGP)- 1.65</p> | <p>Yes; 0 violation</p> | <p>GI absorption- High<br/> BBB permeant- Yes<br/> P-gp substrate- NO<br/> CYP1A2 inhibitor- Yes<br/> CYP2C19 inhibitor-NO<br/> CYP2C9 inhibitor-NO<br/> CYP2D6 inhibitor-NO<br/> CYP3A4 inhibitor- NO<br/> Log K<sub>p</sub> (Skin permeation) - 6.29 cm/s</p> |

|   |  |   |   |                              |  |
|---|--|---|---|------------------------------|--|
|  <p><b>DP151</b></p> | <p>Formula: C<sub>7</sub>H<sub>5</sub>ClN<sub>2</sub>O<br/> Molecular weight:<br/> 168.58g/mol<br/> Molar refractivity: 43.93</p>            | <p><i>Log S (Ali):</i> -<br/> 2.39<br/> Solubility-<br/> 6.89e-01 mg/ml;<br/> 4.08e-03 mol/l<br/> Class- Soluble</p>                  | <p><i>Log P<sub>o/w</sub> (I LOG P)</i>- 1.32</p> | <p>Yes; 0<br/> violation</p> | <p>GI absorption- High<br/> BBB permeant- Yes<br/> P-gp substrate- NO<br/> CYP1A2 inhibitor- Yes<br/> CYP2C19 inhibitor-NO<br/> CYP2C9 inhibitor-NO<br/> CYP2D6 inhibitor-NO<br/> CYP3A4 inhibitor- NO<br/> Log K<sub>p</sub> (Skin permeation) -<br/> 6.09 cm/s</p> |
|  <p><b>DP154</b></p> | <p>Formula: C<sub>7</sub>H<sub>5</sub>N<sub>3</sub>O<sub>3</sub><br/> Molecular weight: 179.13<br/> g/mol<br/> Molar refractivity: 47.74</p> | <p><i>Log S (Ali):</i> -<br/> 1.94<br/> Solubility-<br/> 2.06e+00<br/> mg/ml; 1.15 e-<br/> 02 mol/l<br/> Class- Very<br/> soluble</p> | <p><i>Log P<sub>o/w</sub> (iLOGP)</i>- 0.73</p>   | <p>Yes; 0<br/> violation</p> | <p>GI absorption- High<br/> BBB permeant- Yes<br/> P-gp substrate- NO<br/> CYP1A2 inhibitor- Yes<br/> CYP2C19 inhibitor-NO<br/> CYP2C9 inhibitor-NO<br/> CYP2D6 inhibitor-NO<br/> CYP3A4 inhibitor- No<br/> Log K<sub>p</sub> (Skin permeation) -<br/> 7.12 cm/s</p> |

|   |  |  |   |                         |   |
|---|--|--|---|-------------------------|---|
|  <p><b>DP260</b></p> | <p>Formula: C<sub>7</sub>H<sub>5</sub>ClN<sub>2</sub>S<br/> Molecular weight: 184.65 g/mol<br/> Molar refractivity: 48.49</p>            | <p><i>Log S (Ali):</i> - 3.26<br/> Solubility- 1.00e-01 mg/ml;<br/> 5.44e-04 mol/l<br/> Class- Soluble</p> | <p><i>Log P<sub>o/w</sub></i><br/> (<i>iLOGP</i>)- 1.73</p> | <p>Yes; 0 violation</p> | <p>GI absorption- High<br/> BBB permeant- Yes<br/> P-gp substrate- NO<br/> CYP1A2 inhibitor- Yes<br/> CYP2C19 inhibitor-NO<br/> CYP2C9 inhibitor-NO<br/> CYP2D6 inhibitor-NO<br/> CYP3A4 inhibitor- NO<br/> Log K<sub>p</sub> (Skin permeation) - 5.80 cm/s</p> |
|  <p><b>DP263</b></p> | <p>Formula: C<sub>7</sub>H<sub>5</sub>N<sub>3</sub>O<sub>2</sub>S<br/> Molecular weight: 195.20 g/mol<br/> Molar refractivity: 52.31</p> | <p><i>Log S (Ali):</i> - 2.88<br/> Solubility- 2.59e-01 mg/ml;<br/> 1.32e-03 mol/l<br/> Class- Soluble</p> | <p><i>Log P<sub>o/w</sub></i><br/> (<i>iLOGP</i>)- 1.11</p> | <p>Yes; 0 violation</p> | <p>GI absorption- High<br/> BBB permeant- NO<br/> P-gp substrate- NO<br/> CYP1A2 inhibitor- Yes<br/> CYP2C19 inhibitor-Yes<br/> CYP2C9 inhibitor-NO<br/> CYP2D6 inhibitor-NO<br/> CYP3A4 inhibitor- NO<br/> Log K<sub>p</sub> (Skin permeation) - 6.79 cm/s</p> |



|   |   |  |   |                         |   |
|---|---|--|---|-------------------------|---|
|  <p><b>DP322</b></p> | <p>Formula: C<sub>13</sub>H<sub>8</sub>N<sub>2</sub>O<sub>5</sub><br/> Molecular weight: 272.21 g/mol<br/> Molar refractivity: 72.17</p>  | <p><i>Log S (Ali):</i> - 4.27<br/> Solubility- 1.47E-02 mg/ml; 5.40e-05 mol/l<br/> Class- Moderately soluble</p> | <p><i>Log P<sub>o/w</sub> (iLOGP)</i>- 1.89</p> | <p>Yes; 0 violation</p> | <p>GI absorption- High<br/> BBB permeant- NO<br/> P-gp substrate- NO<br/> CYP1A2 inhibitor- Yes<br/> CYP2C19 inhibitor-NO<br/> CYP2C9 inhibitor-Yes<br/> CYP2D6 inhibitor-NO<br/> CYP3A4 inhibitor- NO<br/> Log K<sub>p</sub> (Skin permeation) - 6.19 cm/s</p> |
|  <p><b>DP330</b></p> | <p>Formula: C<sub>13</sub>H<sub>10</sub>N<sub>2</sub>O<sub>3</sub><br/> Molecular weight: 242.23 g/mol<br/> Molar refractivity: 67.76</p> | <p><i>Log S (Ali):</i> - 3.32<br/> Solubility- 1.15-01mg/ml; 4.76e-04 mol/l<br/> Class- Soluble</p>              | <p><i>Log P<sub>o/w</sub> (iLOGP)</i>- 1.90</p> | <p>Yes; 0 violation</p> | <p>GI absorption- High<br/> BBB permeant- NO<br/> P-gp substrate- NO<br/> CYP1A2 inhibitor- Yes<br/> CYP2C19 inhibitor-NO<br/> CYP2C9 inhibitor-NO<br/> CYP2D6 inhibitor-NO<br/> CYP3A4 inhibitor- NO<br/> Log K<sub>p</sub> (Skin permeation) - 6.36 cm/s</p>  |

|   |   |  |   |                         |  |
|---|---|--|---|-------------------------|--|
|  <p><b>DP422</b></p> | <p>Formula: C<sub>13</sub>H<sub>9</sub>N<sub>3</sub>O<sub>4</sub><br/> Molecular weight: 271.23 g/mol<br/> Molar refractivity: 75.20</p>  | <p><i>Log S (Ali):</i> - 4.34<br/> Solubility- 1.25e-02 mg/ml;<br/> 4.6e-05 mol/l<br/> Class- Moderately soluble</p> | <p><i>Log P<sub>o/w</sub> ( ILOG P)</i>- 1.59</p> | <p>Yes; 0 violation</p> | <p>GI absorption- High<br/> BBB permeant- NO<br/> P-gp substrate- NO<br/> CYP1A2 inhibitor- Yes<br/> CYP2C19 inhibitor-NO<br/> CYP2C9 inhibitor-NO<br/> CYP2D6 inhibitor-NO<br/> CYP3A4 inhibitor- NO<br/> Log K<sub>p</sub> (Skin permeation) - 6.17 cm/s</p> |
|  <p><b>DP442</b></p> | <p>Formula: C<sub>13</sub>H<sub>10</sub>N<sub>4</sub>O<sub>3</sub><br/> Molecular weight: 270.24 g/mol<br/> Molar refractivity: 77.29</p> | <p><i>Log S (Ali):</i> - 3.79<br/> Solubility- 4.38e-02 mg/ml;<br/> 1.62e-04 mol/l<br/> Class- Soluble</p>           | <p><i>Log P<sub>o/w</sub> (iLOGP)</i>- 1.40</p>   | <p>Yes; 0 violation</p> | <p>GI absorption- High<br/> BBB permeant- NO<br/> P-gp substrate- NO<br/> CYP1A2 inhibitor- Yes<br/> CYP2C19 inhibitor-NO<br/> CYP2C9 inhibitor-NO<br/> CYP2D6 inhibitor-NO<br/> CYP3A4 inhibitor- NO<br/> Log K<sub>p</sub> (Skin permeation) - 6.58 cm/s</p> |

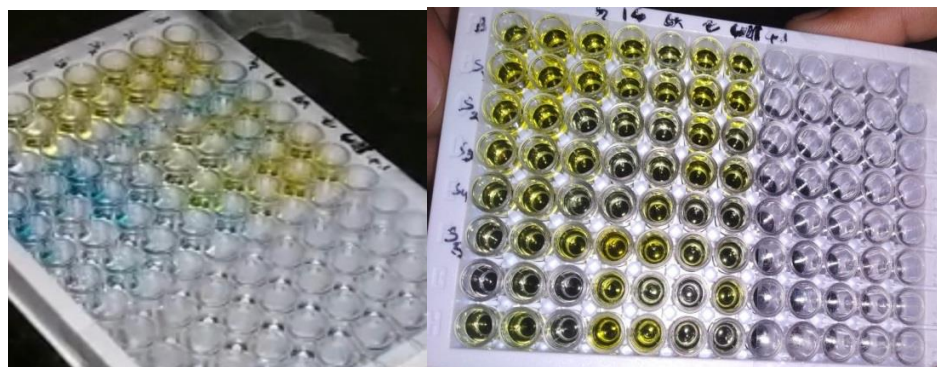
The result of *in silico* ADME indicated, Good-solubility of compounds in water and High GI absorption with no penetration in Blood Brain Barrier except few molecules. Calculated value of lipophilicity (*I Log P*) was observed in between 0.73 to 1.90.

### **6.9: *In vitro* evaluation by Insulin secretion assay:**

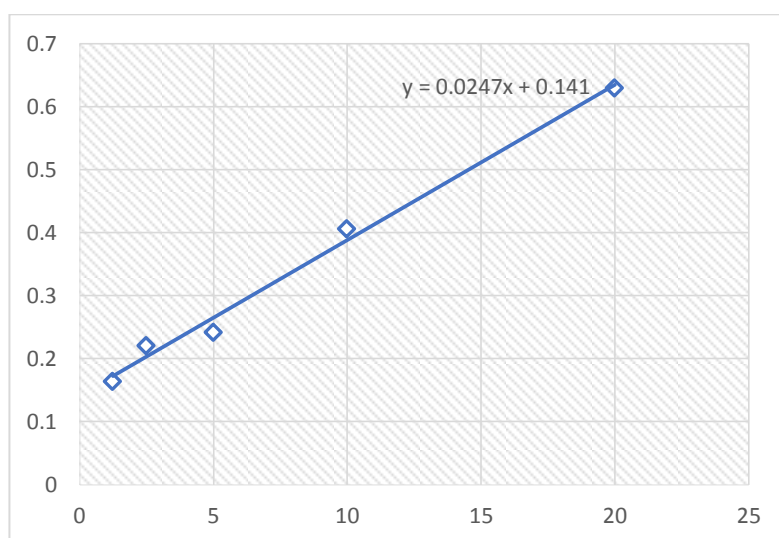
For the *in vitro* analysis of twelve synthesized potential antidiabetic compounds, Rat insulin ELISA kit from Abbkines was chosen.<sup>6</sup> For the evaluation, pancreatic islets from Sprague-Dawley rats were isolated by using collagenase IV enzymatic breakdown. The separated islets were kept in Hanks balanced salt solution (HBSS) without calcium, magnesium and phenol-red and purified through cold centrifugation at 4°C using rpm 1000 for about 1 min which was further filtered through a 70 mm thickness cell-strainer. Thereafter, the separated islets were pre incubated for 30 min at 37°C in Kreb's Ringer-bicarbonate buffer containing 3 mM glucose and 0.1% BSA. After that, different batches were prepared for similar size islets that were kept in KRB buffer medium and later incubated with 3 mM (basal) and 16.7 mM (stimulatory) glucose for 1 hour in the absence and presence of test samples. At the end of the incubation, 150-200 µL samples from each tubes were quickly freeze at -80°C till ELISA assay. To check the insulin release effect of test samples, A rat Insulin ELISA kit was used. For *in vitro* insulin secretion assay, Glibenclamide was utilised as a standard at concentration 100-200 µM.

**6.9.1: ELISA Insulin assay procedure:** The ELISA assay was initiated with the addition of diluted standards maintained at different concentrations i.e. 1.25, 2.5, 5, 10 and 20 mU/L into separate wells. Each well was filled with 50 µL of standard diluents of different concentrations. Similarly, remaining wells were filled with 40 µL of sample diluents and named as sample wells. Thereafter, 10 µL amount of 12 different test samples were added at triplicate concentration in different sample wells except the blank well. All the wells were covered with a plate cover provided with the kit and incubated at 37°C for about 45 minutes. Post incubation, Each wells were aspirated and washings were given four times for 1-3 minutes each times. The washings was given by the wash buffer (250 µL each time) provided with the kit. After the washing, each well was again aspirated to remove any traces of wash buffer. Following the addition of 50 µL HRP conjugated detection antibody into each well

except the blank. All the wells were covered again and incubated at 37°C for 30 minutes. Later on, individual plates were again aspirated and washed to minimum five times with wash buffer. Thereafter, added 50 µL Chromogen solution-B into each well which was gently mixed allowed re-incubating the wells at 37°C for the duration of 15 minutes. At last, added 50 µL stop solution into individual well and observed a colour change from blue to yellow (Figure 116). Once the colour turned to yellow, immediately measured the optical absorbance at 450 nm wavelength with a micro plate ELISA reader and calculated the insulin release concentration of the test samples.



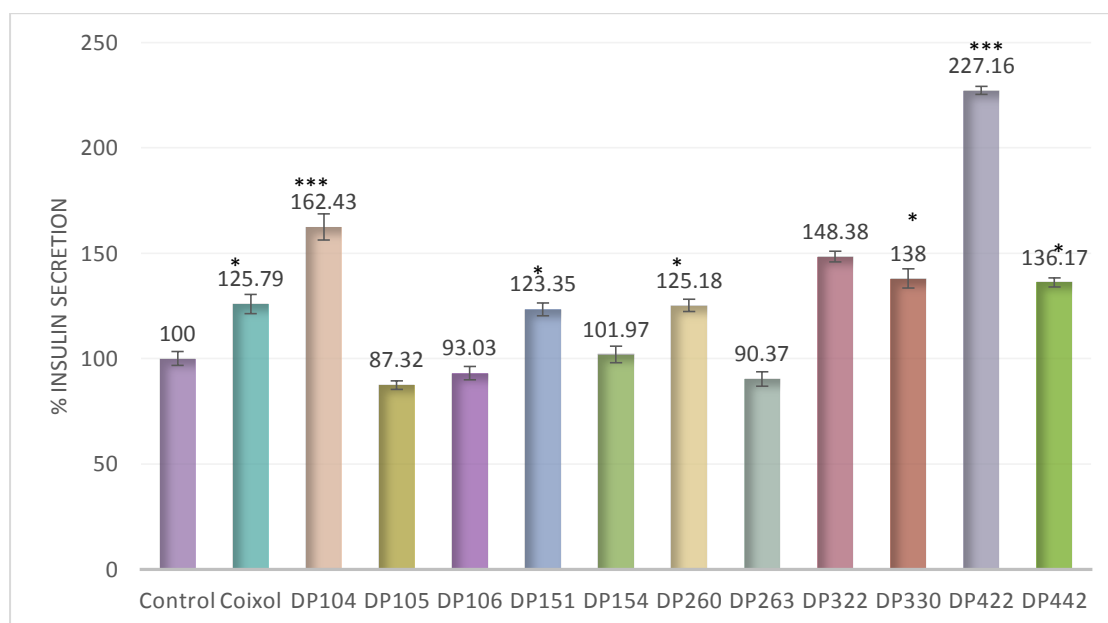
**Figure 116:** ELISA kits representing change in colour while adding stop solutions into test sample wells.



**Figure 117:** Standard plot between the absorbance and concentrations of standard reagent at 1.25, 2.5, 5, 10 & 20 mU/L

**Table 16:** % Insulin concentrations of test compounds with reference to Standard drug Glibenclamide. \*All values are mentioned as the mean±SEM and determinations were carried out in triplicate manner Where\*\*\*p<0.001, \*\*p<0.01 and \*p<0.05, vs control.

| S. NO. | Test Compound  | Insulin Mean Concentration | % Insulin Concentration |
|--------|----------------|----------------------------|-------------------------|
| 1      | GBM (Standard) | 6.63± 3.33                 | 100.00                  |
| 2      | Coixol         | 8.34 ± 4.50                | 125.79*                 |
| 3      | DP104          | <b>10.76± 8.74</b>         | <b>162.43***</b>        |
| 4      | DP105          | 5.78± 3.48                 | 87.32                   |
| 5      | DP106          | 6.23± 5.41                 | 93.03                   |
| 6      | DP151          | 8.17± 5.19                 | 123.35*                 |
| 7      | DP154          | 6.76± 6.66                 | 101.97                  |
| 8      | DP260          | 8.29± 3.39                 | 125.18*                 |
| 9      | DP263          | 5.99 ± 4.35                | 90.37                   |
| 10     | DP322          | 9.83± 2.58                 | 148.38**                |
| 11     | DP330          | 9.14± 4.60                 | 138.00*                 |
| 12     | DP422          | <b>15.06 ± 1.86</b>        | <b>227.16***</b>        |
| 13     | DP442          | 9.02± 2.12                 | 136.17*                 |



**Figure 118:** Effect of Test compounds & GBM as control in triplicate conc. of 100 µM on Glucose stimulated insulin secretory assay on isolated S.D rat islets. Percentage of comparative insulin release of the test samples was calculated where Glibenclamide was taken as standard for 100%. Values are represented as mean ± SEM in triplicate concentrations. p<0.001, \*\*p<0.01 and \*p<0.05 compared with Glibenclamide (standard).

The analysis of assay result revealed that Compound **DP422** and **DP104** had the most potent on the membrane receptor ATP-sensitive potassium channel or  $K_{ATP}$  channel. Therefore for further *in vivo* evaluation we used **DP422** and **DP104** in spite of better binding affinity of other molecules than **DP104**.

**6.10: In vivo evaluation:** For the *in vivo* evaluations of synthetic compounds, an insulin resistance type-II diabetes model was developed in Female Sprague Dawley rats in combination of High-Fat-Diet feed and administering minimum dose of STZ (35mg/kg). The rats were maintained at two different dietary plan i.e. Normal Pellet Diet and High Fat Diet which is composed 17% carbohydrate, 25%-protein and 58% fat as a total kcal percentage of *ad libitum* respectively, for the duration of 15 days. The NPD and HFD rats were divided into NPD (Vehicle) , NPD + Coixol, NPD + synthesized compound-1(i.e. **DP104**), NPD + synthesized compound-2(i.e. **DP422**), HFD + STZ (Negative control) , HFD + STZ + Glibenclamide (Positive control-1), HFD + STZ + Coixol (Positive control-2), HFD + STZ + synthesized compound-1 (low and high dose), HFD + STZ + synthesized compound-2 (low and high dose). After 15 days of dietary changes, the HFD-fed group were injected the minimum dose of STZ (35 mg/kg; i.p.) via intraperitoneal route and NPD-fed groups were injected with 0.5% CMC as a vehicle. After, 7 days of streptozotocin administration, the rats were analysed for different parameters like: change in body weight, total cholesterol and blood glucose levels to ensure the induction of disease. On day 22<sup>nd</sup>, The disease induced animals were given treatment for 7 day continuously. On Day 28<sup>th</sup>, again different parameters were carried out including body weight and biochemical estimations (i.e. blood glucose, insulin and cholesterol) to check the effect of treatment. The result of *In vivo* study indicates that Coixol, DP104 & DP422 have significant effect in decreasing blood glucose level and total plasma cholesterol level in diabetic animals. It also showed no significant effect on the body weight when compared with positive control and negative control groups. Animals were sacrificed on Day 30<sup>th</sup> and pancreas of each sacrificed animals were stored in 10% formalin solution for histopathology evaluation. The test compounds Coixol, DP104 & DP422 has showed dose dependent effect in all the parameters evaluated. The histopathological results confirmed that pancreatic cells of DP104 & DP422 treated animals almost undergone

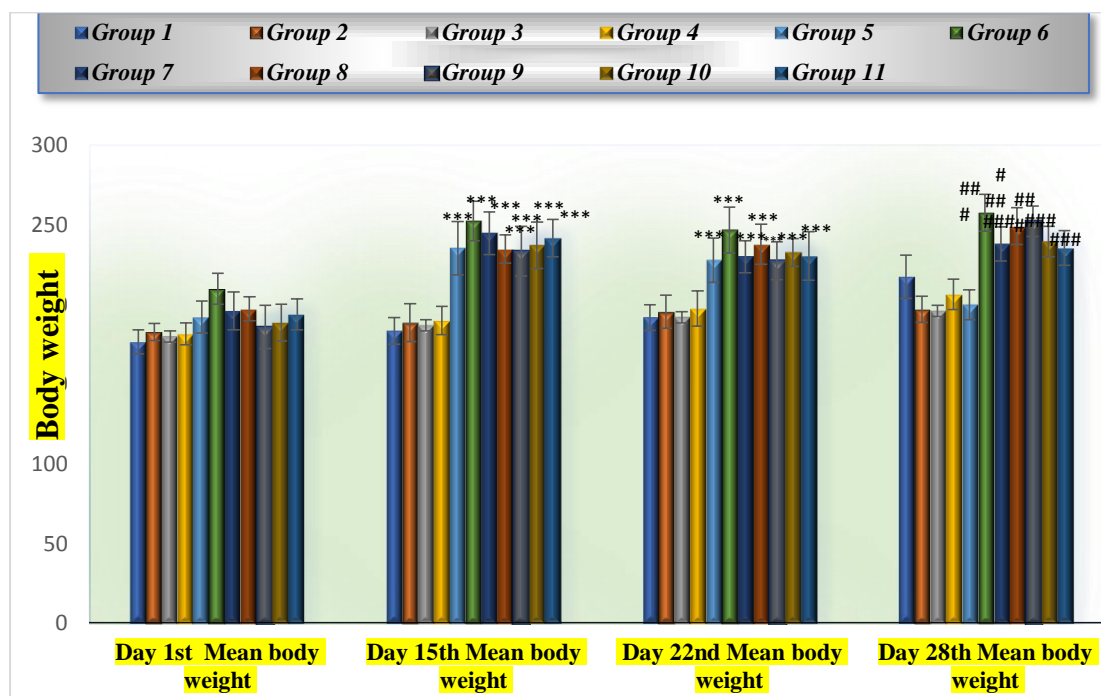
regeneration of their normal structure after 7 days of treatment, which was comparable to the normal control and positive control groups.

**6.10.1: Body weight Estimation:** Experimental animals female Sprague-dawley rats were evaluated on their body weight respectively on Day1<sup>st</sup>, 15<sup>th</sup>, 22<sup>nd</sup> and 28<sup>th</sup> of the study protocol (Table 1). No significant difference was observed in the average body weight of rats on Day 1 in different groups. Whereas, On day 15<sup>th</sup>, noticed a relative increase in body weight specifically on rats maintained on High fat diet (i.e. Group 5 to 11) as compared to the rats maintained on Normal pellet diet (Group 1 to 4). Likewise on day 22<sup>nd</sup> (after 1 week of STZ i.p. administration) in Diabetes control groups those were maintained on High-Fat-Diet, noticed a substantial lower in body weight as compared to rats who were not given streptozotocin and maintained on Normal pellet diet. On day 28<sup>th</sup>, i.e. 7 days after treatment, a slight increase in the body weight was observed in group 6 to 11 with respect to group 5 (i.e. negative control). The change in body weight during diseased state to recovery is mentioned in Table 17 and Figure no.119.

**Table 17:** Change in body weight (gm±SD) during diseased state to recovery state

| S. No.   | Groups Name                          | 1 <sup>st</sup> Day | 15 <sup>th</sup> Day        | 22 <sup>nd</sup> Day         | 28 <sup>th</sup> Day        |
|----------|--------------------------------------|---------------------|-----------------------------|------------------------------|-----------------------------|
| Group 1  | Vehicle control                      | 176.31±7.52         | 183.33±8.33                 | 191.710±8.07                 | 217.33±13.64                |
| Group 2  | Synthesized molecule-1 <i>per se</i> | 182.58±5.39         | 188.33±11.91                | 195.28±10.51                 | 196.83±8.25                 |
| Group 3  | Synthesized molecule-2 <i>per se</i> | 179.67±9.36         | 186.83±10.08                | 191.71±8.99                  | 195.83±12.95                |
| Group 4  | Synthesized Coixol <i>per se</i>     | 181.34±6.78         | 189.83±8.84                 | 197.280±11.01                | 206.20±9.44                 |
| Group 5  | Negative control                     | 191.9±10.16         | 235.28±16.79 <sup>***</sup> | 227.57±13.85 <sup>***</sup>  | 199.71±9.44                 |
| Group 6  | Positive control-1                   | 209.62±9.71         | 252.28±12.49 <sup>***</sup> | 246.57±14.44 <sup>***</sup>  | 257.42±11.64 <sup>###</sup> |
| Group 7  | Positive control-2                   | 195.9±11.89         | 244.57±13.46 <sup>***</sup> | 229.85±10.12 <sup>***</sup>  | 237.83±10.75 <sup>###</sup> |
| Group 8  | Synthesized molecule-1 low dose      | 197.23±7.57         | 234.71±8.88 <sup>***</sup>  | 237.57±12.55 <sup>***</sup>  | 249±11.48 <sup>###</sup>    |
| Group 9  | Synthesized molecule-1 high dose     | 185.64±13.62        | 233.28±15.64 <sup>***</sup> | 227.20±11.903 <sup>***</sup> | 252±9.53 <sup>###</sup>     |
| Group 10 | Synthesized molecule-2 low dose      | 188.41±11.41        | 237±14.73 <sup>***</sup>    | 232.57±8.68 <sup>***</sup>   | 239.28±9.46 <sup>###</sup>  |
| Group 11 | Synthesized molecule-2 high dose     | 193.7±9.80          | 241.57±11.85 <sup>***</sup> | 230.28± 15.14 <sup>***</sup> | 233.33±10.98 <sup>###</sup> |

Data represented as mean±SD(n=6 for group1-4, n=7 for group 5-11). Where,\*\*,\*\*\* represents  $p < 0.01$  and  $p < 0.001$ , respectively, compared to vehicle control group (Group-1); ### represents  $p < 0.001$  as compared to negative control group (Group-5).



**Figure 119:** Effect on rats body weight (g) due to the treatment protocol. Data represented as mean±SD. \*\*, \*\*\* represents  $p < 0.01$ ,  $p < 0.001$ , respectively, compared to vehicle control group; ##, ### represents  $p < 0.01$ ,  $p < 0.001$ , respectively, compared to negative control group.

#### 6.10.2: Plasma Glucose estimation:

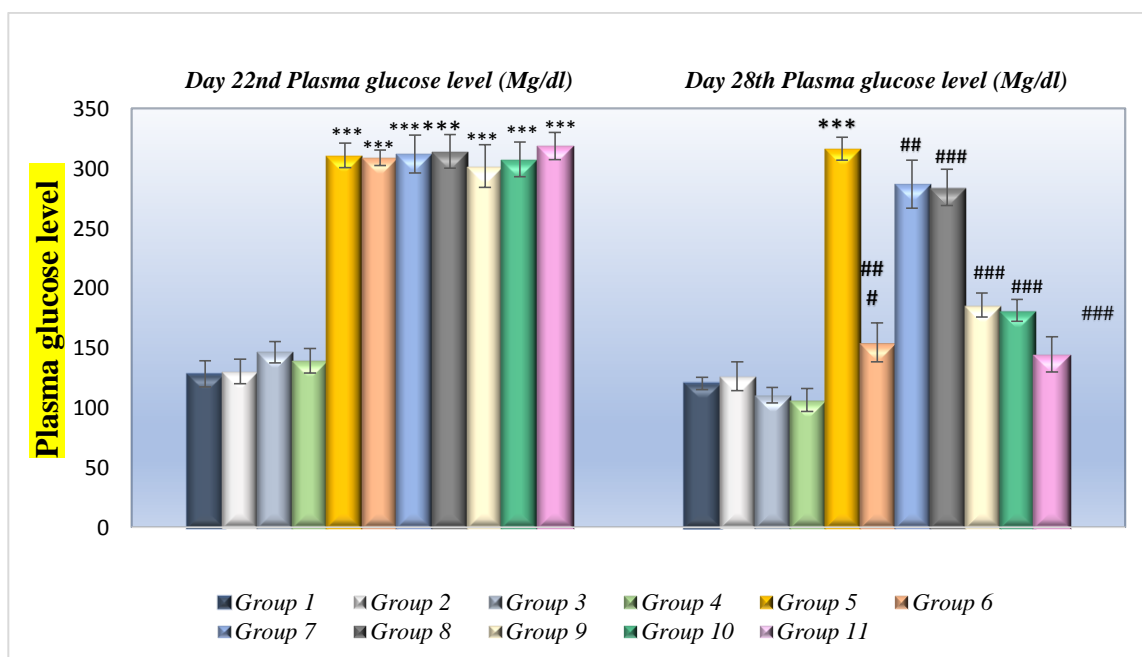
It was evaluated on day 22<sup>nd</sup> and 28<sup>th</sup> using Erba GOD-POD kit. The glucose levels (mg/dL) were checked on day 22<sup>nd</sup> (Before treatment ) and 28<sup>th</sup> (After treatment) respectively. The results revealed that on day 22<sup>nd</sup>, The blood glucose level of group-5 to 11 (HFD + STZ administered groups) were significantly increased as compared to group-1 (i.e. vehicle control). On Day 28<sup>th</sup>, after 7 days of respective treatment in HFD+STZ administered groups, plasma glucose level was significantly reduced as relative to group 5 (negative control group). The effect of different treatments on plasma glucose levels of rats are mentioned in Table no. 18 and Figure 120.



**Table 18:** Effect on Plasma glucose level before and after the treatment (mg/dL±SD).

| S. No.          | Group Name                           | 22 <sup>nd</sup> Day        | 28 <sup>th</sup> Day        |
|-----------------|--------------------------------------|-----------------------------|-----------------------------|
| <b>Group 1</b>  | Vehicle control                      | 128.33±10.76                | 120.25±4.99                 |
| <b>Group 2</b>  | Synthesized molecule-1 <i>per se</i> | 130.33±10.4                 | 126.33±12.01                |
| <b>Group 3</b>  | Synthesized molecule-2 <i>per se</i> | 146.33±8.71                 | 110.33±6.429                |
| <b>Group 4</b>  | Synthesized Coixol <i>per se</i>     | 139.33±10.26                | 106.33±9.50                 |
| <b>Group 5</b>  | Negative control                     | 310.57±16.37 <sup>***</sup> | 316.4±12.87 <sup>***</sup>  |
| <b>Group 6</b>  | Positive control-1                   | 308.85±6.42 <sup>***</sup>  | 154.6±16.21 <sup>###</sup>  |
| <b>Group 7</b>  | Positive control-2                   | 311.85±15.7 <sup>***</sup>  | 286.75±20.03 <sup>##</sup>  |
| <b>Group 8</b>  | Synthesized molecule-1 low dose      | 314.14±13.94 <sup>***</sup> | 284±15.04 <sup>###</sup>    |
| <b>Group 9</b>  | Synthesized molecule-1 high dose     | 301.8±17.86 <sup>***</sup>  | 185.75±10.14 <sup>###</sup> |
| <b>Group 10</b> | Synthesized molecule-2 low dose      | 307.57±14.55 <sup>***</sup> | 181.2±9.23 <sup>###</sup>   |
| <b>Group 11</b> | Synthesized molecule-2 high dose     | 318.57±11.31 <sup>***</sup> | 145.5±14.70 <sup>###</sup>  |

Data represented as mean±SD (n=6 for group1-4, n=7 for group 5-11). Where, <sup>\*\*\*</sup> represents, p < 0.001 as compared to vehicle control group and <sup>##, ###</sup> represents p < 0.01, p < 0.001 as compared to negative control group.



**Figure 120:** Effect on Plasma glucose level (mg/dL) before and after the treatment.

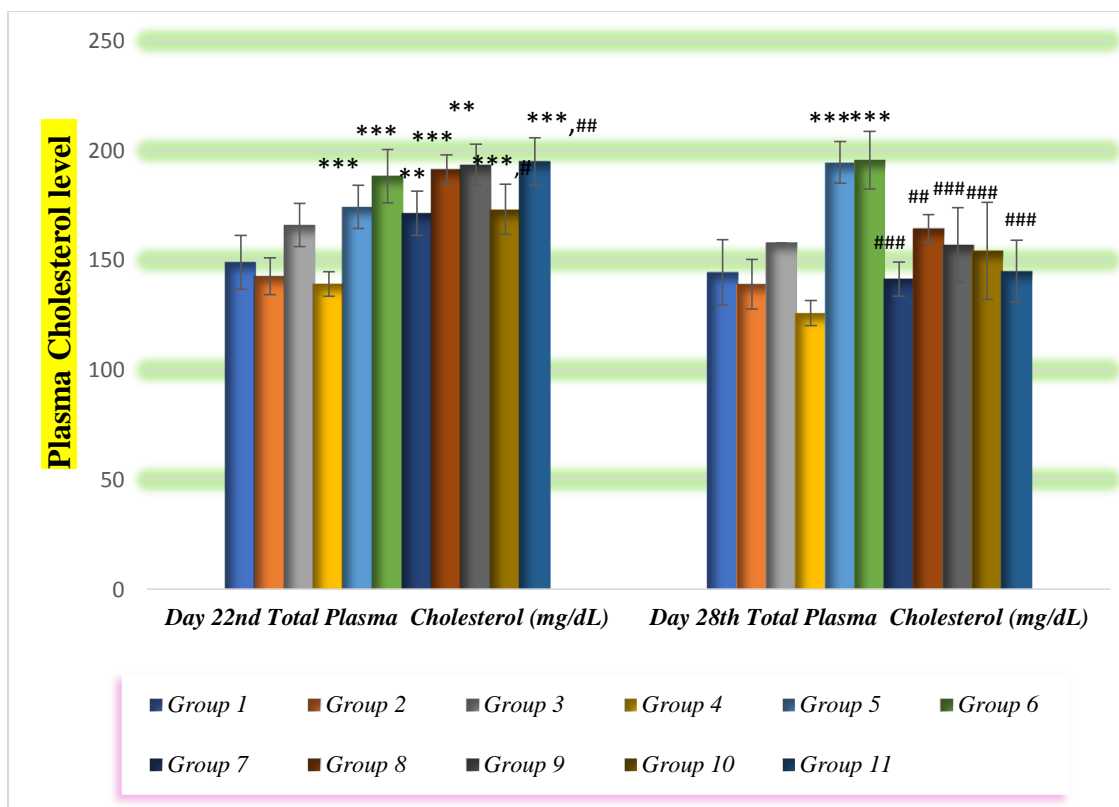
Data represented as mean±SD. <sup>\*\*\*</sup> represents p < 0.001, when negative control group compared to vehicle control group; <sup>##, ###</sup> represents p < 0.01, p < 0.001 when HFD treated groups compared to negative control group.

**6.10.3: Total Cholesterol estimation:** Plasma total cholesterol level was examined through a commercially available Erba cholesterol kit by using 10 $\mu$ L of serum from the fasting animals. The total cholesterol levels was evaluated on day 22<sup>nd</sup> and 28<sup>th</sup> respectively. The results indicated that the cholesterol levels of group 5 to 11 (HFD + STZ administered groups) were significantly increased on day 22<sup>nd</sup> compared to group 1 to 4 (NPD and without STD). After 7 days of respective treatments i.e. day 28<sup>th</sup>, A slight decrease in plasma cholesterol levels was observed in group 6 to 11 as compared to group 5 (negative control). The effect of various treatments on plasma cholesterol levels of rats are represented in Table no. 19 and Figure no. 121.

**Table 19:** Effect on plasma total cholesterol levels before and after the treatment:

| Group No. | Group Name                           | Day 22 <sup>nd</sup>                 | Day 28 <sup>th</sup>              |
|-----------|--------------------------------------|--------------------------------------|-----------------------------------|
| Group 1   | Vehicle control                      | 149.05 $\pm$ 12.36                   | 144.5 $\pm$ 14.84                 |
| Group 2   | Synthesized molecule-1 <i>per se</i> | 142.76 $\pm$ 8.31                    | 139 $\pm$ 11.31                   |
| Group 3   | Synthesized molecule-2 <i>per se</i> | 166 $\pm$ 9.82                       | 158 $\pm$ 14.14                   |
| Group 4   | Synthesized Coixol <i>per se</i>     | 139.23 $\pm$ 5.64                    | 125.5 $\pm$ 5.65                  |
| Group 5   | Negative control                     | 174.34 $\pm$ 9.82 <sup>***</sup>     | 194.66 $\pm$ 9.5 <sup>***</sup>   |
| Group 6   | Positive control-1                   | 188.43 $\pm$ 12.18 <sup>***</sup>    | 195.66 $\pm$ 13.05 <sup>***</sup> |
| Group 7   | Positive control-2                   | 171.49 $\pm$ 10.02 <sup>**</sup>     | 141.5 $\pm$ 7.77 <sup>###</sup>   |
| Group 8   | Synthesized molecule-1 low dose      | 191.31 $\pm$ 6.73 <sup>***</sup>     | 164.5 $\pm$ 6.36 <sup>##</sup>    |
| Group 9   | Synthesized molecule-1 high dose     | 193.52 $\pm$ 9.37 <sup>***,#</sup>   | 157 $\pm$ 16.97 <sup>###</sup>    |
| Group 10  | Synthesized molecule-2 low dose      | 173.24 $\pm$ 11.47 <sup>**</sup>     | 154.33 $\pm$ 22.12 <sup>###</sup> |
| Group 11  | Synthesized molecule-2 high dose     | 195.01 $\pm$ 10.91 <sup>***,##</sup> | 145 $\pm$ 14.14 <sup>###</sup>    |

Data represented as mean $\pm$ SD (n=6 for group1-4, n=7 for group 5-11). Where, <sup>\*\*\*</sup>, <sup>\*\*</sup> represents p < 0.01, p < 0.001, compared to vehicle control group; #, ##, ### represents p < 0.02, p < 0.01, p < 0.001, compared to negative control group.



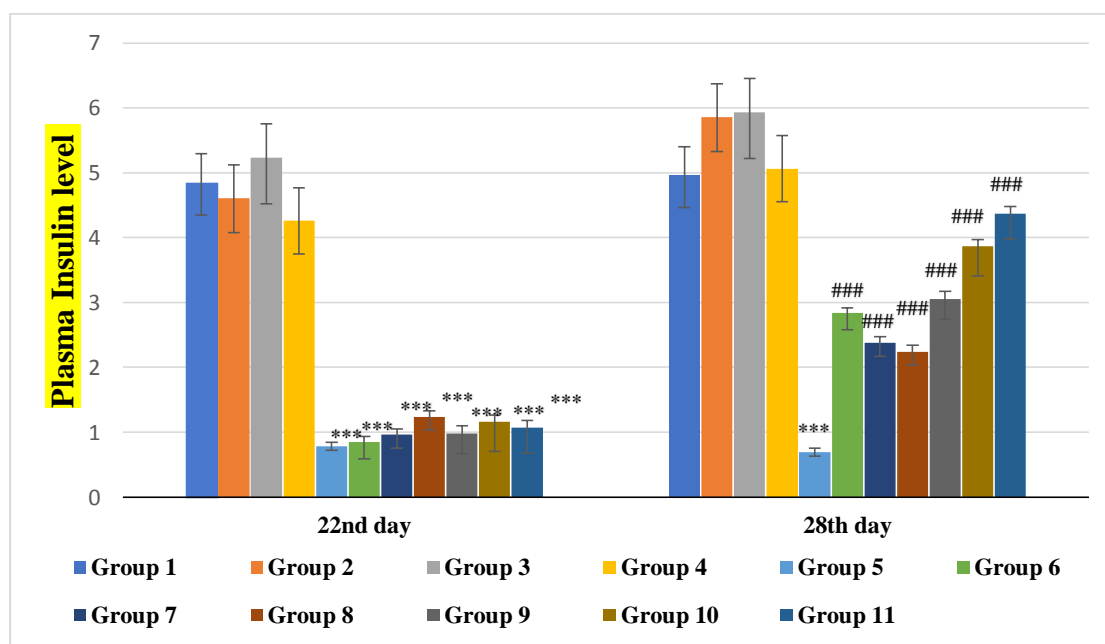
**Figure 121:** Effect on total cholesterol level (mg/dL) before and after treatment . Data represented as mean±SD (n=6 for group1-4, n=7 for group 5-11. Where \*\*,\*\*\* represents  $p < 0.01$ ,  $p < 0.001$ , When negative control group compared to vehicle control group; #, ##, ### represents  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ , when HFD treated groups compared to negative control group.

**6.10.4: Plasma Insulin estimation:** : Plasma Insulin level was examined through a rat insulin ELISA kit by collecting Plasma of fasting rats on Day 22<sup>nd</sup> (After Streptozotocin administration) and Day 28<sup>th</sup> (After treatment). The results revealed that on day 22<sup>nd</sup>, The Plasma insulin level of Group-5 to 11 (HFD + STZ administered groups) were significantly decreased as compared to group-1 (i.e. vehicle control). On Day 28<sup>th</sup>, after 7 days of respective treatment in HFD+STZ administered groups, plasma insulin level was significantly increased as compared to group 5 (negative control group). The effect of different treatments on plasma insulin level of rats are mentioned in Table no. 20 and Figure 122.

**Table 20:** Effect on Plasma insulin levels ( $\mu\text{U/ml}$ ) before and after the treatment:

| Group No. | Group Name                           | Day 22 <sup>nd</sup>     | Day 28 <sup>th</sup>     |
|-----------|--------------------------------------|--------------------------|--------------------------|
| Group 1   | Vehicle control                      | 4.85±0.44                | 4.96±0.5                 |
| Group 2   | Synthesized molecule-1 <i>per se</i> | 4.61±0.51                | 5.86±0.53                |
| Group 3   | Synthesized molecule-2 <i>per se</i> | 5.23±0.52                | 5.93±0.71                |
| Group 4   | Synthesized Coixol <i>per se</i>     | 4.26±0.51                | 5.06±0.51                |
| Group 5   | Negative control                     | 0.78±0.06 <sup>***</sup> | 0.69±0.06 <sup>***</sup> |
| Group 6   | Positive control-1                   | 0.85±0.08 <sup>***</sup> | 2.84±0.26 <sup>###</sup> |
| Group 7   | Positive control-2                   | 0.96±0.09 <sup>**</sup>  | 2.38±0.21 <sup>###</sup> |
| Group 8   | Synthesized molecule-1 low dose      | 1.23±0.1 <sup>***</sup>  | 2.24±0.2 <sup>###</sup>  |
| Group 9   | Synthesized molecule-1 high dose     | 0.98±0.12 <sup>***</sup> | 3.05±0.31 <sup>###</sup> |
| Group 10  | Synthesized molecule-2 low dose      | 1.16±0.1 <sup>**</sup>   | 3.87±0.46 <sup>###</sup> |
| Group 11  | Synthesized molecule-2 high dose     | 1.07±0.11 <sup>***</sup> | 4.37±0.39 <sup>###</sup> |

Data represented as mean±SD. Where <sup>\*\*\*</sup> represents  $p < 0.001$ , when vehicle control group compared to STZ administered groups; <sup>###</sup> represents  $p < 0.001$  when negative control group compared to HFD treated groups.

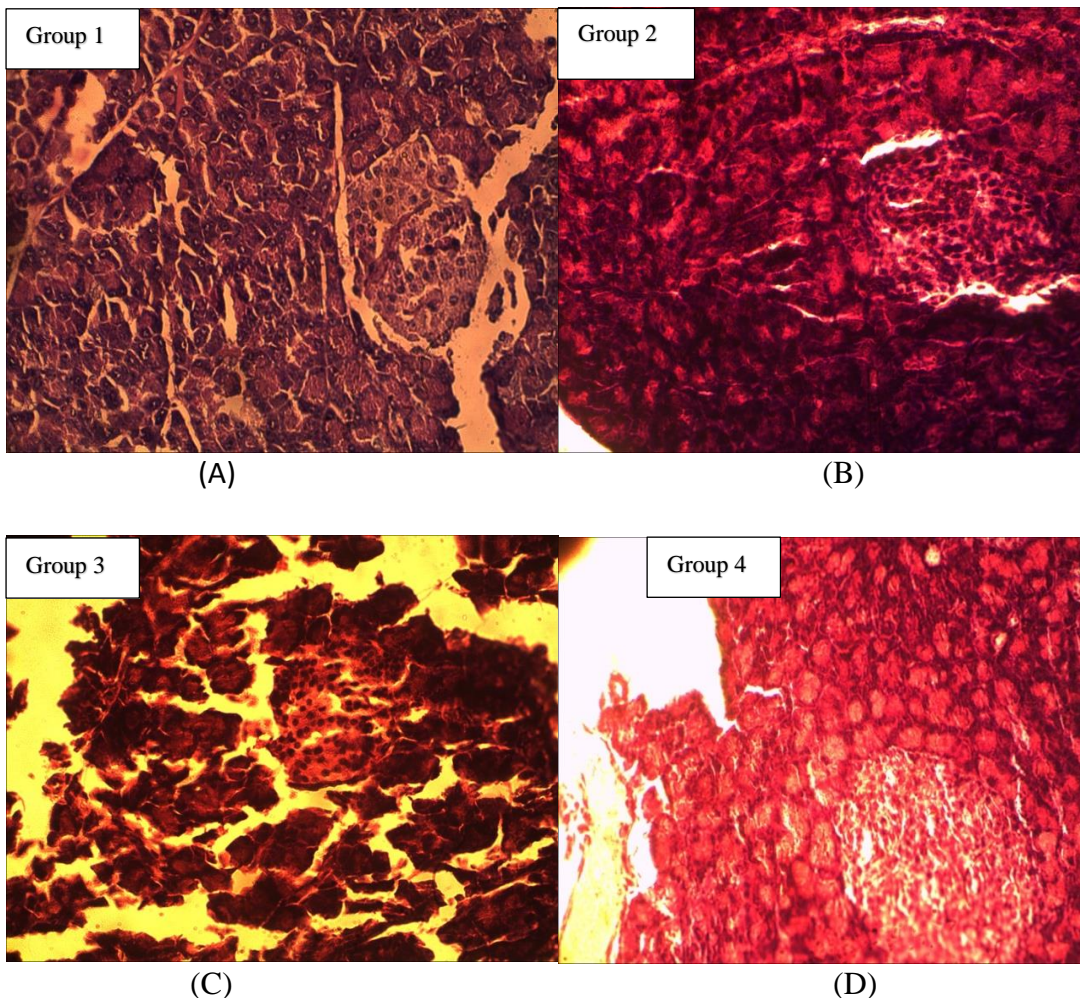


**Figure 122:** Effect on Plasma insulin level ( $\mu\text{U/ml}$ ) before and after the treatment.

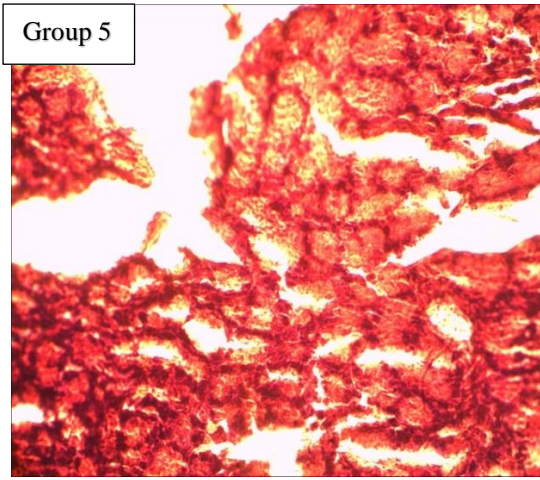
Data represented as mean±SD. <sup>\*\*\*</sup> represents  $p < 0.001$ , when vehicle control group compared to STZ administered groups; <sup>###</sup> represents  $p < 0.001$  when negative control group compared to HFD treated groups.

### 6.10.5: Histopathological Estimation:

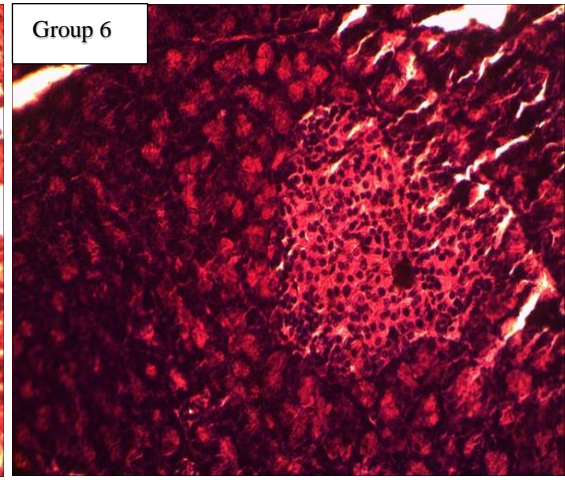
Histopathological estimation of different pancreatic tissue sections were performed at Gargi Diagnostic Lab, Jalandhar. After the grossing of tissue, all the sections were stained with haematoxylin (H) and Eosin (E) which was checked at 400X magnification. Histopathological examination of pancreatic tissue revealed that non diabetic groups maintained on normal pellet diet throughout the study (i.e. group 1-4) shown normal structure of pancreas and exocrine acini surroundings the islets of Langerhans. Whereas in the pancreas of negative control (i.e. Group 5) rats, islets of Langerhans appeared disorganised along with the presence of clusters of slight inflammation. In contrast, in diabetes treated groups i.e. 6 to 11 the islets of Langerhans had satisfactory number of  $\beta$  cells with no degenerative changes but few inflammatory cells were present in low dose (2.5 mg/kg) treated groups whereas high dose (5 mg/kg) treated groups reported moderate recovery with the absence of inflammatory cells. (Figure 123)



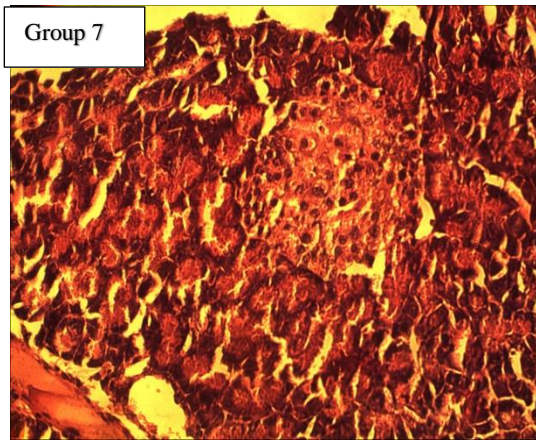




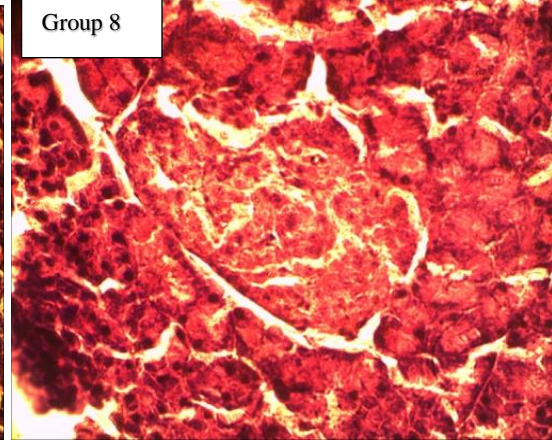
(E)



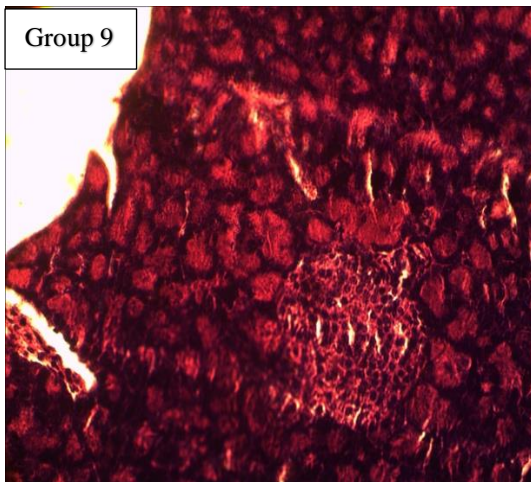
(F)



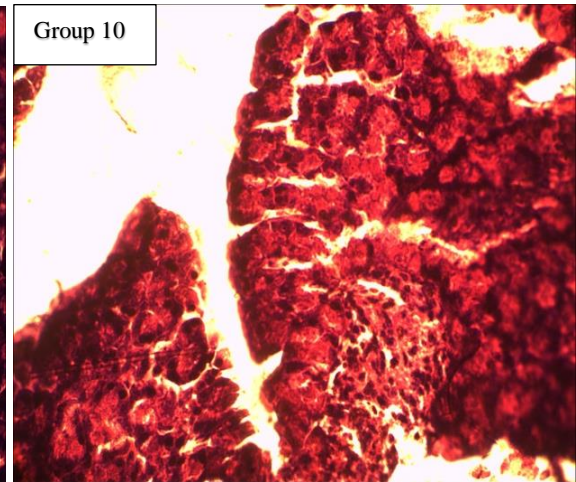
(H)



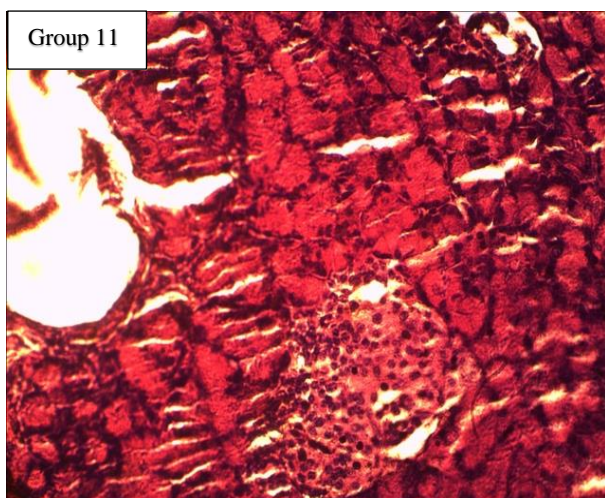
(G)



(I)



(J)



(K)

**Figure 123:** Photomicrographs of a section of the endocrine pancreatic tissue of rats on 28<sup>th</sup> day. Image represents (A-Group 1) NPD + vehicle, (B- Group 2) NPD + DP104, (C- Group 3) NPD + DP422, (D- Group 4) + Coixol, (E- Group 5) HFD + Negative control, (F- Group 6) HFD + Positive control-1 GBM (10mg/kg), (G- Group 7) HFD + Positive control-2 Coixol (5mg/kg), (H- Group 8) HFD + DP104 at low dose (2.5mg/kg), (I- Group 9) HFD + DP104 at high dose (5mg/kg), (J- Group 10) HFD + DP422 at low dose (2.5mg/kg), (K- Group 11) HFD + DP422 at high dose (5mg/kg)

*In vivo* study results indicated the successfully development of type-II diabetes model incorporating high fat diet and low dose administration of streptozotocin (35mg/kg). The *in vivo* evaluation of Natural product, coixol and synthesized compounds DP104 and DP422 estimated the release of insulin by inhibiting ATP sensitive potassium channels. Furthermore; all the selected test molecules shown better results as compared to the standard drug Glibenclamide for managing type-II diabetes and also showed positive results in histopathological analysis. Thus, the entire study suggest that coixol and It's synthesized derivatives having good potential to be utilised for the treatment and management of type-II diabetes.

## CONCLUSION

Diabetes is continuously increasing and a serious illnesses developing worldwide. A pharmacophore is designed to include the skeleton of a potent phytoconstituent, i.e. coixol, which is a powerful antidiabetic agent that inhibits ATP-sensitive potassium channels. The designed scaffold consists of a polar head consisting heterocycle, a linker and an aryl lipophilic tail containing aryl alcohols and aryl amines. Total 254 novel promising anti diabetic molecules were screened following substitution of various electron withdrawing and releasing groups on proposed pharmacophore head and attachment of aryl alcohols and aryl amines tail with ether or amine linkage. The designed molecules were analysed and docked by molecular docking software's i.e. Auto dock vina and PyMOL with the protein 5yw7 (A pancreatic ATP-sensitive potassium channel. The twelve best molecules were identified based on their binding score and feasibility of the synthesis that were coded as **DP102 (Coixol)**, **DP104**, **DP105**, **DP106**, **DP151**, **DP154**, **DP260**, **DP263**, **DP322**, **DP330**, **DP422**. The *in-silico* toxicity studies confirmed the non-carcinogenic effect of synthesized compounds. The result of *in silico* ADME showed good solubility in water with high GI absorption and no penetration into blood brain barrier and the calculated lipophilicity (*iLog P*) was observed in the range of 0.73-1.90. The selected compounds were synthesized with good yield and characterization was done via analysing their Melting point, TLC and spectroscopic data's of IR, Mass, <sup>1</sup>HNMR and <sup>13</sup>C NMR. The *in vitro* study data suggest **DP105** (5.78± 3.48), **DP106** (6.23± 5.41) and **DP263** (5.99±4.35) with least effect. Compound **DP102/Coixol**(8.34± 4.50), **DP151**(8.17± 5.19), **DP154** (6.76± 6.66) and **DP60** (8.29± 3.39) showed similar effect to standard drug **Glibenclamide** (6.63± 3.33). Compounds **DP322** (9.83± 2.58), **DP330** (9.14± 4.60) and **DP442** (9.02± 2.12) showed moderately better effect and **DP104** (10.76± 8.74), **DP422** (15.06 ± 1.86) were the most potent among other test molecules at Statistical calculations for relative insulin secretion at 100 µM conc. were significantly p<0.001, \*\*p<0.01 and \*p<0.05 compared with the value for Glibenclamide as control. Further *in-vivo* evaluation on HFD induced diabetic model showed good insulin secretory properties of tested compounds i.e., **DP104**, **Coixol**, and **DP422**. Coixol and compounds **DP104** and **DP422** showed potent insulin



secretagogue effects along with a reduced level of plasma glucose and total cholesterol in the treated groups compared to standard drug glibenclamide. The hypoglycaemic effect of the test compounds is possibly due to the stimulation of the pancreas to secrete insulin as these are the derivatives of the potent secretagogue, Coixol. In addition, a notable effect on lipid profile was observed as a significant reduction in total cholesterol indicates the hypocholesterolemic effect of test compounds. Further, result also showed improvement in the histopathological analysis of the diabetic model. Overall, result indicated that the presence of aromatic tail primarily substituted with nitro functional group and attachment of amine linker is important for improving the potency of the compounds. Thus the entire study suggests that **coixol**, **DP104** & **DP422** have significant effect in releasing insulin, managing plasma glucose level and plasma total cholesterol level and observed as successful clinical candidates for the treatment and management of type-II diabetes. Thus, due to the significance of this research work, it would be beneficial for social and industrial purpose and the potent molecules would be formulated to serve as anti-diabetic agents in the future.

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## ANNEXURES

### List of Patent, Awards, Publications, Conferences and Workshops:

**Patent:** Patent Published on: NOVEL COIXOL DERIVATIVES AND PROCESS THEREOF. Application number: TEMP/E- 1/67994/2021-DEL. Ref No: 202111059880.

Contributors: Deepshikha Patle, Paranjeet Kaur, Navneet Khurana

**Award:** Best oral presenter award on “ Identification of 6-methoxy benzoxazolinone derivatives as the potential antidiabetic agents via Molecular docking studies” in the international conference on ‘New trends in chemical research’(ICNTPCR-2022) held on April 29-30, 2022.

**Copyright filed :** Deepshikha Patle, Paranjeet Kaur, Navneet Khurana, Jeena Gupta, Neha Sharma; Exploring the potential of coixol like derivatives for the management of diabetes. Application ID: 6167

### Publications from current research work:

1. Patle D, Vyas, M, Khatik, G. L. (2021). A Review on Natural Products and Herbs Used in the Management of Diabetes. *Current diabetes reviews*, 17(2), 186–197. <https://doi.org/10.2174/1573399816666200408090058>
2. Patle D, Khurana N, Gupta J, Kaur P, Khatik GL. Design, synthesis, and biological evaluation of coixol-based derivatives as potential antidiabetic agents. *J Mol Struct.* 2023;1277:134861. doi:<https://doi.org/10.1016/j.molstruc.2022.134861>
3. Deepshikha Patle, Paranjeet Kaur, & Navneet Khurana. (2022). Identification of Novel Coixol-Based Derivatives as the Potential Anti-diabetic Agents Through Molecular Docking Studies. *Asian Pacific Journal of Health Sciences*, 9(4), 127–134. <https://doi.org/10.21276/apjhs.2022.9.4S1.22>

### **Abstract published in conference:**

1. Patle D., Kaur P., Khurana N., (2022), Design & identification of novel 2-benzoxazolinone derivatives as potential candidates for the management of Type-II diabetes through molecular docking studies NIPiCON-IPS-2022, International Conference Jointly Organized with Indian Pharmacological Society, February 17-19, 2022.

### **List of Conferences & Seminars**

1. Presented a Paper entitled on ‘‘Identification of novel 6-methoxy benzoxazolinone derivatives as the potential antidiabetic agents via molecular docking studies’’ in A Two-day international conference on New trends in chemical research (ICNTCR-2022) and selected as best Oral presenter (OP-69).
2. Presented Paper entitled on ‘Design & identification of novel 2- benzoxazolinone derivatives as potential candidates for the management of Type-II diabetes’’ in Emerging Opportunities and Challenges in Pharmacology and Pharmaceutical Sciences for Drug Discovery and Healthcare Innovations’’ held on February17-19, 2022 by NIPiCON-IPS 2022, International Conference jointly organized with Indian Pharmacological Society.
3. Presented Paper on ‘‘Exploring the potential of S. dulcis and developing the rational for the drug designing of antidiabetic derivatives’’ in the International Conference on Materials for Emerging Technologies (ICMET-21) held on February 18-19, 2022, organized by Department of Research Impact and Outcome, Division of Research and Development, Lovely Professional University, Punjab.
4. Presented Poster on ‘‘A review on rationalization of natural compound in the treatment of Diabetes mellitus’’ in a Pharmacy Practice Summit- 2019 on ‘Advanced clinical and community pharmacy services in qualitative therapeutics and Healthcare delivery: The serious missing link’, held on 19-20<sup>th</sup> April, 2019.

5. Participated in International Conference on “commercialization of Medicinal Plant Products: Lab Techniques to Trade” PHYTOCON 2018, held on April 14, 2018.
6. Participated in 21st Punjab Science Congress on “Scientific Advances for inclusive development and Environmental protection” Organized by Punjab Agricultural university, Ludhiana on February 7-9,2018.

**Workshop attended:**

1. Attended an Author Workshop jointly organized by Lovely Professional university, Punjab and Springer Nature on “How to write and publish scientific Articles and manuscripts” on 15<sup>th</sup> April,2018 at Lovely professional university, Punjab
2. Attended One day Workshop on “Funding opportunities for research, Innovation and career development” under aegis of AICTE (SWAYAM) ,July 22,2018.
3. Participated in one day workshop on “Animal Experimental models and Ethics in preclinical research” Sponsored by Springer nature at School of Pharmaceutical sciences, Lovely professional university held on 6<sup>th</sup> July 2019.

Deepshikha Patle  
Registration Number: 41700208  
Program Name: Ph.D. - Pharmaceutical Chemistry (Part Time)

**Subject: Letter of Candidacy for Ph.D.**

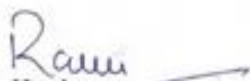
Dear Candidate,

We are very pleased to inform you that the Department Doctoral Board has approved your candidacy for the Ph.D. Programme on September 22, 2018 by accepting your research proposal entitled: "DESIGN, SYNTHESIS & EVALUATION OF COIXOL LIKE DERIVATIVES AS ANTIDIABETIC AGENTS" under the supervision of Dr. Gopal Lal Khatik.

As a Ph.D. candidate you are required to abide by the conditions, rules and regulations laid down for Ph.D. Programme of the University, and amendments, if any, made from time to time.


We wish you the very best!!

In case you have any query related to your programme, please contact Center for Research Degree Programmes.

  
Head

Center for Research Degree Programmes

### Student Result Details

|                       |   |   |
|-----------------------|---|---|
| Prov. Regd.: 41700208 | Student Name: Deepshikha Patle                                      |  |
| Batch Year: 2017      | Program: P5D0-TN3 : Ph.D.<br>(Pharmaceutical Chemistry) [Part Time] |   |
| CGPA: 9.78            |   |   |

In case of any query/issue related to "Result", [Click Here.](#)

|       |        |                        |                    |
|-------|--------|------------------------|--------------------|
| Marks | Grades | ReAppear(s)/Backlog(s) | Graphical Analysis |
|-------|--------|------------------------|--------------------|

|             |                  |
|-------------|------------------|
| Course Wise | Question No Wise |
|-------------|------------------|

### Course Wise Marks

TermId:

| Description                                       | Maximum Marks | Marks Obtained | Weight Maximum Marks | Weight Marks Obtained |
|---|---------------|----------------|----------------------|-----------------------|
| ▼ Course: GEN703::RESEARCH METHODOLOGY            |               |                |                      |                       |
| Continuous Assessment                             | 100           | 87             | 25                   | 22                    |
| Theory Mid Term                                   | 40            | 35             | 20                   | 18                    |
| Theory End Term                                   | 70            | 61             | 50                   | 44                    |
| Attendance Marks                                  | 5             | 5              | 5                    | 5                     |
| ▼ Course: GEN730::DATA ANALYSIS AND REPORTING     |               |                |                      |                       |
| Continuous Assessment                             | 100           | 90             | 45                   | 41                    |
| Practical End Term                                | 100           | 78             | 50                   | 39                    |
| Attendance Marks                                  | 5             | 5              | 5                    | 5                     |
| ▼ Course: PHR782::MODERN BIOANALYTICAL TECHNIQUES |               |                |                      |                       |
| Continuous Assessment                             | 100           | 74             | 25                   | 19                    |
| Theory Mid Term                                   | 40            | 37             | 20                   | 19                    |
| Theory End Term                                   | 70            | 61             | 50                   | 44                    |
| Attendance Marks                                  | 5             | 5              | 5                    | 5                     |



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S.C.O. 202/5, GUJRAL NAGAR, NEAR T.V. CENTRE, JALANDHAR CITY  
Phones : 0181-2258034, 4618034 Mobile : 98158-09352  
E-mail : gargidiagnosticslaboratory@gmail.com

Ms. Deepshikha Patle, Lovely Professional University, Phagwara (Punjab)-144411

| Group No. | Group Name                           | Result  |
|-----------|--------------------------------------|---|
| Group 1   | Vehicle control                      | Normal pancreatic structure and exocrine acini surrounding the islets of Langerhans           |
| Group 2   | Synthesized molecule-1 <i>per se</i> | Normal appearance of the islets of Langerhans located in the exocrine tissue                  |
| Group 3   | Synthesized molecule-2 <i>per se</i> | Normal appearance of the islets of Langerhans located in the exocrine tissue                  |
| Group 4   | Synthesized Coixol <i>per se</i>     | Normal appearance of the islets of Langerhans located in the exocrine tissue                  |
| Group 5   | Negative control                     | Disorganized islets of Langerhans and clusters of inflammatory cells                          |
| Group 6   | Positive control-1                   | Moderate recovery to normal appearance of the islets of Langerhans                            |
| Group 7   | Positive control-2                   | Mild recovery to normal appearance of the islets of Langerhans with few inflammatory cells    |
| Group 8   | Synthesized molecule-1 low dose      | Mild recovery to normal appearance of the islets of Langerhans with few inflammatory cells    |
| Group 9   | Synthesized molecule-1 high dose     | Moderate recovery to normal appearance of the islets of Langerhans with no inflammatory cells |
| Group 10  | Synthesized molecule-2 low dose      | Mild recovery to normal appearance of the islets of Langerhans with few inflammatory cells.   |
| Group 11  | Synthesized molecule-2 high dose     | Moderate recovery to normal appearance of the islets of Langerhans with no inflammatory cells |

*Received  
Project  
21/04/22*

**Facilities Available :**  
Fully Automated Immunoassay System (Chemiluminescence) for Thyroid, Fertility, TORCH, Cancer Markers, Hepatitis Markers & Drug Assays.  
Fully Automated - Hematology Cell Counter, Fully Automated - Biochemistry & Electrolyte Analyser (also for Lithium), Elisa system for Elisa tests  
\* Quantitative Serology (CRP, ASO, RA) \* Histo Pathology \* Micro Biology

**GARGI DIAGNOSTICS  
LABORATORY**  
**Dr. (Mrs.) Gargi Sharma**  
M.D.  
Consultant Pathologist

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Lovely Institute of Technology (Pharmacy), Lovely Professional University  
Ludhiana- Jalandhar G.T. Road, Phagwara (Punjab), 144411  
Registration Number -954/PO/ReRcBiBt/S/06/CPCSEA

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**CERTIFICATE**

This is to certify that the project titled "*Pharmacological evaluation of Coixol like derivatives as Antidiabetic agents. (Thesis Title: Design, Synthesis and Evaluation of Coixol like derivatives as Antidiabetic agents)*" has been approved by the IAEC.

Name of Principal Investigator: Dr. Paranjeet Kaur

IAEC approval number: LPU/IAEC/2021/89

Date of Approval: 24<sup>th</sup> September 2021

Animals approved: 73 SD rats, Either Sex

Remarks if any: - NA

  
Dr. Monica Gulati

Biological Scientist,  
Chairperson IAEC

  
Dr. Navneet Khurana

Scientist from different discipline

  
Dr. Bimlesh Kumar

Scientist In-Charge of Animal House,  
Member Secretary IAEC





Office of the Controller General of Patents, Designs & Trade Marks  
Department of Industrial Policy & Promotion,  
Ministry of Commerce & Industry,  
Government of India

सत्यमेव जयते



#### Application Details

|                                  |  |
|----------------------------------|--|
| APPLICATION NUMBER               | 202111059880                                 |
| APPLICATION TYPE                 | ORDINARY APPLICATION                         |
| DATE OF FILING                   | 22/12/2021                                   |
| APPLICANT NAME                   | Lovely Professional University               |
| TITLE OF INVENTION               | NOVEL COIXOL DERIVATIVES AND PROCESS THEREOF |
| FIELD OF INVENTION               | BIO-MEDICAL ENGINEERING                      |
| E-MAIL (As Per Record)           | dip@lpu.co.in                                |
| ADDITIONAL-EMAIL (As Per Record) | dip@lpu.co.in                                |
| E-MAIL (UPDATED Online)          |  |
| PRIORITY DATE                    |  |
| REQUEST FOR EXAMINATION DATE     | --   |
| PUBLICATION DATE (U/S 11A)       | 16/12/2022                                   |



## A Review on Natural Products and Herbs Used in the Management of Diabetes



Deepshikha Patle<sup>1,2</sup>, Manish Vyas<sup>3</sup> and Gopal L. Khatik<sup>2,\*</sup>

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**Abstract:** *Aim:* We aimed to review the importance of the natural products and herbs used in the management of diabetes mellitus (DM) as medicinal agents.

**Background:** Naturally occurring phytoactive compounds and herbs are very important because they are found to be effective against several diseases. DM is a commonly occurring endocrinological disorder, with the incidences increased four times in the last 34 years. There are several oral hypoglycemic agents available in the market, which in the long term, may lead to a high risk of secondary failure rate.

**Objectives:** This review focuses on natural products and herbs application for effective management of diabetic conditions, and natural products that can be utilized as alternative therapy.

**Methods:** We searched the various online databases (PubMed, Bentham, ScienceDirect) and scientific publications from the library using a qualitative systematic review. The criteria of the review were based on natural products and herbs application for possessing medicinal value against diabetes and the literature of previous thirty years has been searched. The inclusion criteria of materials were based on the quality and relevancy with our aim.

**Results:** We observed that owing to the potential of natural products and herbs, different research groups are searching for the potent natural antidiabetic agents with minimal side effects. Recent research showed that there is a decline in a number of new molecules that fail in clinical trials because of toxicity thus, natural products and herbs are considered as the alternative. Currently, some of the natural products and herbs like coixol, andrographolide, *Tinospora cordifolia*, polypeptide p, charantin, *Annona squamosa*, and *Nigella* are being explored for their potential to be used successfully for the management of type 2 diabetes.

**Conclusion:** The significance of natural products and herbs in the anticipation of diabetes and allied complications are being described herein. We observed that a huge amount of work is being done to explore the natural products and herbs to manage the diabetes and this review gives the highlights of them.

### ARTICLE HISTORY

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**Keywords:** Natural products, herbs, flavonoid, diabetes mellitus, phytoactive compounds, endocrinological disorder.

### 1. INTRODUCTION

Diabetes Mellitus (DM), in the current years, has been observed to be a serious issue, recognized through an imperfection in insulin development or in insulin activity on fringe tissues promoting metabolic variations from the norm and hyperglycemia [1, 2]. In 2015, it was assessed that 415 million individuals had diabetes. However, this figure is relied upon to ascend to 642 million in the following 25 years [3].

The WHO evaluated that around 346 million individuals around the globe experience the effects of diabetes, which will be multiplied constantly by 2030, representing a major face up to the health care system [4]. The disease is isolated in 2 major classes: insulin-dependent, also called type 1 diabetes and non-dependent, also known as type 2 diabetes [5]. It is characterized by anomalous large amounts of plasma glucose in fasting situation or past association of glucose, all through an oral glucose tolerance test. Diabetes is observed by an absolute or relative insufficiency in the discharge of insulin [6]. Diabetes type 1 consequences in insulin insufficiency are caused by a cell linked immune damage in  $\beta$ -cells of the pancreas and found to be most developed in young people [7]. Approximately 90 percent of diabetes is type 2,

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## Design, synthesis, and biological evaluation of coixol-based derivatives as potential antidiabetic agents



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### ABSTRACT

Natural products have great potential for structural modifications to achieve the desired biological effect. In addition, these are considered safer, less expensive, and easily accessible compared to synthetic drugs. The natural compound coixol is a potent insulin secretagogue, obtained from the plant parts of *Scoparia dulcis* and *Coix lacryma Jobi*. In the present research work, we synthesized coixol-based derivatives that could potentially emerge as successful antidiabetic agents. Our previous work explains exclusive molecular docking studies based on the natural product coixol, which led to the current selection of twelve molecules comprising 2-benzoxazolinones, 2-benzimidazolinone, and benzimidazole-2-thiones with a good binding affinity toward ATP-sensitive potassium channel. All the synthesized molecules were screened for *in vitro* studies using a rat insulin ELISA assay. Wherein, Compounds **14a** and **34** were identified as the most potent molecules with percentage insulin secretory concentrations of 162.43 and 227.16  $\mu\text{U}/\text{mL}$  respectively which was reportedly higher than the standard drug Glibenclamide. The *in vivo* antidiabetic effect was checked using the streptozotocin-induced Type-II diabetes rat model, which revealed that test compounds **14a** and **34** significantly reduced plasma glucose and total cholesterol levels and a marked increase in plasma insulin response was observed in diabetic rats. The histopathological examination at the end of the protocol also showed an improvement in the pancreatic tissue of diseased animals treated with test compounds **14a** and **34**. Thus, this research work indicates that the synthesized derivatives of coixol, **14a** and **34** have good potential to be used for treating and managing Type-II diabetes in the future.

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### 1. Introduction

Diabetes mellitus (DM) has been considered a life-threatening metabolic disorder leading to hyperglycaemia [1]. This disease can be classified as Type-I DM, insulin-dependent DM, and Type-II DM, insulin-independent DM [2–4]. It has been seen that approximately 90% of the cases are Type-II DM, either due to peripheral aversion, resistance towards insulin, or insufficiency of insulin [5]. Nevertheless, Type-II DM could also develop amongst the younger population and is said as MODY (Maturity Onset Diabetes of the Young). It is currently impacted by an excess of genetic or surrounding elements, resulting in the improvement of insulin resistance and

dysfunction of  $\beta$  cells [6–8]. Therefore, it has also been considered a persistent disorder related to high mortality risk. Especially the cases of non-insulin-dependent diabetes mellitus (NIDDM) are growing at a worrying level [9–11]. Recently, WHO has accessed that approximately 346 million patients worldwide have been diagnosed with diabetes. This count will continue to rise at a multiplying rate by 2030, which has appeared as the biggest challenge to the healthcare systems [12,13]. Meanwhile, The existing antidiabetic drugs (Fig. 1) are associated with several consequential adverse effects. Subsequently, finding novel, potentially active therapeutic drugs with high efficacy and fewer adverse effects is in high demand [14,15]. Natural products contributed a major role in managing several diseases and finding leads to support drug discovery studies [16]. These naturally driven products are considered drug-like molecules and continue to exist as the best sources for drug leads [17]. Medications from naturally driven products have been considered safer, less expensive, and easily accessible compared to pure synthetic drugs [18].

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# Identification of Novel Coixol-Based Derivatives as the Potential Anti-diabetic Agents Through Molecular Docking Studies

Deepshikha Patle\*, Paranjeet Kaur\*, Navneet Khurana

## ABSTRACT

**Objective:** The objective of this study was to design and identify the novel promising anti-diabetic agents based on the naturally existing potent insulin secretagogue coixol that can improve the potency overcome the adverse effects of existing medicines. **Methods:** The Auto Dock Vina (ADT) 1.5.6 and PyMOL software were used for molecular docking and visualization purposes. The molecular structures were drawn in Chem Draw 16.0 and by the help of Chem Bio draw three dimensions, all structures were energy minimized by MM2 method and converted to PDB extension file which is readable at the ADT interface. **Result:** Total 254 designed molecules from each series 1–4 were checked for binding score with the receptor 5yw7. Out of that total 12 molecules from each series were selected on the basis of their binding affinity in each series. Among these coixol (Natural product), DP322, DP330, and DP422 were studied in-depth. **Conclusion:** Coixol-based derivatives which scored best binding affinity such as DP332, DP330, and DP422 were shown promising result on the interaction with the K<sup>+</sup> ATP sensitive Potassium channel protein (5yw7). The entire study suggests that these novel coixol-based derived molecules could be a promising lead for the further discovery and investigation of insulin sensitizing agents for the treatment of diabetes.

**Keywords:** Drug designing, Insulin secretagogues, Natural product, Potential molecules, Type II diabetes mellitus

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## INTRODUCTION

Among the several metabolic diseases, diabetes has been featured as one of the major concern throughout the world.<sup>[1,2]</sup> In 2015, it was assessed that 415 million individuals had diabetes. However, this figure is relied on to ascend to 642 million in the following 25 years.<sup>[3]</sup> It is characterized by anomalous large amounts of plasma glucose in the fasting state or after the organization of glucose during an oral glucose tolerance test. DM is caused by a relative or absolute insufficiency in insulin discharge, a protection from insulin secretion or both.<sup>[4,5]</sup> About 90% of diabetes cases are type 2 diabetes mellitus, which is characterized by peripheral insulin resistance and insulin lack.<sup>[6]</sup> Type 2 is currently thought to be impacted by in excess of a single gene or environmental factors resulting in improvement of insulin resistance and  $\beta$ -cell dysfunction.<sup>[7-9]</sup>

Natural products have a long tradition as invaluable sources of inspiration for chemistry, biology, and medicine. An overwhelming number of studies have established strategies for obtaining diverse natural products and their analogues and constructed powerful compound libraries for the development of drugs.<sup>[10]</sup> Their libraries contain specialized metabolites derived from plants, animals, and microorganisms that play a pivotal role in drug discovery due to their immense structural diversity in terms of wide range of functional groups, high degree of stereochemistry, and pharmacophores and wide variety of biological activities.<sup>[11,12]</sup>

Natural products and their analogues are meaningful for illustrating the structure-activity relationships that are key for the optimization of pharmaceutical properties of clinically relevant compounds.<sup>[13,14]</sup>

## Coixol as a Potential Anti-diabetic Agent

In the recent studies, it is stated that coixol exhibited insulin secretory activity (Insulin secretagogues) on MIN-6 cells lines

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which make it an active chemical constituent for the treatment of diabetes mellitus.<sup>[15,16]</sup> It is chemically 6 methoxy benzoxazolinone (6-MBOA) which is an alkaloidal flavonoid, this potent insulin secretagogue presents majorly in the plant part of *S. Dulcis* and the seeds of *Coixlacryma-jobi*.<sup>[17]</sup> Perhaps, Among the available anti diabetics, sulfonyleurea drugs show the mechanism as insulin secretory agents and these drugs are also commonly called as insulin secretagogues.<sup>[18]</sup> These drugs inhibit the ATP-sensitive potassium channel (K-ATPase) in the beta-cells of Pancreas. Sulfonyleureas stimulate the secretion of insulin regardless of blood glucose levels. On the other side, coixol has already been reported as insulin secretagogue.<sup>[19]</sup> Considering this, we selected a protein coded as 5yw7 (K ATPase-sensitive potassium channel). All the molecules were designed by considering the structural backbone of coixol and docked with the protein 5yw7 to check insulin secretory effect of the designed ligands.



NIPiCON-IPS- 2022  
6<sup>th</sup> Nirma Institute of Pharmacy International Conference  
Jointly Organized with Indian Pharmacological Society,  
February 17-19, 2022

*“Emerging Opportunities and Challenges in Pharmacology  
and Pharmaceutical Sciences for Drug Discovery and  
Healthcare Innovations”*



## F002

### **Design & identification of novel 2- benzoxazolinone derivatives as potential candidates for the management of Type-II diabetes through molecular docking studies**

Patle Deepshikha, Kaur Paranjeet, Khurana Navneet

School of Pharmaceutical sciences, Lovely Professional university, Phagwara,  
Punjab

*dpatle16@gmail.com*

Diabetes mellitus is observed to be a serious metabolic issue for ages and the current treatment for the disease is associated with some serious side effects, and henceforth there is a need to find new potent therapeutic agents with high potency and fewer side effects. In this report, we present a novel class of benzoxazolinone derivatives involved with inhibitory activity against membrane bound ATP-sensitive potassium channels. 2-benzoxazolinones (BOA) is a molecule of interest due to its origin from nature & diverse pharmacological importance. This research is based on identification of 2- benzoxazolinone derivatives by studying the SAR via varying electron donor, electron withdrawing and other possible substitution that could potentially emerge as a successful clinical candidates for the treatment of type-2 diabetes. The structures were designed & analyzed by molecular docking software; Auto dock vina. The designed molecules were also analyzed by molecular modeling software, in order to resemble the highest binding affinity molecule with selected protein (5yw7) ATP-sensitive potassium channel bound with Glibenclamide as a drug target. The research methodology included downloading A Pdb for insulin protein(5yw7) from protein data bank Thereafter a protein-ligand docking with Auto dock-Vina by using following steps: 1.Ligand and Protein Set up 2. Mapping of the binding site 3. Docking & 4. Analysis.. we synthesized Five derivatives based on their binding scores i.e. 1b, 1d, 1g, 1h & 1J (-6.8, -7.1, -6.7, -7.4, -7.2). After the docking studies all the best scored molecules were further investigated for In silico Toxicity and ADME predictions which revealed the non-carcinogenicity, good absorption as well as solubility characteristics through substrate binding sites & drug-likeness of the selected compounds. The selected compounds showed better-calculated lipophilicity (iLogP) was found to be 0.76 to 1.88.



**DEPARTMENT OF CHEMISTRY**  
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(AFFILIATED TO KURUKSHETRA UNIVERSITY, KURUKSHETRA)

Organized

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**Dr. Suresh Kumar**  
Organizing Secretary

**Dr. Gulab Singh**  
Convener

**Dr Satish Kumar Bhardwaj**  
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**DEPARTMENT OF CHEMISTRY**  
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**"New Trends in Chemical Research"**

(ICNTR-2022)

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It is to certify that Ms. Deepshikha Patle, Scholar/Student from Lovely Professional university, has participated in a two-day Online International Conference on "New Trends in Chemical Research" (ICNTR - 2022) organized by Department of Chemistry, S.U.S GOVT. COLLEGE MATAK MAJRI, KARNAL on April 29-30, 2022. He/She has also presented a paper entitled Identification of novel 6-methoxy benzoxazolinone derivatives as the potential Anti diabetic agents via Molecular docking studies (OP-69)

Dr. Suresh Kumar  
Organizing Secretary

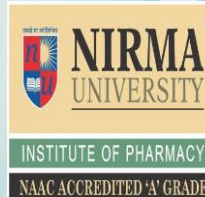
Dr. Gulab Singh  
Convenor

Dr. Satish Kumar Bhardwaj  
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6<sup>th</sup> Nirma Institute of Pharmacy International Conference  
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February 17-19, 2022

## Certificate

This is to certify that Dr. / Mr. / Ms. **Patle Deepshikha** attended 6<sup>th</sup> Nirma Institute of Pharmacy International Conference jointly organized with Indian Pharmacological Society held during February 17-19, 2022 as **Delegate** and **Oral paper presenter** and secured ----- prize.

Prof. Tejal Mehta  
Coordinator,  
Scientific Committee

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No.240281

## Certificate of Participation

This is to certify that **Ms. Deepshikha Patle** of **Lovely Professional University, Phagwara, Punjab, India** has presented paper on **Exploring the potential of *S. dulcis* and developing the rational for the drug designing of antidiabetic derivatives** in the **International Conference on Materials for Emerging Technologies (ICMET-21)** held on February 18-19, 2022, organized by Department of Research Impact and Outcome, Division of Research and Development, Lovely Professional University, Punjab.

Date of Issue: 16-03-2022  
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Prepared by  
(Administrative Officer-Records)



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# 21<sup>st</sup> Punjab Science Congress

SCIENTIFIC ADVANCES FOR INCLUSIVE DEVELOPMENT  
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*Monica Gulati*

Prof. Monica Gulati  
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**ONE DAY WORKSHOP ON  
"FUNDING OPPORTUNITIES FOR RESEARCH, INNOVATION & CAREER DEVELOPMENT"  
UNDER AEGIS OF AICTE (SWAYAM)**

**JULY 22, 2018**

*Certificate*

This is to certify that Prof./Dr./Mr./Ms. Deepshikha Patle  
has participated as a Delegate / Poster Presenter / Resource Person / Chair Person in One Day Workshop on  
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