STUDIES DIRECTED TOWARDS THE SYNTHESIS OF IMATINIB MESYLATE ((GLEEVEC), 4-(4-METHYL-PIPERAZIN-1- YLMETHYL)-N-[4-METHYL-3-(4-PYRIDIN-3-YL-PYRIMIDIN-2- YLAMINO)-PHENYL]-BENZAMIDE METHANESULFONATE) ANALOGS

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NEŞET BATUHAN GÜNAY

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ABSTRACT

STUDIES DIRECTED TOWARDS THE SYNTHESIS OF IMATINIB MESYLATE (GLEEVEC, 4-(4-METHYL-PIPERAZIN-1- YLMETHYL)-N-[4-METHYL-3-(4-PYRIDIN-3-YL-PYRIMIDIN-2- YLAMINO)-PHENYL]-BENZAMIDE METHANESULFONATE) ANALOGS

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Imatinib mesylate is indicated for the treatment of chronic myeloid leukemia (CML) and unresectable and/or metastatic malignant gastrointestinal stromal tumors (GIST). By the application of this anticancer drug, problems occurs in terms of stability and activity. Computer assisted design (CAD) works showed that the modification of the B and C part molecule can increase the

effectivity of the drug. The new derivatives of the drug will be obtained to change some part of the B and C segments. The total synthesis of a new imatinib mesylate will be done and the activity tests are going to be determined in collaboration with other groups.

Keywords: Imanitib, Gleevec, CML, Chronic Myeloid Leukemia.

ÖZ

İMATİNİB MESİLAT (GLEEVEC, 4-(4-METİL-PİPERAZİN-1-İLMETİL)-N-[4-METİL-3-(4-PİRİDİN-3-İL-PİRİMİDİN-2-İLAMİNO)-FENİL]-BENZAMİD METANSÜLFONAT) ANALOGLARININ SENTEZİNE YÖNELİK ÇALIŞMALAR

Günay, Neşet Batuhan
Yüksek Lisans, Kimya Bölümü
Tez Yöneticisi: Prof. Dr. Ayhan S. Demir

Kasım 2008, 47 sayfa

İmatinib mesilat kronik myeloid leukemia, metastatic malignant gastrointestinal stromal tümörler için kullanılan anti kanser bir ilaçtır. İlacın kullanımında kararlılık ve etkenlik konusunda çıkan sorunlar bu ilacın değişik türevlerini sentezlemeyi gerektirmiştir. Computer assisted design (Bilgisayar destekli tasarım) modellerinde yapılan araştırmalar ilacın B ve C segmentlerinde yapılacak bazı değişiklikler sayesinde ilaç ile ilgili sorunların giderilebileceğini

öngörmektedir. Öngörülen yeni türevler ilacın B ve C segmentlerinde değişiklikler yapılmasını gerektirmektedir. Bu nedenle yeni türevlerin baştan itibaren total sentezinin gerçekleştirilmesi denenecektir. Sentezlenmesi planlanan türevlerin biyolojik testleri başka gruplarla ortak çalışılarak yapılacaktır.

Anahtar kelimeler: İmanitib, Gleevec, CML, Kronik Miyeloit Lösemi.

To My Mother and Brother,

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CHAPTER 1

INTRODUCTION

1.1 Myeloid leukemia

Cancer develops when cells in a part of the body begin to grow out of control. Although there are many kinds of cancer, they all start because of out-of-control growth of abnormal cells.

Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide more rapidly until the person becomes an adult. After that, cells in most parts of the body divide only to replace worn-out or dying cells and to repair injuries. Because cancer cells continue to grow and divide, they are different from normal cells. Instead of dying, they outlive normal cells and continue to form new abnormal cells.

Cancer cells develop because of damage to DNA. This substance is in every cell and directs all activities. Most of the time when DNA becomes damaged the body is able to repair it. In cancer cells, the damaged DNA is not repaired. People can inherit damaged DNA, which accounts for inherited cancers. More often, though, a person's DNA becomes damaged by exposure to something in the environment, such as radiation or smoking [1,2].

Cancer, usually forms as a tumor. Some cancers, like leukemia, do not form tumors. Instead, these cancer cells involve the blood and blood-forming organs and circulate through other tissues where they grow. Not all leukemias are the same [2].

Leukemias are divided into 4 main types with several subtypes in order to better predict each patient's prognosis and help doctors select the best treatment for each patient.

In acute leukemia, the bone marrow cells are unable to properly mature. Immature leukemic cells, which are often called blasts, continue to reproduce and accumulate. Without treatment, most patients with acute leukemia would live less than a few months. Some subtypes of acute leukemia respond well to treatment and many patients are cured, while other types of acute leukemia have a less favorable outlook [1].

In chronic leukemia the cells can mature partly but not completely. They are not really normal. They generally do not fight infection as well as do normal white blood cells. And, of course, they survive longer, build up, and crowd out normal cells.

The second factor to consider in classifying leukemia is the type of bone marrow cells that are affected. If granulocytes or monocytes are involved, the leukemia is classified as myeloid leukemia (also known as myelogenous or myelocytic leukemia).

If the cancer develops from bone marrow lymphocytes, it is called lymphocytic (or lymphoblastic) leukemia. (Malignant lymphomas are also cancers of lymphocytes. But, unlike lymphocytic leukemias which develop in the bone marrow, lymphomas develop from lymphocytes in lymph nodes or other organs) [2].

By considering whether they are acute or chronic, and whether they are myeloid or lymphocytic, leukemias can be divided into 4 main types:

- acute myeloid leukemia (AML)
- chronic myeloid leukemia (CML) or chronic myelogenous leukemia
- acute lymphocytic leukemia (ALL) or acute lymphoblastic leukemia
- chronic lymphocytic leukemia (CLL)

Our CAD models are analogs of a previously market available drug used for the treatment of Chronic Myeloid Leukemia. The reason of need for the synthesis of new analogs for this drug is the undesired mutagenic side effect of this drug in long term usage.

If we consider again, Chronic myeloid leukemia (also called CML or chronic granulocytic leukemia) is a disease in which too many white blood cells are made in the bone marrow. The bone marrow is the spongy tissue inside the large bones in the body. The bone marrow makes red blood cells (which carry oxygen and other materials to all tissues of the body), white blood cells (which fight infection), and platelets (which make the blood clot).

Normally, bone marrow cells called blasts, develop into several different types of blood cells that have specific jobs to do in the body. CML affects the blasts that are developing into white blood cells called granulocytes. The blasts do not mature and become too numerous. These immature blast cells are then found in the blood and the bone marrow. In most people with CML, the genetic material (chromosomes) in the leukemia cells have an abnormal feature called a Philadelphia chromosome (Ph).

The Ph chromosome, is an abnormally short chromosome 22, that is one of the two chromosomes involved in a translocation (**Figure 1.1**) (an exchange of material) with chromosome 9. This translocation takes place in a single bone marrow cell and through the process of clonal expansion (the production of many cells from this one mutant cell), it gives rise to the leukemia [3].

The discovery of the Ph chromosome in Philadelphia in 1960 was a landmark. It was the first consistent chromosome abnormality found in any kind of

malignancy. The discovery led to the identification in CML cells of the BCR-ABL fusion gene and its corresponding protein. ABL and BCR are normal genes on chromosomes 9 and 22, respectively. The ABL gene encodes a tyrosine kinase enzyme whose activity is tightly regulated (controlled). In the formation of the Ph translocation, two fusion genes are generated: BCR-ABL on the Ph chromosome and ABL-BCR on the chromosome 9 participating in the translocation. The BCR-ABL gene encodes a protein with deregulated (uncontrolled) tyrosine kinase activity. The presence of this protein in the CML cells is strong evidence of its pathogenetic (disease-causing) role. The efficacy in CML of a drug that inhibits the BCR-ABL tyrosine kinase has provided the final proof that the BCR-ABL oncoprotein is the unique cause of CML [1,4].

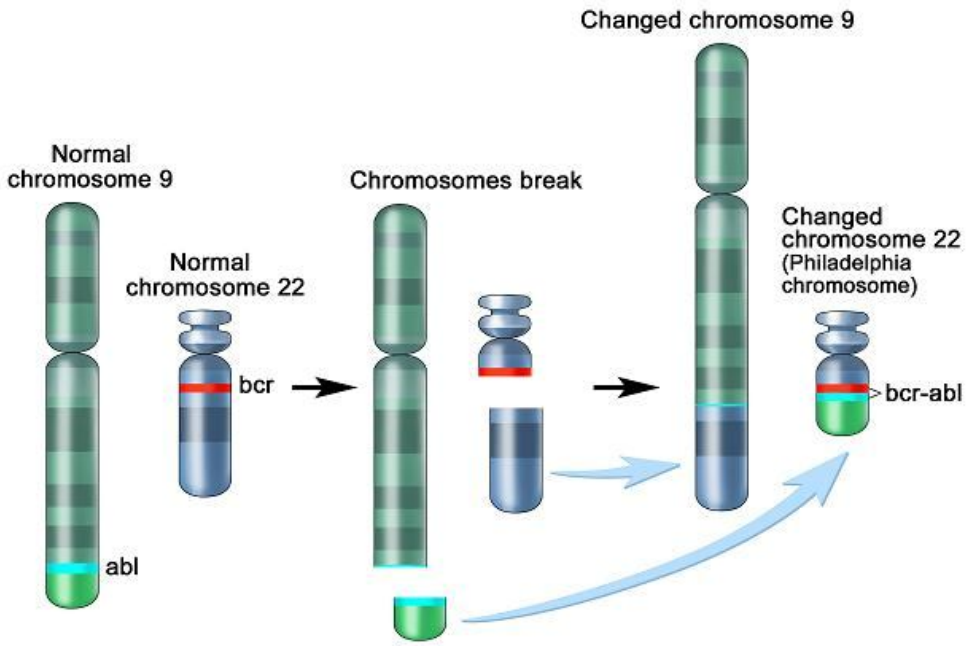


Figure 1.1. Abnormal translocation forming Philadelphia chromosome.

This chromosome usually does not go away, even after treatment. Leukemia can be acute (progressing quickly with many immature blasts) or chronic (progressing slowly with more mature-looking cancer cells). Chronic myeloid leukemia progresses slowly and usually occurs in people who are middle-aged or older, although it can also occur in children. In the first stages of CML, most people don't have any symptoms of cancer. A doctor should be seen if any of the following symptoms appear: tiredness that won't go away, a feeling of no energy, fever, not feeling hungry, or night sweats. Also, the spleen (the organ in the upper abdomen that makes other types of white blood cells and filters old blood cells from the blood) may be swollen.

For the treatment of CML imanitib mesylate is used which inhibits the tyrosine kinase activity of BCR-ABL by displacing ATP and trapping BCR-ABL in an inactive conformation [2].

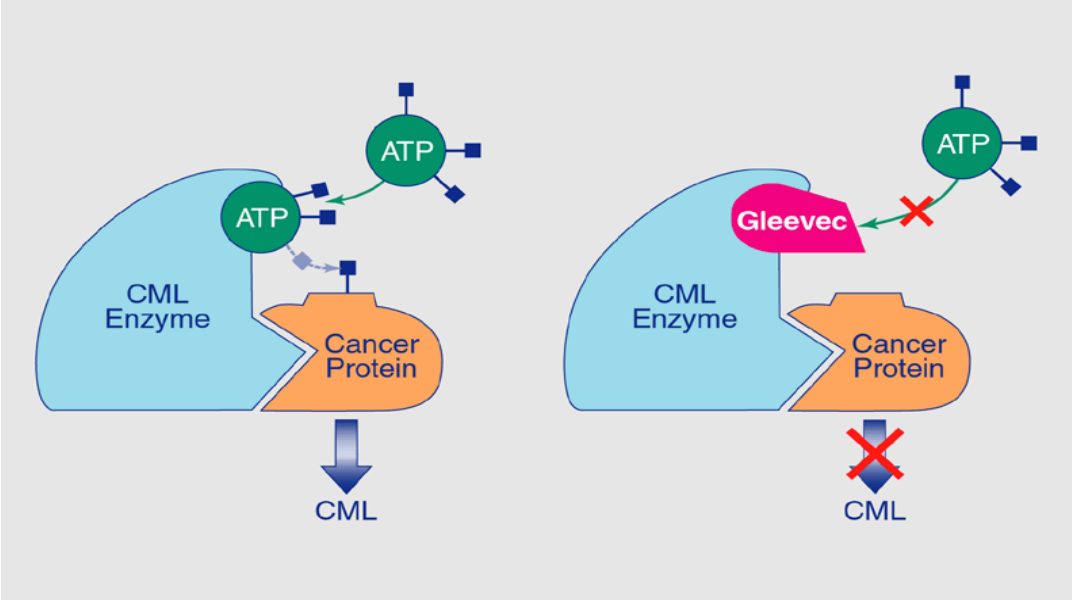


Figure 1.2. Working mechanism of Gleevec.

The idea behind this purpose is to slow down the cell functions such as reproduction and abnormal cell behaviours and even to kill the cancer cells by preventing the energy usage in a long time period. However, this ATP mimic activity **(Figure 1.2)** caused undesired mutagenic side effects on the cells for the long term usage [6]. As a consequence of this fact the need for a new and more efficient analog without none or less side activity become important.

Thus, the aim of this study is the total synthesis of BMA 152, which is an analog of Imanitib, has shown succesful results in CAD studies for the role of ATP mimic activity.

1.2 Imanitib (Gleevec)

Imatinib (Gleevec, 4-(4-methyl-piperazin-1- ylmethyl)-*N*-[4-methyl-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-phenyl]-benzamidemethanesulfonate) **(Figure 1.3)** is indicated for the treatment of chronic myeloid leukemia (CML) and unresectable and/or metastatic malignant gastrointestinal stromal tumors (GIST). CML is a clonal hematopoietic stem cell disorder characterized by the presence of the Philadelphia chromosome. A hallmark of this leukemia is a reciprocal chromosomal translocation involving the bcr gene from chromosome 9 and the c-abl gene from chromosome 22. The resulting Bcr-Abl fusion proteins have elevated nonreceptor tyrosine kinase catalytic activity, causing cell transformations and the concomitant malignancy. The most potent, selective inhibitor of Bcr-Abl is imatinib, signal transduction inhibitor 571 (STI 571). This ATP mimic drug has a high affinity for Abl kinase, while being essentially inactive against Ser/Thr-kinases and most of the tyrosine kinases [1].

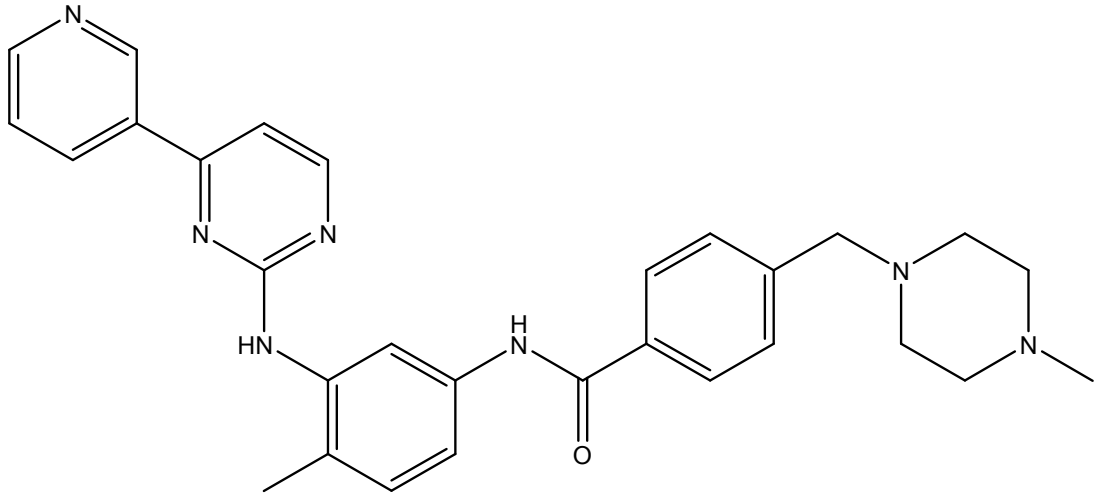


Figure 1.3. Imanitib (Gleevec, STI 571)

One of the most celebrated triumphs of basic cancer research in recent years has been the development of Gleevec for chronic myelogenous leukaemia (CML) therapy. The active ingredient in Gleevec (imatinib myselate, also known as STI571) inhibits the oncogenic BCR– ABL tyrosine kinase in CML cells. BCR–ABL is a fusion protein between part of the BCR (breakpoint cluster region) protein and the ABL (Abelson) tyrosine kinase. Gleevec directly binds to the tyrosine kinase domain of BCR– ABL to inhibit its catalytic activity. This orally administered drug exerts its therapeutic effect rapidly and without any of the side effects commonly associated with chemotherapy. Gleevec is a shining example for how basic research can transform clinical practice. Gleevec also benefits basic research because it provides a tool to explore the biological function of the cellular ABL tyrosine kinase. Gleevec-resistant BCR–ABL kinase mutants have been identified in relapsed CML patients2; when transferred to the cellular ABL kinase, these mutants can be used to distinguish between the ABL-inhibitory versus off-target effects of Gleevec. Using Gleevec and a drugresistant ABL kinase, now show that ABL mediates the tumour suppressive

effects of the EphB4 receptor in breast cancer cells, and that Gleevec disables this cellular defence mechanism [2].

As a consequence of the side effects of Gleevec; a new and more effective drug is in the scope of scientist. Kompela et al. [1] synthesized several analogs of gleevec to obtain a better biological activity for the treatment of CML. In **Figure 1.4** there are some types of gleevec which has been synthesized by Kompela et al. by changing the C part of Imanitib and patented. In our study, we synthesized our analogs by replacing the B part of imanitib with substituted benzimidazole substructure and the agreed conformation of imanitib is is held by changing the position of N- methyl piperazin from para to meta in part C. The idea behind this is study is the CAD works, where our analogs has shown better enzyme substrate interaction for bcr-abl tyrosine kinase inhibition [1].

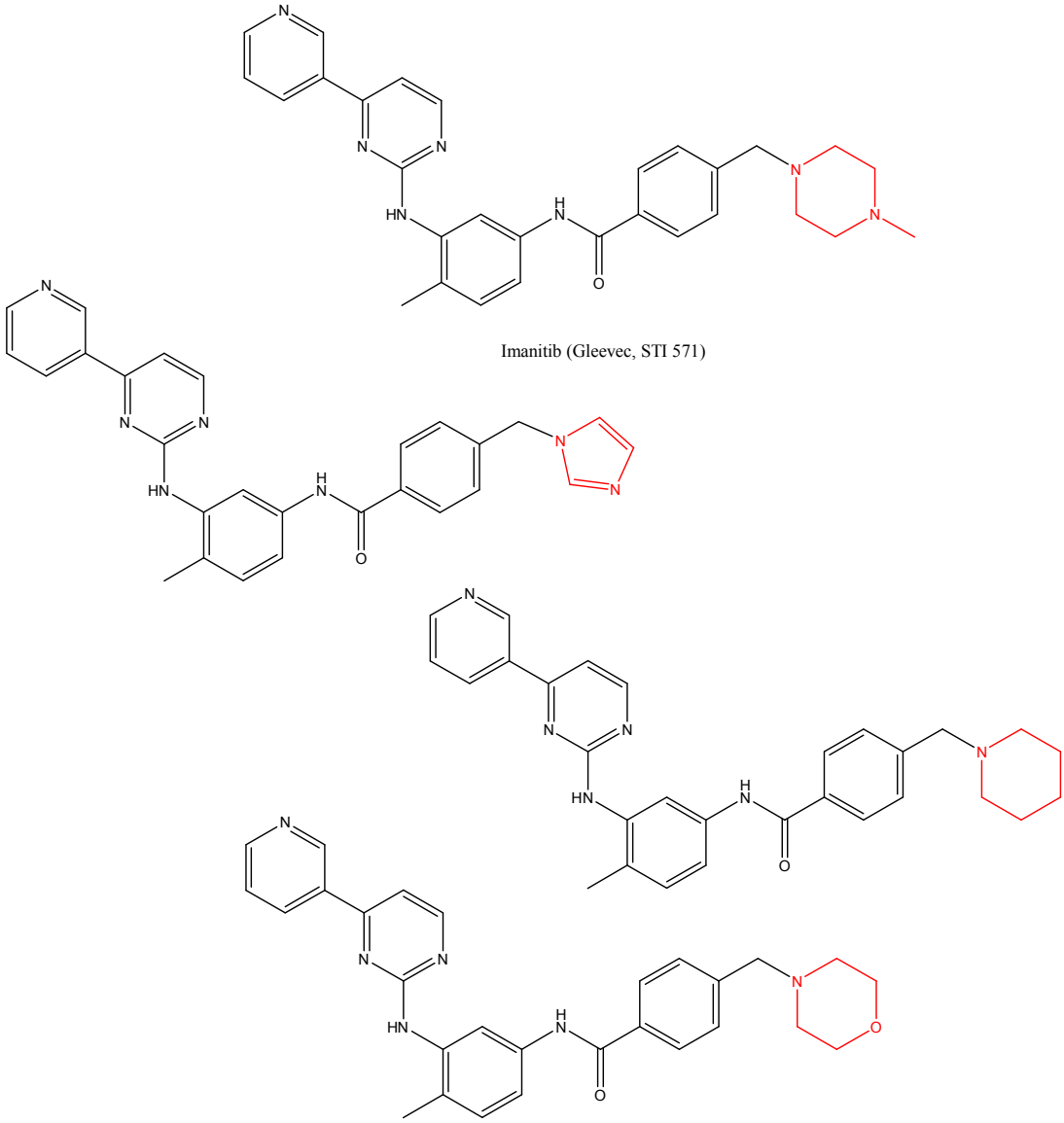


Figure 1.4. Imanitib analogs synthesized by Kompela et al. [1].

1.3 Synthesis of Imanitib and its biological activities

Synthesis of Imanitib Mesylate (Gleevec) has 2 main reactions for the combination of three fragments synthesized separately, called as A, B and C. **Figure 1.5** shows the retro-synthetic pathway for the formation reaction.

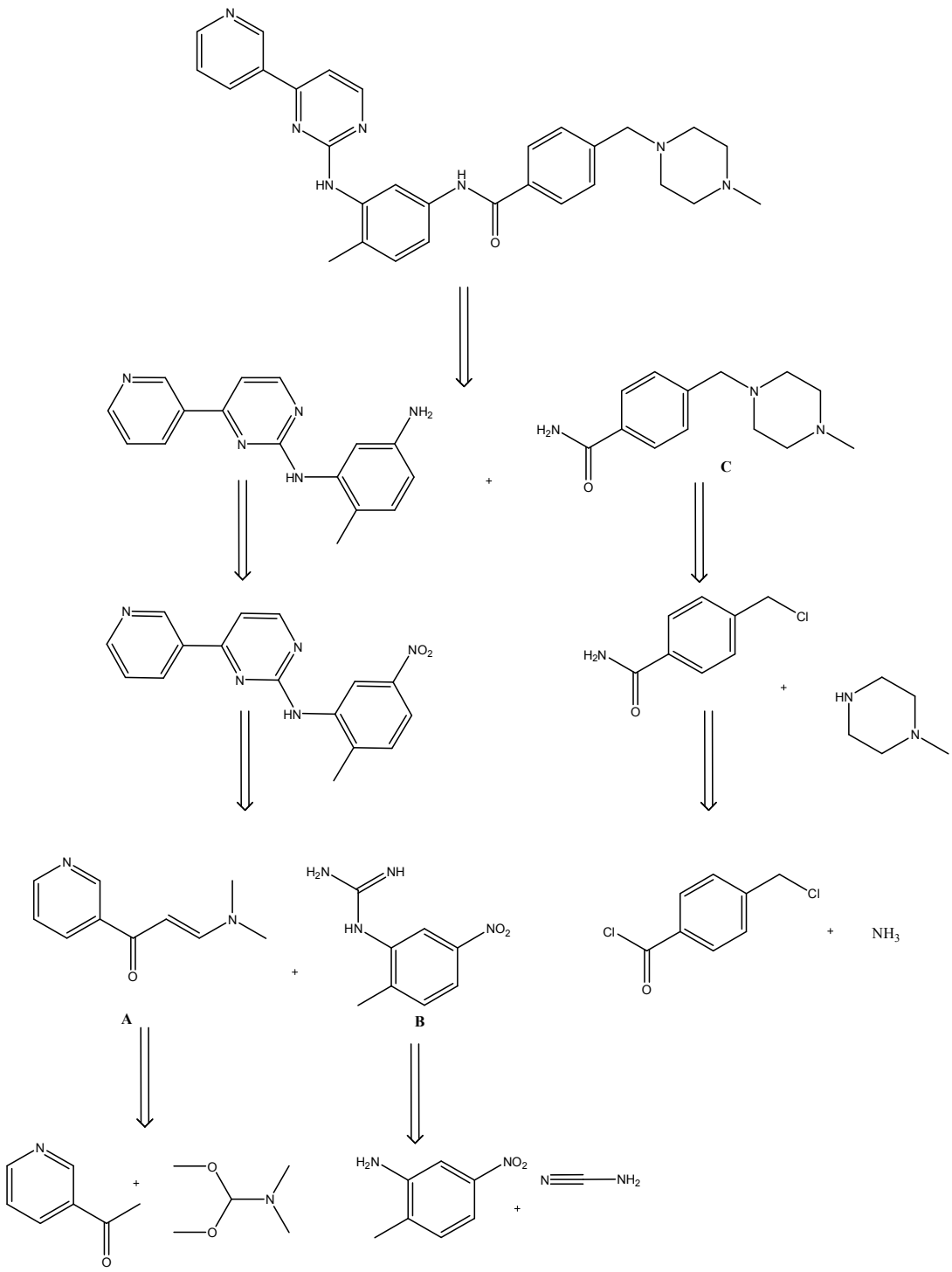


Figure 1.5. Retro-synthetic approach for the synthesis of Imanitib (Gleevec).

1.4 Problems associated with the application of Imanitib

Gleevec (STI 571) inhibits the aberrant ABL tyrosine kinase activity in CML, and has demonstrated remarkable single-agent activity during CML blast crisis. However, the appearance of clinical resistance to Gleevec has begun to be a significant clinical problem. The majority of CML patients that relapse after an initial response have reactivated BCR-ABL kinase activity, which frequently results from mutations rendering the kinase less sensitive to pharmacological inhibition by Gleevec [7].

To appreciate this newly discovered dark side of Gleevec, it is necessary to understand how ABL kinase can promote, as well as suppress, cancer. ABL is conserved in multicellular eukaryotes and is activated by a variety of cell extrinsic and intrinsic signals (including growth factors, cytokines, extracellular matrix, oxidative stress and DNA damage). Current knowledge suggests that ABL is partitioned among signalling complexes by different interacting proteins that can hold it in a latent conformation. With this highly compartmentalized arrangement, different signals can selectively activate a subfraction of the cellular ABL pool to achieve a specific response. As a result, the biological output from ABL is variable and can be modulated by the activating signal, its subcellular localization and the cell context [7,8].

The tumour suppression function of ABL has been deduced by its ability to activate the p53-family of tumour suppressors and to induce apoptosis under conditions of stress. However, the genetic ablation of ABL does not increase tumour risk in mice, probably because ABL also promotes the mitogenic response to growth factors such as PDGF. Interestingly, the studies shows that the tumour suppressor function of the ephrin–Eph pathway requires ABL, and thus suggests caution in the clinical application of Gleevec [8].

1.5 Aim of the work

The aim of this study is to synthesize two new analogs of Imanitib Mesylate (Gleevec) which has shown successful enzyme substrate interaction in CAD studies. Our analogs should exit biological activity, in order to be used for further anti cancer studies. Thus, BMA 152 and its derivatives **Figure 1.6.** will be tested in collaboration with other groups for biological activity, after the accomplishment of our work.

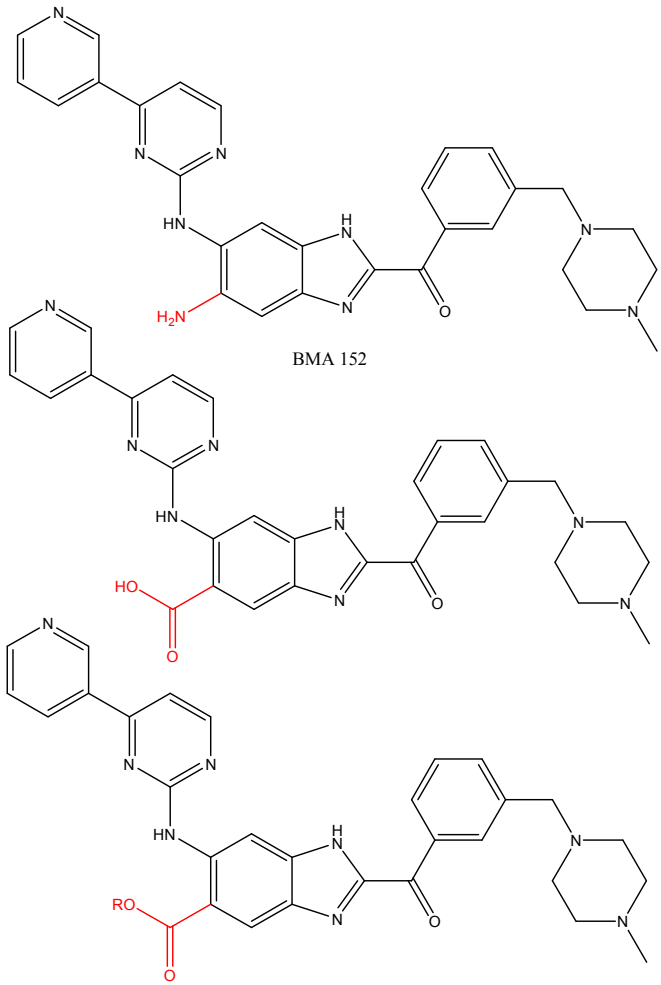


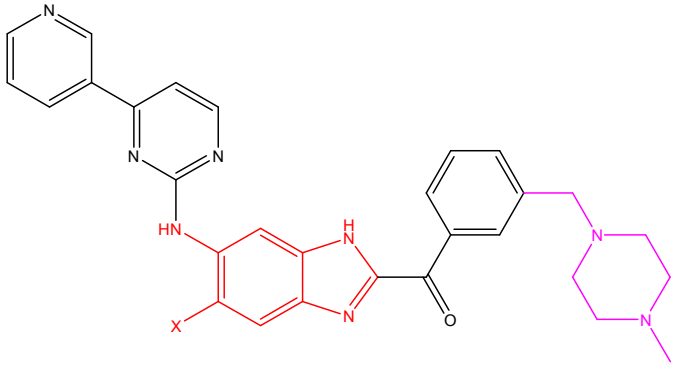
Figure 1.6. BMA 152 and its analogs.

CHAPTER 2

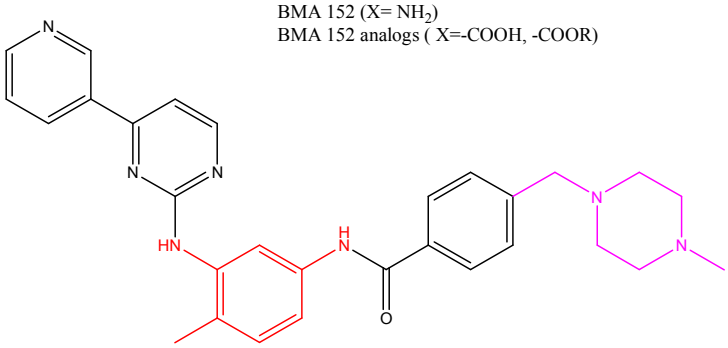
RESULTS AND DISCUSSION

2.1 The new Imanitib analogs

In this study, the analogs of the Imanitib (Gleevec) are built up on the basis of Imanitib, by replacing the central B part of the molecule by X (-COOH, $-NH_2$, -COOR) substituded benzimidazole substructure (**Figure 2.1.)** and the possible comformation assumption for the Imanitib is preserved by changing the substituents position in fragment C.



BMA 152 (X= NH_2)
BMA 152 analogs (X=-COOH, -COOR)



Imanitib (Gleevec, STI 571)

Figure 2.1. Structural differences between Imanitib and BMA 152 and its analogs.

2.2 Retrosynthetic pathway for the synthesis of BMA 152 starting from benzimidazole & 5,6-dimethylbenzimidazole

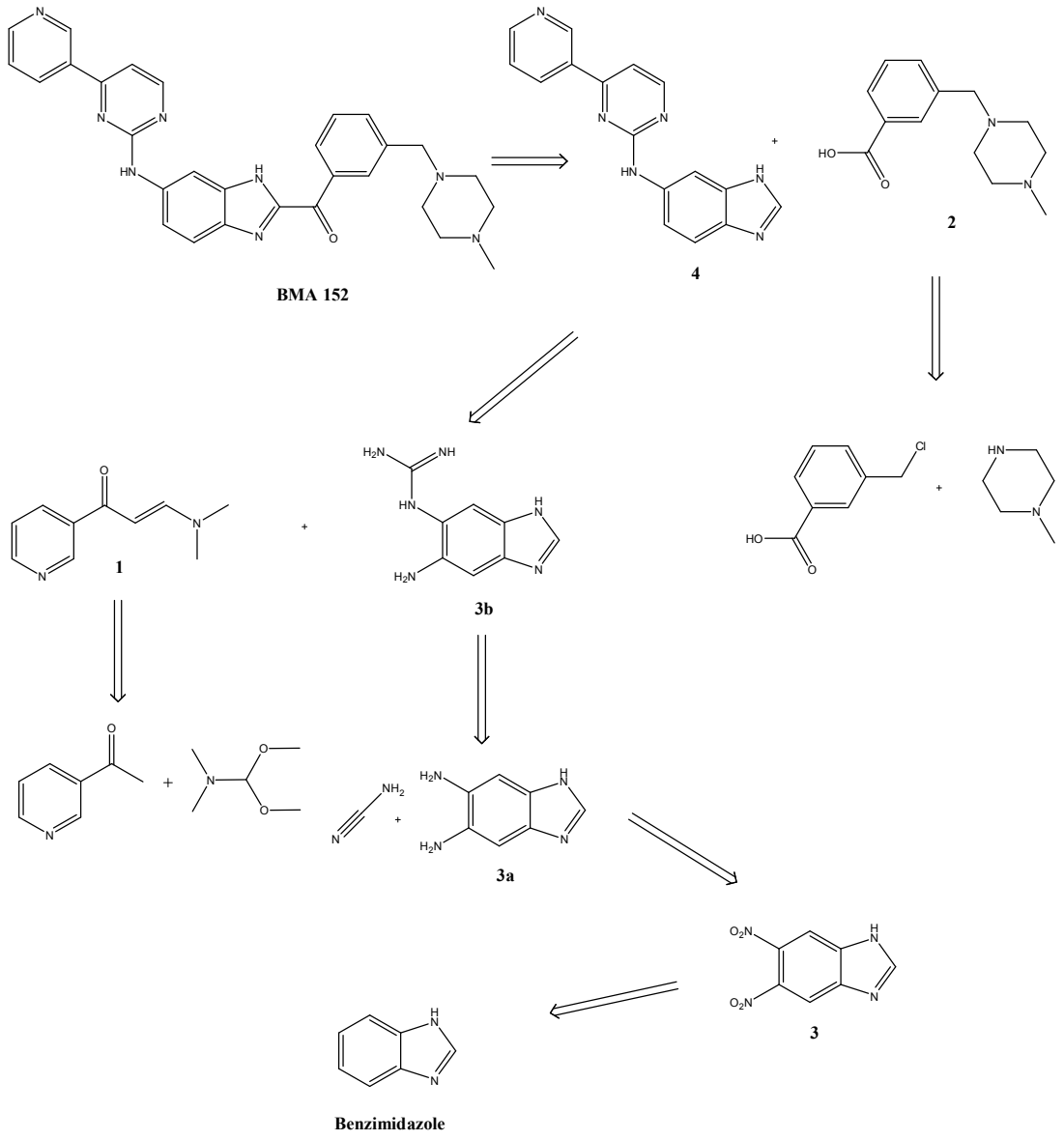


Figure 2.2. Retro-synthetic pathway for BMA 152 starting from benzimidazole.

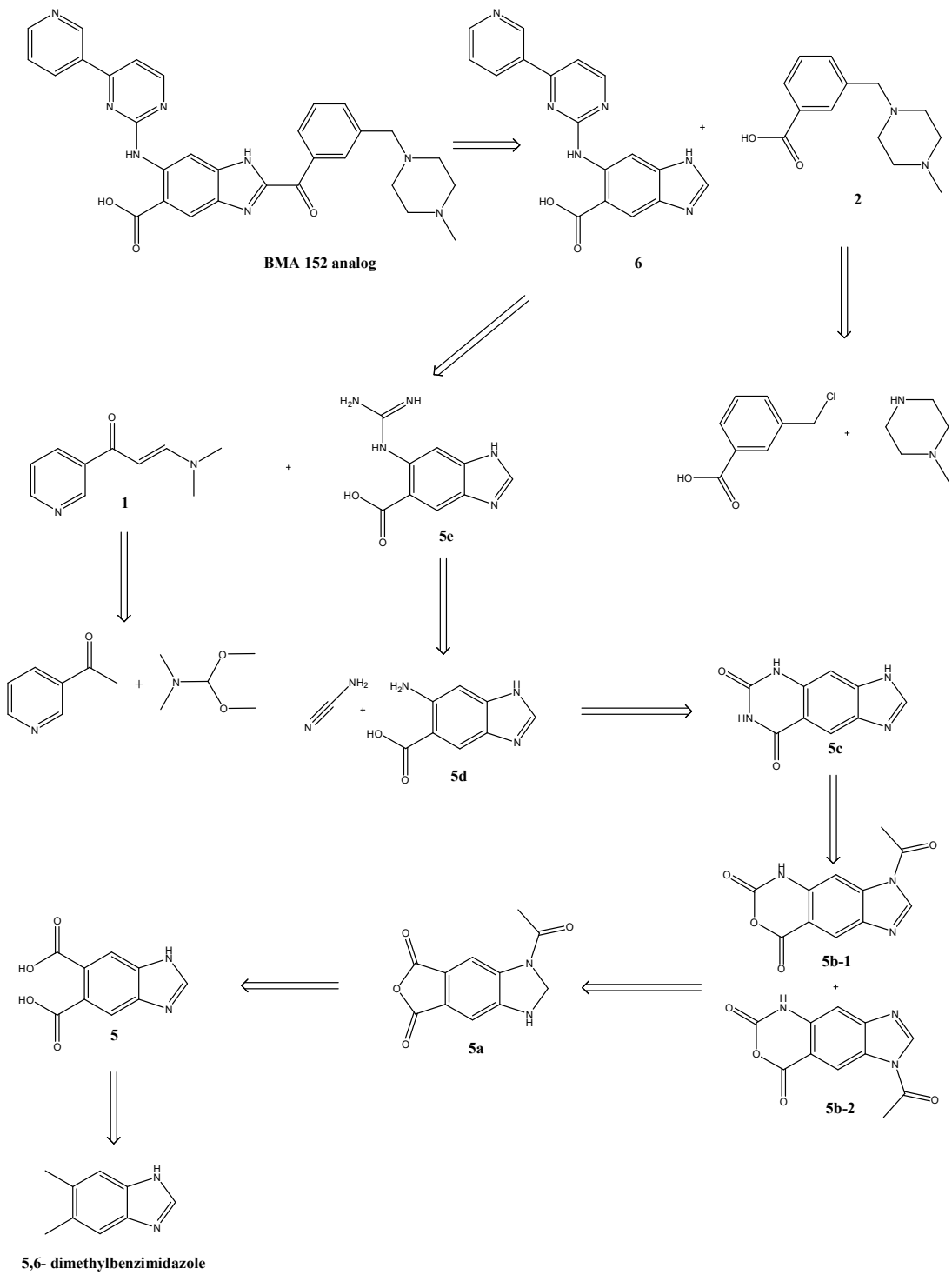


Figure 2.3. Retro-synthetic pathway for the synthesis of BMA 152 analog starting from 5,6- dimethylbenzimidazole.

2.3 Synthesis of fragment A

For the synthesis of fragment A, the same procedure is applied as it is described for the synthesis of Imanitib by Szakacs et al. [2]. The product was obtained as yellow solid with 89% yield. Since, new analogs has the same fragment without any change made on it. Synthesis of fragment A is shown below in the **Figure 2.4**.

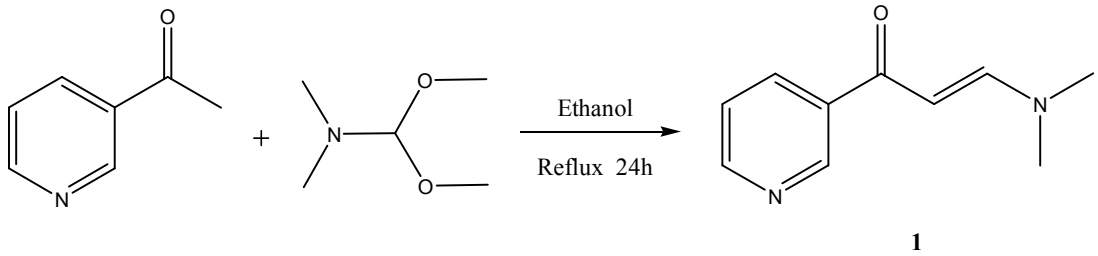


Figure 2.4. Synthesis of Fragment A ((E)-3-(dimethylamino)-1-(pyridin-3-yl) prop-2-en-1-one) (**1**)

2.4 Synthesis of fragment B

2.4.1 Synthesis of fragment B starting from benzimidazole

For the synthesis of fragment B, which differs our analogs from imanitib is carried out by starting from benzimidazole. For this purpose addition of nitro groups to benzimidazole [8], reduction of the 5,6-dinitrobenzimidazole to 5,6-diamino benzimidazole·HCl [10,11] and then mono cyanamide addition [2,12] to the

5,6-diaminobenzimidazole.HCl gave the fragment B in 82 % yield for further connection steps. (**Figure 2.5**)

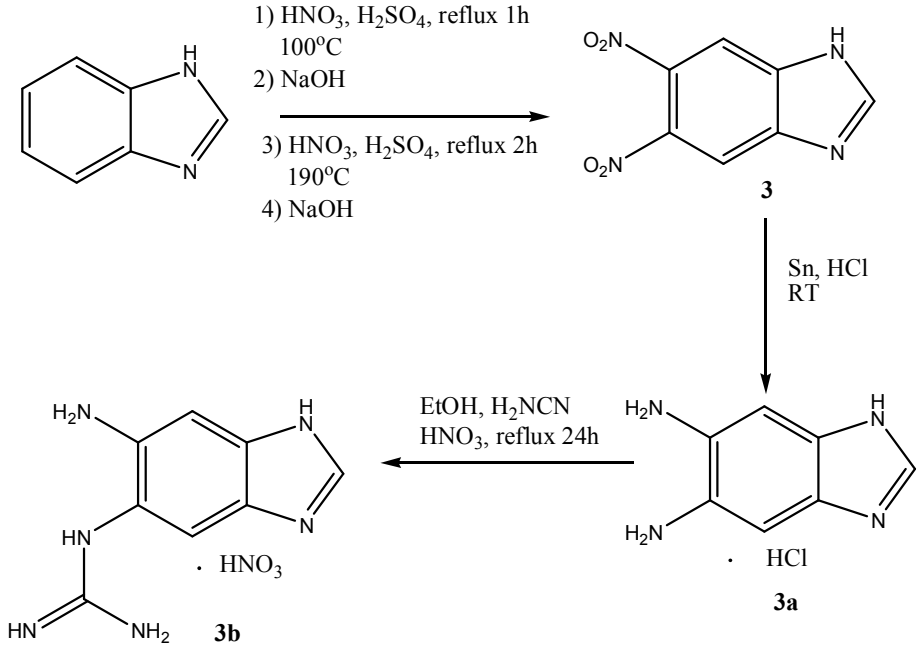


Figure 2.5. Synthesis of Fragment B (6-Amino-1H-benzo[d]imidazol-5-yl) guanidine .HNO_3. (**3b**)

2.4.2 Synthesis of fragment B (X = -COOH) starting from 5,6-dimethylbenzimidazole.

For the synthesis of fragment B, as a second way of a new BMA 152 analog with carboxylic acid fuctionality, is carried out by starting from 5,6-dimethylbenzimidazole. For this purpose, oxidation of two methyl groups carried out by $KMnO_4$ and then, cyclization of the di-carboxylic acid fuctionality is obtained by

acetic anhydride [13]. Amine functionality in the final structure for the formation of guanidium nitrate salt is carried out by using azido trimethyl silane [14,15], which introduced a nitrogen via a ring enlargement reaction. Mixture of isomers obtained from the ring enlargement reaction is further treated with urea to give a quinazoline derivative which will be further treated with NaOH to give a ring opening reaction and carbamic acid elimination [16-18]. 6-Amino-1*H*-benzo[d]imidazole-5-carboxylic acid obtained from the latter step, is further treated with cyanamide [12] to give fragment B with carboxylic acid functionality with .

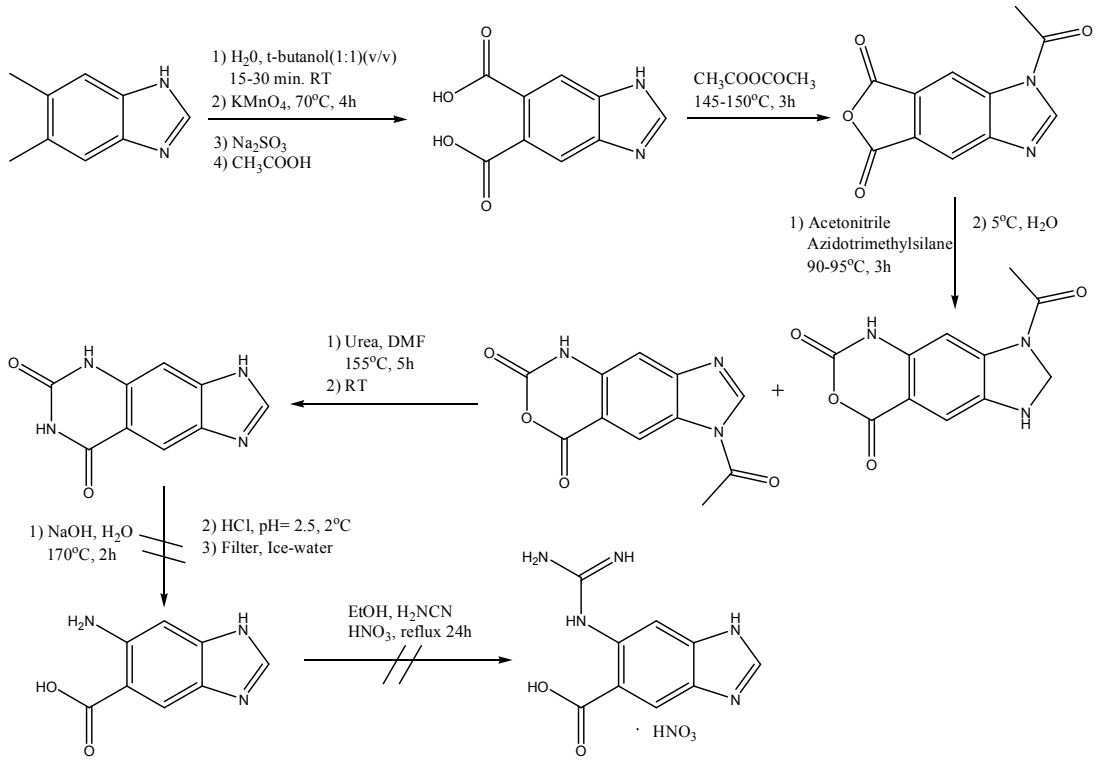


Figure 2.6. Synthesis of fragment B, 6-Guanidino-3*H*-benzo[d]imidazole-5-carboxylic acid.HNO_3. (**5e**)

2.5 Synthesis of fragment C

Synthesis of fragment C, which also differs our analogs from imanitib is the meta substitution of N-methylpiperazin. However; it is ortho substituted in imanitib. The reason of this is position difference is to minimize the conformational differences between imanitib and our analogs which has changed by the central benzimidazole subsutructure [2-19]. The reaction is carried out in benzene and the product is obtained in 95% yield as brownish yellow solid.

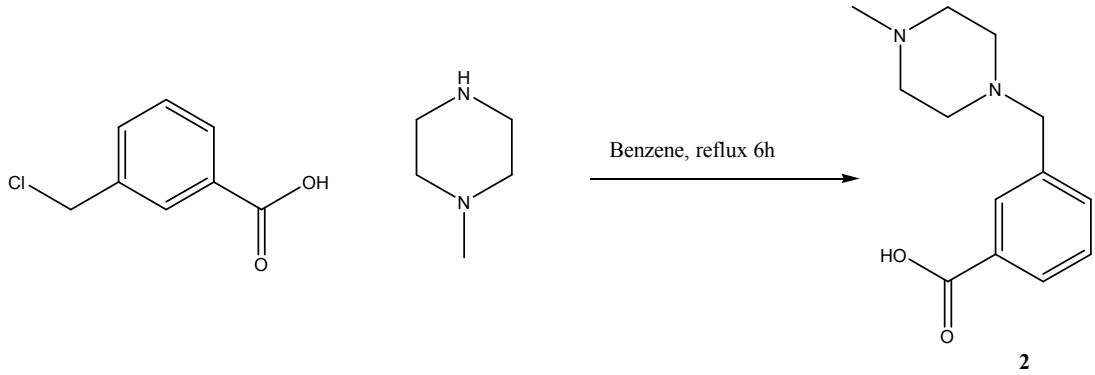


Figure 2.7. Synthesis of fragment C, 3-((4-Methylpiperazin-1-yl)methyl) benzoic acid. (**2**)

2.6 Connection of the fragments

For the synthesis of new imanitib analogs, the last two step is the connection of previously synthesized fragments. First, connection of fragment A and B, is planned to be followed by further connection of C.

2.6.1 Connection of fragment A and B

Connection of fragment A (X = -NH_2, - COOH) and B, is carried out by refluxing A and B in n-propanol in the presence of KOH [20]. Formation of the product is identified by crude NMR spectrum. However, the product decomposed during purification step.

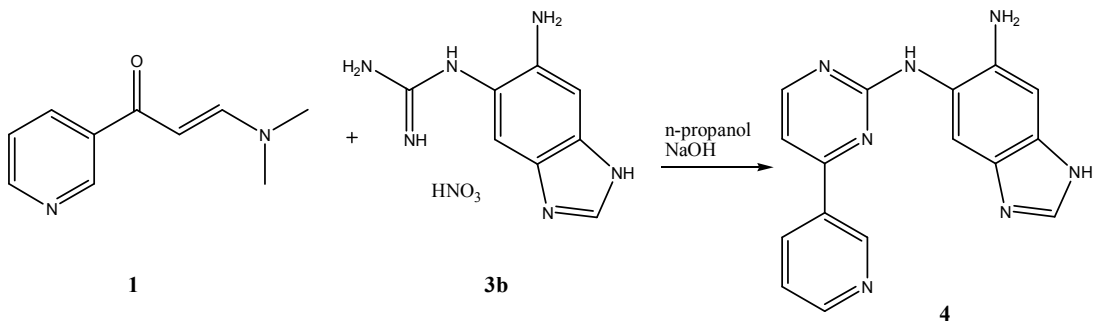


Figure 2.8. Connection of fragment A and B

2.6.2 Connection of fragment C

Connection of fragment C to the previously connected A and B fragments is planned to be carried out by refluxing in DMF (**Figure 2.9)**, as a last step for the synthesis of our new imanitib analog [21]. However, our starting material which is a product of previous step decomposed during purification.

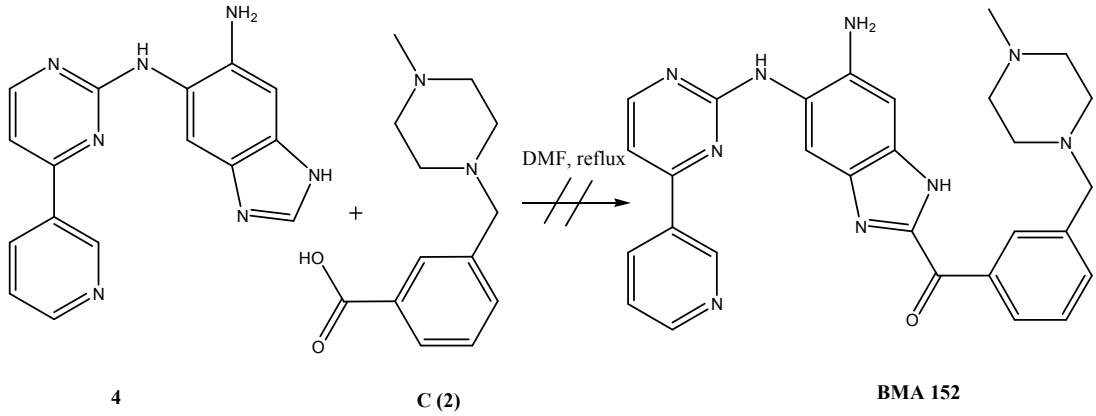


Figure 2.9. Connection of fragment C (**2**)

CHAPTER 3

EXPERIMENTAL

3.1 Materials and methods

In this study all compounds were identified by using Nuclear Magnetic Resonance Spectometer (NMR) (Bruker DPX 400 MHz) by using tetramethylsilane (TMS) as an internal standard and deutereo chloroform and dimethylsulfoxide as solvents.

Flash column chromatographies were done for purifying the products by using Merck Silica Gel 60 (partical size 40-63 μm)

3.2 General procedures

3.2.1 Synthesis of (E)-3-(Dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one (1)

3-Acetylpyridine 2.0 g (16.5 mmol) and 1.98g (16.5 mmol) of *N*,*N*-dimethyl-formamide dimethylacetal were refluxed in 25 cm^3 of ethyl alcohol overnight. The reaction mixture was evaporated under reduced pressure, 15 cm^3 of diethyl ether was added to the residue, and the reaction mixture was cooled to 0 °C. 3-Dimethylamino-1-pyridin-3-ylpropenone was filtered off with 82 % yield as

yellow crystals. This material was used in the subsequent steps without further purification.

^{1}H-NMR ($CDCl_3$+CCl_4):

δ (ppm): 2.95 (s, 3H)
3.18 (s, 3H)
5.66 (d, *J* = 12.3 Hz, 1H)
7.22-7.43 (m, 1H)
7.79 (d, *J* = 12.2 Hz, 1H)
8.16 (d, *J* = 7.7 Hz, 1H)
8.65 (d, *J* = 3.8 Hz, 1H)
9.05 (s, 1H)

^{13}C-NMR ($CDCl_3$+CCl_4):

δ (ppm): 185.8, 154.3, 151.3, 148.8, 137.2, 135.5, 134.9, 123.1, 91.8

3.2.2 Synthesis of 3-((4-Methylpiperazin-1-yl)methyl)benzoic acid (2)

0.250 g 3-(Chloromethyl)benzoic acid (1.46 mmol) and 0.700 g 1-methyl piperazine (6.98 mmol) was refluxed in 20 mL benzene for 6 hours. The product is then filtered off with 87 % yield as brownish yellow solid and used without further purification.

^{1}H-NMR (DMSO+$CDCl_3$+CCl_4):

δ (ppm): 2.26 (s, 3H)
2.52 (t, *J*= 4.97, 4H)
3.05 (t, *J*= 4.96Hz, 4H)
3.53 (s, 2H)
7.39 (t, *J* = 7.62 Hz, 1H)

7.49 (d, J = 7.46 Hz, 1H)
7.81 (d, J = 7.63 Hz, 1H)
7.88 (s, 1H)

^{13}C-NMR (DMSO+$CDCl_3$+CCl_4):

δ (ppm): 167.7, 133.3, 131.5, 130.1, 128.4, 54.4, 51.6, 45.9, 43.2

3.2.3 Synthesis of 5,6-Dinitro-1*H*-benzo[d]imidazole (3)

(A) 10 g Benzimidazole (84.6 mmol) in 45 mL HNO_3 (70%) is cooled with ice and 30 mL conc'd sulphuric acid is added slowly. The reaction is then stirred at 100 °C for 2 h, poured on ice and neutralized with NaOH. The solid precipitated is vaccuum filtered and the filtrate is extracted with 500 mL ethylacetate in 5 portions, filtered, dried over $MgSO_4$ and concentrated under vaccuum.

(B) Solid obtained from **A** in 30 mL HNO_3 (70%) and 18 mL conc'd sulphuric acid is refluxed at 190 °C for 3.5 h, poured on ice and neutralized with NaOH. The solid precipitated is vaccuum filtered and the filtrate is extracted with 250 mL ethylacetate in 5 portions, filtered, dried over $MgSO_4$ and concentrated under vaccuum. Recrystallization from ethanol gave a light yellow solid with 59 % yield.

^{1}H-NMR (DMSO+$CDCl_3$+CCl_4):

δ (ppm): 8.7 (s, 1H)
8.8 (s, 1H)
8.9 (s, 1H)
13.9 (bs, NH)

^{13}C-NMR (DMSO+$CDCl_3$+CCl_4):

δ (ppm): 148.9, 141.4, 120.9, 114.4

3.2.4 Synthesis of 1*H*-Benzo[d]imidazole-5,6-diamine.HCl (3a)

2.5 g (12.0 mmol) **(3)** and 5.0 g (42.1 mmol) Sn in 50 mL conc'd HCl is vigorously stirred until no more Sn is present in the mixture. The white precipitate is then vaccuum filtered and used without further purification. The product was obtained in 78 % yield.

^{1}H-NMR (DMSO+$CDCl_3$+CCl_4):

δ (ppm): 6.2 (s, 1H)
6.4 (s, 1H)
9.04 (s, 1H)

^{13}C-NMR (DMSO+$CDCl_3$+CCl_4):

δ (ppm): 144, 137, 136.5, 133.7, 115.6, 99, 87.6

3.2.5 Synthesis of (6-Amino-1*H*-benzo[d]imidazol-5-yl)guanidine . HNO_3 (3b)

1.25 g (6.8 mmol) of **(3a)** is dissolved in 15 mL ethanol and 1.5 mL HNO_3 is followed by 3 mL 50% (w/w) aqueous cyanamide solution. The reaction mixture is refluxed overnight and then cooled to 0 °C. The red guanidinium nitrate salt precipitate is then filtered and washed with diethylether and used without further purification. The product was obtained in 82 % yield.

^{1}H-NMR (DMSO+$CDCl_3$+CCl_4):

δ (ppm): 7.10 (s, 1H)
7.23 (s, 1H)
7.36 (s, 1H)

^{13}C-NMR (DMSO+$CDCl_3$+CCl_4):

δ (ppm): 161.7, 142.0, 129.5, 124.2, 115.9, 111.6, 79.0, 67.0.

3.2.6 Synthesis of N^5-(4-(Pyridin-3-yl)pyrimidin-2-yl)-1*H*-benzo[d]imidazole-5,6-diamine (4)

0.6 g (2.4 mmol) (6-Amino-1H-benzo[d]imidazol-5-yl)guanidine . HNO_3 **(3b)** and 0.42 g (2.4 mmol) of (E)-3-(dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one **(1)** in 15 ml of 2-propanol was added 0.1 g (2.6 mmol) of NaOH and refluxed for 24 h. The product is concentrated under vaccuum and crude product charactherized NMR spectrometry. We tried to purify the product by using flash column chromatography. However, the product decomposed during the purification step.

3.2.7 Synthesis of Benzimidazole -5,6- dicarboxylic Acid (5)

Into a 2-L, three-necked, round-bottomed flask equipped with condenser, thermometer, mechanical stirrer, dropping funnel, and oil bath was poured 350 mL of a 1:l (v/v) mixture of water and tert-butyl alcohol followed by 20.0 g (0.137 mol) of 5,6-dimethylbenzimidazole (Aldrich). Stirring of this heterogeneous mixture at room temperature for 15-30 min. gave a homogeneous, slightly brown solution, to which was added dropwise during 4 h a solution of 216.2 g (1.37 mol) of $KMnO_4$, dissolved in 1.5 L of H_2O from a dropping funnel at 60±2 °C. This was best accomplished by adding a homogeneous solution of $KMnO_4$, prepared at 68-70 °C, to the funnel in 50-mL portions. The temperature of the reaction mixture increased slowly to 70 ± 2 °C, and the rate of addition of the $KMnO_4$, solution and heating were regulated so as to keep the temperature at this level. The heat was turned off, and stirring was continued for 15 min. Anhydrous Na_2S0_3 (75 g, 0.595 mol) was added in five portions to decompose unreacted $KMnO_4$, while the temperature of the reaction mixture rose to 78-80 °C. The hot mixture was stirred 30 min. and filtered, and the MnO_2, cake was washed with 250 mL of boiling water. The combined filtrates were concentrated to approximately 0.75 L at 40-45 °C under vacuum and then diluted to

1.5 L with distilled water. To the solution cooled at 0-2 °C was added 300 mL of cold aqueous acetic acid (2:1, v/v). The white solid that precipitated, was recrystallized from boiling water. After drying in vacuum at 70 °C, compound **4** was obtained as a colorless powder in 59 % yield that was hygroscopic.

^{1}H-NMR (DMSO+$CDCl_3$+CCl_4):

δ (ppm): 8.21 (s, 2H)
8.51 (s, 1H)
13.32 (bs, 2× COOH)

^{13}C-NMR (DMSO+$CDCl_3$+CCl_4):

δ (ppm): 172.2, 169.5, 145.4, 139.1, 127.8, 116.6.

3.2.8 Synthesis of l-Acetylbenzimidazole-5,6-dicarboxylic anhydride (5a)

A suspension of 10.35 g (50 mmol) of benzimidazole-5,6-dicarboxylic acid **5** in 100 mL of acetic anhydride was stirred for 3 h at 145-150 °C (oil bath) with exclusion of moisture. After cooling to 5 °C, the white precipitate was collected by filtration, washed with 75 mL of anhydrous ether, and dried at 80 °C under vaccuum to provide compound **5a** as white solid in 79 % yield.

^{1}H-NMR (DMSO+$CDCl_3$+CCl_4):

δ (ppm): 2.84 (s, 3H)
8.38 (s, 1H)
8.69 (s, 1H)
9.27 (s, 1H)

^{13}C-NMR (DMSO+$CDCl_3$+CCl_4):

δ (ppm): 169.2, 163.2, 163.1, 149.46, 149.43, 136.6, 127.8, 127.4, 118.1, 113.1, 24.2

3.2.9 Synthesis of mixture of 1-N and 3-N-acetylimidazo[4,5-g]-7,5 benzoxazine-6,8(5*H*)-dione (5b-1,2)

To a suspension of 2.5 g (10.85 mmol) of **5a** in 150 mL of anhydrous acetonitrile was added 5 mL (45 mmol) of azidotrimethylsilane at room temperature with the exclusion of moisture. The mixture was stirred vigorously while the temperature of the oil bath was raised gradually to 90-95 °C. After an additional 3 h of stirring, the solution was cooled to 5 °C and 0.75 mL of water was added dropwise. Removal of the solvent at reduced pressure provided a mixture of **5b-1** and **5b-2** in approximately equal proportions as white solid in 67 % yield.

^{1}H-NMR (DMSO+$CDCl_3$+CCl_4):

δ (ppm): 2.45 (s, 3H)
2.77 (s, 3H)
8.01 (s, 1H)
8.11 (s, 1H)
8.18 (s, 1H)
8.23 (s, 1H)
8.36 (s, 1H)
8.56 (s, 1H)

^{13}C-NMR (DMSO+$CDCl_3$+CCl_4):

δ (ppm): 170.6, 170.3, 140.4, 140.2, 124.4, 118.2 , 114.3, 104.9, 61.5, 61.2, 55.6, 55.5. 31.5, 30.0.

3.2.10 Synthesis of *lin* -Benzoxanthine (imidazo[4,5-g]quinazoline-6,8- (5*H*,7*H*)-dione (5c)

A solution of crude **(5b-1,2)** obtained from 2.5 g (10.85 mmol) of (**5b-1,2)** mixture and 1.89 g (31.5 mmol) urea in 30 mL of DMF was heated in an oil bath at 155 °C for 5 h. The reaction mixture was cooled to room temperature, and the solid that precipitated was collected by filtration and washed with 50 mL of water to give, after drying at 100 °C under vaccuum **5c** as a light cream powder in 79 % yield.

^{1}H-NMR (DMSO+$CDCl_3$+CCl_4):

δ (ppm): 7.57 (s, 1H)
8.33 (s, 1H)
9.47 (s, 1H)
11.29 (s, 1H)
11.34 (s, 1H)

^{13}C-NMR (DMSO+$CDCl_3$+CCl_4):

δ (ppm): 162.8, 150.4, 143.8, 138.6, 136.5, 128.6, 114.5, 113.7, 99.5

3.2.11 Synthesis of 6-Amino-3*H*-benzo[d]imidazole-5-carboxylic acid (5d)

0.5 g **5c** (2.47mmol) and 0.6g NaOH (15mmol) in 5 ml H_2O was heated in a bomb at 170^{0}C for 2 hours. The mixture is then cooled to room temperature and acidified with con'c HCl to pH 2.5 and cooled to 2^{0}C and filtered. The residue is then extracted with chloroform dried with $MgSO_4$ and concentrated under vaccum.

An alternative method for the preceeding reaction for the formation of **5d** is as follows;

1.3 g **5c** (5.2 mmol) and 9 g KOH in 200mL n-propanol is refluxed for 45h. The reaction mixture is then poured into 150mL water and pH is adjusted between 5

and 6. The solid precipitated is then extracted with chloroform, dried over $MgSO_4$ and recrystallized from ethanol-water.

CHAPTER 4

CONCLUSION

The discovery of the Ph chromosome in Philadelphia in 1960 was a landmark. It was the first consistent chromosome abnormality found in any kind of malignancy. The discovery led to the identification in CML cells of the Bcr-Abl fusion gene and its corresponding protein. Abl and Bcr are normal genes on chromosomes 9 and 22, respectively.

Imatinib mesylate (Gleevec) is synthesized in 1992 by Novartis company and approved by FDA in 2003 as a second line treatment of CML patients. Gleevec was designed to affect the underlying cause of most cases of the Philadelphia (Ph) chromosome. The Ph chromosome produces an abnormal protein, called the Bcr-Abl protein, that tells the bone marrow to keep making abnormal white blood cells.

In 2004 researchers found out that, Gleevec (STI 571) inhibits the Bcr-Abl tyrosine kinase activity in CML, and has demonstrated remarkable single-agent activity during CML blast crisis. However, the appearance of clinical resistance to Gleevec begun to be a significant clinical problem. The majority of CML patients that relapse after an initial response, reactivated bcr–abl kinase activity, which frequently results from mutations rendering the kinase less sensitive to pharmacological inhibition by Gleevec.

In this study, we tried to synthesize new and effective imanitib analogs which has shown excellent ATP mimic property in CAD works. For this purpose, modification of B and C fragments was essential. Fragment B, which differs our analogs from imanitib, is the central benzimidazole substructure with amine and carboxylic acid functionality, providing excellent enzyme substrate interaction compared to imanitib. Another modification was done on the fragment C by keeping the conformation without making any change on the original imanitib structure, is accomplished by changing the N–methyl piperazine substitution from para to meta. For this purpose, we succesfully synthesized fragment A, B and C seperately for further connection steps. However, during the purification of amino substituted imanitib analog, target molecule decomposed to give 5,6- Diaminobenzimidazole and 4-(Pyridin-3-yl)pyrimidine.

In the synthesis of fragment B (with X = -COOH), ring opening reaction of 1*H*-imidazo[4,5-g]quinazoline-6,8(5*H*,7*H*)-dione did not give the desired 6-amino-3*H*-benzo[d]imidazole-5-carboxylic acid product.

To conclude, new and milder conditions for the purification of amino functional imanitib analog and ring opening reaction for the carboxylic acid fuctional analog will be further investigated and tried as a future work.

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APPENDIX A

NMR DATA

NMR spectra were recorded on a Bruker DPX 400.

Chemical shifts δ are reported in ppm relative to $CHCl_3$ (^1H: δ=7.27), $CDCl_3$ (^{13}C: δ=77.0) and CCl_4 (^{13}C: δ=96.4) as internal standards.

^1H and ^{13}C NMR spectra of products are given below.

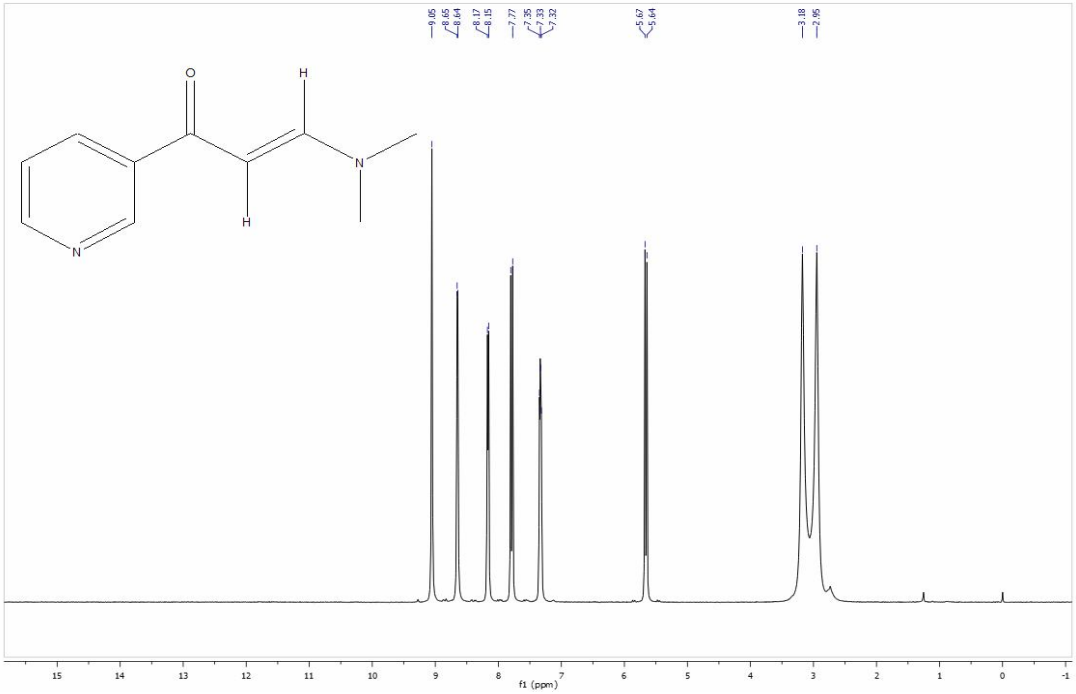

Figure A.1. ^{1}H-NMR spectrum of (E)-3-(Dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one **(1)**

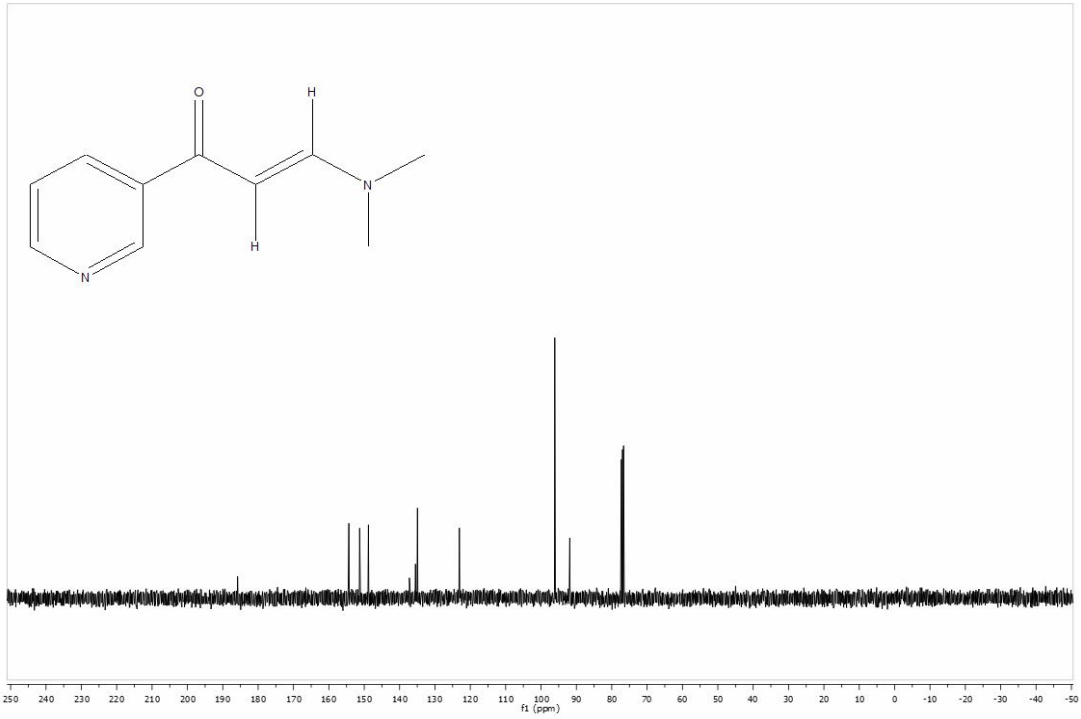

Figure A.2. ^{13}C-NMR spectrum of (E)-3-(Dimethylamino)-1-(pyridin-3-yl)prop-2-en-1-one **(1)**

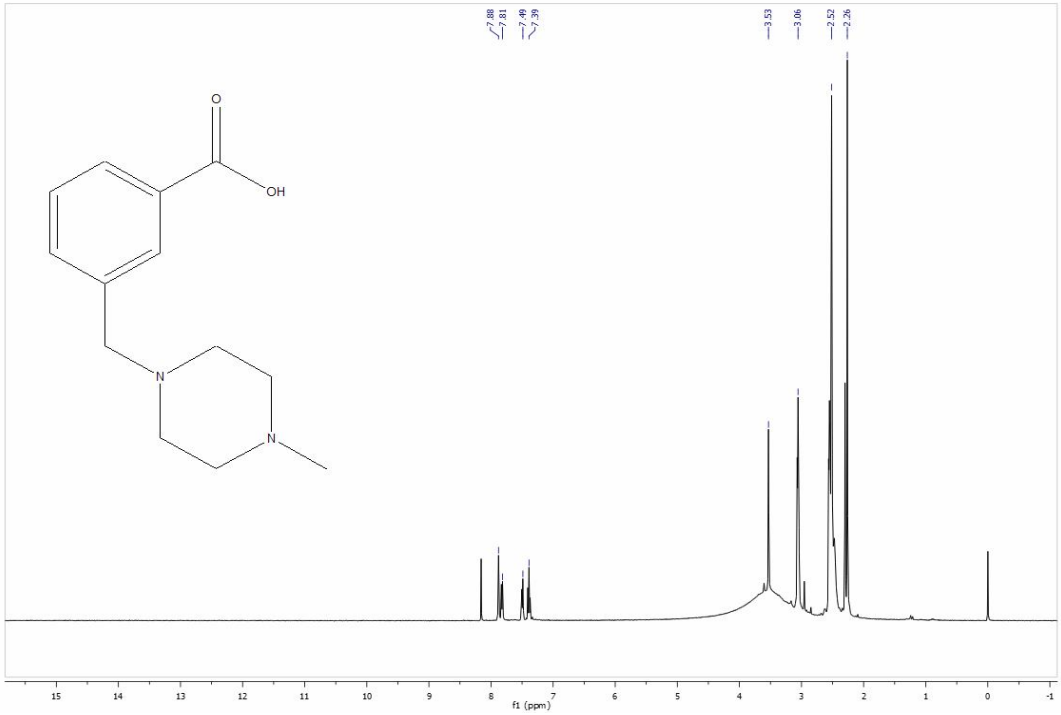

Figure A.3. ^{1}H-NMR spectrum of 3-((4-Methylpiperazin-1-yl)methyl)benzoic acid **(2)**

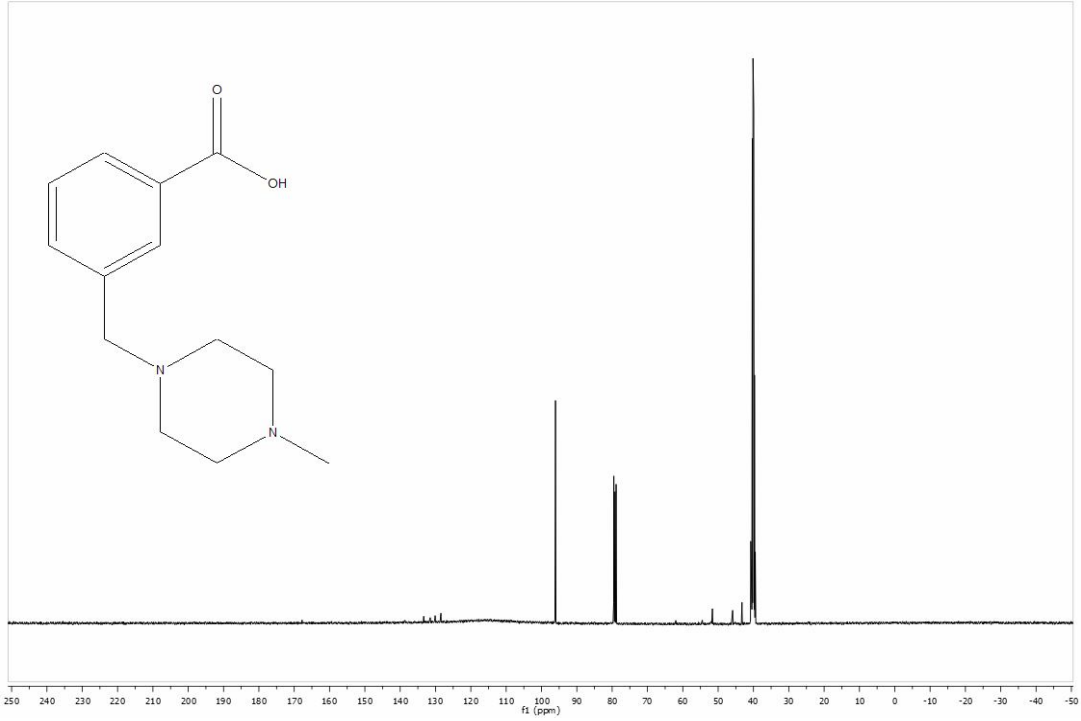

Figure A.4. ^{13}C-NMR spectrum of 3-((4-Methylpiperazin-1-yl)methyl)benzoic acid **(2)**

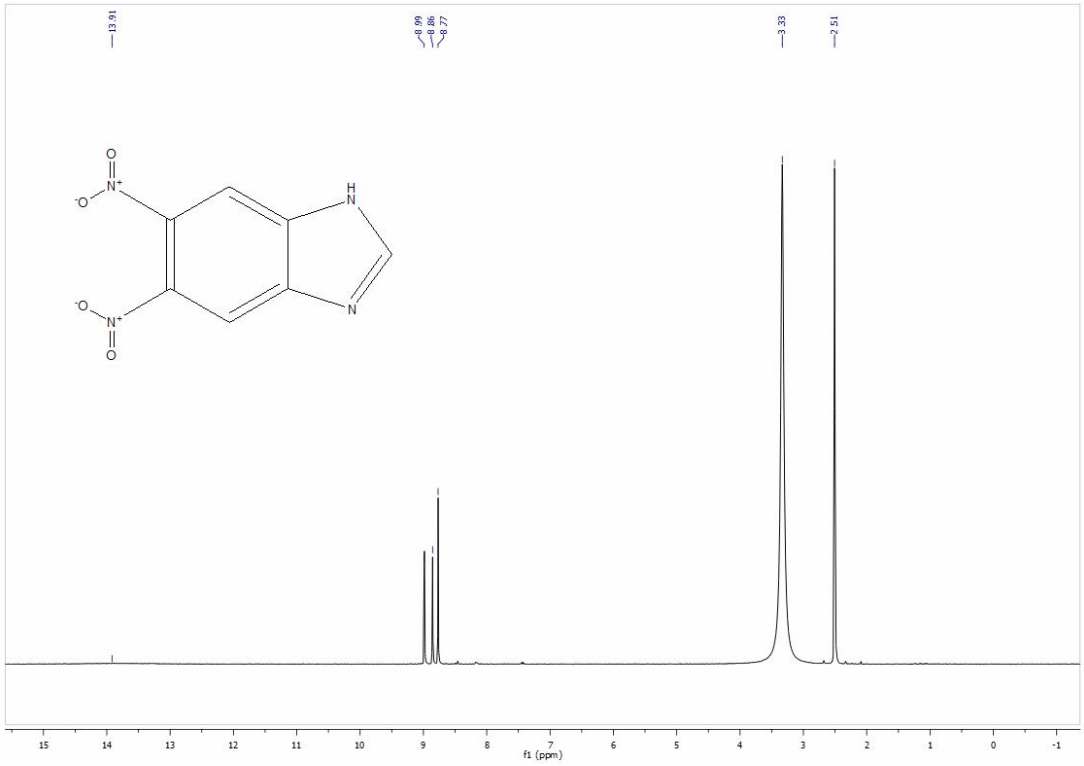

Figure A.5. ^{1}H-NMR spectrum of 5,6-Dinitro-1*H*-benzo[d]imidazole **(3)**

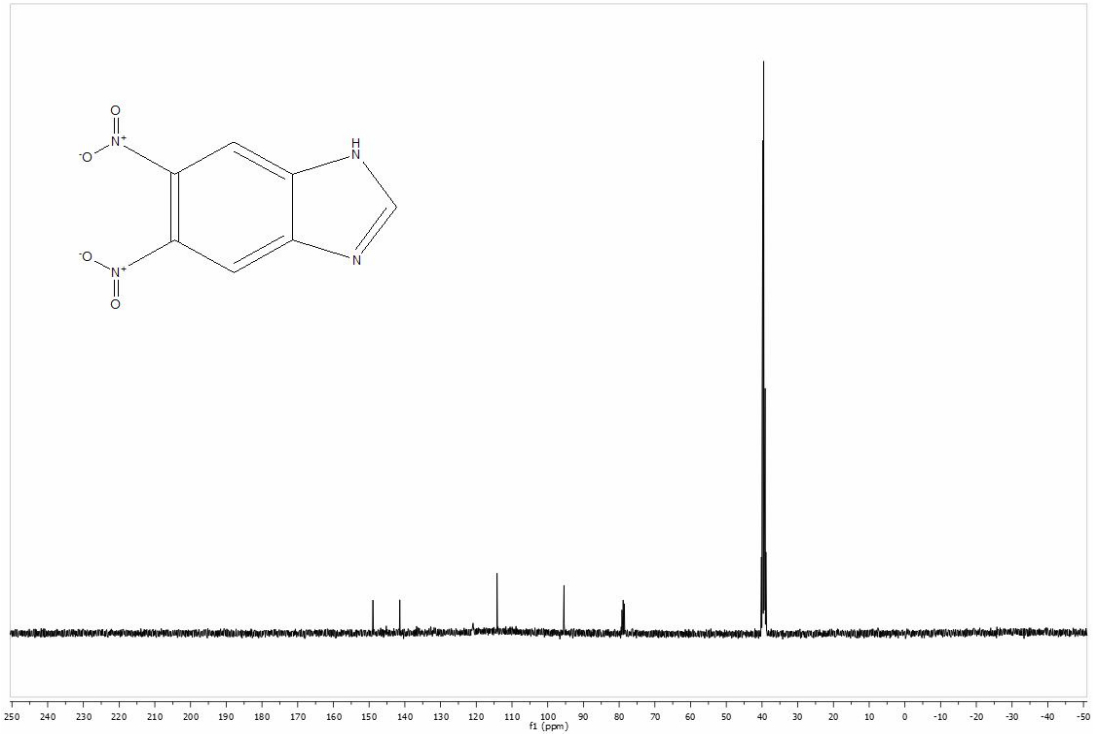

Figure A.6. ^{13}C-NMR spectrum of 5,6-Dinitro-1*H*-benzo[d]imidazole **(3)**

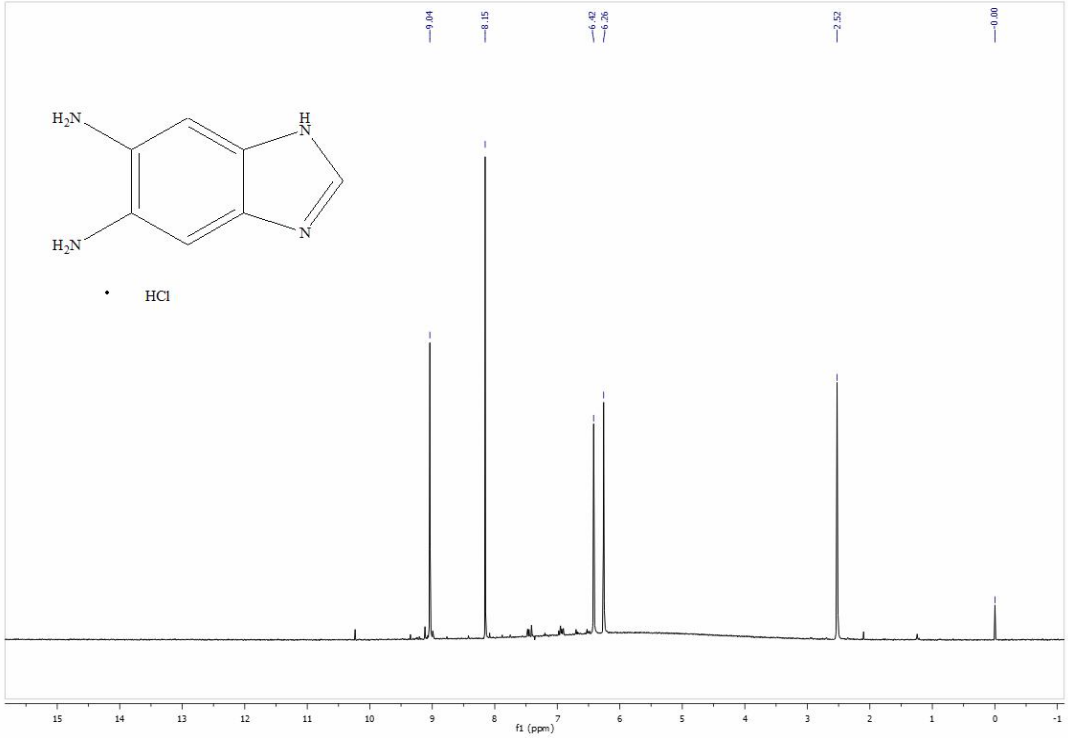

Figure A.7. ^{1}H-NMR spectrum of 1*H*-Benzo[d]imidazole-5,6-diamine.HCl **(3a)**

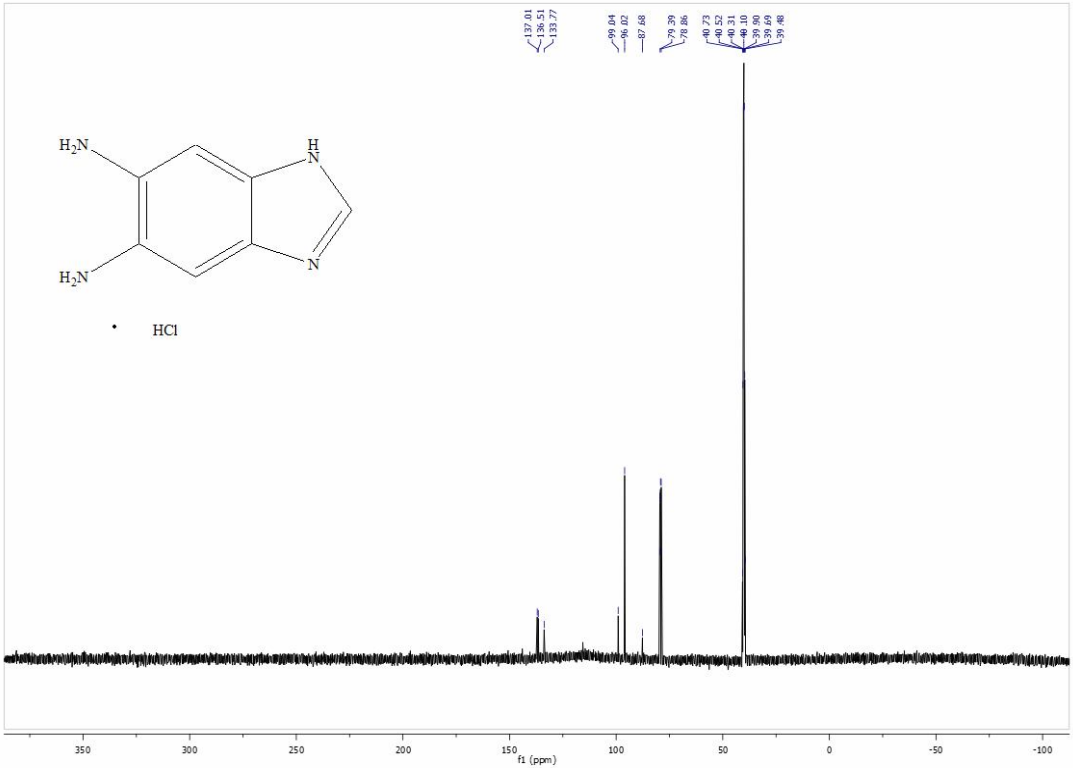

Figure A.8. ^{13}C-NMR spectrum of 1*H*-Benzo[d]imidazole-5,6-diamine.HCl **(3a)**

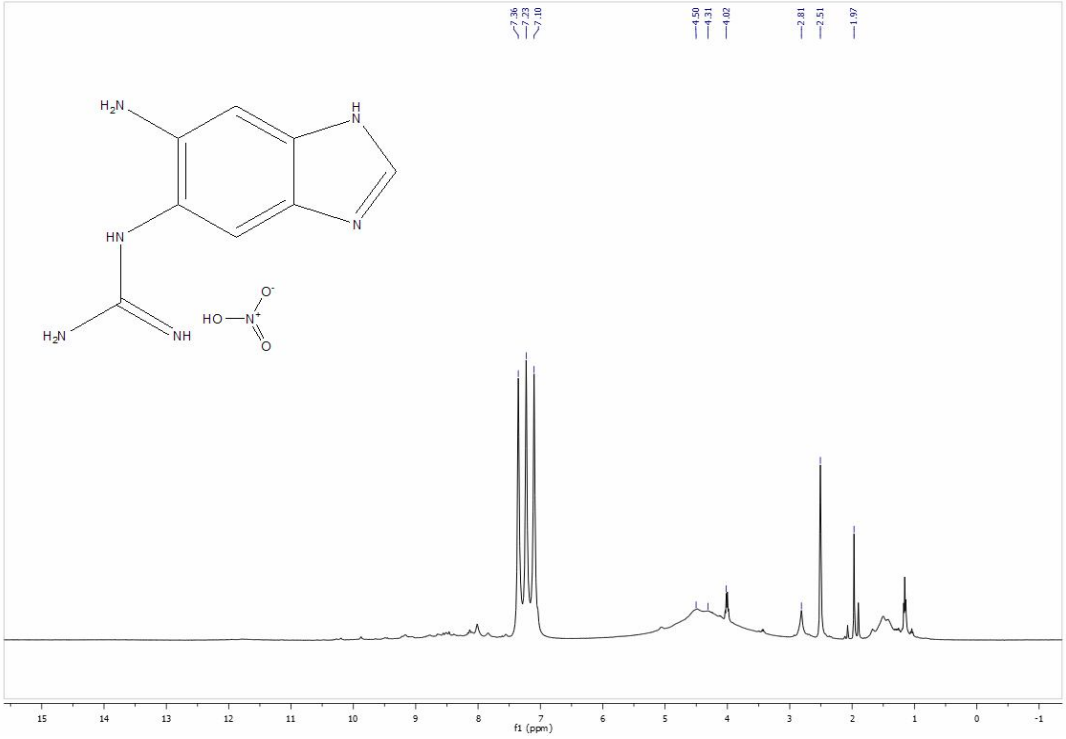

Figure A.9. ^{1}H-NMR spectrum of (6-Amino-1*H*-benzo[d]imidazol-5-yl)guanidine . HNO_3 **(3b)**

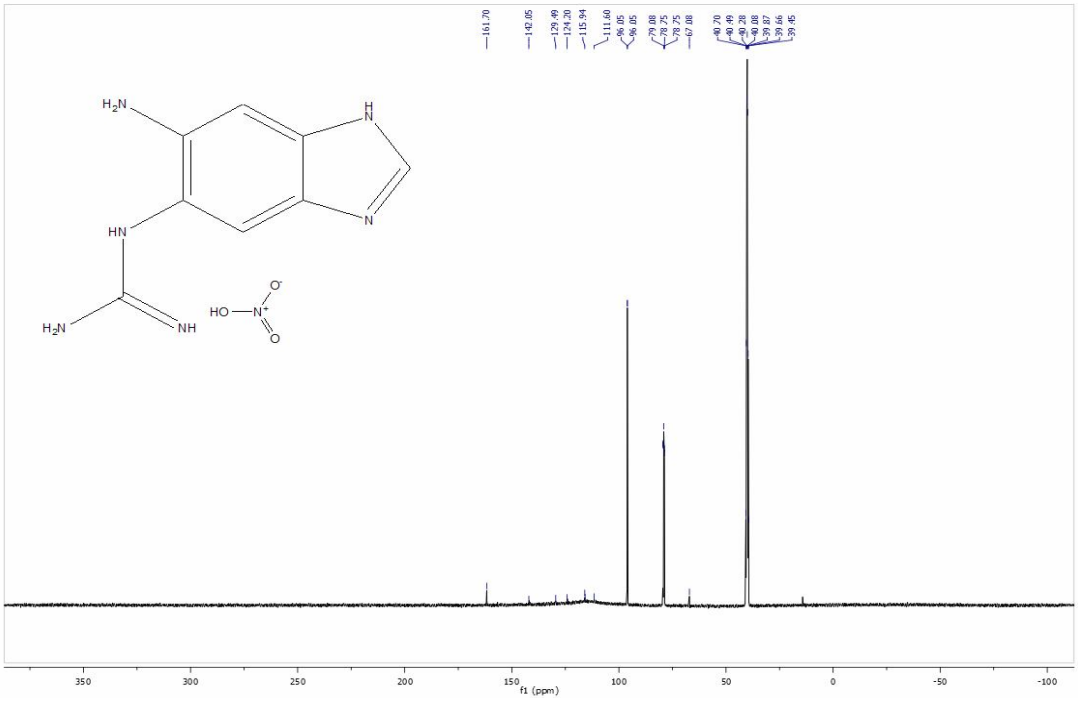

Figure A.10. ^{13}C-NMR spectrum of (6-Amino-1*H*-benzo[d]imidazol-5-yl)guanidine . HNO_3 **(3b)**

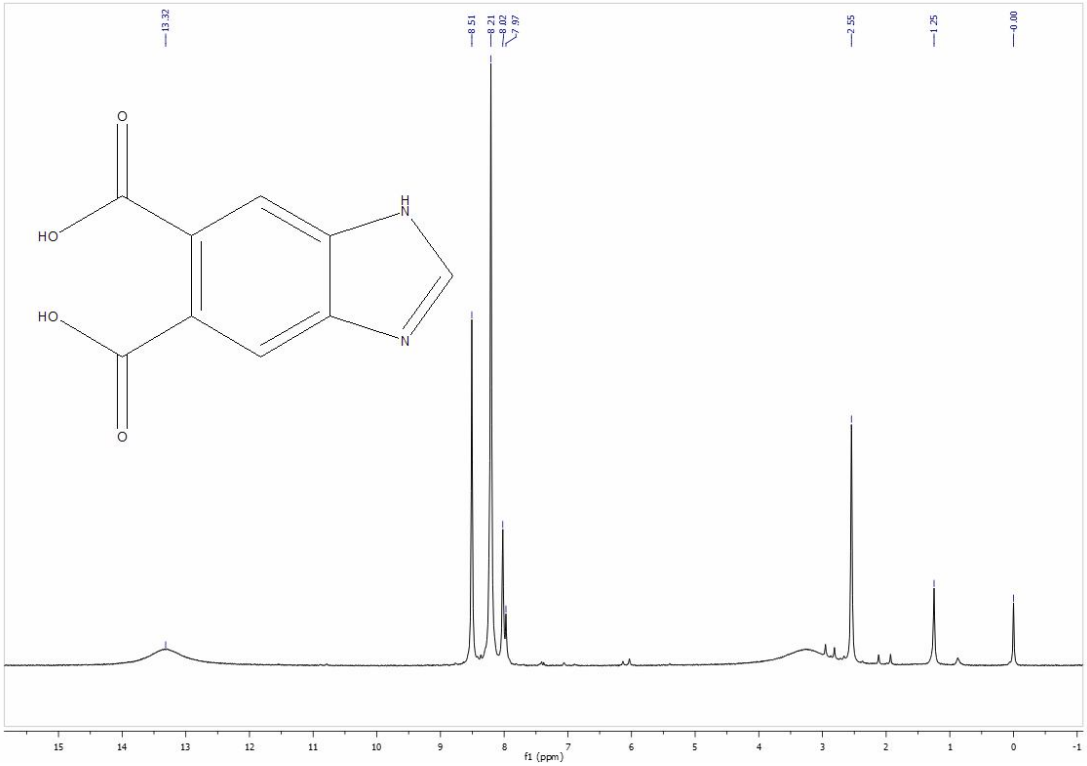

Figure A.11. ^{1}H-NMR spectrum of Benzimidazole -5,6- dicarboxylic Acid **(5)**

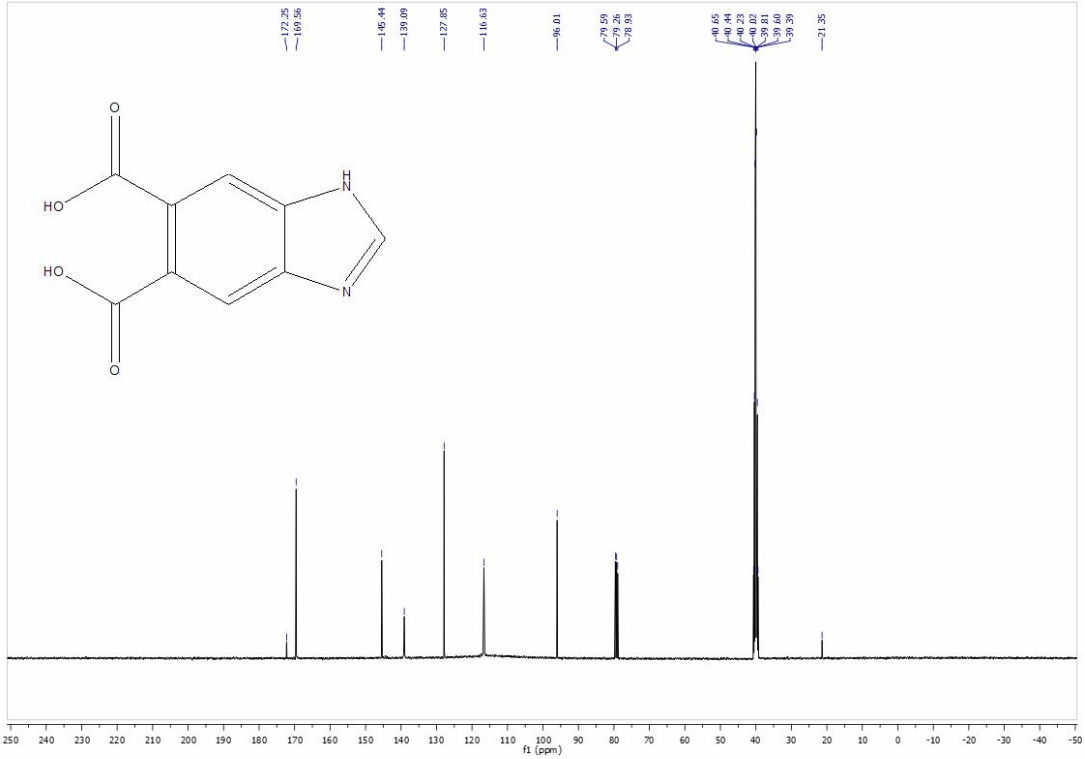

Figure A.12. ^{13}C-NMR spectrum of Benzimidazole-5,6- dicarboxylic Acid **(5)**

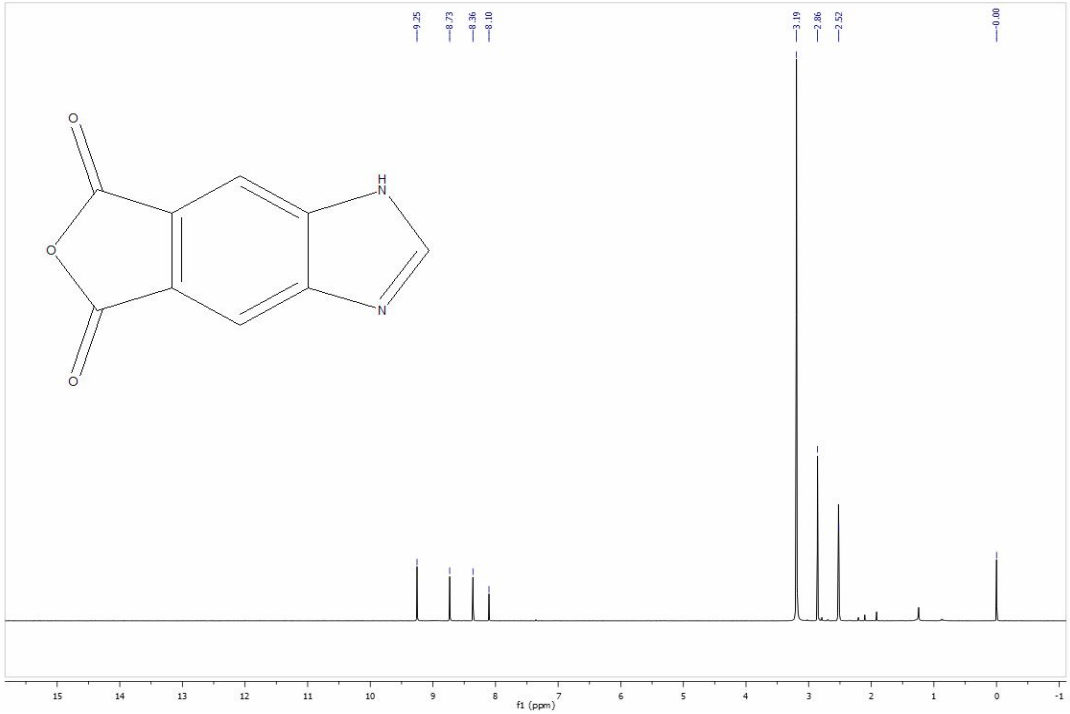

Figure A.13. ^{1}H-NMR spectrum of l-Acetylbenzimidazole-5,6-dicarboxylic anhydride **(5a)**

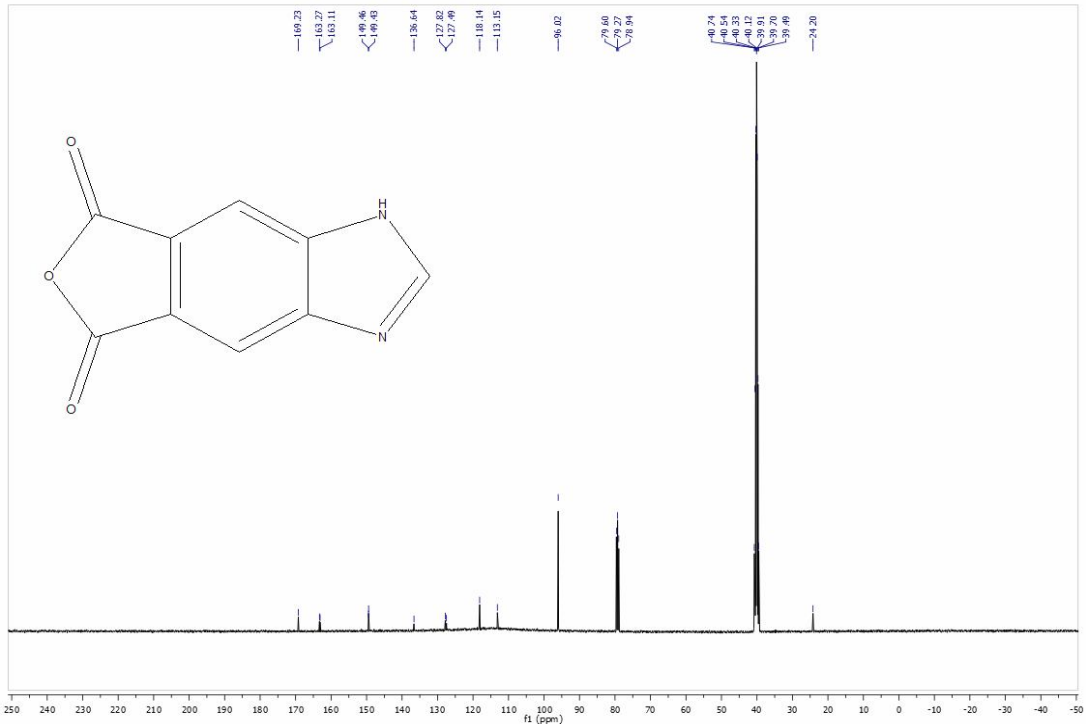

Figure A.14. ^{13}C-NMR spectrum of l-Acetylbenzimidazole-5,6-dicarboxylic anhydride **(5a)**

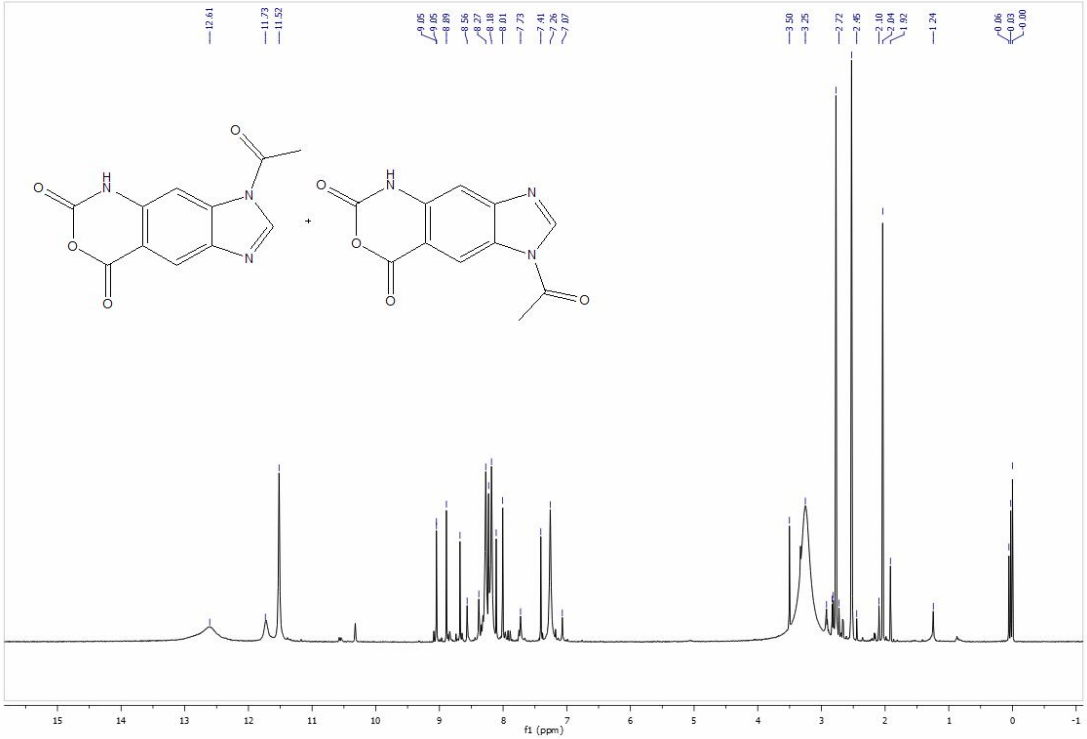

Figure A.15. ^{1}H-NMR spectrum of 1-N and 3-N-acetylimidazo[4,5-g]-7,5 benzoxazine- 6,8(5*H*)-dione **(5b-1,2)**

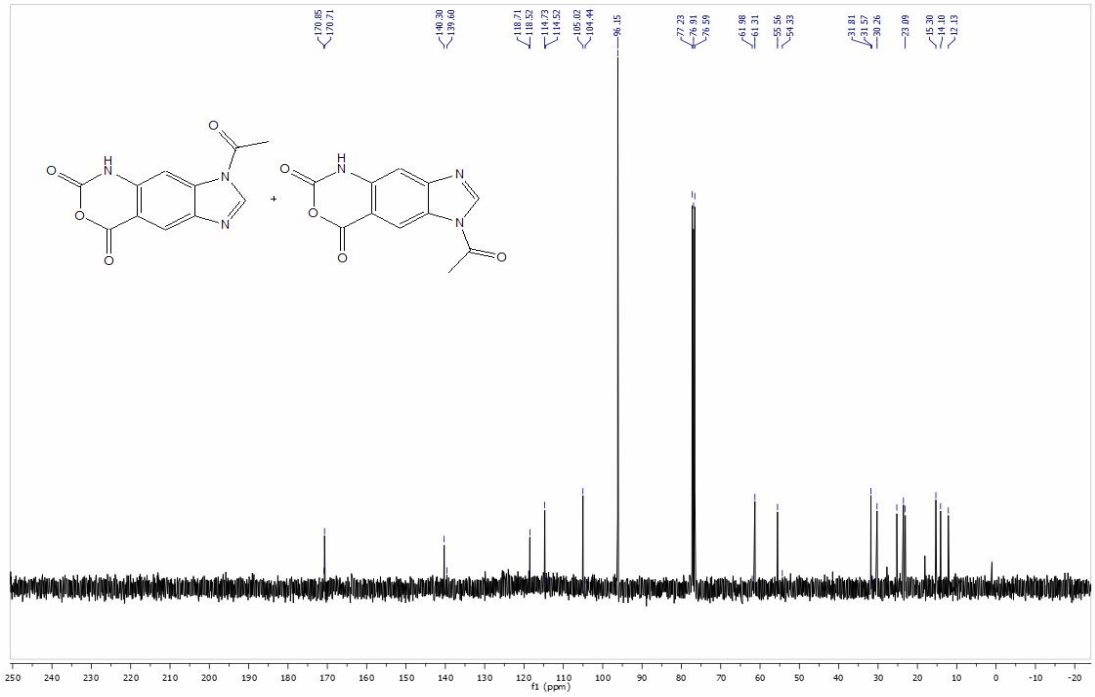

Figure A.16. ^{13}C-NMR spectrum of 1-N and 3-N-acetylimidazo[4,5-g]-7,5 benzoxazine- 6,8(5*H*)-dione **(5b-1,2)**

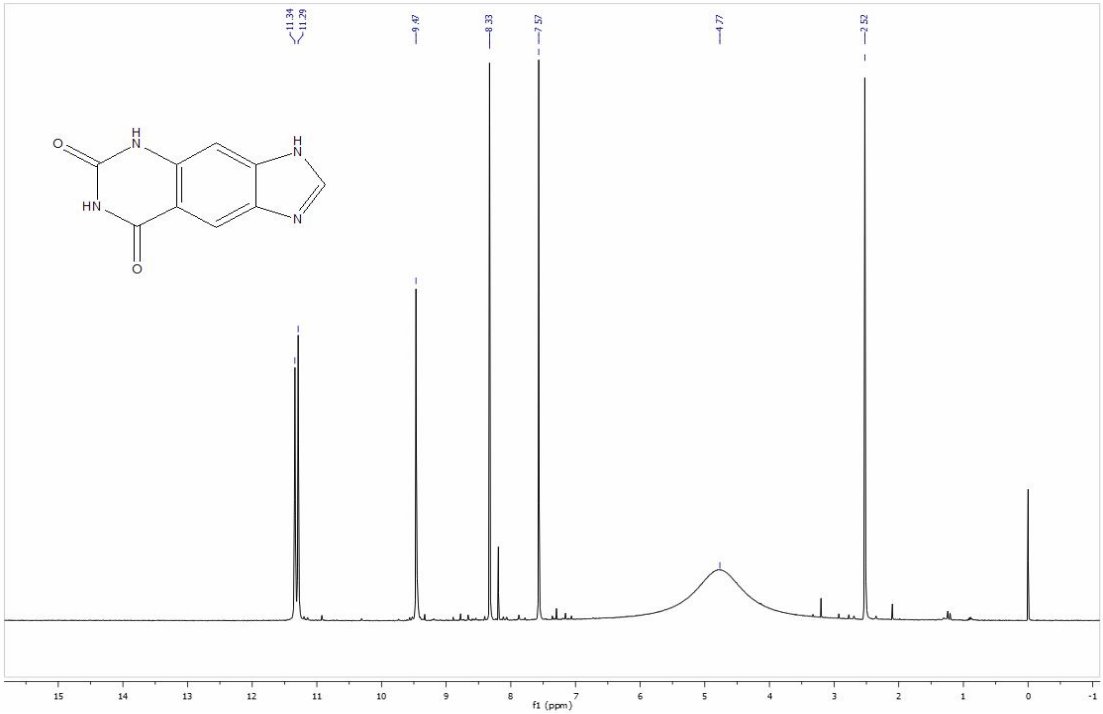

Figure A.17. ^{1}H-NMR spectrum of *lin* -Benzoxanthine (imidazo[4,5-g]quinazoline-6,8- (5*H*,7*H*)-dione **(5c)**

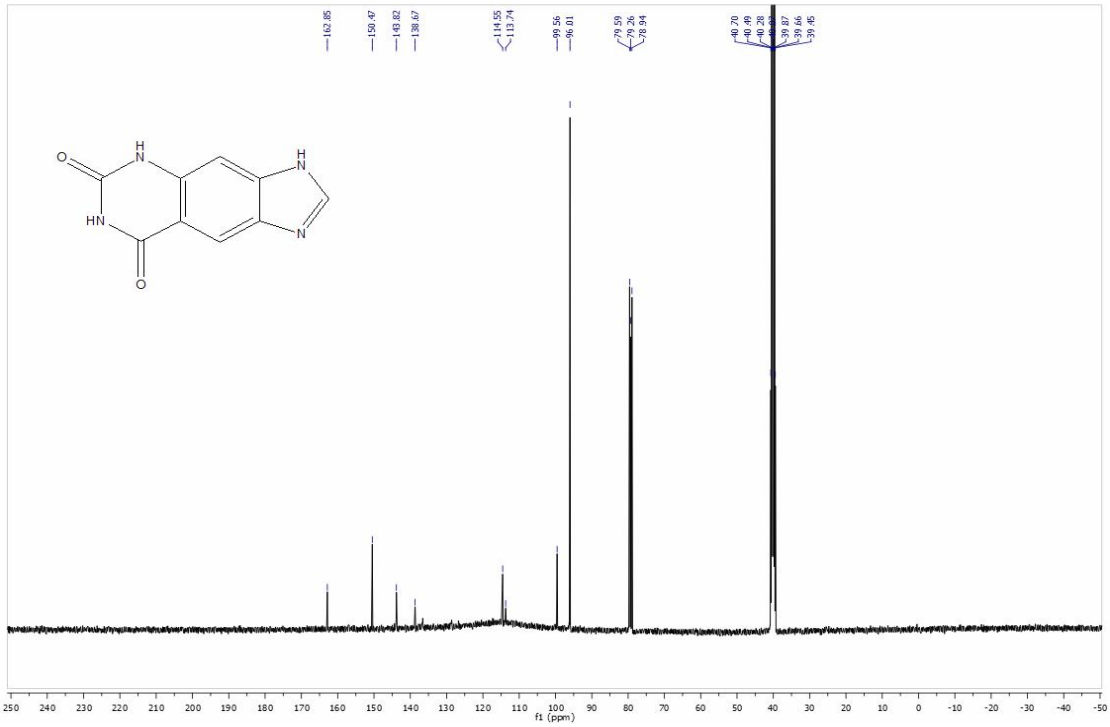

Figure A.18. ^{13}C-NMR spectrum of *lin* -Benzoxanthine (imidazo[4,5-g]quinazoline-6,8- (5*H*,7*H*)-dione **(5c)**