Synthesis and Characterization of Potential Impurities in Imatinib Mesylate

Poojashree P, Pramila T*

Department of Pharmaceutical Chemistry, Bharathi College of Pharmacy, Bharathinagara, Mandya- 571422 Karnataka, India.

***Corresponding author:** Mrs. Pramila T
Email: supratm2001@gmail.com

ABSTRACT

Imatinib mesylate is a potent antineoplastic agent that acts as a tyrosine kinase inhibitor (TK inhibitor). Specifically, it is used for the treatment of different types of cancers such as chronic Myelogenous leukemia (CML), acute lymphocytic leukemia (ALL), Philadelphia chromosome-positive (ph+), certain types of gastrointestinal stromal tumors (GIST), hypereosinophilic syndrome (HES). Genotoxicity describes the property of chemical agents that damage the genetic information or DNA within a cell causing mutations, which may lead to cancer, Impurities which will cause the mutation are known as genotoxic impurities. During the process of Imatinib mesylate synthesis, the formation of various Imatinib impurities was observed, which may or may not act as genotoxic on cells or DNA and will cause mutation which was not reported earlier. Although, the structures and analytical procedures of these impurities have been already reported in the literature, surprisingly their synthesis and genotoxic evaluation were not accounted for. In the present investigation, the synthesis of Imatinib impurity A, B, C, F and J were achieved by designing a new and simple method, the synthesized compounds were characterized and confirmed by using IR, ^{1}H-NMR and Mass Spectrometry for evaluation of genotoxic nature of imatinib impurities.

Keywords: Characterization, Imatinib mesylate, Imatinib impurities, Synthesis.

1. INTRODUCTION

Imatinib is a 2-phenyl amino pyrimidine derivative that functions as a specific inhibitor of many tyrosine kinase enzymes. There are a large number of tyrosine kinase enzymes in the body, including the insulin receptor and it is specific for the BCR-ABL (Abelson proto-oncogene-break point cluster region) platelet-derived factor receptor (PDGF-R) and C-kit. specifically, it is used for chronic Myelogenous leukemia (CML), acute lymphocytic leukemia (ALL), Philadelphia chromosome-positive (ph+), certain types of gastrointestinal stromal

tumors (GIST), and hypereosinophilic syndrome (HES). Chemically imatinib is a 4-[(4-methyl piperazin-1-yl) methyl]-N-(4-methyl-3-{[4-(pyridine-3-yl) pyrimidine-2-yl] amino} phenyl) Benzamide[1].
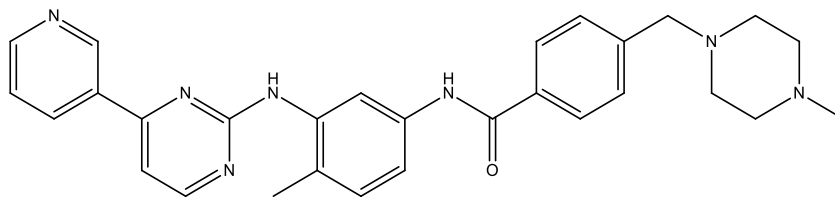

Figure 1: Structure of Imatinib Mesylate

Impurity removal is a critical task in pharmaceutical process research, where the final product meets stringent purity requirements. The presence of impurities in an active pharmaceutical ingredient (API) can have a significant impact on the quality and safety of the drug product. The guidelines recommended by ICH state that the acceptable levels for a known and unknown impurity in an API should be less than 0.15 and 0.10%, respectively. These impurities are also required in pure form to understand the impurity profile and develop an accurate analytical method during the research and development phase [2]. The impurity profile of a drug is a description of the identified and unidentified impurities present in a new drug product and also it provides information about the presence of maximum possible types of impurities but estimates the definite amount of various kinds of impurities present in the final product [3].

Several liquid chromatography (LC) methods have been reported for the quantitative determination of Imatinib mesylate and its related impurities in drug substances, drug products and dosage forms [4-12]. Some LC methods with UV detection have been described for separation and LC method with Mass Spectrometric detection for related substances has also been reported. There have been a few attempts to study the detection of traces of degradation products of Imatinib mesylate in human serum samples [13-21]. In the field of pharmaceutical chemistry, impurities are considered to be unwanted substance(s) present in therapeutically active pharmaceutical compounds. They are expected to have unusually potent, toxic or unexpected pharmacological effects which are harmful to human health and they may interfere with the drug action. The control of impurities is currently a critical issue for the pharmaceutical industry. The most possible source of impurities is the synthesis that involves various stages, *i.e.,* from starting material to finished products through intermediate steps. Impurities in drug substances and drug products are key regulatory issues in the office of generic drugs and have a significant impact on the approvability of drugs hence International Conference on Harmonization (ICH) and Food and Drug Administration (FDA) guidelines introduced some guidelines for the identification and qualification procedures for the preparation of chemical compound, by using various analytical techniques we can control the formation of such type of impurities.

The impurity profile of a drug is defined as "A description of the identified and unidentified impurities present in a new drug products"[22]. In the present era, there is a tremendous upsurge in the impurity profiling of pharmaceutical products. The presence of impurities in trace quantities in drug substances or drug products is inevitable. Therefore, their level should be controlled and monitored. Sometimes, the effect produced by the impurities can be teratogenic, mutagenic or carcinogenic [23].

Genotoxic impurities induce genetic mutations, chromosomal rearrangements, and chromosomal breaks and act as carcinogenic compounds that interact with DNA and it is associated with cellular components or enzymes [24].

Hence, this can jeopardize human health by affecting the quality, safety and efficacy (QSE) of the product. Therefore, there is an ever-interesting interest in controlling and monitoring impurities present in API/pharmaceutical products.

At the time of the development of Imatinib mesylate, various processes related to impurities were observed. Literature reports include an increasing number of publications on the detection of impurities and the development of analytical methods for their analysis indicating the significance of impurities in Imatinib mesylate. However, no synthetic details have been reported yet. In this context, described for the identification, characterization and synthesis of imatinib mesylate impurities namely 3-dimethylamino-1-(pyridine-3-yl) prop-2-en-1-one (Imatinib Impurity-A) (Figure 2), N-[4-Methyl-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-phenyl]-4-chloromethyl Benzamide (Imatinib impurity-B) (Figure 3), N-(3-4-(pyrimidin-3-yl) pyrimidin-2-ylamino)-4-(piperazine-1-yl) methyl) Benzamide (Imatinib impurity- C) (Figure 4), 4-Methyl-N³-[4-(pyrimidin-2-yl] benzene-1, 3-diamine (Imatinib impurity-F) (Figure 5) and 4-[(4-Methyl-4-oxido-1-piperazinyl) methyl-N⁻-[4-methyl-3-[[4-(3-pyridinyl) pyrimidin-2-yl] amino] phenyl]benzamide(Imatinibimpurity-J)(Figure 6)
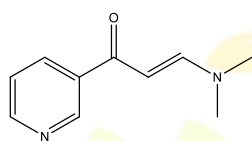

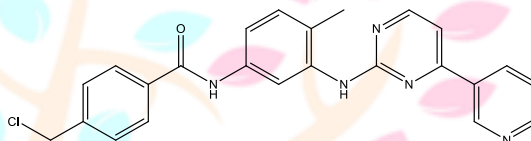**Figure 2: Structure of Imatinib Impurity-A**
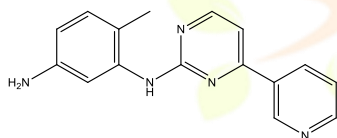

Figure 3: Structure of Imatinib impurity-B

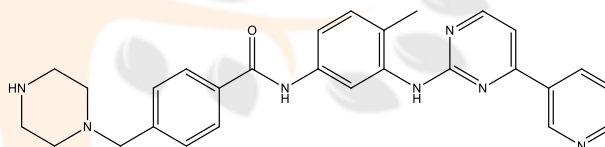**Figure 4: Structure of Imatinib impurity-F**

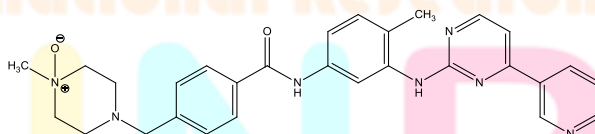**Figure 5: Structure of Imatinib impurity-C**

Figure 6: Structure of Imatinib impurity-J

2. MATERIALS AND METHODS

The chemicals and reagents used in the present project were of LR grade, purchased from S.D. Fine Chem. Ltd., Mumbai, India.

Methods

Infrared spectroscopy: The infrared spectra for the synthesized compounds were recorded using SHIMADZU FTIR 8400 spectrometer using potassium bromide pellet technique. The infrared spectroscopy is one of the most powerful analytical techniques used, which offers the possibility of identification of functional groups present in the compound.

Nuclear magnetic resonance: 1H NMR spectra of the synthesized compounds were taken using BRUKER SPECTROSPIN-400MHz spectrometer using tetra methyl silane (TMS) as an internal standard.

^{1}H NMR spectra were recorded with DMSO as a solvent and the chemical shift data were expressed as delta values related to TMS in ppm.

Mass spectroscopy: The mass spectra of the synthesized compound were taken using GCMS-QP5050 SHIMADZU instrument.

Synthesis of Imatinib impurity-A: Imatinib impurity-A is a potential impurity of Imatinib mesylate, which have not been reported previously. Imatinib impurity- A was synthesized by taking 3-acetyl pyridine with (0.0056 mol) and Dimethyl formamide diethylacetol (0.025mol) in nitrogen condition. Kept the reaction mixture was stir for 3-4 hours at 105°C. After completion of the reaction, (reaction was monitored by TLC) solvent was distilled out under vacuum and diethyl ether (0.40 mol) was added brown colored suspension was formed, separated the solid by filtration with ether and dried, finally recrystallized from diethyl ether to get 1.42 g (88.75%) of red colored crystals of 3-dimethylamino-1-(pyridine-3-yl) prop-2-en-1-one (Imatinib impurity-A) was collected. The synthetic scheme of imatinib impurity-A was shown below (Figure 7)
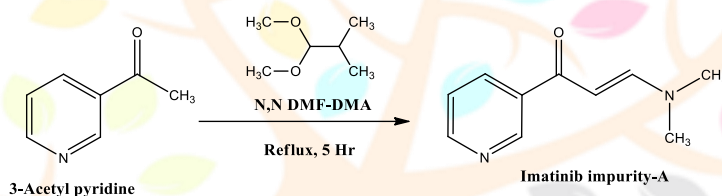

Figure 7: Synthetic scheme of imatinib impurity-A

Synthesis of Imatinib impurity-B: Imatinib impurity B was synthesized by taking Imatinib imp- F (0.003605mol) dissolved in 20ml of chloroform and added Triethylamine (0.0054 mol) drop wise in cooling condition. The reaction mixture was stir for 10 – 15 minutes. Then added drop wise 4-cloromethyl benzyl chloride (0.003605mol) dissolved in 10 ml of chloroform. The reaction mixture was stir for 24 hours at 0°C. After completion of the reaction (reaction was monitored by TLC), solvent was distilled out under vacuum. The obtained product was quenched with water, extracted with ethyl acetate. The organic layer was collected and dried. Recrystallized with ethyl acetate to get 700 mg (45.16%) of light-yellow colored crystals of N-[4-Methyl-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-phenyl]-4-chloromethyl Benzamide (Imatinib impurity-B) was collected. The synthetic scheme of imatinib impurity-B was shown below (Figure 8)
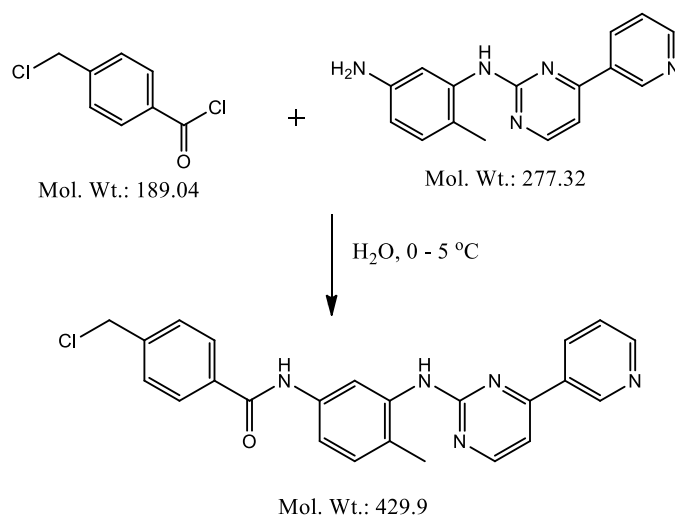
Figure 8: Synthetic scheme of imatinib impurity-B

Synthesis of Imatinib impurity-C: The 100 ml RBF was charged with Imatinib impurity B (0.000465mol), piperazine (0.0003mol), potassium carbonate (0.000418mol) and 20 ml of Dioxane. After the addition, reaction mixture was heated to 100°C about 3 hrs. After completion of the reaction (reaction was monitored by TLC), solvent was distilled out under vacuum. The obtained product was quenched with water, extracted with ethyl acetate. The organic layer was collected and dried. Recrystallized with ethyl acetate to get 200 mg (45.16%) of pale-yellow colored crystals of N-(3-4-(pyrimidin-3-yl) pyrimidin-2-ylamino)-4-(piperazine-1-yl) methyl) Benzamide (Imatinib impurity-C) was collected. The synthetic scheme of imatinib impurity-C was shown below (Figure 9)
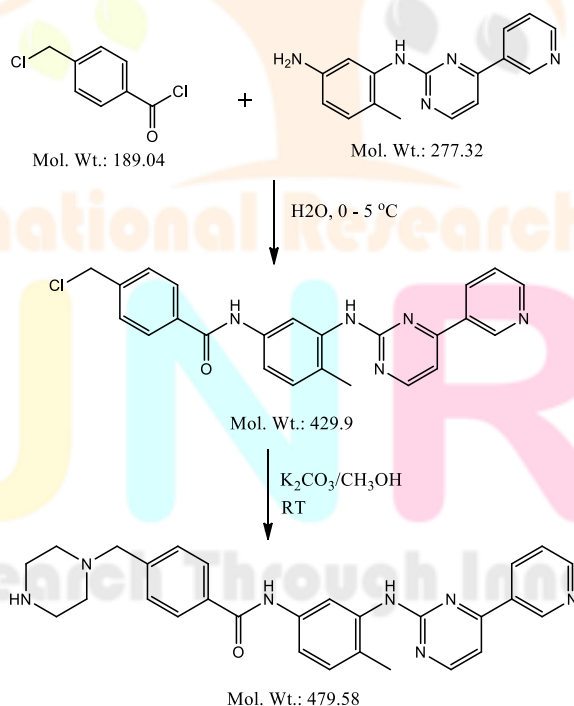

Figure 9: Synthetic scheme of imatinib impurity-C

Synthesis of Imatinib impurity-F:

The 250ml RBF charged with 2- chloro 4-(pyridin-3-yl) pyrimidine (0.00678mol) and 2- methyl 5- nitro aniline (0.0067mol). Then reaction mixture was refluxed for 15 minutes at 170°C about10 minutes. After that 20 ml of Dioxane and 5 ml of methane sulfonic acid were added to the above reaction mixture and kept for refluxing about 6 hours at 170°C. After completion of the reaction (reaction was monitored by TLC),

solvent was distilled out under vacuum and extracted with methanol, solids were separated by filtration. And the compound was identified as N-(2-methyl-5-nitrophenyl)-4-(pyridin-3-yl) pyrimidin-2-amine**.** To the above reaction mixture sodium borohydride, calcium chloride and methanol was added and then refluxed for 2 hours. After completion of the reaction (reaction was monitored by TLC), solvent was distilled out under vacuum and extracted with methanol, solids were separated by filtration. Pale yellow colored compound of 4-Methyl-N^3-[4-(pyrimidin-2-yl] benzene-1, 3-diamine (Imatinib impurity- F) was collected. The synthetic scheme of imatinib impurity-F was shown below (Figure 10).
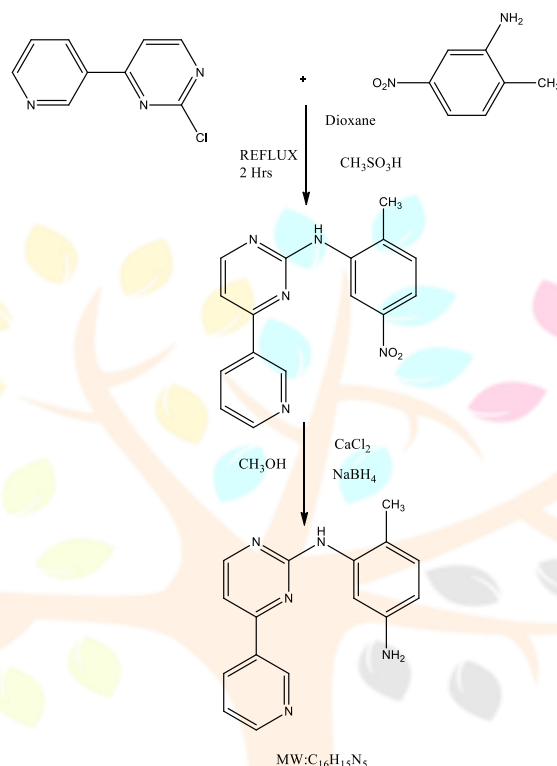

Figure 10: Synthetic scheme of imatinib impurity-F

Synthesis of Imatinib impurity-J: Imatinib API was dissolved in Dichloro methane and cooled to 0°C. After cooling m-Chloroperoxybenzoicacid was added portion wise and kept to stir overnight. After completion of the reaction, (reaction was monitored by TLC) solvent was distilled out under vacuum. The obtained product was quenched with water, extracted with DCM. The organic layer was collected and dried. Pale yellow colored compound of 4-[(4-Methyl-4-oxido-1-piperazinyl) methyl-N$^-$-[4-methyl-3-[[4-(3-pyridinyl) pyrimidin-2-yl] amino] phenyl] benzamide (Imatinib impurity-J) was collected. The synthetic scheme of imatinib impurity-J was shown below (Figure 11).
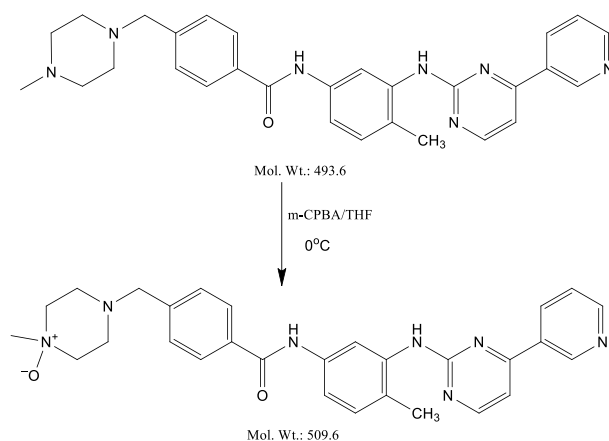
Mol. Wt.: 493.6

m-CPBA/THF
0°C

Mol. Wt.: 509.6

Figure 11: Synthetic scheme of imatinib impurity-J

3. RESULTS AND DISCUSSION

Imatinib impurity - A was synthesized in single step and purified by successive recrystallization using Diethyl Ether. The purity was checked by performing TLC. The synthesized impurity has been characterized using FTIR, H^1NMR, and Mass spectroscopy for m/z. The Imatnib impurity – A exhibited IR (KBr): λmax (cm^{-1}) at 1720 cm^{-1} – C=O , 1635 cm^{-1} – C=C and C=N, 3404 cm^{-1} – CH stretching (Hetero aromatic CH stretching), 2916, 2840 cm^{-1} – CH stretching (of CH$_3$ group) ; H^1NMR (DMSO): δ 3.3- S, 6H [2 CH$_3$ of N(CH$_3$)], δ 5.8 to 7.4 - D, 2H (of -CH=CH- group), δ 7.7 to 9.0 – M, 4H (Hetero aromatic ring) with Mass Spectrum: m/z 175 (M$^+$).

Imatinib impurity - B was synthesized in single step and purified by successive recrystallization using Ethylacetate. The purity was checked by performing TLC. Imatinib impurity - C was synthesized in two steps and purified by successive recrystallization using Ethylacetate. The purity was checked by performing TLC. The structures of the synthesized compounds were determined on the basis of their IR and ^{1}HNMR and Mass spectra. The Imatnib impurity – B exhibited IR (KBr): λmax (cm^{-1}) at 2856 cm^{-1} – NH-C=O, 1413, 1361 cm^{-1} – CH bending (of CH$_3$), 1361, 1265 cm^{-1} - CN stretching, 844.82 cm^{-1} – 1,4 disubstituted phenyl ring, 812 cm^{-1} – substituted hetero aryl ring; H^1NMR(DMSO): δ 2.18 - 2.67 – M, 8H (4 CH$_2$ group of piperidine), δ 3.1 – 3.47 – S, 5H, δ 7.1 – 9.263 – M, 14H (7H of Aromatic ring , 6H of Hetero Aromatic ring and 1H of NH group), δ 10.1 – S, 1H (NH of CONH) with Mass Spectrum: m/z 428 (M$^+$).

Imatinib impurity - F was synthesized in single step and purified by successive recrystallization using methanol. The purity was checked by performing TLC. The Imatnib impurity – F exhibited IR (KBr): λmax (cm^{-1}) at 3441 cm^{-1} – NH stretching (of NH$_2$), 3200 cm^{-1} – NH stretching, 3050 cm^{-1} – Ar-CH stretching, 2950 cm^{-1} – CH stretching (of CH$_3$), 1260 cm^{-1} – C=N, 1556, 1525, 1463 cm^{-1} – C=C ring stretching (of aromatic and hetero aromatic ring); H^1NMR (DMSO): δ 2.1 – S, 3H (3H of CH$_3$), δ 4.9 – S, 2H (2H of NH$_2$), δ 6.7 – S, 1H (1H of NH), δ 7.2 – 9.2 – M, 9H (3H of Aromatic and 6H of Hetero aromatic ring) with Mass Spectrum: m/z 276 (M$^+$);

Imatinib impurity - J was synthesized in single step and purified by successive recrystallization using Dichloromethane. The purity was checked by performing TLC. The Imatnib impurity – F exhibited IR

(KBr): λmax (cm^{-1}) at 3419 cm^{-1}- NH Stretching, 3296 cm^{-1}- CH Stretching (N-CH$_3$), 3018-CH Stretching (Ar-CH$_3$), 2966-2314 cm^{-1}- NH Stretching (NHC=O), 1647 cm^{-1}- C=O (NH-C=O).

H^{1}NMR (DMSO): with Mass Spectrum: m/z 510 (M$^+$+1);

4. CONCLUSION

Imatinib impurity A, B, C, F and J have been synthesized and the synthesized impurities were characterized by using IR, ^{1}H-NMR and Mass Spectrometry for m/z ratio and confirmed that the synthesized compounds were imatinib impurities.

ACKNOWLEDGMENT

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