An improved synthesis of Teriflunomide and Identification, synthesis and characterization of its Critical Process Impurities

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ABSTRACT

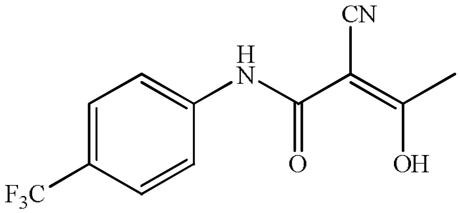
Teriflunomide has an impeccable drug used to treat multiple sclerosis. Ample of literature is available for its synthesis, however, it does not reveals the potential impurities formation during synthesis,The chief intent of the article is to highlightthe key impurities formation in Teriflunomide synthesis that yet to have to be documented in the literature and are not mentioned in any monograph. It also provides an overview of the process flow for the formation of new contaminants and their fate in the carryover study. The generation of corresponding impurities from impurity-I, i.e. ((Z)-4-Amino- N, N'-bis[(4-(trifluoromethyl)phenyl] benzimidamide, which generates in starting material (4-TFMA) and has a genotoxic alert, is discussed in every stage of Teriflunomide (I) synthesis, and a characterization study is carried out for corresponding impurities.The paper also provides a brief overview of the development of isomericimpurities from the beginning material 4-TFMA and equivalent impuritiesfrom the precursor of another starting material, cyano acetic acid. All of the impurities were identified, and the data is presented in this publication. The primary goal of the invention is to reveal the crucial impurity creation and elimination from the process, resulting in a highly efficient and scalable method.Also the invention introduces the new concept for amidationprocess to well-optimized reaction conditions. The synthesis avoids the use of unsafe chemicals and pyrophoric bases, and new the boric acidconcept is introduced foramidation in Teriflunomide synthesis.

Keywords

Teriflunomide, Boric acid, Impurity-I, Process Impurity synthesis,Impurity Characterization

INTRODUCTION

2-cyano-3-hydroxy-N-[4-(trifluoromethyl)phenyl]but-2-enamide, is an immunomodulator drug and has been used as anti-inflammatory drug and inhibits reversibly the mitochondrial enzyme (DHO-DH) essentialfor de novo pyrimidine production reversibly[1] USFDA (Federal Drug Administration) on September 12, 2012 gave an approval to Teriflunomide under the trade name AUBAGIO, and the target patients with multiple sclerosis has been treated/ Teriflunomide (I)



Teriflunomide (I)

The latest research highlights Teriflunomide (I) medication with Capone et al [2] states that Teriflunomide (I) therapy be continued to the patient who develops the SARS-CoV-2 infection. In his conclusion he states that the additional studies to be carried out to prove the protective nature of Teriflunomide (I) against the SARS-CoV-2 infection.

Various techniques to Teriflunomide (I) synthesis have been documented.

The two-step synthesis of Teriflunomide (I) described by Metro and et al [3] begins with 5-Methylisoxazole-4-carboxylic acid and ends with CDI activation of the acid group.In 2012, Ding and colleagues [4]highlighted synthetic techniques in new medication development, with Teriflunomide (I) being one of the drugs studied. According to the literature, the synthesis begins with 4 –TFMA, ethyl- 2-oxo butanoate, and xylene solvent. This synthesis also demonstrates that sodium cyanide was used in the process.

Mulakayala et.al[5]also provide an overview for therapeutic agents for the treatment of multiple sclerosis, in which he describes the Teriflunomide (I) synthesis with use of ethereal solvent THF and pyrophoric base sodium hydride.

Bartlett et al.[6]reported lower yield,After acid amine coupling, the product was treated with acetyl chloride. The acid chloride is generally very unstable and quickly changes to the equivalent acid, resulting in partial conversion and a decreased yield

Hirth et al. [7]describe the synthesis of Teriflunomide (I) in which the reaction of 4-trifluromethyl aniline and ethyl ester of cyano acetic acid was carried out at 180°C. The obtained product after acid amine coupling was treated with Acetyl chloride. Numerous literature with variable method of preparation are available for the teriflunomide (I) synthesis.

We continued our literature search with Thomas –Xavir metro et al[8],who described a one-pot synthesis of Teriflunomide (I), starting with 5-methylisoxazole-4-carboxylic acid, preparing a CDI catalysed active imidazole ester of 5-methylisoxazole -4- carboxylic acid, and finally coupling Teriflunomide (I) with the starting material 4-TFMA to give Teri (I). During development, one-pot synthesis is always preferred to save manufacturing cost. We noticed that the utilised reagent, 5-methylisoxazole-4- carboxylic acid, is expensive and a significant cost contributor. The use of this 5-methylisoxazole-4-carboxylic acid will not result in a cost-effective process that is suitable for industrial production.

Palle venkata et al.[9]published a synthesis that started with the identical starting material, 5-methylisooxazole-4-carboxylic acid, and used thionyl chloride to make the equivalent acid chloride. This acid chloride was combined with 4-TFMA to produce the desired amide bond. Again, this synthesis has limitations because it uses a pricey starting material and the reagent thionyl chloride, which is moisture sensitive and lacrymetric in nature, limiting the process.

To make a synthesis cost efficient, it is vital to choose inexpensive raw materials that are readily available, non-hazardous, and easy to handle on a large scale. With this in mind, we continued our literature search and came across a reference in which Qui Wei tangyouzhi et al[10]started with cyano acetic acid and prepared active ester of cyano acetic acid using pepatide coupling reagent HATU, which is (1 [Bis(dimethylamino)methylene]-1H-1,2,3- triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate This is the most effective approach for peptide bond creation because the reagent is specifically designed for it; but, on a big scale, the cost of the reagent will affect the total cost of the

API. The API obtained will not be cost competitive with the market. Furthermore, the recommended storage temperature for the HATU reagent is 2-8°C, which is out of date for bulk scale storage and adds to the product's cost.

Ju xiulian and et.al[11] used cyano acetic acid as a starting material which is comparatively cheap and easily available. In his effort the converted cyanoacetic acid to cyano acetyl chloride by using phosphorous pentachloride and further coupling with another starting material 4-TFMA.In next step of synthesis sodium Hydride base is used

The selection of starting material in this synthesis was good and ruled out the use of costlier raw material. But on the other hand, PCl_5 and sodium hydride is being used in the process which is hazardous, lacrymetric, not suggestible to use on bulk scale production, and again the synthesis falls in shortcomings of moisture sensitivity.

Gudhua Chen et al. [12],also reported the synthesis of Teriflunomide (I), in which the starting material cyano acetic acid is converted to cyano acetic anhydride using phosphorous pentaoxide as a dehydrating agent in THF as a solvent, and the reaction is carried out for 24 hours at elevated temperature. This cyano acetic anhydride is then treated with 4-TFMA and the reaction took 36 hr for completion at 81°C to yield in to 2- cyano-N-(4-trifluromethylphenyl) acetamide. To the entire synthesis of Teriflunomide, this approach adds an additional conversion step and intermediate (I). In addition, the reaction conditions utilised are too slow for plant-scale manufacture. This will increase the overall cost of API synthesis. The intermediate 2- cyano-N-(4-trifluromethyl-phenyl) acetamide is then treated with acetyl chloride using THF as a solvent and sodium hydride as a base during the manufacturing process. The discussed procedure has several flaws, including an extra step, the use of flammable solvent, and the use of pyrophoric base. The amidation reaction between 4-TFMA and cyano acetic acid ethyl ester is described by Klaus Peter Hirth et al [7, 13]and the synthesis is carried out at 180°C. Acetyl chloride is employed as an acyl group source and sodium hydride is used as a base in the next steps of the synthesis. These are extremely difficult circumstances to carry out on a plant size. These conditions can also lead to tragic accidents in large-scale manufacturing. We couldn't identify a suitable non-hazardous and cost-effective reagent for bulk scale production after a thorough literature search. To resolve this issue, we decided to conduct a literature search for a commonly used reagent that is not specifically stated for Teriflunomide (I) synthesis. A literature search yields numerous compounds, including Disoproyl carbodiimide (DIC)[14,15]. DEPBT[16] 1-hydroxy-7-aza-1H-benzotrazole) (HOAt)[17](benzotrazole-1-yloxy-tris(dimethylamino)-phosphonium hexafluorophosphate) (BOP)[18,19]PyBOP (benzotriazol-1-yloxytripyrrolidino- phos-phonium hexafluorophosphate)[20,21] HCTU (benzotriazol-1-yloxy-tripyrrolidino-phos-phonium hexafluorophosphate)[22]These are chemicals that are employed specifically in peptide synthesis and have unique features for the coupling reaction. The reagents mentioned above are not inexpensive, and several of them have explosive qualities. As a result of the high cost and explosive nature of certain reagents, we chose to skip them. We are unable to meet our requirement for bulk scale Teriflunomide (I) manufacture on a 100 kg batch size due to a reagent shortage. Keeping this in mind, we considered classic reagents for coupling reactions and demonstrated a few in table-I.After a thorough search, we were unable to locate any literature thatrevealed the optimal coupling reagent and process parameters. Furthermore, there is little literature that provides a complete understanding of Teriflunomide impurity profiling (I). We have introduced a new aspect of amidation with the use of catalytic reagents, which is cost effective and removes the risk from the process. In addition, the study provides a detailed picture of Teriflunomide impurity profiling (I). It also explains how impurity synthesis is done. Overall, the study discusses the cost-effective and non-hazardous synthesis of Teriflunomide (I), as well as the synthesis and characterisation of key impurities, which is the study's main goal.

MATERIALS AND METHODS

Until any stated one, the commercial source for raw material purchasing has been Materials and Methods. All solvents and reagents were supplied by the manufacturer. In $CDCl_3$, DMSO-d6, and mass spectra on an API-2000LCMS mass spectrometer, variable (400 MHz) 1H-NMR spectra and (100 MHz) ^{13}C-NMR spectra were acquired.

EXPERIMENTAL

Preparation of 2-cyano-N-[4-(trifluoromethylphenyl) acetamide (II)

4-trifluoromethyl aniline (2.5 kg, 1.0 mol), cyanoacetic acid (1.78 kg, 1.35 mol), boric acid (0. 950 kg, 0.1 mol) and toluene (22.5 L) charged to a flask and heated at 110 to 115°C and remove water azeotropically for 12 h. After monitoring reaction progress by HPLC distilled out toluene completely under vacuum, water was added (22.5 L) and agitated for 2 h at 25 to 30°C. The observed solid was isolated by filtration and washed by H2O (2.5 L), dried under vacuum to get title compound (II), white to yellow solid (3.03 kg, 99.49 % purity) in 73% yield.

Elemental analysis: Calculated: C (52.54 %), H (3.24 %), and N (12.19 %) Found.

FT-IR: 3288, 3031, 3100, 2262, 1682, 1560, 1113 cm^{-1};

^{1}H-NMR in (400 MHz ,DMSO-d6,) δ: 3.97 (s, 2H), 10.65 (brs, 1H), 7.70–7.77 (m, 4H);

^{13}C-NMRin (DMSO-d6, 100 MHz): δ161.78, 141.91, 128.29, 126.08, 120.19, 115.64, 26.99;

Mass 228.18, (M-H); 227.0

Preparation of Teriflunomide (I)

Acetic acid (1.20 kg, 1.0 mol), Ethyl acetate (35 L) followed by N, N-carbonyl diimidazole (CDI, 3.88kg, 1.2 mol) charged to a flask. Stirred at 0 to 10°C for 2 h added this solution to an ice-cooled solution of 2-cyano-N-[4-(trifluoromethylphenyl) acetamide II (3.5 kg, 1.0 mol) containing sodium tert butoxide (3.68 kg, 2.5 mol) and Toluene (35 L) at 5 to 10°C. Stirring was continued at 25 to 30°C for 2 h, and checked by HPLC, water (65 L) and conc. hydrochloric acid (7 L) was added. separated Layers, after neutralizing aqueous layer by sodium carbonate solution crude Teriflunomide was filtered and crystallized from acetone to give title compound I (3.5 kg, 99.96 % purity) in 84.8 % yield.

Elemental analysis: Calculated: C (53.34 %), H (3.36 %), and N (10.37 %). Found.

FT-IR: 3302, 3136, 3069, 2218, 1632, 1593, 1555, 1115 cm−1.

^{1}H-NMR in (400 MHz, DMSO-d6) δ: 10.91 (s, 1H), 9.89 (s, 1H), 7.77(d, 2H, J=8.56 Hz), 7.77(d, 2H, J = 8.64 Hz), 2.25 (s, 3H);

^{13}C-NMR (DMSO-d6, 100 MHz): δ186.99, 166.40, 141.53, 125.84 & 125.87, 125.76 & 125.80, 124.04 & 124.35, 123.40, 123.72, 121.05, 125.66 & 128.36, 120.26, 122.96, 118.13, 80.89, 23.04. ESI-Mass: For C12H9F3N2O2, is 270.2, Found: (M−H)/z: 268.7.

Preparation of ((Z)-4-Amino-N, N'-bis[(4-(trifluoromethyl)phenyl] benzimidamide. (Impurity-I)

Compound-III i.e 4-TFMA is stored at room temperature for longer period of time and the solid obtained is filtered outh through the Buchner funnel. Isolated solid further dried to give ((Z)-4-Amino-N,N'-bis[(4-(trifluoromethyl)phenyl] benzimidamide. (Impurity-I)

FT-IR: 3309, 3271, 3080, 2638, 2216, 1917, 1791, 1627, 1608, 1409, 1325, 1271, 1157, 1109, 1070, 1016, 840, 759, 680 cm−1.

^{1}H-NMR in (400 MHz, DMSO-d6,) δ: 2.08 (s, 3H), 7.61-7.64 (d, 2H), 7.77-7.79 (d, 2H), 10.27 (br. s, 1H);

^{13}C-NMR (100 MHz, DMSO-d6,): δ 168.91, 142.81, 128.40, 125.95, 125.70, 123.46, 122.51, 120.31, 118.72, 40.11, 39.90, 38.85, 24.02;

Mass: - 203.14, found (M+H)-. 204.4

preparation of N-(4-(N,N'-bis(4-trifluromethyl)Phenyl)carbamimidoyl)phenyl)-2-cyanoacetamide. (Impurity-II)

(Z)-4-Amino-N,N'-bis[(4-(trifluoromethyl)phenyl]benzimidamide (impurity I) (0.5 g, 1.0 mol), cyanoacetic acid (1.3 g, 1.35 mol), boric acid (0.07 g, 0.1mol) and toluene (50 mL) charged to a flask and heated at 110 to 115°C and remove water azotropically for 12 h. Distilled out toluene completely under vacuum, water was added (10 mL) and agitated for 2 h at 25 to 30°C. The solid was isolated and washed by H2O (10 mL) dried under vacuum and purified on coloumn packed with silica, title impurity 2 (0.3g) (Purity 80%). The impurity was not further purified and use for impurity –III synthesis. The impurity was not further purified and use for impurity –III synthesis.

Elemental analysis: Calculated: C (53.19 %), H (3.97%), and N (10.13 %).

^{1}H-NMR in (400 MHz, DMSO-d6) δ: 12.46(brs, 1H), 10.83(brs, 1H), 6.71-8.03(m, 12H), 3.99 (s, 2H);

^{13}C-NMR (DMSO-d6, 100 MHz): δ165.26, 161.57, 142.89, 141.60, 133.78, 131.46, 129.36, 128.94, 126.25, 126.21, 125.97, 125.86, 125.83, 123.92, 123.60, 120.05, 118.61, 118.48, 115.71, 115.62, 113.86, 113.15, 26.88.

ESI-Mass: For $C_{24}H_{16}F_6N_4O$ is 490.41,found (M+H): 491.3.

Preparation of N-((E)-N, N'-bis (4-trifluromethyl) phenyl) carbamimidoyl) phenyl)-2-cyano3-hydroxybut-2-enamide (Impurity III)

Acetic acid (0.036g, 1.0 mol), Ethyl acetate (10 mL) followed by N, N-carbonyldiimidazole (CDI, 0.07g, 1.2 mol) charged to flask. Stirreded at 0 to 10°C for 2 h added this solution to an ice-cooled solution of N-(4-(N,N'-bis (4-trifluromethyl)Phenyl) carbamimidoyl)phenyl)-2-cyanoacetamide.(II) (0.2 g, 1.0 mol) containing sodium tert butoxide (0.096g, 2.5 mol) and Toluene (10 L) at 5 to 10°C. Stirring was continued at 25 to 30°C for 2 h, checked by HPLC, water (10 mL) and conc. hydrochloric acid (1 mL) was added. Separated Layers, after neutralizing aq.layer by sodium carbonate solution impurity III was filtered and crystallized from acetone to give title impurity III (0.1 g).

Elemental analysis: Calculated: C (55.72 %), H (4.38 %), and N (9.24)

^{1}H-NMR in (400 MHz, DMSO-d6) δ: 12.20 (brs, 2H), 9.80 (brs, 1H), 6.86-7.98 (m, 12H), 2.04 (s; 3H);

^{13}C-NMR (100 MHz, DMSO-d6,): δ188.59, 166.52, 165.85, 152.11, 143.89, 143.07, 128.99, 126.35, 126.31, 125.97 125.94, 124.35, 124.23, 123.56, 123.24, 123.20, 120.05, 117.89, 117.73, 113.14, 26.97,

ESI-Mass: For $C_{26}H_{18}F_{6}N_{4}O_{2}$ is 532.45 found (M+H): 533.4.

SYNTHESIS OF IMPURITY IV

preparation of ((Z)-4-Amino-N,N'-bis[(4-(trifluoromethyl) phenyl] benzimidamide. (ImpurityIV)

Impurity-I (0.5g, 1.0 mol), triethyl amine (0.16 g, 1.5 mol) and THF (5 mL) charged to flask and cooled at 5 to 10°C. After addition of Acetyl chloride (0.14 g, 1.2 mol) at 5 to 10°C stirring was continued at 25 to 30°C for 2, checked by TLC, water (30 mL) Ethyl acetate (50 mL) was added. After layer separation to an upper organic layer, H2O (50 ml) charged and separated. Organic layer was concentrated under vacuum give title compound as an off white color solid (0.2g). The formation of impurity is confirmed by LCMS (M+H):- 466.0.

Preparation of N, N'- bis [4-(trifluoromethyl) phenyl] malonamide. (Impurity V)

Malonic acid (0.5 g, 1.0 mol) methylene dichloride (5 mL) was added to the flask and, maintained 0° to 10°C. PCl5 (1.0 g 1.0 mol) charged and stirred for 2-4 h at 0 to 10°C. Added 4-TFMA (0.92 g, 1.2 eq) into reaction mixture and stirred for 4-5 h. and checked TLC. Finally reaction mass was distilled out and quenched into water and stirred for 30-60 min. 0.2 g Solid was filtered

^{1}H-NMR in (400 MHz, DMSO-d6,) δ: 10.56 (s,2H) 7.83-7.68(m, 8H), 3.57(s, 2H),
^{13}C NMR (100 MHz ,DMSO-d6,): 165.76, 142.37, 142.36, 128.33, 126.12, 126.09, 126.05, 126.01, 125.63, 123.87, 123.56, 123.24, 122.93, 122.90, 120.23, 118.94, 46.09.
Mass 390.3 (M-H) - : 389.4 m/z
FT-IR: 3271, 1671, 1654, 1650, 1540, 1341, 841, cm^{-1}.

Elemental analysis: Calculated: C (51.48%), H (4.02%), N (10.50%).

Preparation of 3-imino-N-[4-(trifluoromethyl)phenyl]-3-{[4-(trifluoromethyl) phenyl]amino} propanamide. (Impurity VI)

Compound II (5g, 1.0 mol) and ethanolic HCl (30 mL) charged to flask. Maintained at 0-10°C Stirred the reaction mixture for 60 min and 4- TFMA (3.52 g, 1.0 m mol) charged to flask. Stirred the reaction mixture for 2-3 h and distilled out the reaction mixture under vacuum below 50°C. Mass was isolated and purified on the column chromatography.

^{1}H-NMR in (400 MHz, DMSO-d6) δ: 9.35 (s, 1H), 8.68 (s, 1H), 6.47-7.81 (m, 10H), 4.58 (s, 1H),

^{13}C NMR (100 MHz, DMSO-d6,): δ 169.22, 156.88, 156.81, 144.73, 143.84, 142.46, 126.48-125.76, 123.64, 123.58, 123.48, 122.39, 122.20, 121.89, 120.90, 120.88, 120.59, 120.47, 120.37, 119.03, 117.69.

Mass: (M+)/z: 390.28, Found: $(M+H)^{+}$: 390.3

FT-IR: 3460, 3319, 3194, 3044, 1682, 1647, 1607, 1117 cm^{-1}.

Elemental analysis: Calculated: C (51.48%), H (4.02%), N (10.50%).

RESULTS AND DISCUSSION

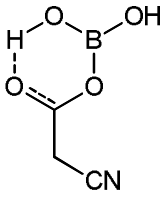
There is a vast requirement and need to have a scalable process which can fulfill the generic requirement; hence as a part of our research we decided to develop a synthesis of Teriflunomide (I). During our study, various literature was evaluated which are encompassed with the use of lethal reagents, bases with pyrophoric in nature. Also it includes the extravagant purification techniques including column chromatography. In the initial stages of synthesis we tried Teriflunomide (I) synthesis. At the beginning of our work we focused on compound II synthesis. It's a simple amidation process and there are several reagents are reported for this type conversion. All these of short fall and non-suitable process lead us to carry out some experiments with cyano acetic acid as a stating material by using traditional methods and coupling reagent like EDAC: HOBt[23], EDAC: DMAP[24]DCC [25]These are the common reagents and should be used on the equimolar basis. Back of the mind we had a thought for bulk scale manufacturing of the Teriflunomide (I) consequently it needed to compound –II manufacturing, hence these reagents may hamper the reaction progress due to its sensitive nature towards moisture. To eliminate this ambiguity of moisture sensitive reagent usages we continued our screening for the suitable reagent. We thought of reagent which is easy to handle, non-sensitive, can be used on the catalytic amount. As a result of our search we found Ravi Kumar et.al[26]reported a new approach for the amide bond formations starting with carboxylic acid. Boric acid is the reagent used for this synthesis. Further literature screening for boric acid use we found Yamamoto et al [27]reported boron reagent based catalytic method for the amide formation from amines and carboxylic acids as the reaction partners. Tang's work [28] also described the use boric acid which is superior to other amidation reagent in terms of availability, nontoxic nature and usage in catalytic amount. All these properties of boric acid, made it as a preferred catalyst for this amidation process. It was observed that highly facile condensation of cyanoacetic acid with 4-Trifluoromethyl aniline was possible when boric acid is present in catalytic amount (0.3 eq.). Boric acid activates the carboxylic acid and reduce the density of electron at carbonyl at the carbonyl function, which makes the "intermediate A" more prone to amine nucleophilic substitution to expedite the transformation in good yield. The screening of the coupling reagents is tabulated the Table –1. The study is carried out in during synthesis of compound –II in the laboratory.

Table 1: Optimization of reagent and mole equivalents.

Entry	Solvent	Reagent	Molar equivalent

01.	Dimethylformamide	EDAC:HOBT	1.1
02.	Dichloromethane	EDAC:DMAP	1.1
03.	Acetonitrile	DCC	1.1
04	Toluene	B(OH)3	0.3
05	THF	PCl_5	1.0

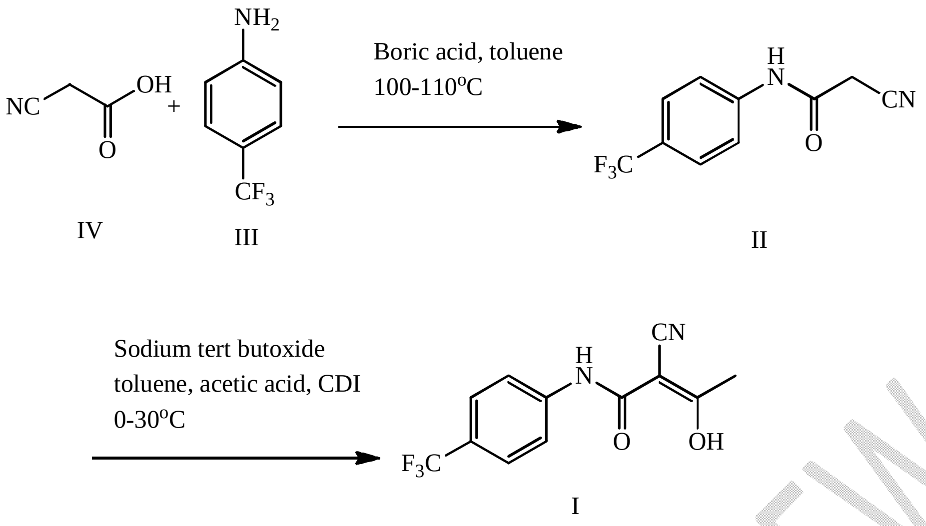
All above the reagents gives desired product formation, however the reagent for interest is boric acid owing the non-hazardous, nature. Therefore, in the first step of the compound II synthesis, acid group activation has been done by boric acid thus use of the EDAC: HOBT, EDAC: DMAP, DCC and CDI, PCl5 reagents were bypassed. Compound II isolated with high purity (99.49 %) and satisfactory yield (73 %)



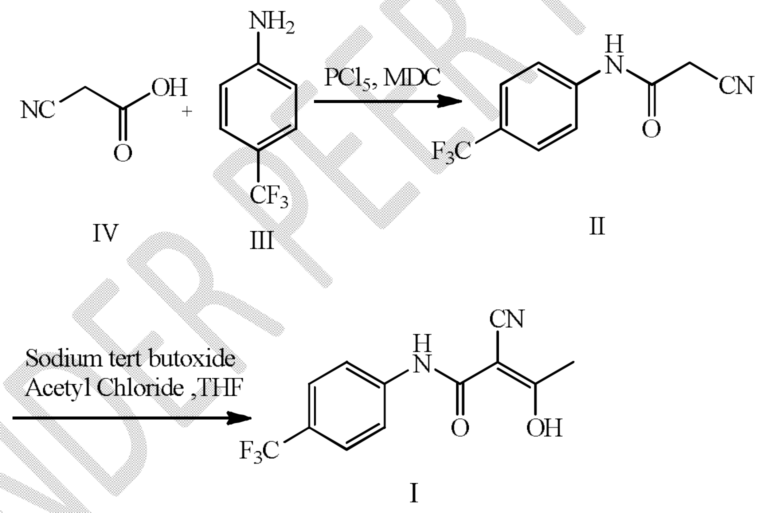
Intermediate A

For synthesis of Compound-I from compound-II, there are limited choices of reagents to get desired compound–I. The conversion is about the addition of acetyl group and numerous references are available for this reaction, mainly acetyl chloride, acetic anhydride and acetic acid reagents were used for conversion. With the limited choice of reagent, we decided to step forward with acetic acid which is superior to acetyl chloride and acetic anhydride in terms of bulk scale production and limitation under the narcotic substance usage. Since, Imidazole is soluble in various solvents including water, alcohol, ether and chloroform etc., its removal from the reaction mixture is easy. The second part is to generate the carbanion on alpha carbon to Cyano group. This carbon is a not sufficiently acidic thus requires a strong base to generate carbanion. Different bases were screened to generate the carbanion. Eventually we find Sodium tert butoxide is the base which suffices our requirement for bulk scale production.Scheme–1give an overview of optimized reaction parameters. The synthesis is simple and avoids the monotonous purification technique. The material produced as per the scheme 1 complies in all regulatory limits. The material is generated from g to kg scale under the same reaction conditions.

Scheme 1.New synthetic route for Teriflunomide synthesis.

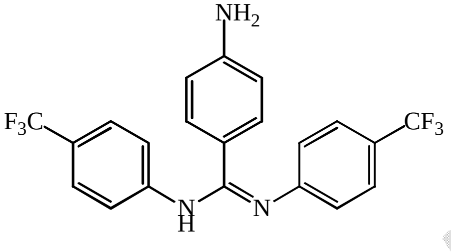


On the basis of the optimized conditions, batches were executed at kg scale. These batches met all quality parameter and desired yield of 84.8 %.The new concept made the process non-hazardous and safe. However, the traditional synthesis of teriflunomide starting with amidation of cyanoacetic acid (Compound –III) and 4-TFMA (compound –IV) by using PCl_5. The next reaction of the compound –II withAcetyl chloride with sodium tertiary butoxide base yields teriflunomide (I).Scheme is depicted below



The development study of Teriflunomide (I) introduce us with several unknown impurities. For impurity identification, LC- MS was used to identify impurities in Teriflunomide (I) by which we confirmed impurities molecular weights. Identification as well as quantification of impurities has been carried out by Proton NMR, Mass, Carbon NMR of an active pharmaceutical ingredient is of fundamental significance in terms of medical safety and for effectiveness of drug.

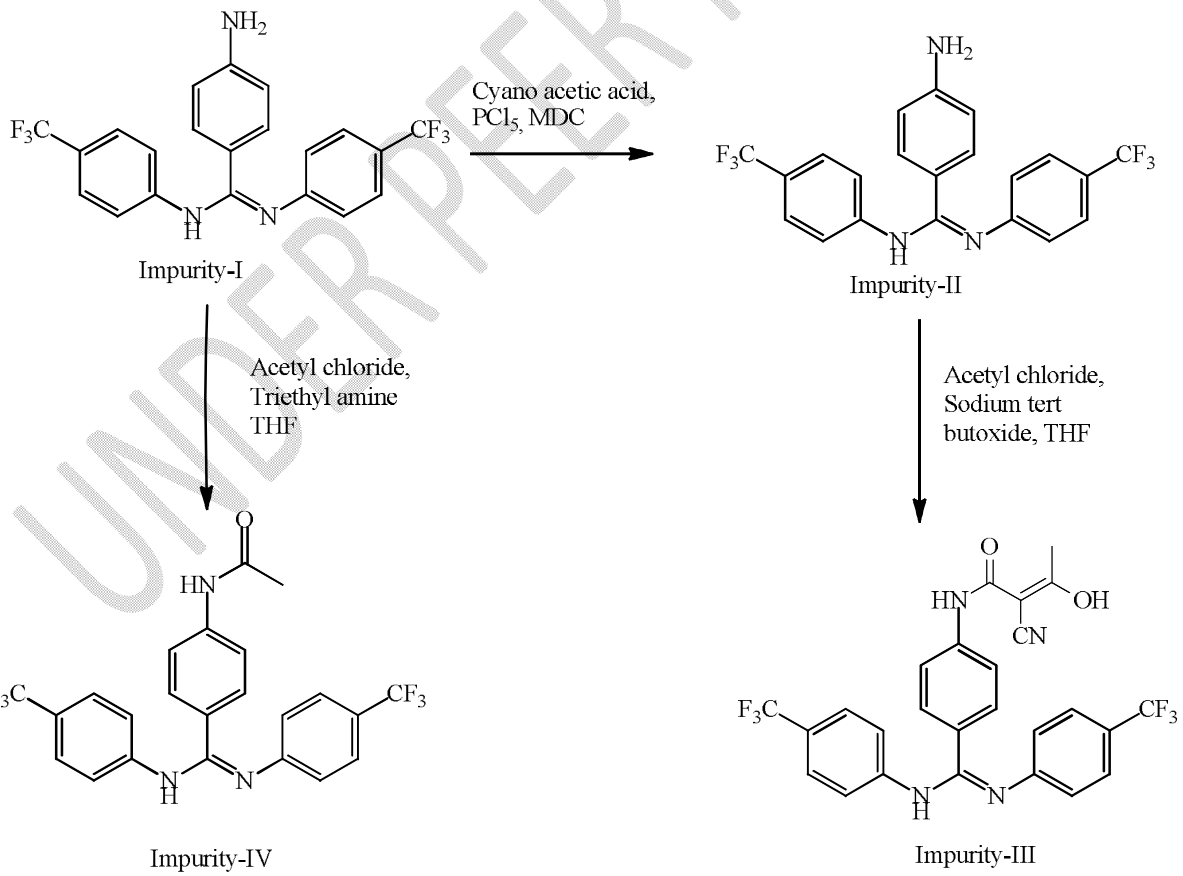
Currently regulatory authorities [29]receiving vital attention for the drug. The above discussion gives a better idea of plant feasible process for bulk scale manufacturing starting with cyano acetic acid and 4-TFMA. The development study of Teriflunomide (I) introduce us with several unknown impurities, which are process related as well as carryover impurities. Derek Tickner and Narasimhan Kasthurikrishnan[30]who describes the new impurity generation in Compound III. This impurity is trimer like structure of compound III and which has a genotoxic alert also.We also observed the solid formation in the drum of compound III which was hold for longer period of time. The obtained solid was purified in ethyl acetate and impurity was characterized by 1H NMR and Mass. As the impurity has a genotoxic alert, we controlled it in drug substance specification to the TTC limit i.e. NMT 107 ppm. Now the question arises for the control of corresponding impurities generated Teriflunomide (I) synthesis. The impurity I have a free amine group which can readily undergo for the chemical transformation and give the corresponding impurities of compound I and II



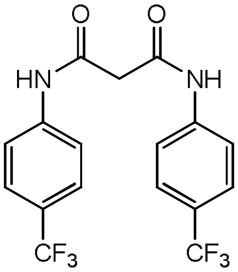
Impurity-I

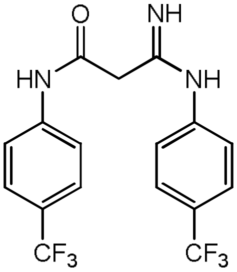
Now the interesting part of the study was to check the fate of the impurities present in 4- TFMA and cyano acetic acid in further course of synthesis

Scheme-2 Impurities present in 4- TFMA and cyano acetic acid in further course of synthesis



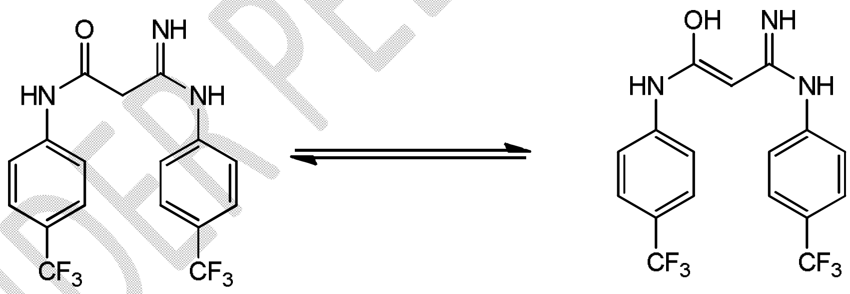
Impurities starting from impurity-I, II, III, IV has the genotoxic alert, hence all the impurities were synthesized on laboratory scale and characterized and controlled it to TTC level i.e. NMT 107 ppm. The synthesis of these impurities is discussed in the experimental section. Apart from the above discussed impurities, two new impurities impurity V and VI were identified and confirmed. Formation of impurity V and VI was observed in compound –II synthesis. The structures were depicted below





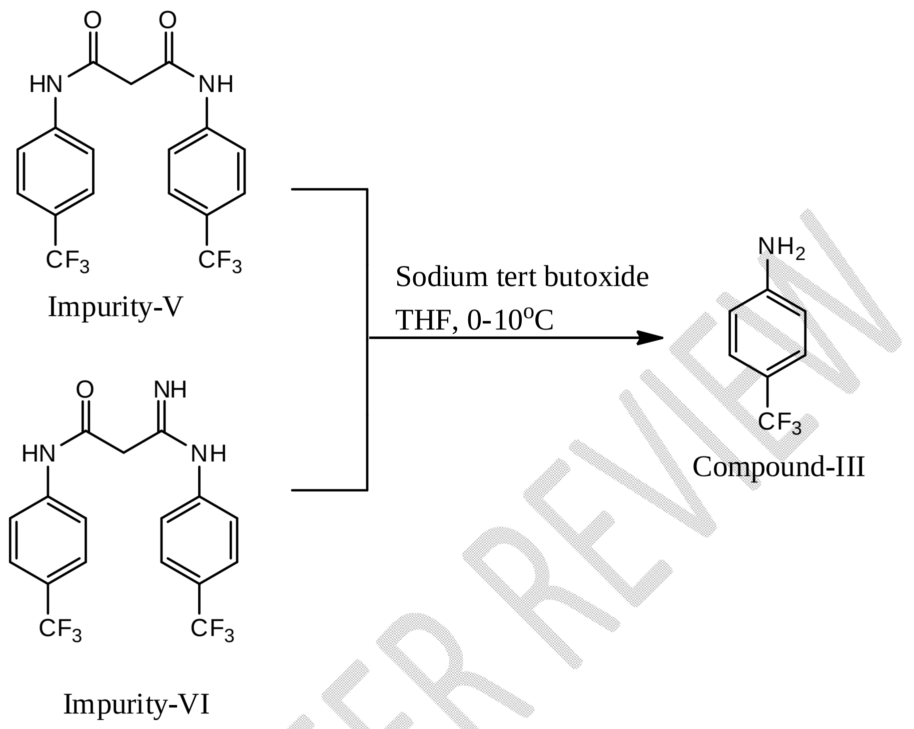
Impurity-V Impurity -VI

Impurity V and impurity VI were isolated by column chromatography and characterized by the NMR and Mass and confirm structure. As a part of interest we started the investigation to find out source of impurity generation. The ongoing study gave us a clue for the malonic acid source. The only possibility is from cyano acetic acid. The hydrolysis of cyano under acidic condition yields in corresponding acid [31]Now to discuss the impurity VI synthesis compound-II and compound III undergoes for chemical reaction under acidic nature and the slight acidic nature of reaction accelerates the impurity VI generation[32]



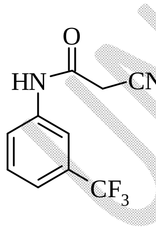
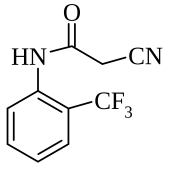
The synthesis of compound -II is discussed in this paper is carried out under milder acidic reaction condition by the use of 0.3 mol of boric acid. Also we have controlled the malonic acid to ICH limit (i.e. NMT 0.15%) in the cyanoacetic acid specification. To check the consequences of impurity V and VI we subjected these impurities under reaction condition of compound I synthesis. As an outcome of the reaction it was observed that both impurities were converted in to the starting material compound III under basic reaction condition. Compound III is a starting material i.e. 4- TFMA which is controlled in specification to the TTC limits

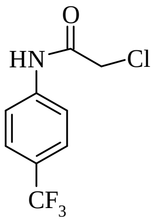
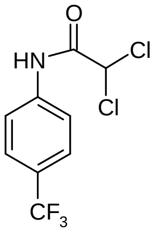
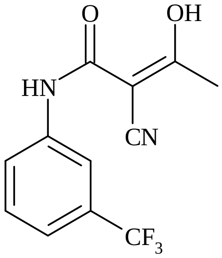
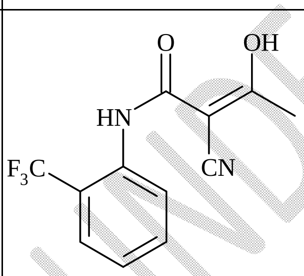
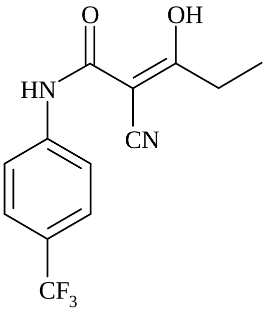
Scheme 3 Impurities during our lab development

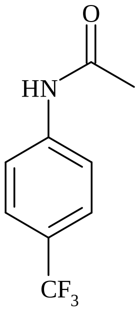


Apart from all this critical impurities, we found 8 more impurities during our lab development study which are tabulated below table.

Table-2 : New process related impurities synthesis and characterization.

Sr. No	Impurity	Characterization data
1.	 Impurity -VII	^{13}**C NMR** (DMSO-d_6, 100 MHz): 161.7, 139.07, 130.18, 130.01, 129.70, 129.38, 129.07, 128.01, 125.31, 122.60, 120.26, 120.24, 120.20, 120.16, 119.89, 115.59, 115.32, 115.28, 115.24, 115.20, 40.12, 39.91, 39.91, 39.70, 39.49, 39.29, 39.08, 39.87, 26.84 **IR:** 3338, 2970, 2929, 2266, 1882, 1797, 1691, 1610, 1570, 1452, 1396, 1338, 1253, 1178, 1128, 1068, 945, 900, 798, 700cm^{-1}. **Mass** 228.18, (M-H)$^-$,226.9
2.	 Impurity -VIII	^{1}**HNMR in DMSO-d6 (400 MHz,)** δ: 3.93 (s, 2H), 7.48-7.53 (m,2H), 7.69-7.77 (m,2H),, 10.02 (brs, 1H); ^{13}**C NMR (DMSO-d6, 100 MHz):** δ162.35, 134.37, 133.17, 130.05, 127.33, 126.44, 126.39, 126.34, 126.29, 124.73, 122.01, 115.70, 4011, 39.90, 39.70, 39.28, 39.07, 38.86, 25.74. **IR:** 3277, 3234, 2736, 2260, 1672, 1591, 1548, 1388, 1340, 1323, 1280, 1165, 1111, 1039, 950, 765 cm−1.

		Mass 228.18, 226.9 (M-H)-;227.2
3.	 Impurity -IX	**^{1}HNMR in DMSO-d6 (400 MHz,)** δ: 4.30 (s, 2H), 7.69-7.71 (d,2H), 7.79-7.81 (d,2H), 10.64 (brs, 1H); **^{13}C NMR (DMSO-d6, 100 MHz):** δ 165.22, 141.99, 126.18, 126.15, 126.11, 126.08, 125.58, 123.95, 123.63, 122.88, 119.24, 43.49, 40.11, 39.91, 39.70, 39.49, 39.28, 39.07, 38.86. **IR**: 3280, 3211, 3091, 2956, 2222, 1923, 1797, 1687, 1612, 1556, 1411, 1332, 1321, 1259, 1193, 1155, 1114, 1068, 844, 729 cm−1. **Mass** :-237.61, (M-H)- 236.2
4.	 Impurity -X	**^{1}HNMR in DMSO-d6 (400 MHz,)** δ: 6.63 (s, 1H), 7.74-7.84 (m,4H), 10.99 (brs, 1H); **^{13}C NMR (DMSO-d6, 100 MHz):** δ 162.22, 141.17, 128.18, 126.35,126.32, 126.28, 126.24, 125.48, 124.46, 122.77, 119.88, 67.15, 40.12, 39.91, 39.70, 39.50, 39.29, 39.08, 38.87 **IR**: 3271, 3211, 3142, 3082, 2999, 1919, 1793, 1685, 1610, 1552, 1411, 1325, 1246, 1157, 1130, 1068, 1018, 974, 869, 840, 810, cm−1. **Mass** 272.06, (M-H)-270.1
5.	 Impurity -XI	**^{1}HNMR in DMSO-d6 (400 MHz,)** δ: 2.26 (s, 3H), 7.39-7.44 (m, 1H), 7.53-7.57 (m, 1H), 7.76-7.78 (d, 1H), 8.04 (s, 1H), 9.10 (brs, 1H), 10.76(brs, 1H); **^{13}C NMR (DMSO-d6, 100 MHz):** δ 187.47, 166.62, 139.18, 130.33, 130.02, 129.70, 129.59, 128.55, 128.55, 125.64, 124.72, 123.13, 120.45, 120.41, 130.37, 119.67, 117.32, 117.25, 117.21, 80.76, 40.12, 39.19, 39.70, 39.49, 39.28, 39.07, 38.86, 23.93; **IR:** 3308, 3138, 3080, 2222, 1611, 1562, 1497, 1132cm−1; **Mass** 270.21, (M-H)- 269.0
6	 Impurity -XII	**^{1}HNMR in DMSO-d6 (400 MHz,)** δ: 2.02 (s, 3H), 7.05-7.09 (m, 1H), 7.48-7.56 (m, 2H), 8.38-8.40 (m, 1H), 12.26 (brs, 1H); **^{13}C NMR (DMSO-d6, 100 MHz):** δ 188.56, 166.48, 137.97, 132.64, 128.32, 125.74,125.69, 125.63, 125.58, 124.72, 123.37, 122.90, 121.60, 120.19, 117.91, 117.05, 117.34, 117.05, 77.13, 40.11,39.90, 39.69, 39.48, 39.27, 39..06, 38.85, 26.86; **IR:** 3308, 3138, 3078, 2222, 1611, 1562, 1331, 1072, 799 cm−1. **Mass** 270.21, (M-H)- 269.1
7		**^{1}HNMR in DMSO-d6 (400 MHz,)** δ: 1.12-1.15 (t, 3H), 2.52-2.58 (q, 2H), 7.65-7.67 (d, 2H), 7.76-7.78 (d, 2H), 10.97 (brs, 1H), 11.28 (brs, 1H); **^{13}C NMR (DMSO-d6, 100 MHz):** δ 191.80, 167.11, 141.72, 125.81,125.77, 125.74, 125.68, 124.09, 123.77, 123.45, 122.98, 120.92, 118.40, 78.91, 40.12, 39.91, 39.70, 39.50, 39.29, 39.08, 38.87, 10.21; **Mass** 284.12, (M-H)- 282.7

	Impurity -XIII	
8	 Impurity -XIV	**^{1}HNMR in DMSO-d6 (400 MHz,)** δ: 2.09 (s, 3H), 7.62-7.64 (d, 2H), 7.78-7.80 (d, 2H), 10.28(brs, 1H); **^{13}C NMR (DMSO-d6, 100 MHz):** δ 168.91, 142.81, 128.40, 125.95, 125.92, 125.88, 125.84, 125.70, 123.46, 123.15, 123.00, 122.83, 122.51, 120.31, 118.72, 40.11, 39.90, 39.69,39.46, 39.27, 39.06,38.85, 24.02; **IR:** 3309, 3271, 3080, 2638, 2216, 1917, 1791, 1627, 1608, 1409, 1325, 1271, 1157, 1109, 1070, 1016, 840, 759, 680 cm−1. **Mass** 203.14, (M+H)- 204.4

Control: The vast no of impurities was observed in the synthesis, which are critical and now it is mandatory to control them to the limit of NMT 0.15% in Teriflunomide (I) specification as per the ICH requirement. By keeping this in view and to avoid the impurity formation, we specified the limit of impurity precursor in the corresponding starting material [33,34].

Also the work-up parameters like under acid followed by base treatment, purification in acetone are well established to remove all impurities from Teriflunomide (I). Thus, this strategy results in an industrially feasible, easy scale up at commercial level and convenient process for Teriflunomide-I.

CONCLUSION

The new boric acid concept is introduced for the amidation process and concept was encompassed in Teriflunomide (I) synthesis. The newly developed Teriflunomide (I) process was demonstrated on bulk scale.

The impurity ((Z)-4-Amino-N, N'-bis[(4-(trifluoromethyl)phenyl] benzimidamide in 4-TFMA was isolated and characterized , also the fate of this impurity was discussed and corresponding impurity formation at every stage of Teriflunomide (I) synthesis. This corresponding impurities were synthesized, isolated and characterized. Along with this researcher introduce the detailed process related impurity profile, which is not discussed earlier in any literature or by any regulatory agencies.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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