World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

A Facile Approach for the Synthesis of Highly Pure Immunomodulator Drugs-Leflunomide and Teriflunomide: A Robust Strategy to Control Impurities

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Received: 27-09-2015 / Revised: 28-10-2015 / Accepted: 30-10-2015

ABSTRACT

A facile synthesis of immunomodulator drugs, Leflunomide (**10**) and its active metabolite, Teriflunomide (**1**) is described. The synthesis is sequential and comprises N-acylation of 4-trifluoromethyl aniline (**9**) with 5-methylisoxazole-4-carboxylic acid chloride (**8**) in dimethoxyethane as a solvent to yield leflunomide (**10**) with an overall yield of 68% and 99.8% purity by HPLC. Sodium hydroxide mediated isoxazole ring scission of leflunomide (**10**) in aqueous methanol furnished teriflunomide with 81% yield and 99.9% purity by HPLC. The work further describes raw material and process attributes which are critical to control the process related, degradation and carryover impurities in both **10** and **1.**

Keywords: Immunomodulator drug, Leflunomide, Teriflunomide, process attribute and Impurity profile.

INTRODUCTION

Identification and quantification of impurities in drug substance is a very intensive activity to be performed during the API development as monitoring and control of impurities in API and drug product is essential to ensure drug safety and quality. It is always preferable to control or minimize the formation of the impurities during the reaction by understanding the kinetics of the reaction and root cause for their formation rather than multiple purifications at the end. This control strategy can be achieved by optimizing reaction conditions, mole ratio of reagents and catalysts, mode of addition of reagents, control of impurities in key starting materials, efficient work up procedure and lastly by means of establishing efficient and robust purification process. The present work described in this article provides an efficient, economic and production friendly process for the preparation of two immunomodulator drugs leflunomide (**10**) and teriflunomide (**1**) in its purest form by the HPLC method developed in our laboratory. The developed HPLC method is superior to the existing USP or EP methods. The process optimization details including spike and purge studies performed to achieve high yield and purity of **10** and **1** are also presented. Leflunomide (**10**) is chemically designated as 5-methyl-N-[4-(trifluoromethyl) phenyl]-isoxazole-4-carboxamide developed by Sanofi as an immunosuppressive disease modifying antirheumatic drug to treat active moderate to severe rheumatoid arthritis and psoriatic arthritis. This drug was approved by U.S. Food and Drug Administration (FDA) for use in the United States [1] on September 10, 1998 and in the European Union [2] on September 02, 1999. Subsequently, Sanofi introduced active metabolite of **10**, teriflunomide (**1**) as a new drug candidate to treat the relapsing forms of multiple sclerosis (MS) under the trade name Aubagio.[3] Teriflunomide (**1**) is chemically designated as (2Z)-2-cyano-3-hydroxy-N-[4-(trifluoromethyl) phenyl]but-2-enamide and was approved by US FDA [3] on September 12, 2012 and in the European Union [4] on August 26, 2013. This medicine does not cure MS, but it may slow some disabling effects and decrease the number of relapses of the disease.[5]
First method reported for the synthesis of **1** involves condensation of 5-methylisoxazole-4-carboxylic acid chloride (**8**) with 4-trifluoromethyl aniline (**9**) in acetonitrile followed by hydrolysis of the obtained **10** using aqueous sodium hydroxide in methanol to provide **1** (Scheme 1). [6-11] The

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reported yield of **10** is ranging from 78% to 94% depending on the process variant used for the synthesis whereas reported yield of **1** is around 90%. These reported processes possess several drawbacks such as high vacuum distillation of **8** which is industrially infeasible, lack of control mechanism for the impurities in both **10** and **1**, etc.[12] Exploration of the reported process for **10** posed several impurities (**1**, **7**, and **11** to 14). When leflunomide (**10**) containing these impurities was further used for the preparation of teriflunomide (**1**), all the impurities present in **10** got derivatized and lead to the formation of corresponding set of new impurities (**7**, **10**, and **14** to **17**) in **1** (Scheme 2). Thus, we felt a need to develop a facile, efficient, economic and scalable process with a proper process control to minimize or even restrict the formation of above said critical impurities in **10** and **1** (Scheme 3).

MATERIALS AND METHODS

Melting points were determined on Analab melting point apparatus, in open capillary tubes and are uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Gemini 400 MHz FT NMR spectrometer. Chemical shifts were reported in parts per million using tetramethylsilane as internal standard and are given in δ units. The solvents for NMR spectra were deuterochloroform and deuterodimethylsulfoxide unless otherwise stated. Infrared spectra were taken on Perkin Elmer Spectrum 100 in potassium bromide pallets unless otherwise stated. Elemental analyses were performed on a Hosli CH-Analyzer and the results were within ±0.35% of the calculated values. High-resolution mass spectra were obtained with a Shimadzu GC-MS QP mass spectrometer with an ionization potential of 70 eV. All reactions were monitored by High performance liquid chromatography (HPLC) on Agilent Technologies 1200 series. Gas chromatography on Agilent Technologies 7683B with head space was used for analyzing the residual solvents. Common reagent grade chemicals used were either commercially available and were used without further purification or prepared by standard literature procedures. TLC was performed on silica-gel plates (60 F254; Merk), and TLC visualizations were performed with ultraviolet light.

Purification of commercially procured 4-(trifluoromethyl) aniline (9): Anhydrous methyl tert-butly ether (25.0 l) was charged to the reactor and cooled to 0-5 °C. To the cooled methyl tert-butly ether was charged commercially available 4-trifluoromethyl aniline (**9,** 5.0 kg, 31 mol). To the obtained solution of **9** was added hydrogen chloride-2-propanol solution (6.55 l, 19 % w/v 34.14 mol) at temperature below 20°C. Precipitated product was stirred for 1-2 h at 15-20 °C, filtered and washed with chilled methyl tert-butly ether (1.0 l).Wet product was dried under vacuum at 40-45 °C for 2-3 h to obtain 6.13 kg of 4-(trifluoromethyl) aniline hydrochloride (**9.HCl**, 100% yield). Dichloromethane (20.0 l), 4-trifluoromethyl aniline hydrochloride (**9.HCl**, 4.5 kg, 22.7 mol) and water (20.0 L) were charged to reactor and stirred at 25-30 °C to obtain clear solution and pH of the resultant solution was adjusted between 9-10 using aqueous ammonia solution. Dichloromethane layer was separated from the aqueous phase, washed with water (20.0 l) and distilled under vacuum at below 40 °C to obtain 3.50 kg of pure **9** as oil. (95% yield); HPLC purity: 99.97%; Content of 9a: 0.02%; Content of 9b: ND; Content of 9c: ND; Single maximum unknown impurity: 0.01; Total impurities: 0.03%; FT-IR (KBr): 3900, 3669, 3399, 3229, 3051, 2933, 2851, 2646, 2221, 2076, 1899, 1769, 1629, 1527, 1437, 1327, 1191, 1103, 1064, 1011, 944, 831 cm^{-1}; ^{1}H NMR (400 MHz, DMSO, ppm): δ 5.79 (s, 2H), 6.62-6.64 (d, 2H), 7.28-7.30 (d, 2H); MS (ESI, m/z): 162 $[M + H]^{+}$.

Synthesis of Leflunomide (10): Anhydrous dimethoxyethane (28.0 l) and 5-methylisoxazole-4-carboxylic acid (7.0 kg, 55.02 mol) were charged to the reactor and mixture was heated to 45-50 °C to obtain clear solution. To the obtained solution was added thionyl chloride (7.0 kg, 82.32 mol) at temperature 45-50 °C. Reaction mixture was then heated to 70-75 °C for 3-4 h. The completion of reaction was monitored by TLC. Reaction mass was cooled to 45 °C and excess thionyl chloride and dimethoxyethane was distilled out under vacuum at temperature below 45 °C to obtain acid chloride **8**. The acid chloride **8** was dissolved in dimethoxyethane (17.5 l) and the obtained solution was added to the pre-cooled solution of purified **9** (13.75 kg, 85.35 mol) in dimethoxyethane (17.5 l) at temperature below 25 °C. Reaction mixture was then stirred for 45-60 m at 20-25 °C, precipitated by-product 4-trifluoromethyl aniline hydrochloride (9.HCl) was filtered, washed with dimethoxyethane (7.0 l) and dried under vacuum at 35-40 °C (Yield: 6.80 kg). Combined filtrate containing leflunomide **(10)** was concentrated under vacuum at 50 °C to obtain residue. Purified water (70.0 l) was added to the residue and obtained slurry was stirred at 45-50 °C for 30-45 m. The slurry was then cooled to 25-30 °C, filtered and washed with purified water (7.0 l). Obtained wet product was dried under vacuum at 50-55 °C for 5-6 h to obtain crude **10** (13.34 kg, 90% yield); HPLC purity: 99.45%, Content of impurity having (m/z) 228: ND.

Purification of Crude Leflunomide (10): Methanol (4.8 l), purified water (1.2 l) and crude

10 (1.5 kg), were charged to the reactor and mixture was heated to 60-70 °C to obtain a clear solution. Toluene (3.0 l) was then charged to the above contents and the solution was cooled to 0-5 °C. The precipitated product was stirred at 0-5 °C for 1-2 h, filtered and washed with water (1.0 l). Wet compound **10** was further decolorized using methanol (7.5 l) and activated charcoal. Charcoal was filtered and methanol layer was evaporated under vacuum at below 50 °C to obtain the residue. Purified water (10.0 l) was added to the residue and slurry was cooled to 25-30 °C, the obtained solid product was filtered, washed with purified water (1.0 l) and dried under vacuum at 50-55 °C for 5-6 h to obtain pure **10** (1.14 kg, 76 % yield); HPLC purity: 99.97%; FT-IR (KBr): 3333, 3205, 3117, 3071, 2802, 2635, 2218, 1693, 1608, 1541, 1522, 1486, 1410, 1388, 1365, 1325, 1264, 1242, 1163, 1187, 1123, 1092, 1065, 899, 884, 854, 825, 764, 671, 592, 512, 424 cm^{-1}; ^{1}H NMR (400 MHz, DMSO, ppm): δ 2.69 (s, 3H), 7.71-7.73 (d, 2H), 7.91-7.93 (d, 2H), 9.09 (s, 1H), 10.35 (s 1H); MS (ESI, m/z): 269 $[M - H]^-$

Synthesis of Teriflunomide (1) using crude (10): Methanol (6.5 l) and crude **10** (1.0kg, 3.7 mol) were charged to the reactor and mixture was stirred to obtain clear solution. To the obtained clear solution was added 0.8 N sodium hydroxide solution (5.0 l) at temperature below 30 °C. Reaction mixture was then stirred at 20-30 °C for about 30 m. Upon completion of reaction by HPLC, activated carbon (40 gm) was charged to the reaction mixture and stirred for 30 m. The reaction mixture was filtered over celite bed and celite bed was washed with methanol (1.0 l). To the combined filtrate was added concentrated hydrochloric acid (0.50 l) and precipitated product was stirred at 25-30 °C for 1.0 to 2.0 h. The crystalline solid was filtered, washed with water (2.0 l) and further suspended in hot water (45-50 °C, 10.0 l) for 60 min to remove the inorganic salts. The crystalline solid was filtered and washed with water (2.0 l) to obtain crude teriflunomide (**1**). Yield: 2.5 kg, Water content: 60 %; HPLC purity: 99.74%. Content of impurity having (m/z) 228: ND; Single maximum unknown impurity: 0.10%.

Purification of teriflunomide (1): Water (0.3 l), wet teriflunomide (2.5 kg, obtained as above) and acetone (7.20 l) were charged to the reactor and mixture was heated to reflux temperature for 1.0 h. The suspension was cooled to 25-30 °C, stirred for 1.0 h and filtered. Wet material was washed with water (1.0 l) and dried under vacuum at 50-55 °C for 6-7 h to obtain 0.90 kg of pure **1** (90% yield); HPLC purity: 99.98%; FT-IR (KBr): 3303, 3137, 2220, 1634, 1594, 1553, 1522, 1406, 1419, 1325, 1269, 1247, 1189, 1158, 1114, 1072, 1019, 971, 843, 686, 595, 425 cm^{-1};^{1}H NMR (400 MHz, DMSO, ppm): δ 2.15 (s, 3H), 7.59-7.61 (d, 2H), 7.71-7.73 (d, 2H), 11.54 (s, 1H); ^{13}C NMR (100 MHz, DMSO ppm): δ = 22.77, 81.22, 117.70, 121.32, 123.69-124.64, 125.80-125.91, 141.37, 166.47, 186.89 ppm; MS (ESI, m/z): 269 $[M - H]^-$ Anal. Calcd. (%) for $C_{12}H_9F_3N_2O_2$: C, 53.34; H, 3.36; N, 10.37; found (%): C, 53.35; H, 3.37; N, 10.45.

RESULTS AND DISCUSSION

Intermediates **7** and **9** were readily available and thus procured form the commercial source. As per our optimization study, acid intermediate **7** was converted to its acid chloride **8** using 1.5 moles of thionyl chloride in dimethoxyethane (4 volumes) as a solvent at 70-75 °C. Condensation of acid chloride **8** with amine **9** was explored using different solvents and bases. Among the several bases explored (viz., triethylamine, diisopropylamine, diisopropylethylamine and pyridine), none of them were found to be suitable as they lead to the degradation of **10** up to 30%, but surprisingly excess use of **9** (around 1.55 moles with respect to **7**) without any additional base provided excellent results. The excess amount of **9** used in the reaction not only served as a scavenger for the liberated HCl gas during the reaction but also avoided degradation of **10** as compared to other bases. Among the different solvents explored [viz., toluene, dimethoxyethane (DME) dichloromethane (DCM), tetrahydrofuran (THF), and acetonitrile (ACN)], DME not only favored easy and early completion of reaction but also favored to recover unreacted **9** in the form of its hydrochloride salt (**9.HCl**) due to its poor solubility in DME (Scheme 3). The optimized process for synthesis of **10** involves use of five volumes of DME, 1.55 moles of **9** and temperature of 20-25 ° C for 2 to 3 h for reaction completion. After completion of reaction, the reaction mass was filtered to recover the **9.HCl** and filtrate was concentrated to obtain crude **10**. To improve the economics and to reduce the effluent generated during the manufacturing process the obtained by-product **9.HCl** was hydrolyzed to recover **9** quantitatively. The HPLC analysis of crude **10** consistently showed an unknown impurity along with the listed impurities (Scheme 2) as per the improved HPLC method developed in our analytical laboratory. [13] The LC-MS study of the unknown impurity in **10** was confirmed to have the molecular mass (m/z) of 228 which was suspected to be carried forward from the raw materials or from previous stages as we could not predict any molecule related structure matching to the molecular mass (m/z) of 228. Moreover when **10** containing the unknown impurity was used for the

synthesis of **1** then the said unknown impurity was also found to be carried over in **1**. A detailed investigation of the reaction progress, work up, isolation part, by-product analysis and re-use of recovered **9** for synthesis of **10** and **1** was performed to understand the root cause for the formation of unknown impurity having molecular mass (m/z) of 228. Surprisingly, when recovered **9** was used for the synthesis of **10** then unknown impurity with molecular mass (m/z) of 228 was not observed even at trace level both in **10** and **1**. Chromatographic purities of both commercially procured **9** and recovered **9** were evaluated to understand the quality difference between the two and data is presented in Table-01. The data clearly indicates that the unknown impurity with molecular mass (m/z) of 228 was formed only when commercially procured **9** was used for the synthesis of **10** and **1**. Further evaluation indicated that the presence of unknown impurity with molecular mass (m/z) of 163 in commercial procured **9** was found to be responsible for the generation of unknown impurity with molecular mass (m/z) of 228 both in **10** and **1**. Several commercial vendors were evaluated to have the purest quality of **9** but none of them could meet our quality requirements. Thus commercially available **9** was purified before using it for manufacturing of **10** and **1.** The purification of **9** involves**,** converting **9** into its HCl salt (**9.HCl**) followed by its hydrolysis to achieve pure **9** (Scheme 3).

Further, the quality of 5-methylisoxazole-4-carboxylic acid (**7**) and aniline compound **9** were evaluated in detail for the control and elimination of the set of other impurities both in **10** and **1** (Figure 1). Presence of positional isomer 3-methylisoxazole-4-carboxylic acid (**7a**) in **7** was found to undergo similar reaction sequence to generate corresponding 3-methyl isomer **14** both in **10** and **1**. Similarly the positional isomers of **9** i.e., 3-trifluoromethyl aniline (**9a**) and 2-trifluoromethyl aniline (**9b**) present in trace level leads to the formation of corresponding impurities **11** and **12** in **10** whereas the presence of 4-methyl aniline (**9c**) in **9** was found to be responsible in formation of 4-methyl impurity **13**. The presence of teriflunomide (**1)** in **10** was mainly due to the degradation of **10** which was controlled by optimizing reaction temperature as its formation was temperature dependent.

Pharmacopeial method (EP) reports impurity **11** and **14** together where as USP method does not cover impurity **14** in its specification thus an in-house HPLC method was developed and validated [13] to estimate both of these impurities (**11** and **14**) independently in **10**. With the developed in-house HPLC method, we analyzed samples of **10** prepared as per literature reported process (Scheme 1) and optimized process (Scheme 3) and compared their impurity profile as per pharmacopeial method (EP) and in-house method (Table-02). The major advantages of in-house HPLC method was in its capability of detecting the unknown impurity with molecular mass (m/z) of 228 in **10** which otherwise could not be detected by pharmacopeial methods (EP and USP). Additionally the in-house method could detect content of **11** and **14** individually. With the proper HPLC method is hand we explored the purification of **10** to have better control on the impurities to achieve pharmaceutically acceptable quality of **10**.

The purification process for crude **10** was explored using different solvent mediated crystallizations. Among the explored solvent combinations, the mixture of methanol, water and toluene was found to be effective to control all the impurities in **10** with an overall yield of 68%. The established purification process for **10** was found to be efficient to control all the process, isomeric and degradation related impurities in **10** (Table-03).

To identify the robustness and ruggedness of the process, a spike and purge study was conducted wherein **9a**, **9b** and **9c** were spiked in **9** up to the level of around 0.4% to 0.6% and such a contaminated **9** was used for the synthesis of **10.** The experimental results for crude and pure **10** are tabulated in Table-04. Spike and purge study revealed that the developed process was capable to control the impurities **11** and **12** as per International Conference on Harmonization (ICH) limits, whereas impurity **13** was observed at higher levels. Impurity **13** could not be controlled even by multiple purifications thus the content of **9c** was restricted to not more than 0.10% in **9.** To control impurity **14** in **10** or **1** the limit of **7a** in **7** was established by spike and purge study. Acid intermediate **7** was spiked with around 0.6% of **7a** and such spiked **7** was used for the synthesis of **10**. The presence of 0.6% of **7a** in **7** generated around 0.10% of **14** in **10** after purifications. Thus the content of **7a** in **7** was restricted to not more than 0.3%.

With pure **10** in hand synthesis of **1** was established as per reported process with minor process variation. The impurities **15, 16, 17** identified in **1** were due to the presence of impurity **11**, **12** and **13** respectively in **10** for which control mechanism was already established in **10.** Finally purification process for **1** was explored to achieve pharmaceutically acceptable quality using different solvent mediated crystallizations. Among all the explored solvent combinations, mixture of acetone and water was found to be effective in controlling

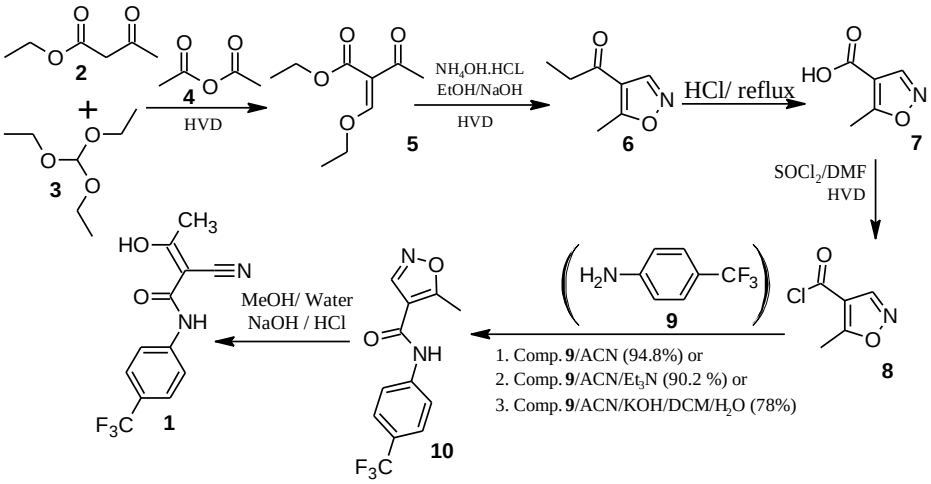
impurities. The quantities of these solvents were further optimized. The optimized quantity of acetone and water are 7.2 Vol. and 1.8 Vol. respectively per gram of **1.** The impurity profile of **1** using optimized process is tabulated in Table-05. Further we also explored the possibility to utilize crude **10** for the synthesis of **1** and surprisingly we observed that the yield and quality of **1** obtained by using crude **10** also furnished **1** with desired quality as per ICH limits.

CONCLUSIONS

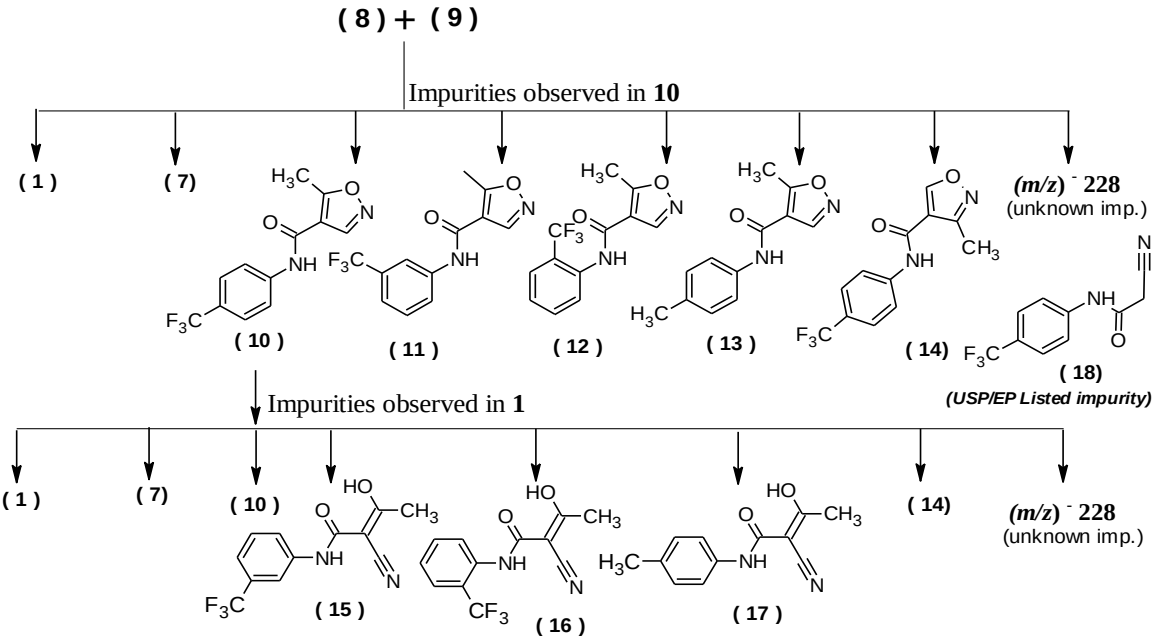
This contribution presents a Facile, sustainable, efficient, economic, production friendly and commercially viable and high yielding process for the production of highly pure leflunomide (**10**) and teriflunomide **(1)** which is substantially free from its potential impurities and meets the regulatory norms in terms of quality with an overall yield of about 68 % and 81% respectively which is a notable advantage of this procedure. We believe that this process will provide better scope and more practical alternative to the existing method for the synthesis of **10** and **1**.

ACKNOWLEDGEMENTS

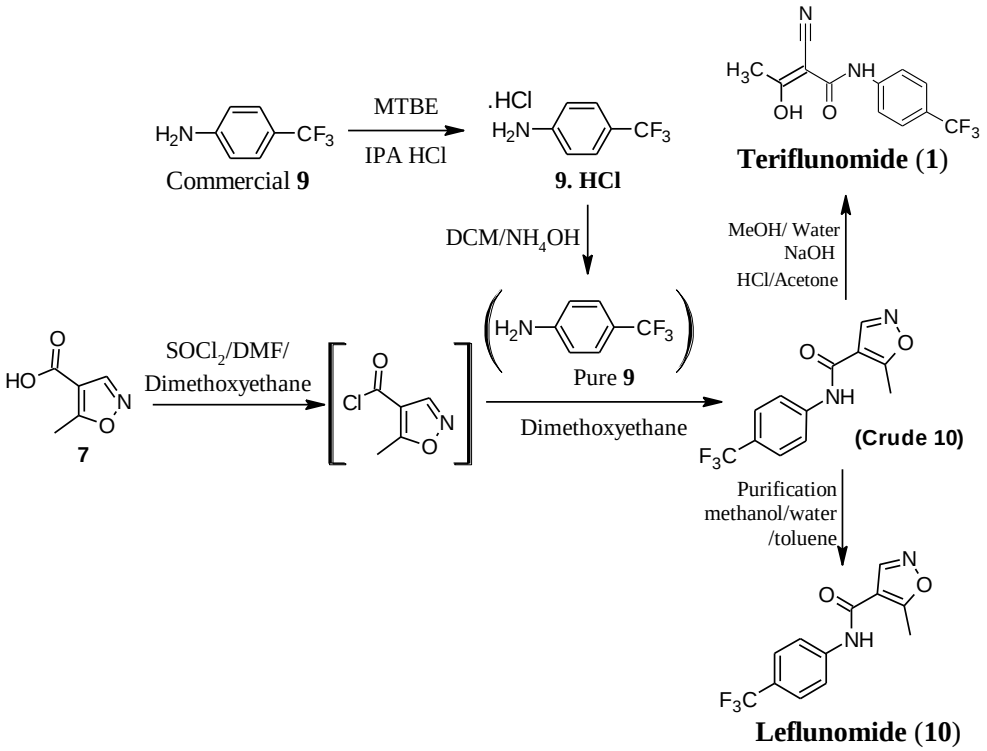
The authors are grateful to the management of Megafine Pharmaceuticals (P) Ltd., for supporting this work. We also thank colleagues of Analytical Research and Development Department, Megafine Pharmaceuticals (P) Ltd., for valuable cooperation in developing the chromatographic methods for establishing the process and identifying the impurities.



Scheme 1. Reported process for preparation of Teriflunomide (1)



Scheme 2. Flowchart representing formation of Impurities in 10 and 1.



Scheme 3. Facile and efficient process for preparation of highly pure 10 and 1.

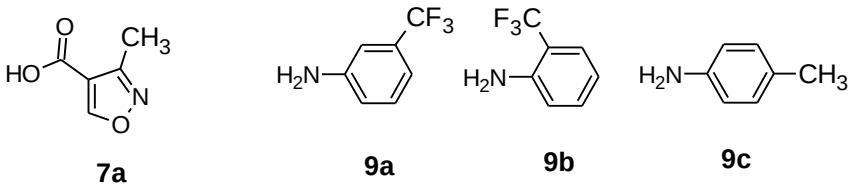


Figure 1. Potential impurities of 7 and 9.

Table-01: Comparison of Impurity profile by G.C for 4-trifluoromethyl aniline procured from commercial source and recovered from our manufacturing process.

Sr. No	Impurities	Commercial batch-01	Commercial batch-02	Recovered lot-01	Recovered lot-02
1	Toluene	0.06%	ND	ND	0.01%
2	4-chlorobenzotriflunomide	0.21	0.17%	ND	ND
3	p-chloro toluene	ND	ND	ND	ND
4	9a	0.24%	0.13%	ND	0.02%
5	9b	0.08%	0.10%	ND	ND
6	9c	0.06	ND	ND	ND
7	UIMP with (m/z) of 163	0.41% (RRT-0.79)	0.24% (RRT-0.79)	ND	ND

Note: UIMP = Unknown impurity; ND = Not detected.

Table-02: Comparison Impurity profile by HPLC for leflunomide as per EP and In-house method obtained as per reported process and improved process.

Sr. No	Impurities	Abbreviation as per EP	Limit as per EP specification	Crude Leflunomide obtained as per Reported Process		Crude Leflunomide obtained as per developed process	
				Results as per EP Method	Results as per In-house Method	Results as per EP Method	Results as per In-house Method
1	9	Imp-A	NMT-0.10%	0.73%	0.48%	ND	0.02%
2	1	Imp-B	NMT-0.3%	0.14%	ND%	ND	ND
3	11	Imp-C	IMP-C & E together: NMT-0.1%	0.22%	0.19%	0.14%	0.13%
4	14	Imp-E			ND		0.09%
5	7	Imp-D	NMT-0.10%	2.00%	2.06%	0.03%	0.07%
6	12	Imp-F	NMT-0.10%	0.01%	ND	ND	ND
7	13	Imp-G	NMT-0.10%	0.10%	0.10%	ND	0.02%
8	18	Not listed in EP	NMT-0.10%	ND	ND	0.02%	ND
9	SMU	--	NMT-0.10%	1.59% (RRT 0.66)	0.29% (RRT-0.6 /*m/z*: 228)	0.02% (RRT-0.72)	ND (RRT-0.6 /*m/z*: 228)
10	Total	--	NMT-0.2% excluding imp-B	4.8%	5.05%	0.27%	0.55

Note: RRT- Relative retention time. ND = Not detected. SMU = Single maximum unknown impurity. NMT = Not more than.

Table-03. Impurities profile in Crude and Pure material of 10.

Batch No	Particulars	Contents of impurities by HPLC (%)									
		9	**11**	**1**	**12**	**13**	**7**	**14**	**(m/z) of 228**	**SMU**	**Purity**
	Crude 10	0.02	0.13	ND	ND	0.02	0.07	0.09	ND	0.10	99.45
	Pure 10	ND	ND	ND	ND	ND	ND	ND	ND	0.01	99.97

Note: SMU = Single maximum unknown impurity; ND = Not detected

Table-04. Results of spike and purge study in 9 and its impact on 10

Sr. No	Details of 9 after spiking with impurities by HPLC		Impurity profile of Crude 10 by HPLC		Impurity profile of Pure 10 by HPLC	
	Impurities	**Content (%)**	**Impurities**	**Content (%)**	**Impurities**	**Content (%)**
1	**9a**	0.46	**11**	0.72	**11**	0.08
2	**9b**	0.37	**12**	0.17	**12**	0.02
3	**9c**	0.06	**13**	0.05	**13**	0.04

Note: Analysis of crude and pure **10** was done as per the in-house HPLC method.

Table-05. Impurity Profile study of 1 at different stages of manufacturing process.

Batch No	Particulars	Contents of impurities by HPLC (%)								
		15	10	16	17	7	14	(m/z) of 228	SMU	Purity
1	RM	0.13	ND	ND	0.02	0.07	0.09	ND	0.10	99.55
	Crude **1**	0.11	ND	ND	0.01	ND	0.04	ND	0.07	99.74
	Pure **1**	0.01	ND	ND	ND	ND	ND	ND	0.01	99.98

Note: ND = Not Detected, *RM = Reaction mixture; #SMU = Single maximum unknown impurity;

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