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 Synthesis of 1-(4-nitrophenyl)-3-morpholine-4-yl-5,6-dihydropyridin-2(1H)-one, A key Intermediate of Apixaban-An anticoagulant Drug

Pavankumar V. Solanki, Pramod A. Dhokrat, Sekhar Babu Uppelli and Vijayavitthal T. Mathad*

Department of Process Research and Development, Megafine Pharma (P) Ltd., 201, Lakhmapur, Dindori, Nashik-422 202, Maharashtra, India

Received: 12-07-2015 / Revised: 21-08-2015 / Accepted: 24-08-2015

ABSTRACT

Described herein is an improved synthesis of 1-(4-nitrophenyl)-3-morpholine-4-yl-5,6-dihydropyridin-2(1H)-one (**1**), a key intermediate for the synthesis of Apixaban. The present work provides robust and commercially viable manufacturing process for the synthesis of pure **1** with an overall yield of 48% over four steps and purity of around ≥ 98% by overcoming the limitations of the literature reported processes.

Keywords: Apixaban, Eliquis, Dihydropyridine, Key Intermediate and Deep Vein Thrombosis

INTRODUCTION

Apixaban (**8**) is an anticoagulant drug chemically known as l-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-l-yl) phenyl]-4,5,6,7-tetrahydro-lH-pyrazolo[3,4-c]pyridine-3-carboxamide and sold under the brand name "Eliquis" to treat the people with atrial fibrillation (a heart rhythm disorder) to lower the risk of stroke caused by a blood clot. It was invented by Aderis pharmaceuticals and developed jointly by Pfizer and Bristol-Myers Squibb.

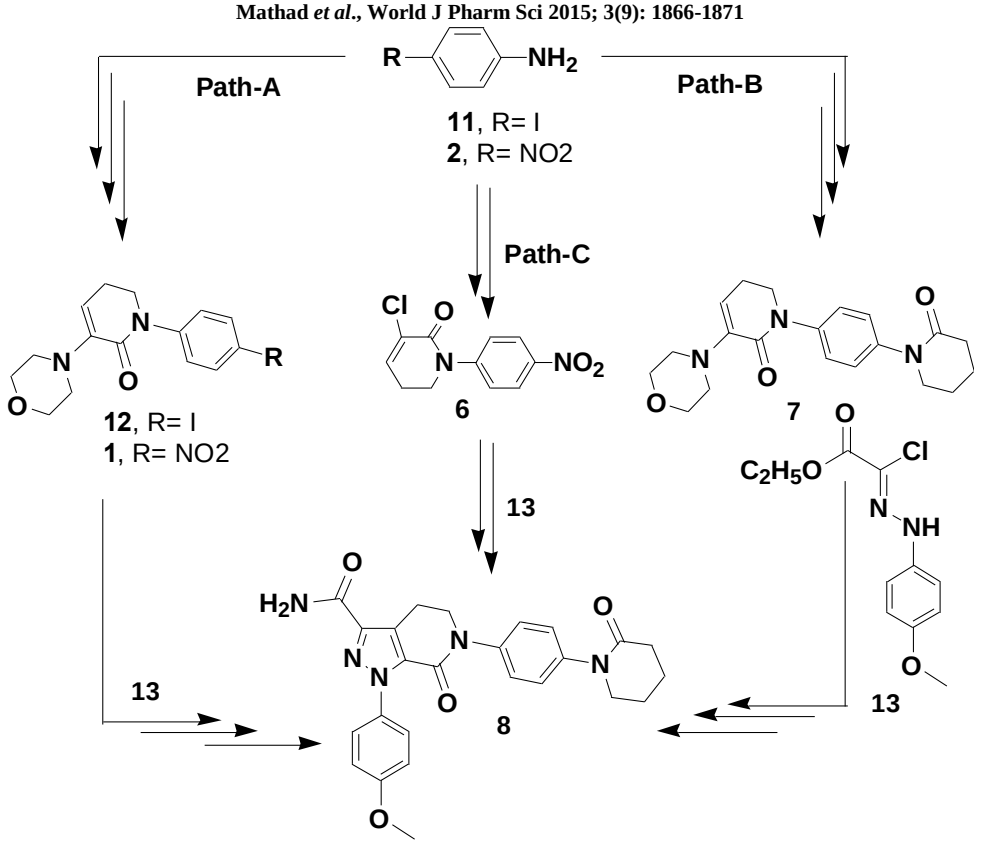
The first synthetic method (Scheme 1, Path A) reported for apixaban [1] involves the use of two key starting materials, namely 1-(4-iodophenyl)-3-morpholin-4-yl-5,6-dihydropyridin-2(1H)-one (**12**) and ethyl chloro[(4-methoxyphenyl) hydrazono]acetate (**13**).

The reported synthetic method is highly expensive and not economic due to the use of expensive starting raw materials like iodoaniline (**11**). Moreover the process is not feasible for commercial manufacturing due to the use of column chromatography purifications at several stages of the process. Several other routes reported for apixaban are also relying on the use of expensive iodoaniline (**11**) and thus economically not feasible [2-3].

In the recent past we have also reported an improved process for preparation of apixaban polymorphs and study of their phase transformation using iodoaniline (**11**) as the starting material [4]. Though alternate routes for the preparation of apixaban (**8**) are reported in the literature using less expensive key starting material p-nitro aniline (**2**, Scheme 1) [5-8] but they have several disadvantages with respect to scalability and quality. In continuation of our research work on this molecule we explored p-nitroaniline (**2**) as a key starting material for the synthesis of apixaban (**8**) via intermediate **1**.

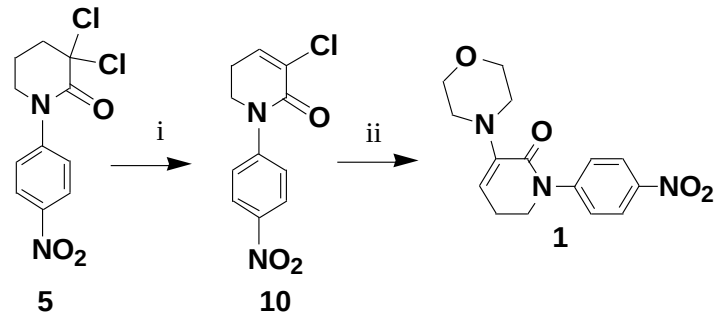
Using p-nitroaniline (**2**) as the key starting material, synthesis of three key intermediates viz., 3-chloro-1-(4-nitrophenyl)-5-6-dihydropyridin-2-1H-one (**6**), 1-(4-nitrophenyl)-3-morpholine-4-yl-5,6-dihydropyridin-2(1H)-one (**1**) and 3-morpholino-1-(4-(2-oxopiperidin-1-yl)phenyl)-5,6-dihydropyridin-2(1H)-one (**7**) are reported in the literature and each of these intermediates are used independently for the preparation of Apixaban as shown in Scheme 1 (Path A, B and C). Among the three intermediates (**1**, **6**, and **7**), we have selected the intermediate **1** for the synthesis of Apixaban (**8**) due to the economics of the process. Several processes reported for the preparation of **1** starting from **2** suffer from major drawbacks [7-14].

***Corresponding Author Address:** Vijayavitthal T. Mathad, Department of Process Research and Development, Megafine Pharma (P) Ltd., 201, Lakhmapur, Dindori, Nashik-422 202, Maharashtra, India.E-mail; vt.mathad@megafine.in, drvtmathad@yahoo.co.in*



Scheme 1. Key Intermediates for the synthesis of Apixaban (8)

Few of the drawbacks of the literature procedure for synthesis of key intermediate **1** include; column chromatography technique used for the purification [6], use of huge excess of morpholine for conversion of **5** to **1** [8, 10, and 13], and formation of mono chloro derivative (**9**, Scheme 3) which is difficult to remove from **5** even after multiple crystallizations [10-12]. There also exists a synthesis of **1** using **5** via **10**, but economics of this route is not favorable due to the use of expensive reagent like lithium carbonate and also involves additional reaction step for the synthesis of **10** (Scheme 2) [7]. Moreover the reaction involved in the conversion of **5** into **1** is found to be very critical to deliver desired quality of **1** because of the formation of several impurities.



i. DMF, Li_2CO_3 ii.THF, Morpholine, Et_3N

Scheme 2. Reported synthesis of 1 by 5 via 10 [7]

To overcome the above disadvantages, we initiated the development of an efficient, scalable and commercially viable process for the synthesis of **1** starting from p-nitroaniline (**2**) with an overall yield of around 48% over four steps and the details are presented in this article.

MATERIALS AND METHODS

Melting points were determined on Analab melting point apparatus, in open capillary tubes and are uncorrected. The ^{1}H NMR (400 MHz) spectra were recorded on a Varian Gemini 400 MHz FT NMR spectrometer. Chemical shifts were reported in parts per million (PPM) using tetramethylsilane

(TMS) as internal standard and are given in δ units. The solvents for NMR spectra were deuterochloroform (CDCl3) and deuterodimethylsulfoxide (DMSO-d6) unless otherwise stated. Infrared spectra were taken on Perkin Elmer Spectrum 100 in potassium bromide pallets unless otherwise stated. High-resolution mass spectra were obtained with a Shimadzu GC-MS QP mass spectrometer with an ionization potential of 70 eV. Reactions were monitored by High performance liquid chromatography (HPLC) on Agilent Technologies 1200 series.

Synthesis of 5-Chloro-N-(4-Nitrophenyl) pentanamide (3): Tetrahydrofuran (6.0L) and p-nitroaniline (**2**, 1.0 kg, 7.24 moles) were charged into the reactor and stirred at 25-30 °C to obtain clear solution. To the obtained clear solution was charged anhydrous potassium carbonate (1.15 kg, 8.32 moles) and the reaction mixture was cooled to 0-5 °C. 5-Chlorovaleryl chloride (1.45 kg, 9.35 moles) was charged to the above mixture at 0-5 °C temperature. Temperature of reaction mass was then raised to 25-30 °C and mixture was stirred for 1.5 hr. Completion of reaction was monitored by TLC (Mobile phase: 10 ml of toluene with one drop of methanol). Upon completion of reaction, tetrahydrofuran was distilled under vacuum at temperature below 45 °C to obtain the residue. To the obtained residue was added purified water (10.0 L) and suspension was stirred at 25-30 °C for 1 hr. The obtained solid product was filtered, washed with purified water (1.0 L) and dried under vacuum at temperature 50-55 °C for 6-8 hr to obtain 1.85 kg (100%) of **3** as a crystalline solid. HPLC purity: 99.83%. Content of **2**: 0.02%, individual unknown impurity: 0.11%, M.p.:133-134°C. FT-IR (KBr): 3290.11, 2908.40, 2938.79, 1682.47, 1511.36, 1559.25, 1598.29, 691.79, 750.57, 774.68, 830.80, 857.30 (cm-1). ^{1}H NMR (DMSO, δ ppm): 1.70-1.78 (m, 4H), 2.40-2.46 (t, 2H), 3.63-3.68 (t, 2H), 7.81-7.84 (Ar H, 2H), 8.17-8.21 (Ar H, 2H), 10.52 (s, 1H). MS (ESI, m/z): 255 [M - H].

Synthesis of 1-(4-nitrophenyl) piperidin-2-one (4): Tetrahydrofuran (8.0L) and **3** (1.0 kg, 3.89 moles), were charged into the reactor and stirred for 5-10 minutes to obtain a clear solution. Solution was then cooled to 0-5 °C and was charged with potassium tertiary butoxide (0.570 kg, 5.08 moles). Temperature of reaction mass was then raised to 25-30 °C and mixture was stirred for 3-4 hr. Completion of reaction was monitored by HPLC. Upon completion of reaction, tetrahydrofuran was distilled under vacuum at temperature below 45 °C to obtain the residue. To the obtained residue was added purified water (5.0 L) and desired product was extracted in dichloromethane (5.0 L x 2). Combined dichloromethane layer was washed with water (5.0 L) and dichloromethane was distilled under vacuum at below 40 °C to obtain 0.712 kg of crude **4** (83%); HPLC purity: 98.78%. M.p.: 96-98°C. FT-IR (KBr): 2956.95, 1655.21, 1519.76, 1588.36, 1604.26, 697.42, 718.88, 859.74 (cm-1). ^{1}H NMR (DMSO, δ ppm): δ 1.80-1.92 (m, 4H), 2.44-2.48 (t, 2H), 3.69-3.72 (t, 2H), 7.58-7.63 (Ar H, 2H), 8.19-8.24 (Ar H, 2H). MS (ESI, m/z): 221 [M + H].

Optional purification of 1-(4-nitrophenyl) piperidin-2-one (4): Ethyl acetate (1.5 L) and methyl tertiary butyl ether (4.0 L), were charged into the reactor containing crude **4** (1.0 kg, obtained as per the above mentioned process), and suspension was refluxed for 1 hr. Mixture was then cooled to 0-5 °C and stirred for 1-2 hr. The solid product obtained was filtered and washed with methyl tertiary butyl ether (0.5 L). Wet material was dried under vacuum at temperature 50-55°C for 3-4 hr to obtain 0.83 kg of pure **4** (83% w/w); HPLC purity: 99.40%

Synthesis of 3, 3-dichloro-1-(4-nitrophenyl) piperidin-2-one (5): To the reactor containing crude **4** (1.0 kg, 4.54 moles, obtained as per the process mentioned above having purity 98.78%) was charge chlorobenzene (8.0L) and solution was chilled to 0-5 °C. To the chilled solution was charged phosphorus pentachloride (3.77 kg, 18.10 moles) in portions at temperature below 10 °C. Temperature of reaction mass was then raised to 50-55 °C and mixture was stirred for 2 hr. Completion of reaction was monitored by TLC (Mobile phase: toluene: ethyl acetate 7:3). Upon completion of the reaction, reaction mass was quenched over chilled water (8.0 L) and desired product was extracted in dichloromethane (5.0 L X 3). Combined organic layer was washed with aqueous sodium bicarbonate solution (5.0 L X 3, prepared by dissolving 1.12 kg sodium bicarbonate in 15 L water) and finally with brine solution (5.0 L, prepared by dissolving 1.0 kg sodium chloride in 5.0 L purified water). Organic layer was then concentrated under vacuum at below 60 °C to obtain crude **5** as a residue. To the obtained residue was charged isopropyl alcohol (1.2 L), water (1.2 L) and mixture was refluxed for 1 hr. Suspension was then cooled to 0-5 °C, stirred for 1-2 hr and the solid product obtained was filtered. Wet cake was dried under vacuum at temperature 50-55° for 6-8 hr to obtain 1.05 kg (80%) of pure **5** as a crystalline solid. HPLC purity: 99.83%. Content of **3**: Not detected, Content of **4**: Not detected, individual unknown impurity: 0.10%, M.p.: 135.1-135.4°C. FT-IR (KBr): 2851.54, 1670.16, 1516.94, 1592.90, 1608.36, 700.11, 754.13, 765.56 (cm-1). ^{1}H NMR (DMSO, δ ppm): 2.11-2.17 (m, 2H), 2.94-2.96 (m,

2H), 3.82-3.85 (t, 2H), 7.60-7.63 (Ar H, 2H), 8.28-8.32 (Ar H, 2H). MS (ESI, m/z): 289 [M + H].

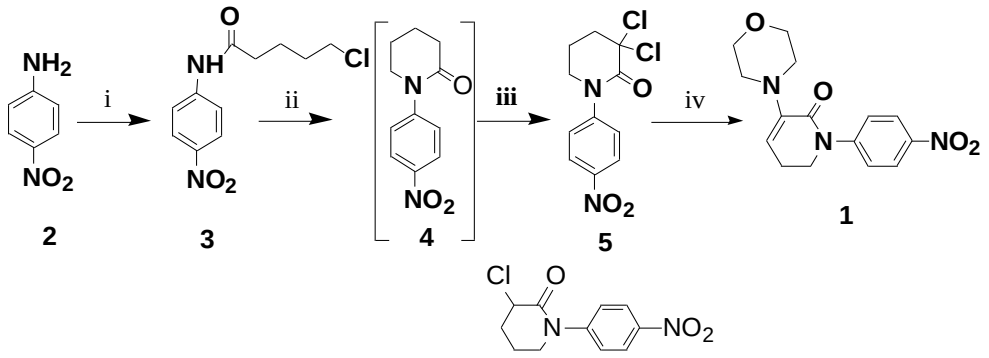
Synthesis of 1-(4-nitrophenyl)-3-morpholine-4yl-5,6-dihydropyridin-2(1H)-one (1): Dimethyl formamide (5.0L) and **5** (1.0 kg, 3.45 moles) were charged to the reactor and contents were stirred at 25-30 °C to achieve clear solution. To the obtained clear solution was charged triethylamine (1.047 kg, 10.35 moles) and morpholine (1.6 kg, 18.36 moles). Mixture was heated to 95-100 °C for 5-6 hr. Completion of reaction was monitored by TLC (Mobile phase: toluene: ethyl acetate 7:3). Upon completion of reaction, reaction mass was cooled to 85-90 °C and purified water (10.0 L) was added at temperature between 50-90°C. Precipitated product was cooled to 0-5 °C and stirred for 1-2 hr. The product precipitated out was filtered and washed with purified water (0.5 L). Wet material was dried under vacuum at temperature 50-55°C for 8-10 hr to obtain 0.755 kg of pure **1** (72%); HPLC purity: 98.67%. Content of **3**: Not detected, Content of **4**: Not detected, Content of **5**: Not detected, individual unknown impurity: 0.45%, M.p.: 165.4-167.0°C. FT-IR (KBr): 3081.75, 2928.53, 2960.20, 1672.78, 1620.88, 1508.11, 1589.96, 1605.23, 1118.68 (cm^{-1}). ^1H NMR (DMSO, δ ppm): 2.52-2.56 (m, 2H), 2.87-2.89 (t, 4H), 3.80-3.88 (m, 6H), 5.72-5.75 (s, 1H) 7.51-7.55 (Ar H, 2H), 8.21-8.25 (Ar H, 2H). MS (ESI, m/z): 304 [M + H].

Optional purification of 1-(4-nitrophenyl)-3-morpholine-4yl-5,6-dihydropyridin-2(1H)-one (1): Ethyl acetate (3.0 L), methyl tertiary butyl ether (3.0 L) and crude **1** (1.0 kg, obtained as per the above mentioned process) were charged to the reactor and suspension was heated to reflux temperature for 1 hr. Mixture was then cooled to 0-5 °C and stirred for 1-2 hr. The crystalline product obtained was filtered and washed with methyl tertiary butyl ether (0.5 L). Wet material was dried under vacuum at temperature 50-55 °C for 3-4 hr to obtain 0.92 kg of pure **1** (92%); HPLC purity: 99.40%

RESULTS AND DISCUSSION

Synthesis of key intermediate **1** was explored from a basic key starting material p-nitroaniline (**2)** by systematic understanding of reaction parameters contributing to the yield and quality and then a systematic optimization study was conducted to establish the scalable, economic and production friendly process (Scheme 3).

N-acylation of **2** with 5-chlorovaleryl chloride was explored using bases like sodium hydride, triethylamine, potassium tertiary butoxide, potassium carbonate, potassium hydroxide and sodium hydroxide in solvents like 2-methyl tetrahydrofuran, tetrahydrofuran, dimethoxyethane, and dimethyl formamide. Among the explored combinations of solvent and base, use of potassium carbonate as a base and tetrahydrofuran as a solvent furnished desired yield and quality of **3**. During the initial exploration reaction was also attempted using 5-chlorovaleryl chloride and 5-bromovaleryl chloride to understand the rate of the reaction, yield and quality of the product **3**. However, 5-chlorovaleryl chloride was selected due to its cost advantage over 5-bromovaleryl chloride as the yield and quality of the product obtained were almost similar in both the cases. Further, volume of tetrahydrofuran and mole ratio of potassium carbonate required for reaction were optimized and as per the optimized process six volumes of tetrahydrofuran and 1.15 moles of potassium carbonate were found to be optimum for the reaction. Since the purity of the isolated material was over 99%, we proceeded to next step without further purification of **3**.



Monochloro impurity (**9**)

i. THF, K_2CO_3, Water, 5-chlorovaleryl chloride

ii.THF, MDC,$tBuO^-k,^+$ iii**.** chlorobenzene, PCl_5, $NaHCO_3$, MDC,IPA, H_2O

iv. Morpholine, Et_3N, DMF, water

Scheme 3. Improved process for the preparation 1

Base catalyzed cyclization of **3** to provide **4** was explored in 2-methyltetrahydrofuran and tetrahydrofuran as solvents and sodium hydride, potassium carbonate, potassium tertiary butoxide, and potassium hydroxide as bases. Among the explored combinations of solvents and bases, use of tetrahydrofuran as a solvent and potassium tertiary butoxide as a base furnished appreciable results and hence were chosen for further optimization. Reaction using potassium carbonate as a base was not initiated even at reflux temperature. As per the optimized process, eight volumes of tetrahydrofuran and 1.3 moles of potassium tertiary butoxide furnished acceptable results. Reaction temperature of 25-30°C was found to be sufficient to bring complete conversion of **3** to **4** within 5 to 6 hours. Further the chlorination of **4** using PCl_5 was explored in chloroform, dichloromethane and chlorobenzene. Formation of mono-chloro impurity **9** was observed when dichloromethane and chloroform were used as a solvents thus chlorobenzene was finalized as a solvent for this reaction. The obtained crude product **5** after usual work-up procedure was subjected for purification using mixture of isopropyl alcohol and water to achieve purity of around 99% by HPLC. Based on the exploration of reactions and subsequent HPLC analysis it was confirmed that to have better quality of **1**, it was necessary to have intermediate **5** with minimum of 98% purity by HPLC.

Finally, a base catalyzed N-alkylation and dehydro-halogenation of **5** was a critical step to achieve highly pure **1**. Exploration of reported process using morpholine alone as a reagent, base and solvent furnished **1** with purity of about 83 to 89% by HPLC along with many unknown impurities. Surprisingly use of morpholine as a reagent, DMF as a solvent and triethylamine as a co-base provided substantial improvements in the rate of reaction and reduced the formation of impurities. We then optimized the mole ratio of morpholine and triethylamine to achieve the optimum yield and purity of **1** (Table-1).

The experimental data indicates that use of Et_3N as a co-base is advantageous probably because of its ability to trap the liberated HCl than the morpholine which helps to maintain the reaction in basic condition and maintain the rate of reaction. Once the reaction was completed, water was added to the reaction mass and obtained solid was stirred, filtered and washed with additional quantity of water to provide **1**.

CONCLUSIONS

In conclusion, the present work provides an efficient manufacturing process for synthesis of **1** which has sufficient purity to be used as a key starting material for the synthesis of apixaban. The work also describes purification process for the purification of **4**, **5** and **1** which is additional advantage of the process. We believe that this process will provide better scope and more practical alternative to the existing method for the synthesis of **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.

Table 1: Impact of varied mole ratio of triethylamine on the yield and quality of 1.

Sr. No	**5** (gm)	DMF (mL)	Et_3N (Moles)	Temp. (°C)	Yield (%)	HPLC Analysis		
						Purity of **1**	Content of **5**	Largest Unknown impurity (%)
1	10	50	Not Used	95-100	57.25	59.17	ND	37
2	10	50	1.0	95-100	49.57	55.50	0.38	10.94
3	10	50	2.0	95-100	51.76	84.31	ND	3.51
4	10	50	3.0	95-100	72	98.74	ND	0.50
5	50	250	3.0	95-100	71.8	98.56	0.03	0.20
6	100	500	3.0	95-100	72.1	98.73	ND	0.21

REFERENCES

1. Pinto D, Quan M, Orwat M, Li YL, Han W, Qiao JX, Lam P. Lactam-containing compounds and derivatives thereof as factor Xa inhibitors. E.P. Patent 1, 427,415. June 16, 2004.
2. Zhou J, Lynette OM, Philip M, Hui-y L. Synthesis of 4,5-dihydro-pyrazolo [3,4-c] pyrid-2-ones.WO. Patent 2003/049681, June 19, 2003.
3. Gant TG, Shahbaz M. Pyrazole carboxamide inhibitors of factor XA. WO. Patent 2010/030983, March 18, 2010.
4. Mathad VT et al. Investigation on Polymorphs of Apixaban, an Anticoagulant Drug: Study of Phase Transformations and Designing Efficient Process for their Preparation. World J Pharm Sci., 2015; 3(3): 663-67.
5. Rafael S, Lucius TR, Boguslaw MM, Nicolas C, Matthew O, Huiping Z, Bang-C C. Process for preparing 4,5-dihydro-pyrazolo[3,4-c]pyrid-2-ones. U.S. Patent 7,396,932, July 08, 2008.
6. Maxwell BD et al. The syntheses and in vitro biotransformation studies of [^{14}C] apixaban, a highly potent, selective, efficacious and orally bioavailable inhibitor of blood coagulation Factor Xa. J. Label Comp. Radiopharm. 2011; 54 (8): 418-25.
7. Cohen M, Yeori A, Mittelman A, Erhlich M. Solid state forms of apixaban. WO. Patent 2013/119328, August 15, 2013.
8. Jia'an J, Yafei J. Alternate synthesis of apixaban (BMS-562247), an inhibitor of blood coagulation factor Xa. Syn comm.2013; 43(1): 72-79.
9. Ji Y, Liu Q, Liuai X, Jiang J, Wang Y, Wang C, Yuyan K. Method for preparing antithrombotic medicament apixaban. CN. Patent 201010277358 .July 04, 2012**.**
10. Huo, S. W.; Guo, K. Y.; Zhong, J.; when, H-L. Preparation method of apixaban intermediates. CN. Patent 201210305258, March 12, 2014.
11. Guo F, Renli X, Xu, He L. A method of preparing intermediates of apixaban CN. Patent. 201410113371. July 16, 2014.
12. Ji Y, Liu Q, Liuai X, Jiang J, Wang Y, Wang C, Yuyan K. An anti-thrombotic drugs apixaban preparation. CN. Patent. 201010277358. July 04, 2012.
13. Dwivedi SD, Singh KK, Tandon N, Ware D. An improved process for the preparation of apixaban and intermediates thereof. WO. Patent 2014/203275, Dec 24, 2014.
14. Donald JPP et al. Discovery of 1-(4-Methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro- 1H-pyrazolo[3,4-c]pyridine-3-carboxamide (Apixaban, BMS-562247), a Highly Potent, Selective, Efficacious, and Orally Bioavailable Inhibitor of Blood Coagulation Factor Xa. J. Med. Chem. 2007; 50 (22): 5339-56