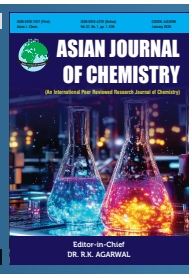
Asian Journal of Chemistry; Vol. 37, No. 7 (2025), 1707-1712

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2025.32993



A Novel Synthetic Route of (*Z*)-Ethyl 2-chloro-2-(2-(4-methoxyphenyl)hydrazono)acetate: A Key Intermediate of Apixaban

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Received: 7 November 2024; *Accepted*: 14 June 2025; *Published online*: 30 June 2025; AJC-22046

The objective of the present study to resolve the technical problem like long reaction time, low product yield for the synthetic method of Eliquis intermediate, (Z)-ethyl 2-chloro-2-(2-(4-methoxyphenyl)hydrazono)acetate and the inconsistency in the purity of product. Thus, a novel synthesis pathway for the Eliquis intermediate, (*Z*)-2-chloro-[(4-methoxyphenyl)hydrazono]ethyl acetate, which is a crucial precursor for apixaban is developed.

Keywords: Diazotization, Japp-Klingemann, Apixaban, Hydrazono, Anticoagulant, Novel process.

INTRODUCTION

Apixaban (Fig. 1a) is a novel oral anticoagulant (NOAC) approved by the US Food and Drug Administration (FDA) in 2012 for use in patients with non-valvular atrial fibrillation to reduce the risk of stroke and blood clots [1,2]. Later, in 2014, it was approved to treat deep venous thrombosis (DVT) and pulmonary embolism (PE). In 2014, it was also approved for use to reduce the risk of blood clots (DVT and PE) in patients following knee and hip replacement surgery. This activity outlines the indications, mechanism of action, safe administration, adverse effects, contraindications, monitoring, overdose management and toxicity of apixaban [3-5].

The synthesis of apixaban involves several key intermediates, with one of the key building blocks being ethyl (Z)-ethyl 2-chloro-2-(2-(4-methoxyphenyl)hydrazono)acetate (also known as Eliquis intermediate, Fig. 1b). This intermediate is a pivotal precursor in the formation of the final drug product, necessitating the development of efficient and scalable synthetic routes to ensure cost-effectiveness and quality control during the large-scale production. Several process for the synthesis of Eliquis intermediate are already reported in the literature [9-11] and mostly the results are unsatifactorily in terms of tedious reaction time as well as the yield and purity of the products. In 2009, Thomas *et al*. [12] used ethyl acetate as solvent for the synthesis of 2-chloro-2-(2-(4-methoxy-phenyl)hydrazono)-acetate. Due to the immiscible nature of ethyl acetate in water, the reaction occurred in two phases, resulting in an incomplete reaction which took longer than 12 h. This process led to a higher formation of impurities, ultimately yielding only 74% product. In a different approach, The Shandong Haoyuan Industry Group Co. Ltd., East China University of Science and Technology [13] uses alcoholic solvents like methanol. Although homogenous, this reaction results in the formation of Eliquis intermediate. After the reaction, a sticky black solid with several impurities is obtained, resulting in the low product purity. The reaction was completed in methyl alcohol, followed by solvent evaporation, repeated ethyl acetate extractions and washing. The organic layer was dried and evaporated to obtain a crude product, which is purified with a suitable solvent to yield 76%.

Keeping in mind about these facts, an economical and feasibale method using Japp-Klingemann reaction, which involves the reaction of 1,3-dicarbonyl molecule with an aromatic diazonium salt to produce an arylhydrazone [14]. Thus, the reaction between diazonium chloride salt of *p*-anisidine and ethyl 2-chloroacetoacetate to form ethyl (2*Z*)-chloro[2-(4-methoxyphenyl)hydrazinylidene]ethanoate (**1**). The advantage of the current methodology is the reduction of reaction time, simple process to enhance yield and the manufacture of a product with high purity as free from process related and isomeric

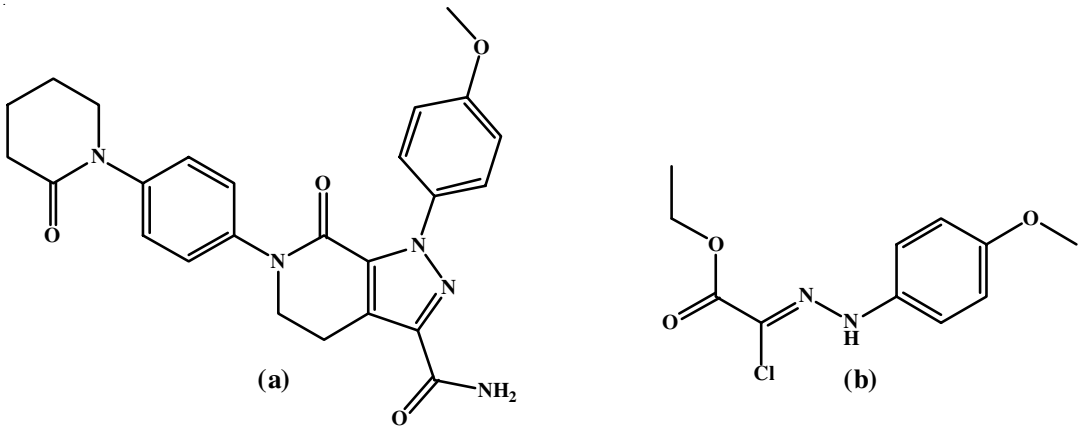


Fig. 1. Structure of the (a) apixaban an anticoagulant and (b) intermediate **1**

impurities, making this material more appropriate for large-scale industrial production.

EXPERIMENTAL

All reagents and solvents were commercially obtained and used as received. Glass-lined reactors, stainless steel reactor, gas induction reactor having variable rate agitation and a –10 to 150 ºC jacket temperature range were used for reactions run on a pilot scale. ^{1}H NMR spectra were recorded on a Bruker 300 MHz FT spectrometer in $CDCl_3$ and/or DMSO as solvent. Purity of compounds was assessed by HPLC on an Agilent Technologies 1200 series. Related impurities of 2-chloro-2-(2-(4-methoxyphenyl)hydrazono)acetate (**1**) were estimated by a gradient HPLC analysis method using Kromasil C8, (250 × 4.6 mm ID), 5 µ column. Mobile phase-A comprising a mixture of phosphate buffer and mobile phase-B consist of acetonitrile.

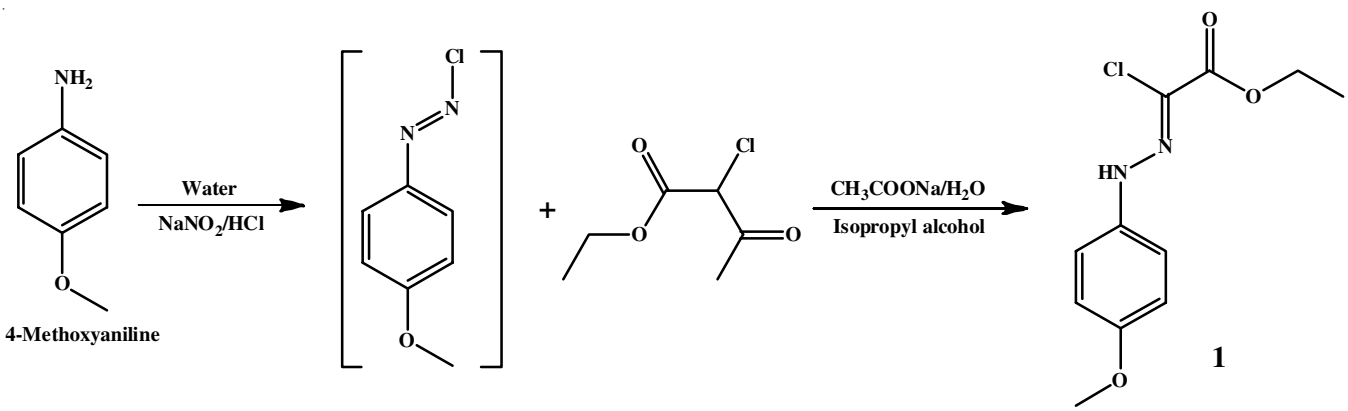
Method: The work in this research article is divided into two sections.

Section A: Development of efficient synthesis of ethyl (*Z*)-2-chloro-[(4-*p*-methoxyphenyl)hydrazone]ethyl acetate.

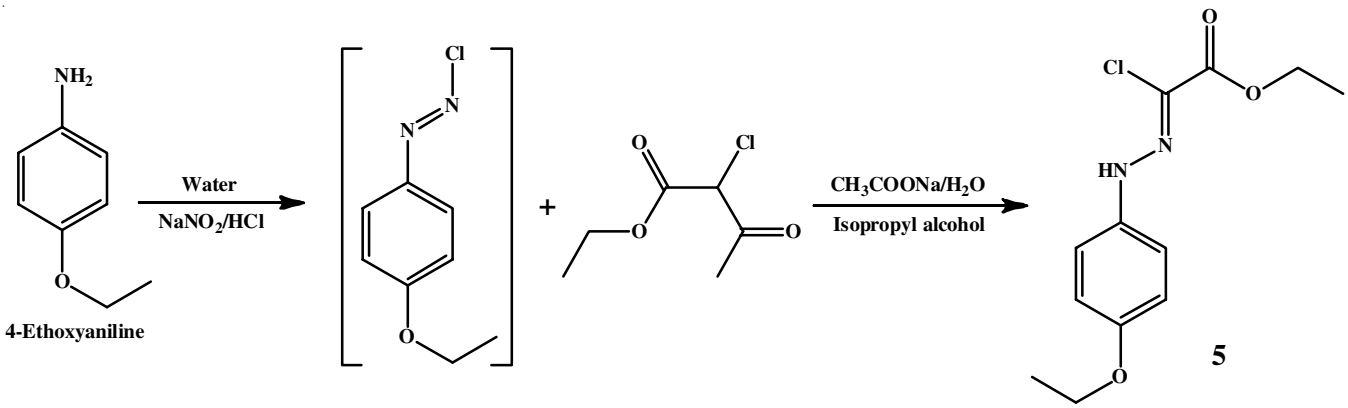
Section B: Control of potential impurities in ethyl (*Z*)-2-chloro-[(4-*p*-methoxyphenyl)hydrazone]ethyl acetate.

Synthesis of ethyl (*Z*)-2-chloro-[(4-*p*-methoxy-phenyl)-hydrazone]ethyl acetate (1): To a solution of *p*-anisidine (**2**) (25 g, 0.26 mol) in water (320 mL) at 0-5 ºC, HCl (35% w/w, 229 g, 2.2 mol) was added followed by the addition of sodium nitrite (16.75 g, 0.24 mol) slowly while stirred for 30 min at the same temperature. Water (100 mL) and sodium acetate (38.25 g 0.46 mol) were added sequentially to a solution of ethyl 2-chloro acetoacetate (**3**) (33.50 g, 0.20 mol) in ethyl acetate (100 mL). The resulting mixture was cooled to 0-5 ºC and stirred again for 1 h. The solution prepared in Part-A was added slowly over a period of 1 h at 0-5 ºC. Thereafter, reaction mass temperature was raised to 25-30 ºC and stirred for 1 h. The organic layer was separated and aqueous phase was extracted with ethyl acetate (100 mL). The combined organic layers were washed with water (150 mL) and concentrated. Finally, the obtained concentrated mass was crystalized in ethanol/water to afford ethyl (*Z*)-2-chloro-[(4-*p*-methoxyphenyl)hydrazone]-ethyl acetate (**1**) (**Scheme-I**). Light yellowish-brown colour solid was obtained with 24 g, yield 96 w/w. Purity by HPLC > 95.00. FT-IR (KBr, ν_{max}, cm^{-1}): 683.89 (C-Cl *str.*), 1714.86 (-C=O), 1708.51 (-C=N), 1225.92 (-C-O-C-), 1605.04 (C=C *str.*), 2981.08 (CH_3-CH_2, *str.*), 3257.05 (-NH *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.28 (s, 1H), 7.4-7.18 (m, 2H), 6.86-6.90 (m, 2H), 4.35-4.40 (q, 2H), 3.79 (s, 3H), 37-1.41 (t, 3H); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 159.70, 155.63, 135.29, 115.61, 114.49, 55.32, 55.31, 14.06. ESI-MS: *m/z* 257 [M+1].

Synthesis of (Z)-ethyl 2-chloro-2-(2-(4-ethoxyphenyl) -hydrazono)acetate (5): Compound **5** was synthesized in the same manner as for compound **1**, while using 4-ethoxy aniline (100 g, 0.81 mol) instead of *p*-anisidine (**2**) (**Scheme-II**). Light yellowish-brown colour solid was obtained with 9.8 g, yield 94%. m.p.: 96-98 ºC. Purity by HPLC > 99.00. FT-IR (KBr, ν_{max}, cm^{-1}):



Scheme-I



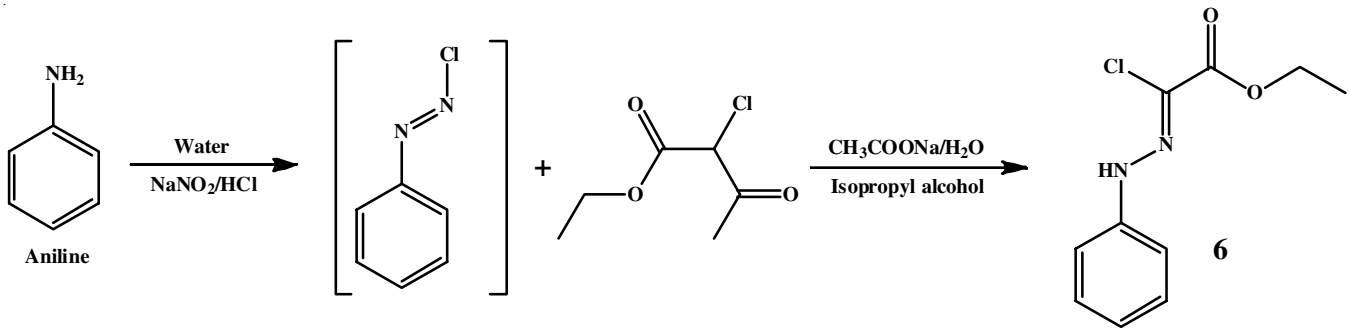
Scheme-II

562.64 (C-Cl *str.*), 1562.39 (-C=O), 1699.34 (-C=N), 1296.10 (-C-O-C- *str.* ether group), 2982.05 (C-H, *str.*), 3259.81 (-NH *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.27 (s, 1H), 7.14-7.18 (m, 2H), 6.68-6.90 (m, 2H), 4.35-4.40 (q, 2H), 3.79 (s, 3H), 1.37-1.41 (t, 3H); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 159.70, 155.63, 135.29, 115.61, 114.49, 55.32, 55.31, 14.06. ESI-MS: *m/z* 256.9.

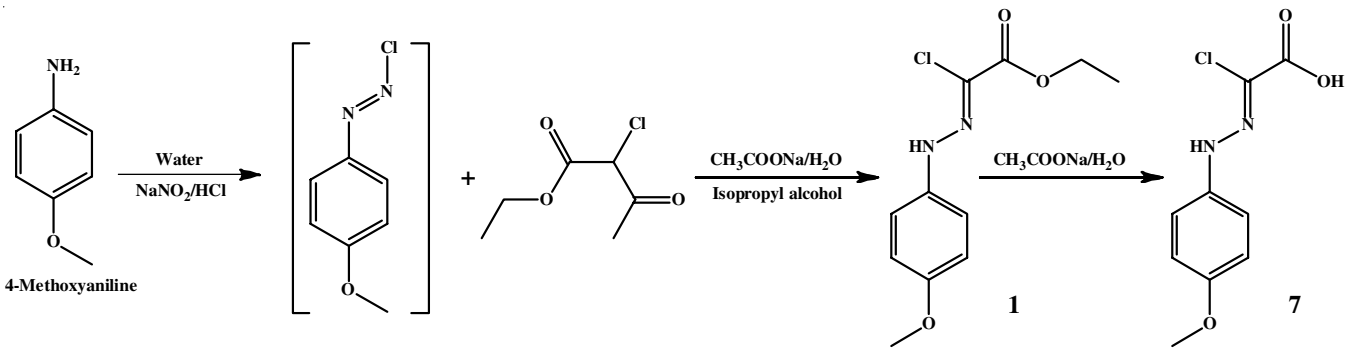
Synthesis of ethyl (2*Z*)-chloro-(phenylhydrazono)acetate (6): Compound **6** was synthesized in the same manner as for compound **1**, while using aniline (**10**, 25 g, 0.26 mol) instead of *p*-anisidine (**2**) (**Scheme-III**). Yellowish-brown colour solid was obtained with 24 g, yield 96 w/w. Purity by HPLC > 95.00. FT-IR (KBr, ν_{max}, cm^{-1}): 748.72 (C-Cl *str.*), 1714.86 (-C=O), 1708.51 (-C=N), 1225.92 (-C-O-C-), 1605.04 (C=C *str.*), 2981.08 (CH_3-CH_2 *str.*), 3279.32 (-CH-N, *str.*); ^{1}H NMR (400 MHz, DMSO, δ ppm): 10.56 (s, 1H), 7.30-7.38 (m, 4H), 6.97-7.02 (m, 1H), 4.25-4.32 (q, 2H), 1.28-1.32 (t, 3H); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 159.37, 142.70, 129.17, 122.37, 114.57, 113.52, 62.13, 14.07. ESI-MS: *m/z* 227.

Synthesis of (2*Z*)-chloro[(4-methoxyphenyl)hydrazono]-acetic acid (7): Following the same method, 4-methoxy aniline (100 g, 0.81 mol) was used (**Scheme-IV**). Light brown solid 150 g, yield 94%. m.p.: 96~98 ºC. Purity by HPLC > 95.00. FT-IR (KBr, ν_{max}, cm^{-1}): 698.80 (C-Cl *str.*), 1708 (-C=O), 1225.90 (-C-O-C- *str.* ether), 2833 (-C-H *str.*), 3259.81 (-NH); ^{1}H NMR (400 MHz, DMSO, δ ppm): 8.27 (s, 1H), 7.15-7.18 (d, 2H), 6.67-6.90 (d, 2H), 4.34-4.41 (m, 2H), 3.79 (s, 3H), 1.37-1.41 (t, 3H); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 159.84, 155.75, 135.43, 115.74, 114.53, 14.06. ESI-MS: *m/z* 226.

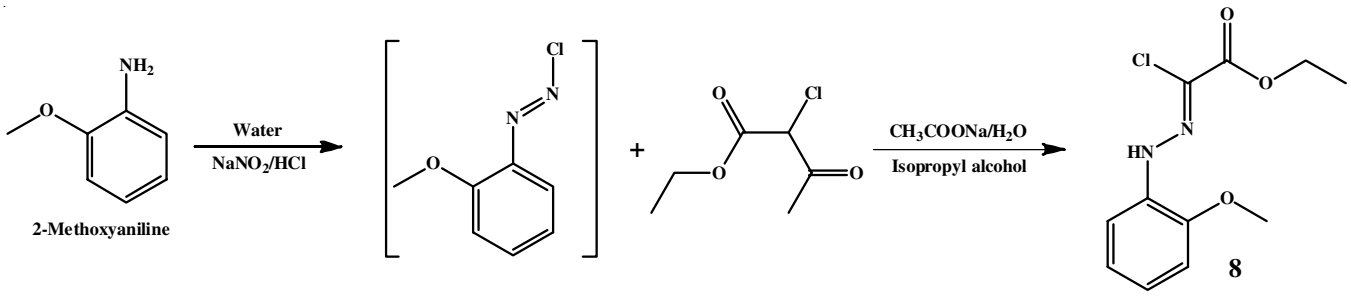
Synthesis of ethyl (2*Z*)-chloro[(2-methoxyphenyl) hydrazono]acetate (8): Following the same method, 2-methoxy aniline (**14**) (100 g, 0.81 mol)was used (**Scheme-V**). Light brownish-yellow solid, 9.8 g, yield 94%. m.p.: 96~98 ºC. Purity by HPLC > 99.00. FT-IR (KBr, ν_{max}, cm^{-1}): 698.80 (C-Cl *str.*), 1708 (-C=O),



Scheme-III



Scheme-IV



Scheme-V

1226.77 (-C-O-C- *str.* ether), 1562.39 (-C=O *str.*), 1699.34 (-C=N *str.*), 2982.05 (-C-H *str.*), 3259.81 (-NH); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.27 (s, 1H), 7.14-7.18 (m, 2H), 6.68-6.90 (m, 2H), 4.35-4.40 (q, 2H), 3.79 (s, 3H), 1.37-1.41 (t, 3H); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 159.70, 155.63, 135.29, 115.61, 114.49, 55.32, 55.31, 14.06. ESI-MS: *m/z* 256.9.

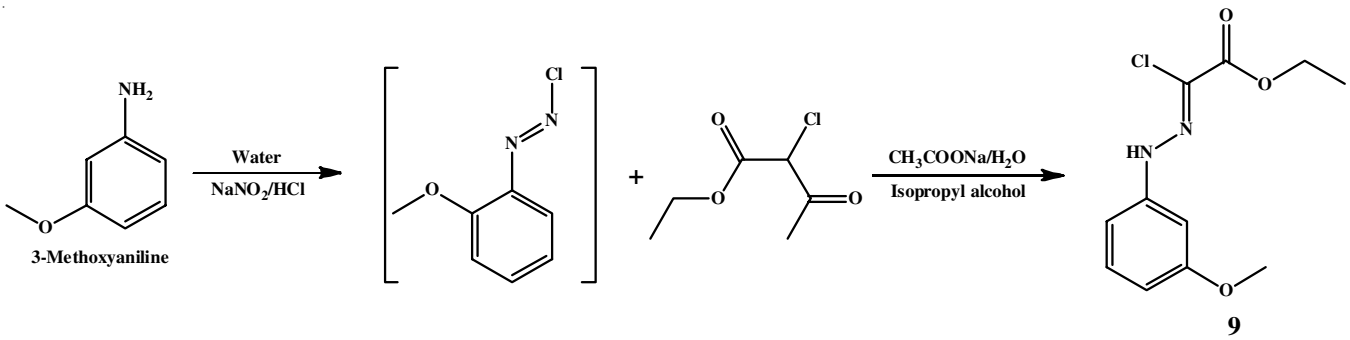
Synthesis of (Z)-ethyl 2-chloro-2-(2-(4-methoxyphenyl) hydrazono)acetate (9): Using 3-methoxy aniline (**16**) (100 g, 0.81 mol), synthesis of compound **9** was achieved (**Scheme-VI**). Tawny solid, 9.8 g, yield 94%. m.p.: 96~98 ºC. Purity by HPLC > 99.00. FT-IR (KBr, ν_{max}, cm^{-1}): 698.80 (Cl *str.*), 1226.77 (-C-O-C- *str.* ether), 1699.34 (-C=N *str.*), 1562.39 (-C=O *str.*), 2982.05 (-C-H *str.*), 3259.81 (-N-H *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.27 (s, 1H), 7.14-7.18 (m, 2H), 6.68-6.90 (m, 2H), 4.35-4.40 (q, 2H), 3.79 (s, 3H), 1.37-1.41 (t, 3H). ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 159.70, 155.63, 135.29, 115.61, 114.49, 55.32, 55.31, 14.06. ESI-MS: *m/z* 256.9.

RESULTS AND DISCUSSION

During the optimization study, compound **1** was synthesized by using different solvents like dichloromethane, chloroform and mixture of dimethoxymethane-water to improve the yield and purity of the product. The reaction did not even complete while using a mixture of dimethoxymethane and water sytsem. whereas the methanol-water system yielded variable results. Therefore, the utilization of these two solvents are discarded. The reaction with dichloromethane achieved completion on a modest scale, yielding a product of satisfactory purity; but, upon increasing the batch size, the reaction failed to reach completion. The usage of purified water for reaction was found to be suitable and reaction went to completion and obtained optimum yield and purity of the product. Hence, based on cost advantage purified water instead of solvent for the synthesis of ethyl (2*Z*)-chloro[2-(4-methoxyphenyl)hydrazinylidene]-ethanoate was freezed. Preliminary during the development, the quantity of ethyl-2-chloro acetoacetate and sodium nitrite used for reaction. Ethyl-2-chloroacetoacetate was around 1.40 mol (w.r.t *p*-anisidine) wherein no adverse effect was observed and satisfactory yield and purity was obtained. Looking at the scope to further reduce the ethyl-2-chloroacetoacetate quantity additional reaction was attempted by using 1.25 moles (w.r.t *p*-anisidine) of ethyl-2-chloroacetoacetate. The usage of 1.20 and 1.30 mol (w.r.t *p*-anisidine) of ethyl-2-chloroacetoacetate furnished the almost same yield and purity of the product. Hence the usage of 1.25 moles (w.r.t *p*-anisidine) of ethyl-2-chloroacetoacetate was selected for the synthesis of ethyl (2*Z*)-chloro[2-(4-methoxyphenyl)hydrazinylidene]ethanoate.

Preliminary during optimization of reaction temperature, reaction was conducted at 25-30 ºC, where in reaction was monitored by TLC. At this temperature reaction was found to be completed in 7-8 h. During the optimization of reaction temperature at 25-30 ºC, the reaction mass was very thick and contents were not dissolved at 25-30 ºC for 1 h. It observed that reaction also did not go to completion hence the reaction mass was heated to 30-35 ºC with this reaction mass was completely dissolved and additionally reaction was completed in 5-6 h. The obtained yield and purity of the product was good. Based on the experiment further optimization of reaction temperature was not performed. Hence, the reaction temperature of 30-35 ºC was selected for the synthesis of ethyl (2*Z*)-chloro-[2-(4-methoxyphenyl)hydrazinylidene]ethanoate, screening of reaction for the synthesis of compound **6** using compounds **12** and **14** as depicted in Table-1.

Though the structure of ethyl (*Z*)-2-chloro-[(4-*p*-methoxyphenyl)hydrazone]ethyl acetate is quite simple molecule, many processes related impurities and impurities due to positional



Scheme-VI

TABLE-1
SCREENING OF REACTION CONDITIONS FOR THE SYNTHESIS OF INTERMEDIATE **1**

Entry	Solvent	Ethyl-2-chloro acetoacetate quantity	$NaNO_2$ + water	Urea + water	CH_3COONa + water	Temp. (ºC)	Time (h)	Yield (%)	HPLC purity crude	HPLC purity pure
1	Purified water: methanol	1.3 eq.	70 + 50	50 + 75	475 + 980 pH = 3.5-4.0	25-30	6	70	89.71	99.57
2	Purified water: IPA	1.2 eq.	80 + 50	55 + 75	490 + 1010 pH = 3.5-4.0	25-30	6	74	91.03	99.68
3	Purified water: DME	1.4 eq.	75 + 50	0	540 + 1090 pH = 4.0-4.5	35-40	9	74	94.92	98.84
4	Purified water	1.25 eq.	70 + 50	0	600 + 1210 pH = 4.5- 5.0	35-40	9	72	94.15	98.37
5	Purified water	1.25 eq.	70 + 50	0	370 + 740 pH = 4.5-5.0	35-40	9	71	91.99	99.91
6	Purified water	1.25 eq.	70 + 50	0	390 + 780 pH = 4.5-5.0	35-40	9	74	90.89	99.75

isomers of *p*-anisidine are expected to be present in the final drug substances along with raw materials and intermediates. Impurities **5**, **6**, **7**, **8** and **9** observed in the crude of ethyl (*Z*)-2-chloro-[(4-*p*-methoxy-phenyl)hydrazone]ethyl acetate are attributed as process impurities as they are formed as byproducts due to side reactions.

Optimization of isopropyl alcohol: Preliminary during initial development around 8 volumes (w.r.t crude) of isopropyl alcohol:water was used for the purification of crude ethyl (2*Z*)-chloro[2-(4-methoxyphenyl)hydrazinylidene]ethanoate to be obtained good yield and purity. Further to increase the yield of product without affecting the purity of product, additional purification was attempted by using 45 and 6 volumes of isopropyl alcohol:water for purification. Based on the experimental results the usage of 6 volumes gave poor yield and good quality of product. Hence, the usage of above said volumes of isopropyl alcohol:water was ruled out for the purification of ethyl (2*Z*)-chloro-[2-(4-methoxyphenyl)hydrazinylidene]ethanoate. The usage of 4.0 volumes of isopropyl alcohol:water for the purification of ethyl (2*Z*)-chloro[2-(4-methoxyphenyl)hydrazinylidene]ethanoate gave good yield and purity. Hence 4.0 vol. of isopropyl alcohol:water for purification of ethyl (2*Z*)-chloro-[2-(4-methoxyphenyl)hydrazinylidene]ethanoate was selected. Table-2 optimization purification and drying conditions of intermediate **1**.

In summary, several observed and potential impurities of ethyl (*Z*)-2-chloro-[(4-*p*-methoxy-phenyl)hydrazone]ethyl acetate have been synthesized and characterized. The origins of formation of all the impurities during the synthesis were also ruled out. ln addition, a suitable purification method to lower the concentration of these impurities to levels accepted by ICH is developed. Green and efficient synthesis of intermediate **1** is developed in water as a solvent at ambient temperature, avoiding hazardous chemical and solvents. Robust and simple isolation procedure is developed to furnish intermediate **1** in 70-85 % yield. The results of lab consistency batch data for synthesis of intermediate **1** is shown in Table-3.

Conclusion

A novel, cost-effective, green and efficient synthesis of intermediate, ethyl (*Z*)-2-chloro-[(4-*p*-methoxyphenyl)hydrazone]ethyl acetate **(1)** is developed in water as a solvent at ambient temperature was achieved as per ICH guidelines. From the selected route of synthesis, we achieved the overall yield of 75-80%, With HPLC purity NLT 99% and single maximum impurity level of NMT 0.2%.

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-2
OPTIMIZATION PURIFICATION AND DRYING CONDITIONS OF INTERMEDIATE **1**

Entry	IPA (mL)	Filtration temperature (ºC)	Purification temperature (ºC)	Drying temperature (ºC)	Crude HPLC purity	Pure HPLC purity
1	800	25-35	55-65	60-65	93.94	99.41
2	600	0-5	25-30	50-55	93.23	99.43
3	400	0-5	40-45	40-45	93.23	99.87

TABLE-3
RESULTS OF LAB CONSISTENCY BATCH DATA FOR SYNTHESIS OF INTERMEDIATE **1**

Exp. No.	Water (mL)	*p*-Anisidine (g)	HCl (mL)	$NaNO_2$ + water	CH_3COONa + water	ECAA (g)	Dry wt. (g)
1	500	200	1000	140 + 300	800 + 1600	320	296
2	500	200	1000	140 + 300	800 + 1600	320	294
3	500	200	1000	140 + 300	800 + 1600	320	290

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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