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FACILE SYNTHESIS OF PROCESS RELATED IMPURITIES OF LOPINAVIR

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ABSTRACT

The Present research is directed towards the synthesis and Characterization of process related impurities appear in Lopinavir active pharmaceutical ingredient as per available pharmacopeias like E.P and USP drug substance monographs. In order to meet the stringent regulatory requirements, we have been synthesized total of seven pharmacopeia specified impurities and these are confirmed by their structural elucidation with the help of sophisticated analytical instruments of FT-IR, Mass, ^{1}H&$^{13}CNMR$ and those were validated with available RPHPLC method with respect to their corresponding retention factors.

Keywords: Lopinavir, Process Related, Impurities Synthesis as per USP, EP and Regulatory Authorities, Impurity B, C, D, F, G, I, Q

INDRODUCTION

The HIV protease inhibitor ABT-378 (Lopinavir) is the antiviral component of Kaletra (approved by FDA in September, 2000), the latest approved HIV protease inhibitor for the treatment of human immunodeficiency virus (HIV) infection. The presence of impurities, also called as, related substances in an active pharmaceutical ingredient (API). The Lopinavir related substances have been mentioned in European Pharmacopoeia and US Pharmacopoeia. The presence of Impurities in a drug substance can have a significant impact on the quality and safety of the drug product. According to the general guidelines on impurities in new drug substances recommended by the International Conference on Harmonization (ICH), the acceptable level for all impurities present should be less than 0.10%[1]. These impurities are also required in pure form to check the analytical performance characteristics, such as system suitability and relative correction factor.

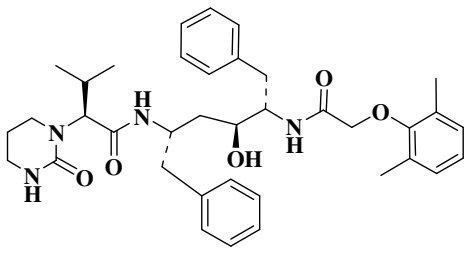

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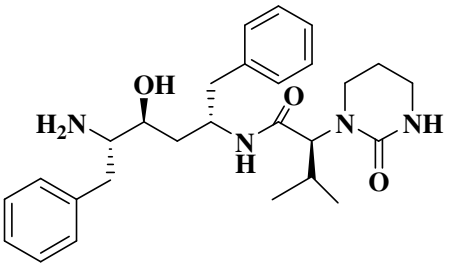
Fig.-1: Structure of Lopinavir

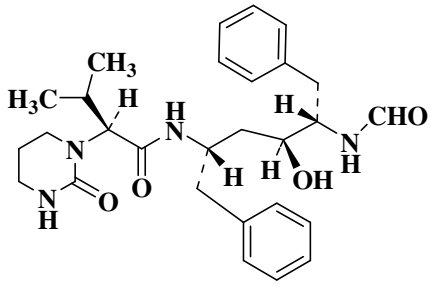
A literature survey revealed that the seven impurities which we mentioned here are not previously synthesized and characterized. None of available literature reported for seven impurities, the seven impurities synthesized were impurity B, C, D, F, G, I, and Q as per European pharmacopoeia. All these impurities are never present together while we are preparing bulk drug. These synthesis involved functional group inter conversion (FGI) such as Peptide coupling, reduction of carbonyl group to

hydroxyl, reduction of olefin bond, de-protection BOC group, formylation, acetylation, sulphonation, and de benzylation. Prior art literature shown that many of the reagents used for acid and amine coupling carbonyldiimidazole (CDI), acyl chloride formation using thionyl chloride[2], oxalyl chloride[3,4], dicyclohexyl carbodiimide (DCC)[5], acyl azides[6], 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)[7], Reductions reactions we done here for keto to hydroxyl functional group transformation and for reduction of double bond, reductions are reveals in many books, research papers[8]. Reduction is normally accomplished by using hydrogen and heterogeneous transition metal catalysts, e.g. Rh/C, Pd/C, Raney Nickel, or Adams catalyst (PtO2)[9]. A wide range of reagents encompassing protic acids, Lewis acids, organosilanes[10], have been proposed for de blocking the Boc group. HCl in organic solvents and TFA are the most frequently employed reagents for N-Boc de-protection function[11]. Various reagents for the synthesis of formamide have been developed including formic acid with activating reagents such as DCC[12], EDCI[13], CDMT[14], PEG-400[15], or Lewis acids [16], formic acid derivatives such as acetic formic anhydride[17], 2,2,2,-trifluoroethyl formate[18], and pentafluorophenyl formate[19] , cyano methyl formate[20] ammonium formate [21, 22]. The acetylation of amines or thiols is usually performed with acid anhydrides or acetyl chlorides in the presence of amine bases such as triethylamine or pyridine along with 4-(dimethyl amino) pyridine (which acts as a co-catalyst) or 4-pyrrolidinopyridine [23]. Sometimes tributylphosphine is also employed as a less basic catalyst for acetylation reactions, particularly for substrates that are relatively sensitive to strong bases[24]. Sulphonation reactions are carried mostly using chlororsulphonic acid. Other sulfonating reagents are sulfur trioxide, oleum, concentric sulphuric acid, sulfamic acid. The most common method of removal of benzyl is through the use of hydrogenation (Pd/C),[25] another method for removal utilizes a strong Lewis acid (such as $AlCl_3$)[26, 27]. Catalytic transfer hydrogenation has been successfully applied for removal of a benzyl group from protected benzyloxycarbonyl, benzyl ester and benzyl ester derivatives of peptides and amino acids using cyclohexene [28, 29], cyclohexadiene, hydrazine hydrate[30] and ammonium formate[31, 32] as the hydrogen donor. Deprotection of the N-benzyl group however is still most often carried out by traditional high pressure catalytic hydrogenation[33, 34].

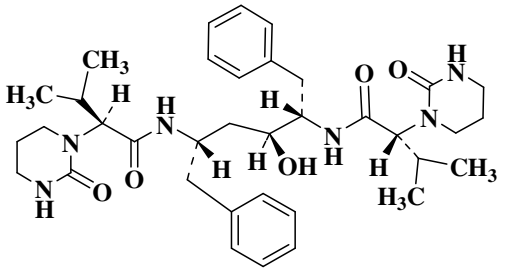
Here we have done all reactions using state of the art organic chemistry synthetic techniques.



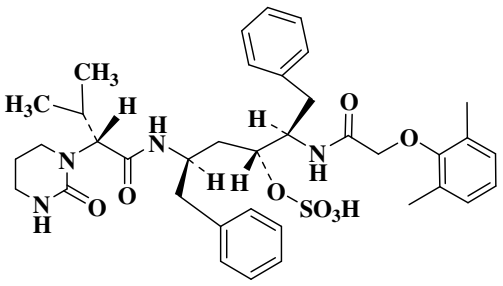
Impurity A (2)



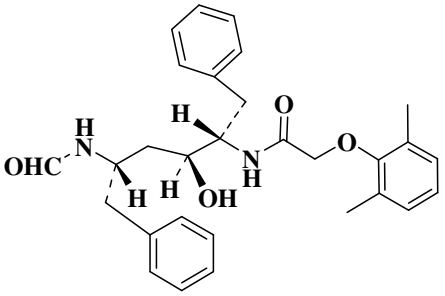
Impurity B (3)



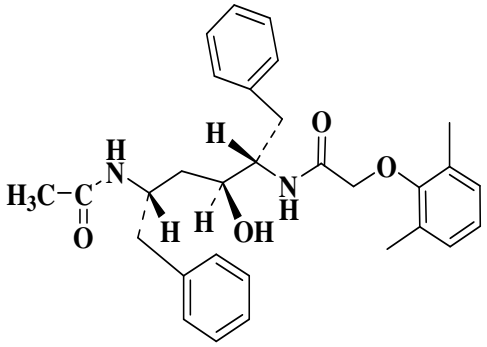
Impurity C (4)



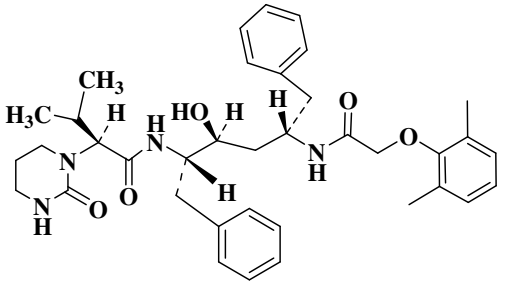
Impurity D (5)



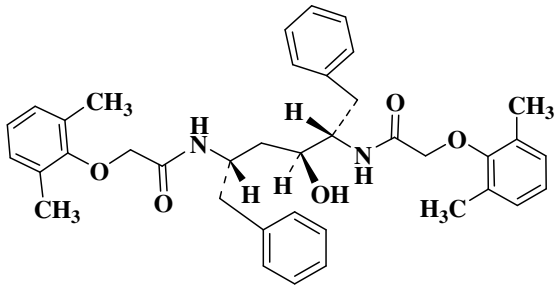
Impurity F (6)



Impurity G (7)



Impurity I (8)



Impurity Q (9)

Fig.-2: Structures of the impurities

EXPERIMENTAL

The FT-IR spectrum was recorded on Perkin Elmer Spectrum 100 FT-IR Spectrometer by using 1% potassium bromide pellet technique. The ^{1}H-NMR spectra were measured in $CDCl_3$ or DMSO-d6 as solvent on Bruker 400MHz NMR spectrometer. The ^{1}H-NMR chemical shifts are reported on δ scale in ppm relative to TMS. The mass spectrum was recorded on Agilent 6110AA ESI and APCI system. The solvents and reagents were used without further purification as received from the vendor.

Synthesis of (2S)-N-[(1S, 3S, 4S)-1-benzyl-4-(formylamino)-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl] butanamide (Impurity-B)

In 500ml three neck round bottom flask taken compound-2 (30 g, 0.064 mol) in 150ml of toluene addition followed by (14.72 g, 0.32 mol) formic acid and (9.9gm, 0.33mol) zinc dust at room temperature. Reactions mass stir it for 30 minutes at room temperature and then slowly raise the temperature for reflux, maintained up to TLC complies. After completion of the reaction filtered the in-organic salts over celite bed, concentrated the organic layer to adjust the pH~10-11 with 40% sodium hydroxide solution and the basified aqueous layer was extracted with methylene dichloride (3x 100 ml), the total organic layer was washed with Brine solution and then dried over anhydrous sodium sulphate. The obtained residue under vacuum distillation was subjected for silica-gel column purification resulting pure Impurity-B, was characterized and its analytical data is FT-IR (KBr): 3394, 2962, 1657, and 1308 cm^{-1}. ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.08-7.26 (m, 10H), 4.85 (s, 1H), 1.19 (d, 6H), 0.81-0.87 (m, 6H). MS (ESI): 495.33 $(M + H)^+$.

Synthesis of (2R)-N-[(1S, 2S, 4S)-1-benzyl-2-hydroxy-4-[[(2S)-3-methyl-2-[2-oxotetrahydro pyrimidin-1(2H)-yl] butanoyl] amino]-5-phenylpentyl]-3-methyl-2-[2-oxo tetra hydro pyrimidin-1(2H)-yl] butanamide (Impurity-C)

To a 400ml solution of toluene and THBA (40 g, 0.199mol) slowly added thionyl chloride (63.9 g, 0.53mol) at room temperature and maintain for 4-5 hours until TLC completion. If TLC once complies distilled out the excess thionyl chloride under reduced pressure and the obtained residue was stripped off with toluene (2x50ml) to ensure the total removal of thionyl chloride and the obtained acid chloride solution in DMF added to a cooled solution of compound 2 (50 g, 0.107 mol), imidazole (39.33g, 0.57mol) in 300ml of ethyl acetate at 0 to 5°C and maintain for 2-3 hours, check TLC. Quench the reaction mass with 300ml of water, separate the organic layer, washed with 100ml of 0.5 N HCl solutions, with 5% sodium carbonate solution, and then washed with brine solution and dried over anhydrous sodium sulphate. Concentrate the organic layer under vacuum, the residue taken for silica-gel column purification with chloroform and methanol used as a mobile phase. The pure compound analytical data is FT-IR (KBr): 3368, 2962, 1634, 1509, 1307, cm^{-1}. ^{1}HNMR (400 MHz, CDCl3): δ 7.14-7.28 (m, 10H), 3.2 (m, 4H), 2.78 (m, 2H), 2.21 (d, 2H), 1.58 (m, 4H), 0.85-1.01 (m, 6H). MS (ESI): 649.44 $(M + H)^+$.

Synthesis of (1R, 3R)-1-[(1R)-1-[[2-(2, 6-dimethylphenoxy) acetyl] amino]-2-phenylethyl]-3-[[(2R)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl] butanoyl] amino]-4-phenylbutyl hydrogen sulfate (Impurity-D)

To a cooled solution of compound-1 (30 g, 0.047mol), pyridine (18.5 g, 0.23mol) in 300ml of methylene dichloride added chlororsulphonic acid (14.08g, 0.08mol) at 0 to 5°C and the whole reaction mass maintained for overnight. After completion of TLC, the reaction mass quenched with 150ml of water, separated the organic layer and is washed with 6N hydrochloric acid (50ml), brine solution and dried over anhydrous sodium sulphate. The obtained residue after vacuum distillation taken in to 100ml of methyl tertiary butyl ether and stirred for 30 minutes then, isolated solid material was filtered, dried and its characterized data is FT-IR (KBr): 3420, 2963, 1639, 1525, 1309, 1197, 766 cm^{-1}. ^{1}HNMR (400 MHz, $CDCl_3$): δ 6.96-7.33 (m, 13H), 2.95 (m, 4H), 2.15 (s, 6H), 1.97 (m, 2H), 0.84-0.86 (d, 6H); MS (ESI): 709.36 $(M + H)^+$.

Synthesis of 2-(2, 6-Dimethylphenoxy)-N-[(2S, 3S, 5S)-5-formamido-3-hydroxy-1, 6-diphenyl hexan-2-yl] acetamide (Impurity-F)

Compound **21** (25g, 0.0559 mol), formic acid (20.57g, 0.44mol) and catalytic amount of zinc dust taken in 150 ml of toluene at room temperature. Slowly heated to reflux, maintained the reaction for 3-4 hours and after completion of the TLC reaction mass cool to room temperature and then filtered over celite. The filtrate was washed with 10% aqueous sodium hydroxide solution followed by brine solution. The separated organic layer was dried over anhydrous sodium sulphate, removed the solvent under vacuum pressure and the resulting compound was purified on silica-gel column chromatography. The pure impurity-F was characterized and its analytical data is FT-IR (KBr): 3315, 3030, 2923, 1660, and 1197 cm^{-1}. ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.06 (s, 1H), 6.98-7.29 (m, 13H), 3.77-4.19 (s, 4H), 2.99 (d, 1H), 2.91 (m, 1H), 2.16 (s, 6H). MS (ESI): 475.27 $(M + H)^+$.

Synthesis of N-[(1S, 2S, 4S)-(4-acetylamino)-1-benzyl-2-hydroxy-5-phenylpentyl]-2-(2, 6-dimethylphenoxy) acetamide (Impurity-G)

To a cooled solution of compound **21** (30 g, 0.064mol) in 300ml of dry chloroform taken in 500ml round bottom flask (0 to 5°C), added 50ml of acetic acid and catalytic amount of aluminum chloride and after 15 minutes stirring slowly added acetic anhydride (18.8g, 0.19mol) ,after addition maintained the reaction mass for overnight at room temperature. The next day upon completion of the TLC the reaction poured in 250ml of ice cold water, separated the organic layer washed with 5% aqueous sodium bicarbonate solution followed by brine solution and dried over anhydrous magnesium sulphate. The residue obtained after distillation under reduced pressure taken in methyl tertiary butyl ether to isolate the pure solid Impurity-G and its characterized data is FT-IR (KBr): 3320, 3029, 2921, 1644, 1198, cm^{-1}. ^{1}HNMR (400

MHz, $CDCl_3$): δ 6.99-7.25 (m, 13H), 4.15 (m, 3H), 2.98 (d, 2H), 2.80 (t, 2H), 2.17 (s, 6H), 1.89 (s, 3H). MS (ESI): 489.27 $(M + H)^+$.

Synthesis of (2S)-N-[(1S, 2S, 4S) -1-benzyl-4-[[2-(2, 6-dimethyl phenoxy) acetyl] amino]-2-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxo-tetrahydropyrimidin-1(2H)-yl] butanamide (Impurity-I)

Preparation of compound-22

The acid chloride of dimethyl phenoxy acetic acid (13 g, 0.072) has prepared as per the procedure mentioned in impurity-C, prepared dimethyl phenoxy acid chloride was slowly added to a solution of compound 12 (21 g, 0.045 mol), imidazole (15.3g, 0.22mol) and dimethyl amino pyridine(2.76g, 0.022mol) in 100ml of ethyl acetate, stir for overnight at room temperature. After completion of reaction the whole mass was quenched in 200ml of water, separate the organic layer washed with dilute hydrochloric acid solution, sodium bicarbonate solution and dried the organic layer. The organic residue obtained after vacuum distillation the compound 22 was isolated with ethyl acetate and heptane mixture ratio (1:1).

Preparation of compound-23

Refluxed a mixture of (10g, 0.016mol) compound-**22**, 1gm of 10% palladium on carbon, Ammonium formate (6g, 0.095mol) in 100ml of methanol for 6-7 hours, after completion of TLC the catalyst was filtered through celite bed and the resulting residue after filtrate distillation was dissolved in 50ml of methylene dichloride, washed with 60ml of 1N sodium hydroxide, brine solution and dried over anhydrous sodium sulphate. The organic layer was distilled under reduced pressure yielded compound-23 and was isolated in n-hexane.

Preparation of Impurity-I

The acid chloride of THBA has prepared as per the procedure mentioned in impurity-C, prepared THBAC (12g, 0.055mol) was slowly added to a solution of compound-**23** (24.5 g, 0.055mol), imidazole (7.4g, 0.11mol) in 100ml of ethyl acetate at 0 to 5°C and after addition the entire reaction mass maintain for overnight under stirring at room temperature. After completion of the reaction quenched in 80 ml of DM water, separated the organic layer, washed with 0.5 N HCl and with 5% sodium bicarbonate solutions. The crude material obtained after distillation taken for Silica-gel column purification (mobile phase: methanol/ chloroform). The pure fraction was characterized with FT-IR (KBr): 3366, 2962, 1664, 1535, 1311, and 1197cm^{-1}. ^{1}HNMR (400 MHz, $CDCl_3$): δ 6.91-7.25 (m, 13H), 5.31 (s, 1H), 2.80-3.10 (m, 6H), 2.11 (s, 6H), 0.83-0.90 (d, 6H). MS (ESI): 629.38$(M + H)^+$.

Synthesis of N-[(1S, 2S, 4S)-1-benzyl-4-[[2-(2, 6-dimethylphenoxy) acetyl] amino]-2-hydroxy-5-phenylpentyl]-2-(2, 6-dimethylphenoxy) acetamide (Impurity-Q)

The acid chloride of 2, 6-dimethyl phenoxy acetic acid (13 g, 0.072) has prepared as per the procedure mentioned in impurity-C, prepared 2, 6-dimethyl phenoxy acid chloride was slowly added to a solution of compound-21(20 g 0.044 mol), imidazole (14.9g, 0.22mol) and N, N-dimethyl amino pyridine (5.37g, 0.004mol) in 200ml of ethyl acetate, after addition was completed the mass leaved for room temperature stirring for overnight. The next day morning checked TLC and it complies, then the reaction mass taken into 100ml of DM water, separated the organic layer washed with 0.5M HCl, 5% sodium bicarbonate and finally with brine solution. Concentrated the organic layer, the resulting residue was purified in 1:1 mixture of Ethyl acetate and heptane and isolated pure impurity characterized by FT-IR (KBr): 3298, 2923, 1650, 1265 cm^{-1}. ^{1}HNMR (400 MHz, $CDCl_3$): δ 6.98-7.29 (m, 16H), 4.21 (s, 4H), 2.10-2.18 (s, 12H). MS (ESI): 609.38 $(M + H)^+$.

RESULTS AND DISCUSSION

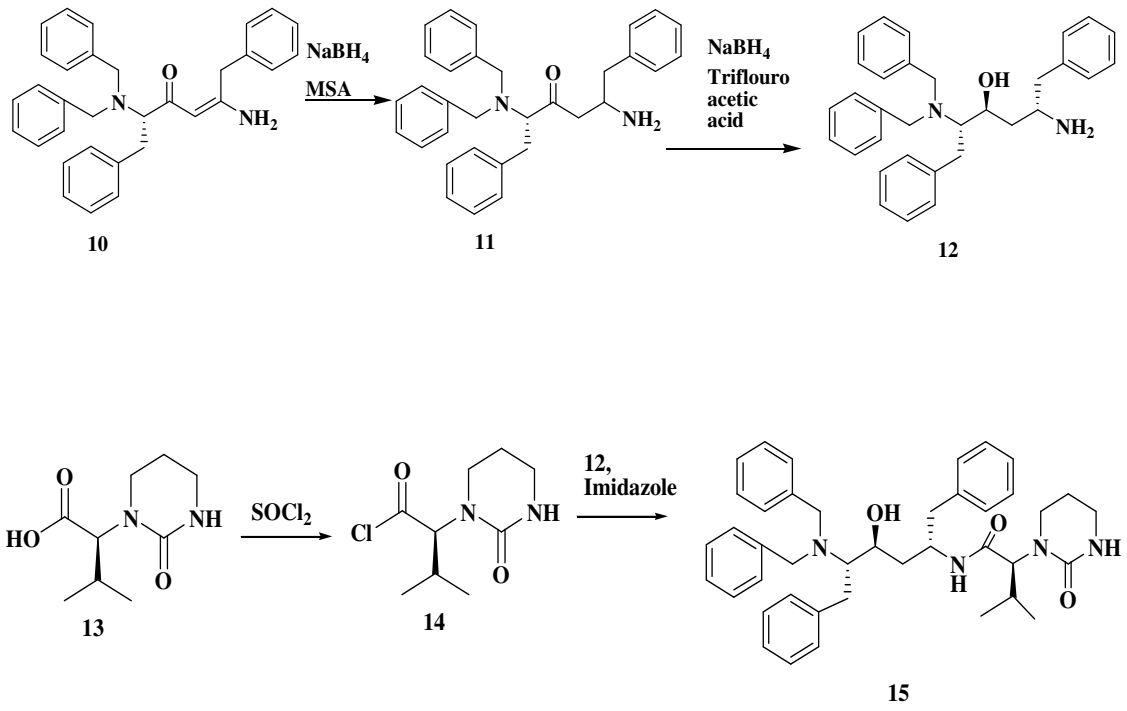
Few literatures are available for synthesis of Lopinavir active pharmaceutical ingredient[35, 36] and its feasible routes of synthesis were described in innovator patent[37], United States Patent no. 5914332 [as shown in the Scheme-1 and Scheme-2].

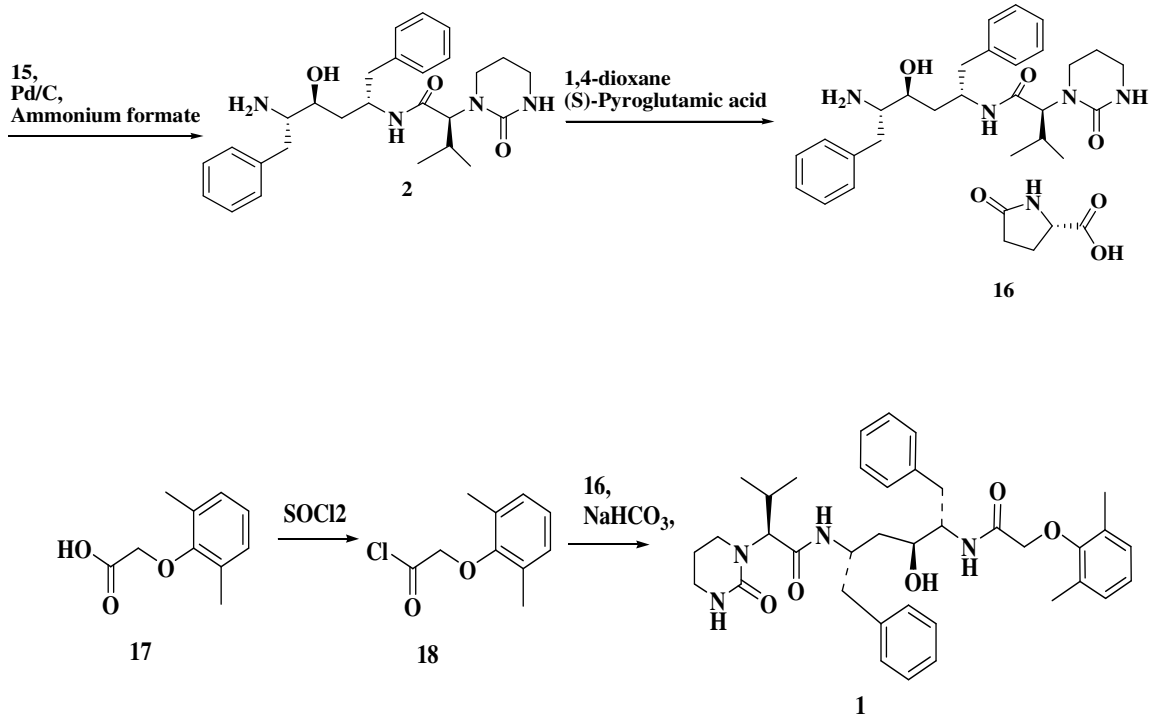
Scheme-1 represents process for the preparation of enaminone which is reacted with metal borohydrides like sodium borohydride in the presence of methane sulphonic acid to get keto intermediate (in situ) is again reacted with another mole of sodium borohydride in presence of triflouro acetic acid offered compound-**12.** The compound-**14** (**THBAC**) is under go condensation reaction with compound-**12** leads to the formation of compound-**15**.

The catalytic reduction of Compound-**15** with 10% palladium on carbon without hydrogen pressure obtained compound-**2**(Impurity-**A**). Finally the pyro glutamic acid salt of Compound-**2** coupled with compound-**18** gives Lopinavir.

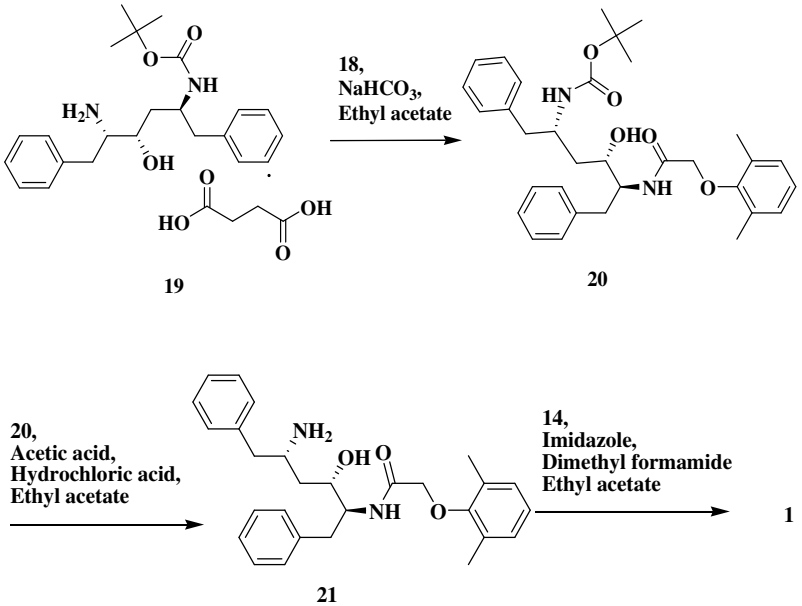
Scheme-2 involved three steps synthesis from compound-**19,** which is first reacts with compound-**18** of 2, 6-Dimethyl phenoxy acid chloride (**DMPAAC**) with 2-(2, 6-dimethylphenoxy) acetyl chloride to give (2s, 3s, 5s)-2-(2, 6-imethylphenoxyacetylamino-3-hydroxy-5-(t-butyloxycarbonylamino)-1, 6-diphenylhexane (Compound-**20**) , which is subjected for BOC deprotection in presence an acid like hydrochloric acid predominates the compound-**21** and is allowed for coupling reaction with (**THBAC**) Compound-14 in presence of base like imidazole and dimethyl formamide leads to the formation of Lopinavir.

During the course of synthesis of Lopinavir with selected route of synthesis some of the unwanted chemicals forming as byproducts in the process, those are still remains with active pharmaceutical ingredient in the final stage. So these by products are called as impurities as per ICH Q3A, ICHQ3C, and as per other regulatory guidelines. The important step in the impurity profiling is the synthesis of the material (Impurity standard) with the proposed structure. The Spectral retention time, characterization data of synthesized material matches with the impurity in question is useful for analytical method development and validation.





Scheme-1: Synthesis of Lopinavir (Route-1)



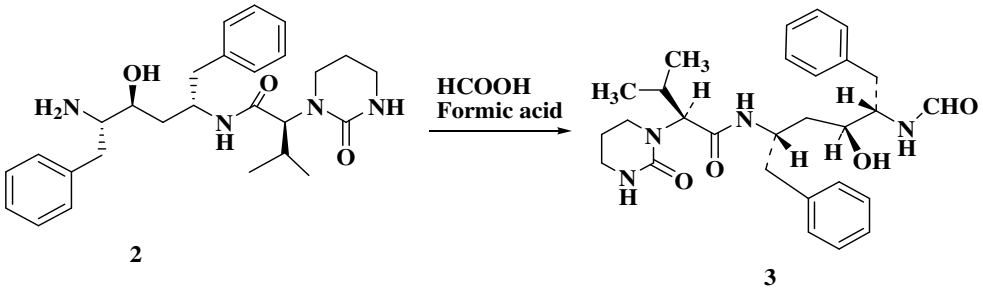
Scheme-2: Synthesis of Lopinavir (Route-2)

Impurity-A (2)

In the monograph of US pharmacopoeia, (2S)-N-[(1S, 3S, 4S)-1-benzyl-4-amino-3-hydroxy-5-phenylpentyl]- 3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl] butanamide chemical name represents the **Compound-2** as a process related impurity and same thing as per European pharmacopoeia known as **Impurity-A**, It is an intermediate of Scheme-1.

Impurity-B (3)

The Chemical name (2S)-N-[(1S, 3S, 4S)-1-benzyl-4-(formylamino)-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl] butanamide is known as **Compound-3** , **Impurity-B** as per US pharmacopoeia and European pharmacopoeia respectively, which is prepared by attempting simple N-formylation reaction of **Impurity-A** by using formic acid and acetic acid as shown as in Scheme-3.



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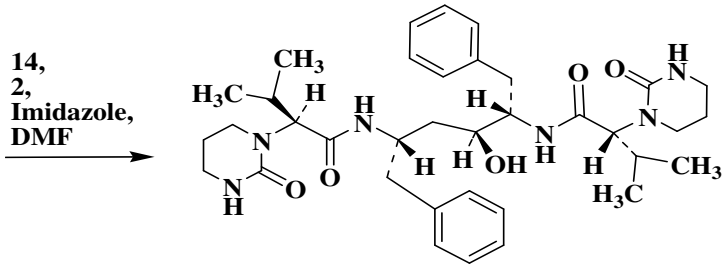
Scheme-3: Synthesis of impurity-B

Impurity-C (4)

As per E.P, USP the Chemical name (2R)-N-[(1S, 2S, 4S)-1-benzyl-2-hydroxy-4-[[(2S)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl] butanoyl] amino]-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2H)-yl] butanamide is known as **Impurity-C** and **Compound-4**. This is synthesized by taking Impurity-A as precursor for coupling reaction with **THBAC**.



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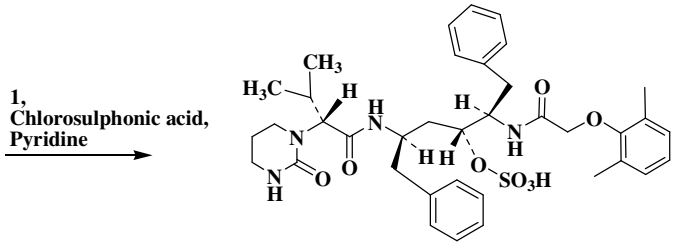
Scheme-4: Synthesis of impurity-C

Impurity-D (5)

Compound-5 is also known as Impurity-D as per European pharmacopoeia and (sulfo Lopinavir) as per US pharmacopoeia. Its Chemical name is (1R, 3R)-1-[(1R)-1-[[2-(2, 6-dimethylphenoxy) acetyl] amino]-2-phenylethyl]-3-[[(2R)-3-methyl-2-[2 oxotetrahydropyrimidin-1(2H)-yl] butanoyl] amino]-4-phenylbutyl hydrogen sulfate. This impurity was prepared by a conventional chemical method; sulphonation of hydroxyl group present in Lopinavir with chlorosulphonic acid in presence of pyridine used as catalyst as mentioned in Scheme-5.



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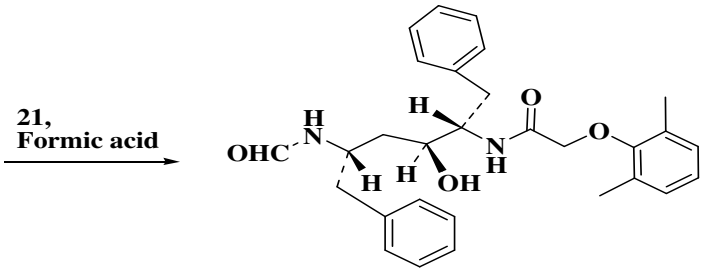
Scheme-5: Synthesis of Impurity-D

Impurity-F (6)

Compound-6 is impurity F as per European pharmacopoeia and **Lopinavir N-Formylphenoxyacetamide** as per US pharmacopoeia. Its Chemical name is N-[(1S, 2S, 4S)-1-benzyl-4-(formylamino)-2-hydroxy-5-phenylpentyl]-2-(2, 6-dimethylphenoxy) acetamide and is synthesized by formylation compound-21 by using formic acid.



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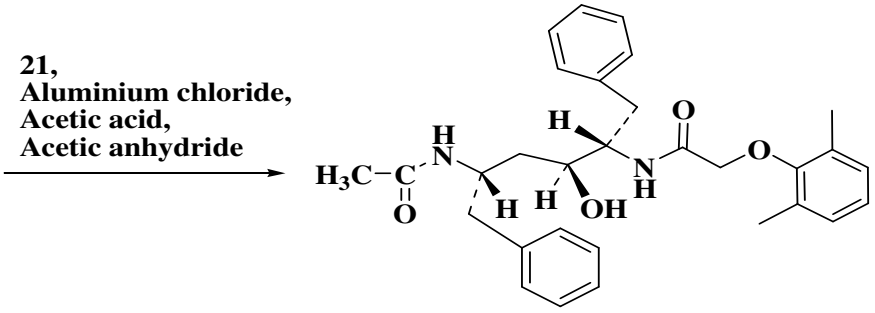
Scheme-6: Synthesis of impurity-F

Impurity-G (7)

The Chemical name of N-[(1S, 2S, 4S)-(4-acetylamino)-1-benzyl-2-hydroxy-5-phenylpentyl]-2-(2, 6-dimethylphenoxy) acetamide (Compound-**7**) is also known as Impurity-G as per E.P and (Lopinavir N-Acetylphenoxyacetamide) US Pharmacopoeia Impurity G is synthesized as mentioned in scheme-VII, Acetylation of compound-21 with the help of acetic anhydride, acetic acid and anhydrous aluminum chloride used as a catalyst.



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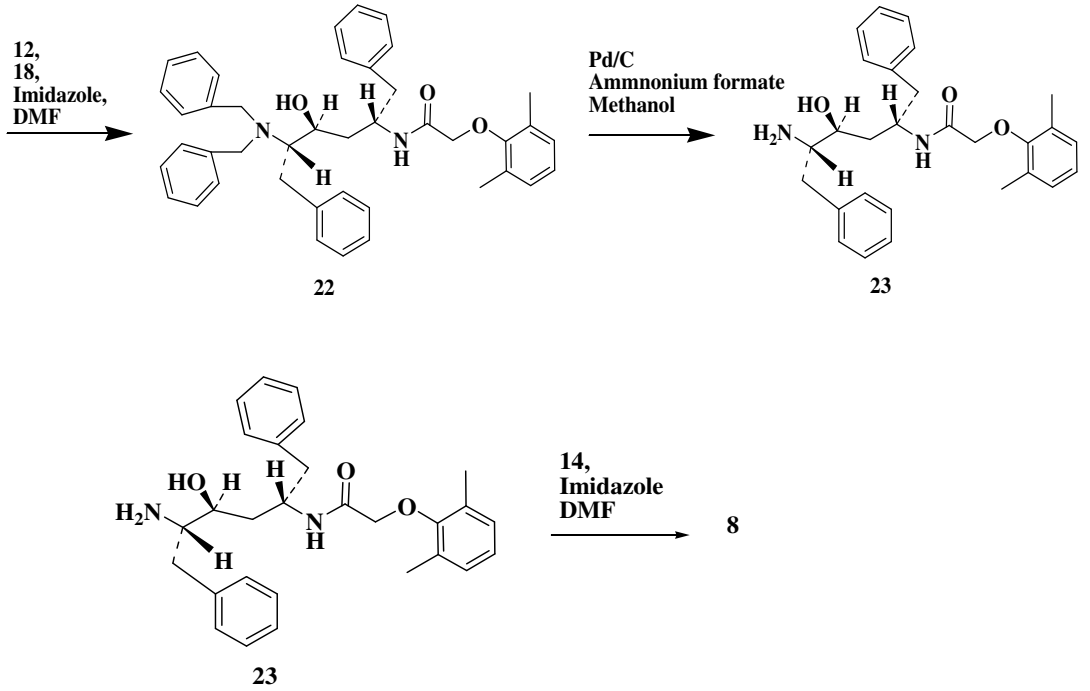
Scheme-7: Synthesis of impurity-G

Impurity-I (8)

Compound-8 is Impurity-I as per European pharmacopoeia, Chemical name is (2S)-N-[(1S, 2S, 4S)-1-benzyl-4-[[2-(2, 6-dimethylphenoxy) acetyl] amino]-2-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxo-tetrahydropyrimidin-1(2H)-yl] butanamide. This Impurity is prepared by conventional coupling method of compound-**12** with compound-**18** followed by de benzylation with Palladium on carbon with Ammonium formate and coupled with compound **23**.

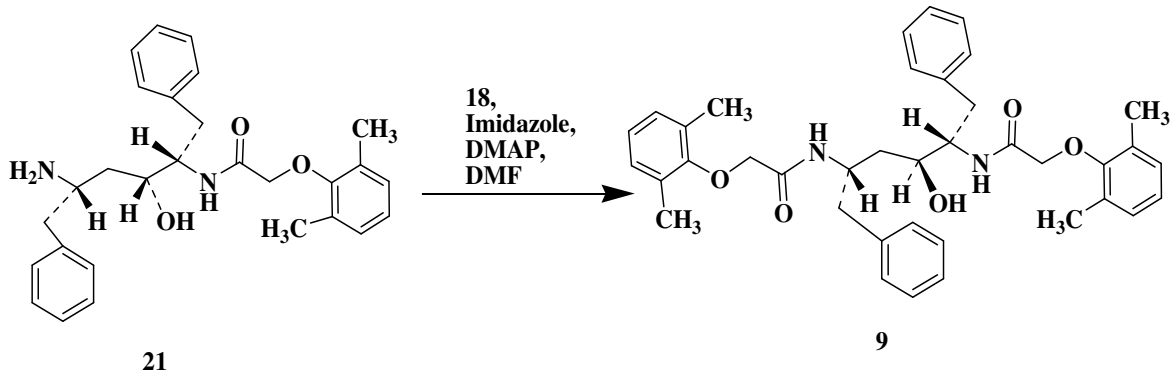


22 23 23

Scheme-8: Synthesis of impurity-I

Impurity-Q (9)

Compound-9, Impurity-Q as per European pharmacopoeia and Lopinavir diamide as per US pharmacopoeia and chemical name is N-[(1S, 2S, 4S)-1-benzyl-4-[[2-(2, 6-dimethylphenoxy) acetyl] amino] -2- hydroxy-5-phenyl pentyl]-2-(2, 6-dimethylphenoxy) acetamide Impurity-Q, which is prepared by coupling of compound-**21** with compound **17.**



21 9

Scheme-9: Synthesis of impurity-Q

CONCLUSION

After come across many of the literatures of Lopinavir and its related compounds synthesis, we finally concluded that our justified route of synthesis of each impurity of Lopinavir is novel and unique. The synthesized all impurities were confirmed by structural elucidation and as well as their respective relative retention times in HPLC; hence the required relative substances of Lopinavir were fulfilled as per Pharmacopeia needs.

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