The Pharma Innovation Journal 2021; 10(12): 2255-2258

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(12): 2255-2258

www.thepharmajournal.com
Received: 18-10-2021
Accepted: 20-11-2021

Poonam Gupta
Department of Chemistry,
M.M.H. College, Ghaziabad,
Uttar Pradesh, India

Ajet Saxena
Menkem Nature Research,
Ghaziabad, Uttar Pradesh, India

Corresponding Author:
Poonam Gupta
Department of Chemistry,
M.M.H. College, Ghaziabad,
Uttar Pradesh, India

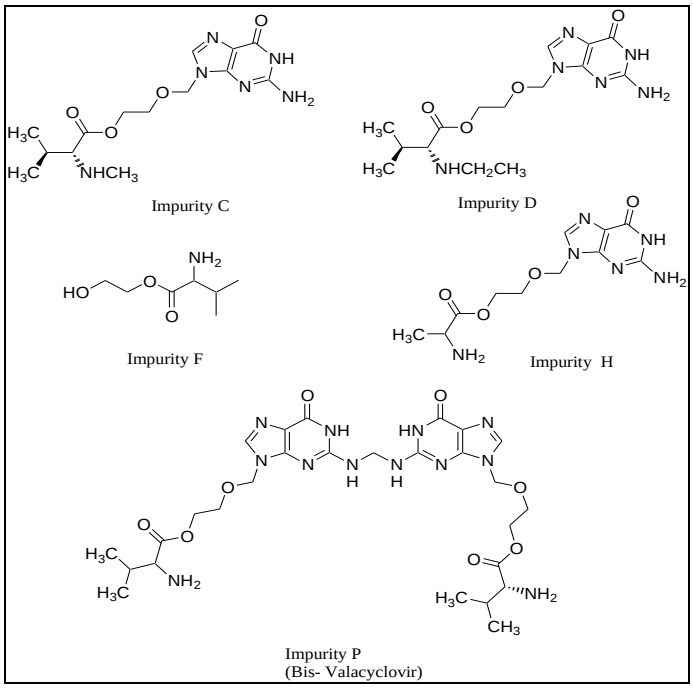
Synthesis of related substances of antiviral drug Valacyclovir

Poonam Gupta and Ajet Saxena

Abstract
Valacyclovir Hydrochloride has the chemical name L-valine -2-[(2-amino-1,6-dihydro-6-oxo-9-H-purin-9-yl-) methoxy] ethyl ester monohydrochloride, is prodrug and the ester derivative of acyclovir, a purine nucleoside analogue, useful in the treatment of anti-viral infections. European pharmacopeia related substances C, D, F, H, I, J, P and O and others impurities have been obtained during its synthesis. The present work describes the detection, origin, synthesis, characterization of some related substances, which may improve the commercial process.

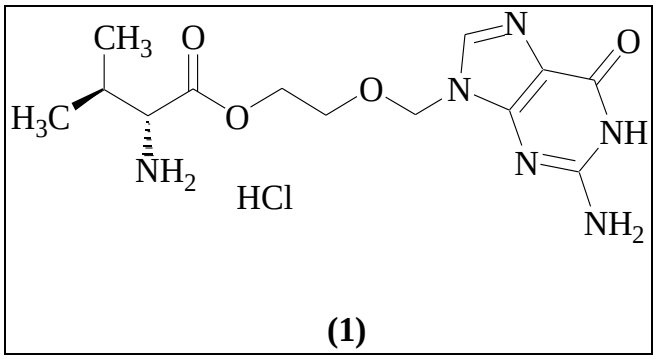
Keywords: Valacyclovir, impurities, antiviral, synthesis

Introduction
Valacyclovir Hydrochloride has the chemical name L-valine -2-[(2amino-1,6-dihydro-6-oxo-9-H-purin-9-yl-) methoxy] ethyl ester monohydrochloride (1), is prodrug and the ester derivative of acyclovir [1], a purine nucleoside analogue, useful in the treatment of antiviral infections[2-3](herpes simplex). A literature [4] survey revealed that in the ICH guidelines and FDA, the valacyclovir drug has impurity profile with more than seventeen impurities in pharmacopeial HPLC method. The synthesis of the most of the impurities are not reported in literature. The development of a drug substance is incomplete without the identification of an impurity profile involved in the process. Thus, in our study we explored the identification, synthesis and characterization of impurities found during synthesis of valacyclovir. This study will be of immense help for organic chemist to understand the potential impurities in valacyclovir synthesis and there by to obtain pure compound. The structures of synthesized impurities are given below



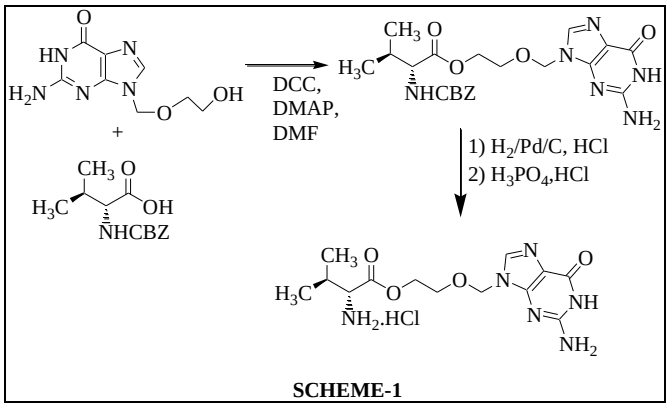
Material and methods

The antiviral drug acyclovir is derived from guanine and has prominent activity against viral infections. But due to the less solubility there is low absorption of acyclovir in gastrointestinal tract in human body whereas Valacyclovir exhibit more favorable and better pharmacokinetic characteristics, requiring less frequent dosing and achieving high blood plasma levels than acyclovir [5-7].



(1)

The literature survey revealed various synthetic method of Valacyclovir [8, 9]. The most common synthesis of valacyclovir is shown in scheme -1 in which N-protected Valine is condensed with acyclovir in presence of 4-dimethyl amino pyrimidine (DMAP) and dicyclohexyl carbodiimide (DCC) in dimethyl formamide (DMF), to give protected valacyclovir. The Carbobenzyloxy group was removed by catalytic hydrogenation using palladium on carbon to give valacyclovir. It was subsequently converted in to hydrochloride salt. The process can be depicted by following reaction scheme.



SCHEME-1

Results and Discussion

In this article we have disclosed our work regarding the synthesis and characterization of the various potential impurity of Valacyclovir Hydrochloride.

Preparation of Impurity C (2-[(2-Amino-6-oxo-1,6-dihydro-9H-purin-9-yl) methoxy]ethyl-N-methyl-L-valinate)

This impurity is formed by the condensation of N-methyl-N-Carbobenzyloxy valine with acyclovir in presence of dicyclohexyl carbodiimide catalyst and 4-dimethyl amino pyrimidine as a base in DMF. The condensed material is hydrogenated using palladium on carbon in presence of conc. hydrochloric acid and methanol under hydrogen pressure. After completion and workup of the reaction, the impurity C is formed as hydrochloride salt. The mass spectrum shows protonated molecular ion peak at m/z 339.The IR spectrum showed the presence of aliphatic and aromatic C-H Stretching (2967 cm^{-1},2927 cm^{-1} and 3111 cm^{-1}) respectively. It also shows the C=O Stretching (1732cm^{-1},1690 cm^{-1}) which corresponding to the ester and amide group. In proton NMR (solvent D_2O) spectrum N-methyl appeared as a singlet at δ2.68, and the terminal methyl group of isopropyl group appeared as a doublet at δ0.826 and δ0.837 and the proton present in guanine ring appeared as a singlet at δ8.07.

Preparation of Impurity D (2-((2-amino-6-oxo-1H-purin-9(6H)-yl) methoxy) ethyl 2-(ethylamino)-3-methylbutanoate)

The synthesis of impurity D involves the N-ethylation of N-Carbobenzyloxy -L-Valine with ethyl iodide in presence of sodium hydride as a base in the mixture of solvent tetrahydrofuran and dimethyl formamide under reflux condition. The resultant intermediate ethyl ester of N-CBZ-L-Valine is hydrolyzed. The hydrolyzed product is condensed with acyclovir in presence of DCC as catalyst and DMAP as base and in solvent DMF. After catalytic hydrogenation, the impurity D was formed. The protonated molecular ion of impurity D appeared as the base peak at m/z 353 in mass spectra. The IR spectrum showed the presence of C=O stretching (1728cm^{-1}, 1633cm^{-1}) corresponding to presence of amide and ester group. In the proton NMR spectrum (solvent D_2O), the peak of N-methylene is showed at δ3.10(multiplet), The peak of 2-CH_3 of isopropyl group showed at δ0.83 and δ0.88 (double doublets), the methyl group of N-ethyl group showed peak at δ1.2 (triplet). The peak of proton of guanine ring is showed at δ8.5 singlet.

Preparation of Impurity F (L-Valine 2-hydroxyethyl ester)

It is acid degradative impurity of valacyclovir drug. This impurity is synthesized by the condensation of N-Carbobenzyloxy -L-Valine with chloroethanol in presence potassium carbonate in DMF solvent. In the next step, the product formed was hydrogenated in the presence of catalyst in methanol to obtained impurity F. The mass spectrum showed protonated molecular ion peak at m/z 162. The IR spectrum showed the presence N-H stretching (3358cm^{-1}), aliphatic C-H Stretching (2968 cm^{-1}) and C=O stretching of ester 1744 cm^{-1} respectively. In ^{1}H-NMR (solvent DMSO), The peak of two methyl group of isopropyl is found as a doublet at δ1.02 & δ1.03. The CH proton of isopropyl is appeared as multiplet at δ2.37. The methylene proton attached to the hydroxyl appeared as triplet at δ2.82 and the peak of methylene linked to ester appeared as multiplet at δ4.35.

Preparation of Impurity H (2-[(2-Amino-6-oxo-1,6-dihydro-9H-purin-9-yl) methoxy] ethyl L-alaninate)

This impurity is present in drug due to contamination in Valine. It is synthesized as such by same step as the process of drug (Scheme-1) where alanine is protected and condensed with acyclovir. The mass spectrum showed protonated molecular ion peak at m/z 297 which is consistent with expected protonated molecular ion. IR spectrum showed the presence of aliphatic and aromatic C-H stretching (3138 cm^{-1}, 2955 cm^{-1}), N-H stretching at 3426 cm^{-1} and C=O Stretching (1741cm^{-1}, 1693 cm^{-1}) and which corresponding to the ester and amide group. In ^{1}H-NMR (DMSO) appeared a doublet and the CH_2 proton present between N and O appeared as singlet δ5.4 and CH proton of guanine ring appeared as singlet at δ7.9 confirm the structure.

Preparation of Impurity P (Bis Valacyclovir) (2,2'-[Methylenebis[imino(6-oxo-1,6-dihydro-9H-purine-9,2-diyl) methyleneoxy]] diethyl di(L-valinate))

This impurity is prepared by the condensation of two molecules of valacyclovir with the CH_2 linkage between NH_2 group of guanine catalyzed by the formic acid during the formation of valacyclovir. The deprotection is carried out by formic acid. The mechanism of the reaction involves the formation of NH_2CHO which undergo reduction to give N-CH_2-OH, which is condensed with the amino group of the second molecule of the valacyclovir to give bis-valacyclovir molecule. The mass spectrum showed protonated molecular ion peak at m/z 661. IR spectrum showed the presence of N-H stretching (3435cm^{-1}), aliphatic C-H Stretching (2924 cm^{-1} and 2965 cm^{-1}) and C-N stretching (1345cm^{-1}). It also showed the C=O stretching (1739cm^{-1}, 1638 cm^{-1}) corresponding to the ester and amide group. In ^{1}H-NMR (solvent DMSO) the CH_2 proton which is linked between two molecules of Valacyclovir appeared as a multiplet at δ4.88. The 12 protons of four methyl group appeared a doublet at δ 0.75 and δ 0.80. The methylene protons present between the ester and amide linkage appeared as singlet of 4 proton, while the 2 proton of guanine moiety also appeared as a singlet.

Conclusions

This work on synthesis of different impurities of Valacyclovir is a prerequisite for better impurity profiling and finding better route of synthesis of Valacyclovir Hydrochloride. Keeping in view the regulatory importance of Valacyclovir Hydrochloride impurities, our efforts to synthesize and characterize them effectively prove to be valuable.

Experimental

Materials and instruments

All solvents and reagents were purchased from the suppliers and used without purification. ^{1}H and ^{13}CNMR spectra of the compound were recorded on 400 MHz Bruker's NMR spectrometer (av400). The chemical shifts were recorded in parts per million (δ) relative to TMS. FT-IR (Perkin Elmer) spectrometer was used to record the IR spectrum of the compound. KBr pellet of the compound was prepared by the standard method and the spectrum was recorded at resolution from 400 cm^{-1} – 4000 cm^{-1}. Mass spectra of the compound were recorded on Mass spectrometer (Waters).

Impurity C (2-[(2-Amino-6-oxo-1,6-dihydro-9H-purin-9-yl) methoxy]ethyl-N-methyl-L-valinate)

The methyl iodide was added slowly in the mixture of N-CBZ-L-Valine and solvents THF-DMF (50:50) at 0-5º C. After complete addition of methyl iodide, sodium hydride was added slowly at same temperature. After that reaction mixture was heated to reflux condition for 24 hours. Reaction mixture was cooled and concentrated under reduced pressure to get crude compound. This was extracted with ether and water to get N-ethyl ester compound. This ester compound was treated with sodium hydroxide in methanol at 50ºC for 3hours. The solvent was removed. Aqueous solution was extracted with ethyl acetate by maintaining its pH 2-3 with aqueous HCl. The organic layer was washed with water and brine solution. This layer was concentrated to get crude oily compound which was purified by column chromatography. Pure N-alkylated N-CBZ-L-Valine was condensed with acyclovir in presence of dicyclohexyldicarbamide (DCC), dimethyl amino pyrimidine (DMAP) in DMF to give protected N-alkylated valacyclovir. The CBZ group was removed by catalytic hydrogenation (Pd/C) in methanol. This free base was treated with 2N HCl solution to give its hydrochloride salt. IR(KBr cm^{-1}) 3438 (N-H), 3111 (C-H Aromatic.C-H), 2967, 2972 (Aliphatic C-H), 1732, 1690 (C=O stretching ester, amide), 1634 (Ar.C=C), ^{1}H-NMR (solvent D_2O, δ) 8.0 (1H,s), 5.49-5.55 (2H, m) 4.295-4.348, 4.49-4.54 (2H, m), 3.89-3.92 (2H,m), 3.79-3.80 (1H, d, J=4.0H_Z), 2.686 (3H,s), 2.14-2.19 (1H, m), 0.82 (3H, d, J=7.2H_Z, 0.85 (3H, d, J=6.8H_Z) . ^{13}C-NMR (solvent D_2O, δ), 16.20, 17.68, 29.08, 32.40, 64.99, 66.51, 67.24, 73.02, 114.29, 139.46, 151.29, 154.33, 157.90, 168.33. Mass (m/z) 339(M+1)

Impurity D (2-((2-amino-6-oxo-1H-purin-9(6H)-yl) methoxy) ethyl 2-(ethylamino) -3-methylbutanoate)

The ethyl iodide was added slowly in the mixture of N-CBZ-L-Valine and solvents THF-DMF (50:50) at 0-5º C. After complete addition of methyl iodide, sodium hydride was added slowly at same temperature. After that reaction mixture was heated to reflux condition for 24 hours. Reaction mixture was cooled and concentrated under reduced pressure to get crude compound. This was extracted with ether and water to get N-ethyl ester compound. This ester compound was treated with sodium hydroxide in methanol at 50 ºC for 3hours. The solvent was removed. Aqueous solution was extracted with ethyl acetate by maintaining its pH 2-3 with aqueous HCl. The organic layer was washed with water and brine solution. This layer was concentrated to get crude oily compound which was purified by column chromatography. Pure N-alkylated N-CBZ-L-Valine was condensed with acyclovir in presence of dicyclohexyl dicarbamide (DCC) and dimethyl amino pyrimidine (DMAP) in solvent DMF to give protected N-alkylated valacyclovir. The CBZ group was removed by catalytic hydrogenation (Pd/C) in methanol. This free base was treated with 2N HCl solution to give its hydrochloride salt. IR (KBr, cm^{-1}) 3395 (N-H), 3117 (Aromatic C-H), 2969 (Aliphatic C-H), 1728, 1633 (C=O), 11572(Aromatic C=C), ^{1}H-NMR (solvent D_2O, δ) 8.52(1H, s), 5.60 (2H, m), 4.51-4.34 (2H, m), 3.95 (2H, m), 3.86(1H, d, J=4.0H_Z), 3.07(2H, m), 2.20(1H, m), 1.26(3H, b), 0.89(3H, d, J=6.8H_Z), 0.843H, d, J=6.8H_Z). ^{13}C-NMR (solvent D_2O, δ), 10.64, 16.56, 19.00, 29.17, 42.84, 64.28, 64.91, 67.02, 72.96, 113.00, 137.73, 150.98, 154.50, 156.18, 167.21.

Impurity F (L-Valine 2-hydroxyethyl ester)

Potassium carbonate and choro ethanol were added in a solution of N-CBZ-L-Valine in DMF and the reaction mixture was refluxed for 12 hours. The reaction was cooled, filtered and washed. The filtrate was diluted with diisopropyl ether and washed it with chilled water and brine. The organic layer was concentrated under vacuo to get viscous mass which was purified by column chromatography. This pure compound was catalytic hydrogenated by palladium on carbon in methanol at 60 psi pressure for 4 hours. The reaction mass was filtered and dried under vacuo to get free base compound. IR (KBr, cm^{-1}). 3358cm^{-1} (N-H), 2968cm^{-1} (Aliphatic C-H),1744cm^{-1}(C=O). ^{1}H-NMR (solvent D_2O, δ), 4.35(m,2H), 4.04(d, J=4.8Hz, 3.82(m,2H), 2.37(m, H), 1.030(d, J=6.4Hz, 3H), 1.023(d, J=6.8Hz, 3H). ^{13}C-NMR (solvent D_2O, δ), 16.83, 17.14, 28.9, 58.2, 59.11, 67.36, 169.48.

Impurity H (2-[(2-Amino-6-oxo-1,6-dihydro-9H-purin-9-yl) methoxy] ethyl L-alaninate)

Alanine was dissolved in water and in this solution, sodium

carbonate was added and was stirred at RT to make its clear solution. The reaction mixture was cooled up to 0-5ºC. The benzyl chloroformate in toluene was added dropwise. After addition the reaction mixture was heated to 30 ºC for 3 hours. After completion of reaction, layers were separated and the aqueous layer was taken. The 3.0 pH of aqueous layer was maintained by addition of conc. HCl. Aqueous layer was extracted with ethyl acetate and organic layer was washed with water and brine solution. Solvent was removed under vacuo to get N-CBZ-L-Alanine. The acyclovir was added in solution of N-CBZ-Alanine in presence of DCC & DMAP in solvent DMF. The reaction mixture was stirred for 16 hours and added 2-3 drops of water and filtered. The cold water was added into the filtrate and was stirred for 1 hour. Filtered and residue was dried as white ppt under vacuo. White powder was treated with five volume of isopropyl alcohol and refluxed for 30 minutes and cooled it with stirring. The white powder appeared which was filtered and dried under vacuum at 50 ºC. This powder was hydrogenated in methanol and 0.5N HCl in presence of Pd/C under nitrogen condition at 3.5 kg pressure of hydrogen at RT for 4 hours. After completion of reaction, the reaction mixture was filtered and solvent was removed under reduced pressure completely. The crude product was crystallized by using water and isopropyl alcohol and was heated to 45-50^{0}C to make solution clear. This solution was cooled at 0-5^{0}C and stirred for 4 hours. The pure product was obtained by keeping overnight. IR (KBr, cm^{-1}) 3426 (N-H), 3138 (Aromatic C-H), 2955 (Aliphatic C-H), 1741, 1693 (C=O stretching ester, amide), 1612 (Ar.C=C). ^{1}HNMR (solvent D_2O, δ) 7.91 (1H, s, CH), 5.46 (2H, s, CH_2), 4.32 (2H, m, CH_2), 4.05 (1H, q, CH), 3.85 (2H, s, CH_2), 1.44 (3H, d, J=7.2Hz, CH_3).^{13}CNMR (solvent D_2O, δ) 14.85, 48.58, 64.96, 66.69, 72.63, 115.61, 139.64, 151.41,153.92, 158.48, 170.42. Mass (M/Z) 297 (M+1)

Impurity P (Bis Valacyclovir) (2,2'-[Methylenebis[imino(6-oxo-1,6-dihydro-9H-purine-9,2-diyl) methyleneoxy]] diethyl Di (L-valinate))

The acyclovir was added in the mixture of N-CBZ-L-Valine and solvent DMF in the presence of DCC and DMAP and stirred the reaction mixture for 6 hours The reaction mixture was filtered. The cold water was added in filtrate and stirred for 1 hour. The white ppt was formed which was dried under reduced pressure. The white powder was treated with isopropyl alcohol then it was filtered and dried under vacuo at 50ºC. This white powder was dissolved in the mixture of acetonitrile and water (70:30) and formaldehyde was added in presence of catalytic amount of formic acid. After the addition, the reaction mixture was refluxed for 24 hours and was cooled and concentrated under reduced pressure to get crude compound. This crude compound was purified by column chromatography using methanol-dichloromethane as eluent. The pure compound was obtained as free base. This pure compound was hydrogenated using mixture of solvent of methanol and water (8:2) in presence of 10% palladium on carbon under nitrogen condition and at 3.5 kg hydrogen pressure at room temperature for 1hour. The reaction mixture was filtered and solvent was removed under vacuum completely. This crude compound was purified by column chromatography on silica gel using solvent system of chloroform: methanol: ammonia (70:28:2). The pure compound was obtained as off-white solid. IR(KBr, cm^{-1}) 3435 (N-H), 2924, 2965 (Aliphatic C-H), 1739, (C=O), 1345 (C-N), 1295(C-O) .^{1}H-NMR (solvent DMSO, δ) 7.88 (2H, s, 2CH), 7.15 (2H, b, NH), 5.47 (4H, s, $2CH_2$), 4.89(2H, m, CH_2), 4.17(2H, m, 2CH), 4.13(2H, m, 2CH), 3.73(4H, m, $2CH_2$), 3.07 (2H, d, 2CH), 1.74 (2H, m, 2CH), 0.80 (6H, d, J=7.2Hz, $2CH_3$), 0.76 (6H, d, J=7.2Hz, $2CH_3$).^{13}C-NMR (solvent DMSO, δ) 18.00, 19.20, 32.20, 48.14, 59.71, 59.88, 63.20, 67.38, 72.17, 117.39, 138.44, 151.08, 153.04, 157.35, 175.41. Mass (M/Z) 661(M+1)

Acknowledgements

The authors are thankful to management of Menkem Nature Research, Ghaziabad, Uttar Pradesh and Frolic Pharmachem Laboratories, Solan, Himanchal Pradesh, for providing necessary support for the work.

References

1. Beauchamp LM, Orr GF, De Miranda P, Burnette T, Krenitsky TA. Amino acid ester prodrugs of acyclovir. Antiviral Chem. Chemother. 1992;3:157-164.
2. Ormrod D, Scott LJ, Perry CM. Valaciclovir: a review of its long-term utility in the management of genital herpes simplex virus and cytomegalovirus infection. Drugs. 2000;59(4):839-863.
3. Perry CM, Faulds D, Valaciclovir. A review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy in herpesvirus infections, Drugs. 1996;52:754-772.
4. European Pharmacopoeia, Valacyclovir. HCl anhydrous. 2011;7(3):4021-4024.
5. Shinkai I, Ohta Y. New drugs reports of new drugs recently approved the FDA Bioorganic & Medicinal Chemistry. 1996;4:1- 2.
6. De Clercq E, Antiviral drugs in current clinical use. Journal of Clinical Virology. 2004;30:115-133.
7. Ganesh M, Narasimharao CV, Saravana KA, Kamala KK, Vinoba M, Mahajan HS. UV spectrophotometer Method for the Estimation of Valacyclovir HCl in Tablet Dosage form, Journal of Chemistry. 2009;6:814-818.
8. Beauchamp LM. Therapeutic nucleosides. EP 0308065, 1988.
9. Beauchamp LM, Raleigh NC. Therapeutic valine esters of acyclovir and pharmaceutically acceptable salts thereof US 4957924 B2, US. 1990.