The spreading of avian flu in winged animals increased the chance of undeviating contamination in people. Till now, transmission through individual to individual has been only from time to time and aligned with close human contact [1]. At present, hostile to flu medicines comprise of two classes of medications: M2 protein inhibitors (amantadine and rimantadine) and neuraminidase inhibitors (oseltamivir and zanamivir) [2-4]. As a class, neuraminidase inhibitors are successful against all neuraminidase subtypes and, hence, against all strains of flu. This is a key point in pestilence and pandemic readiness and an imperative favourable position over the M2 protein inhibitors which are successful just against touchy strains of flu A2. Among every single antiviral medication, oseltamivir is the prescribed antiviral to treat patients tainted with avian flu (H5N1) incorporating chemoprophylaxis in high-hazard populaces [5]. To protect people from the attack of pandemic human influenza or H5N1 Avian flu, it is recommended that oseltamivir phosphate (Tamiflu, Fig. 1) should be manufactured and stocked in every country all over the world [6,7]. As a continuous requirement of this drug in bulk stock, in recent years, many researchers have reported many synthetic sequences towards the preparation of oseltamivir phosphate [8-18]. Furthermore, in order to improve the efficacy oseltamivir drug, continuous efforts has been put into action for the development of new derivatives of oseltamivir [19-21].

The existence of impurities in an active pharmaceutical ingredient can have a substantial influence on the quality and safety of the drug pr oducts. Therefore, it is essential to understand the impurity profile of the API to be castoff in the manufacturing of the drug product. A level of impurity profile ≥ 0.1 % [22]. Guidelines was endorsed by International Conference on Harmonization (ICH) for the identification and characterization of all impurities. In this perspective, we have been taken to synthesize and characterization of the two potential WHO impurities: 5-acetylamino-4-amino-3-(1-ethyl propoxy)cyclohex-1-enecarboxylic acid ethylester (9, WHO Impurity-F) and 5-acetylamino-4-amino-3-(1-ethyl propoxy)cyclohex-1-enecarboxylic acid (10, WHO impurity-A) (Fig. 2).
Ethyl-(3R,4S,5R)-5-azido-4-hydroxy-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (2): To a stirred solution of epoxide 1 (30 g, 118.1 mmol) in a mixture of methanol (920 mL) and water (120 mL) was added sodium azide (13.64 g, 259.8 mmol). The reaction mixture was heated to 65-70 °C for 16 h. After completion of the reaction, the solvents were stripped from the reaction mixture under vacuum, the residue was purified by column chromatography to obtain Boc compound 5. White solid; Yield: 65%; Anal. calcd (%) for C19H32N4O5: C, 57.60; H, 8.16; N, 14.00. 1H NMR (CDCl3, 300 MHz): δ 9.3, 9.5, 14.1, 25.6, 26.2, 28.3 (3C), 30.7, 57.4, 58.3, 60.9, 73.4, 79.8, 82.2, 128.0, 138.1, 152.5, 165.8; ESI-MS m/z calculated (found) for C19H32N4O5: (397.6) 397.3.

Ethyl-(3R,4S,5S)-5-amino-4-[[(tert-butoxycarbonyl)amino]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (6): To a solution Boc-compound 5 (7 g, 17.67 mmol) in THF (70 mL) was added triphenyl phosphine (5.5 g, 21.21 mmol) in portions at 25-30 °C. The reaction mixture was stirred at 25-30°C for 16 h. The reaction mixture was diluted with water (70 mL) and extracted with ethyl acetate (2 × 50 mL) and triethylamine (8.1 g, 37.11 mmol) at 0 °C. The reaction mixture was allowed to stir at room temperature for 16 h. After completion of the reaction, checked by TLC, water (100 mL) was added to the reaction mixture under vacuum up to 4 vol. Water (130 mL) was added to the reaction mixture and the product was extracted with ethyl acetate (2 × 100 mL). The organic layer was separated and evaporated under vacuum, the residue was purified by column chromatography to obtain crude compound 6. The crude compound was utilized in the next step without any purification. Anal. calcd (%) for C19H32N4O5: C, 61.60; H, 8.14; N, 14.13. Found: C, 61.60; H, 8.16; N, 14.03. 1H NMR (CDCl3, 300 MHz): δ 0.92 (t, J = 6.6 Hz, 6H), 1.30 (t, J = 7.2 Hz, 3H), 1.42 (s, 9H), 1.45-1.57 (m, 4H), 2.15-2.36 (m, 1H), 2.74-2.87 (m, 1H), 3.07-3.18 (m, 1H), 3.64-3.74 (m, 1H), 4.21 (q, J = 7.2 Hz, 2H), 4.4-4.5 (brs, 1H), 4.8-4.9 (brs, 1H), 6.75 (brs, 1H); 13C NMR (300 MHz, DMSO-d6): δ 9.3, 9.5, 14.1, 25.6, 26.2, 28.3 (3C), 30.7, 57.4, 58.3, 60.9, 73.4, 79.8, 82.2, 128.0, 138.1, 152.5, 165.8; ESI-MS m/z calculated (found) for [M+H+] for C19H32N4O5: (397.6) 397.3.

Ethyl-(3R,4R,5S)-5-amino-4-[(tert-butoxycarbonyl)amino]-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (7): A mixture of amine 6 (7 g, 18.89 mmol), potassium carbonate (3.91 g, 28.33 mmol) and acetic anhydride (2.14 mL, 22.67 mmol) in THF (70 mL) was stirred at room temperature for 4 h. Water (56 mL) was added to the reaction mixture and evaporated with ethyl acetate twice (2 × 42 mL) and evaporated under vacuum, the residue was purified by column chromatography to obtain acetyl compound 7. Pale-yellow syrup liquid; Yield: 60%; Anal. calcd (%) for C17H26NO5: C, 61.14; H, 8.80; N, 6.79. Found: C, 61.19; H, 8.76; N, 6.82. 1H NMR (CDCl3, 300 MHz): δ 0.94 (t, J = 6.6 Hz, 6H), 1.28 (t, J = 7.2 Hz, 3H), 1.43 (s, 9H), 1.46-1.58 (m, 4H), 1.94 (s, 3H), 2.25-2.31 (m, 1H), 2.72-2.79 (m, 1H), 3.38-3.42 (m, 1H), 3.72-3.78 (m, 1H), 3.93-3.96 (m, 1H), 4.23 (q, J = 7.2 Hz, 2H), 4.69.
Synthesis and Characterization of Potential Pharmacopeial Impurities of Oseltamivir 2005

The synthesis of two potential WHO impurities of oseltamivir phosphate, (i) ethyl-(3R,4R,5S)-5-(acetylamino)-4-amino-3-(pentan-3-yl-oxycyclohex-1-ene-1-carboxylate (WHO-IMP-F, 9), and (ii) (3R,4R,5S)-5-(acetylamino)-4-amino-3-(pentan-3-yl-oxycyclohex-1-ene-1-carboxylic acid (WHO-IMP-A, 10) is illustrated in Scheme-I. These two impurities were prepared starting from epoxide compound 1 [6,7].

The ring opening of epoxide 1 was carried out in presence of sodium azide, ammonium chloride in methanol:THF at 65-70 °C for 16 h produced azide-hydroxy 2. Reduction of azide 2 followed by cyclization in presence of triphenyl phosphine in acetonitrile at 80 °C for 3 h produced aziridine 3 [23-25]. Ring opening of aziridine 3 [26] in presence of NaN₃, NH₄Cl in methanol:THF, DMF at 65-70 °C for 16 h yielded azidine-4. Boc-tection of azide-amine 4 in presence of Boc anhydride, triethyl amine in dichloromethane at room temperature for 16 h yielded N-Boc-azidodecompound 5. Reduction of N-Boc-azide 5 was carried out in presence of triphenyl phosphine in THF at 25-30 °C for 16 h produced N-Boc-amine 6. Acetylation of N-Boc-amine 6 was done in the presence of acetic anhydride, potassium carbonate in THF at room temperature for 4 h gave compound 7. De-tection of compound 7 in presence of trifluoroacetic acid in dichloromethane, at 25-30 °C for 16 h resulted in the formation of impurity WHO-IMP-F, 9 [27]. Initial attempts to prepare WHO-IMP-A, 10 by LiOH hydrolysis of WHO-IMP-F, 9 was unsuccessful mainly due to its high soluble property in water and difficulty in isolating the product. Therefore, as an alternative approach, Boc-protected ethyl ester compound 7 was hydrolyzed in presence of LiOH and isolated compound 8. After purification, it was directly used in the next step. Boc de-tection of compound 8, in presence of IPA-HCl resultant in the formation of desired compound WHO-IMP-A, 10.

The structural determination of newly synthesized WHO-IMP-F, 9 and WHO-IMP-A, 10 was established by ¹H NMR, ¹³C NMR, mass and IR techniques. Characterization of ethyl (3R,4R,5S)-5-(acetylamino)-4-amino-3-(pentan-3-yl-oxycyclohex-1-ene-1-carboxylate (WHO-IMP-F, 9) ¹H NMR description: The protons resonating at 1.21 ppm as triplet (3H) and 4.16 ppm as quartet (2H) is assigned to the methyl ester group. The protons resonating at 0.84-0.89 as multiplet (6H), 1.38-1.58 as multiplet (4H) and 3.67-3.72 ppm is assigned to the pental group. The protons resonating at 6.66 ppm (1H), 1.84 ppm (3H) as singlets corresponds to olefinic proton and acetyl group, respectively. The D₂O exchangeable protons of -NH₂ and -NH groups resonated at 1.63 and 7.79 ppm. The protons associated with the cyclohexene ring resonated at 1.95-2.04 ppm.

**RESULTS AND DISCUSSION**

**Synthesis and Characterization of Potential Pharmacopeial Impurities of Oseltamivir 2005**

The synthesis of two potential WHO impurities of oseltamivir phosphate, (i) ethyl-(3R,4R,5S)-5-(acetylamino)-4-amino-3-(pentan-3-yl-oxycyclohex-1-ene-1-carboxylic acid (WHO-IMP-F, 9) and (ii) (3R,4R,5S)-5-(acetylamino)-4-amino-3-(pentan-3-yl-oxycyclohex-1-ene-1-carboxylic acid (WHO-IMP-A, 10) is illustrated in Scheme-I. These two impurities were prepared starting from epoxide compound 1 [6,7].

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and 2.49-2.57 ppm (-CH2 adjacent to ethyl ester group), 2.66-2.72 ppm (-CH flanked to acetyl group), 3.33-3.44 ppm (-CH flanked to -NH2 group) and 3.85 ppm (-CH flanked to -O-pentanal group). Thus, above 1H NMR description is in agreement with the desired number of protons in the structure.

**13C NMR:** The carbon signals resonating at 169.1 ppm, 165.6 ppm is assigned to the characteristic carbonyl groups viz., ethylester carbonyl (C-12) and acetyl carbonyl group (C-15), respectively. The carbon signals resonating at 128.3 and 137.8 ppm is assigned to oelfin carbons (C-2 and C-1), respectively. The methylene carbons signals (-CH2, C-6, C-8, C-10 and C-13) resonated in the region 25.3 ppm, 25.7 ppm, 30.3 ppm and 60.2 ppm, respectively and were confirmed by carbon DEPT experiment. The methyl carbons viz., C-9, C-11, C-14 and C-16 resonated at 9.2, 9.5, 14.0 and 22.8 ppm, respectively while tertiary carbon signals of cyclohexene ring such as C-5, C-4, C-3 and C-7 resonated at 48.8, 54.8, 78.2 and 80.0 ppm, respectively. Thus, above 13 C NMR description is in agreement with the desired number of carbons in the structure.

**IR:** IR data suggest that strong characteristic bands appeared at 3383, 1714, 1634 and 1281 cm⁻¹ are due to the following characteristic groups -NH, -C=O, -C=C-, -C-N associated with the desired compound.

**Mass spectra:** Mass spectrum of WHO Imp-F, 9 showed M+1 peaks at m/z 313 in positive mode and is in agreement with its molecular formula. Similarly, the structural determination of WHO Imp-A, 10 was established as per the above description.

**Conclusion**

The synthesis and characterization of two potential WHO listed impurities of oseltamivir phosphate viz., (i) ethyl-(3R, 4R,5S)-5-(acetylamino)-4-amino-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (WHO-IMP-F, 9) and (ii) (3R,4R,5S)-5-(acetylamino)-4-amino-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (WHO-IMP-A, 10) are demonstrated. These impurities were characterized by IR, mass and NMR spectroscopic techniques. Synthesis of these impurities helps in obtaining a good-quality output of API and formulation, also helps in establishing the impurity profile of API by understanding the cause of its origin and control.

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**CONFLICT OF INTEREST**

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