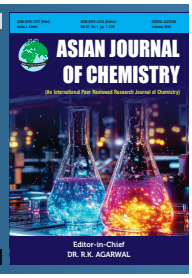
Asian Journal of Chemistry; Vol. 37, No. 3 (2025), 698-706

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2025.33265



Identification, Synthesis and Characterization of Novel Palbociclib Impurities

NANDYALA MALYADRI REDDY[1,2], K.R.S. PRASAD[1,*], RAVI SEKHAR REDDY NANDIPATI[2], KATAM REDDY VINOD KUMAR REDDY[2], KATAM REDDY VINEELA[3], G. PAVANA KUMARI[4] and NAVEEN MULAKAYALA[2,*]

[1]Department of Chemistry, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur-522302, India
[2]SVAK Life Sciences, ALEAP Industrial Area, Hyderabad-500090, India
[3]Department of Periodontology, Indira Gandhi Institute of Dental Sciences, Sri Balaji Vidyapeeth, Pondicherry-607402, India
[4]Depatment of Chemistry, Sri Sathya Sai Institute of Higher Learning-Anantapur Campus Anantapur-515001, India

*Corresponding authors: E-mail: krsprasad_fed@kluniversity.in; naveen071280@gmail.com

Received: 27 December 2024; *Accepted*: 11 February 2025; *Published online*: 28 February 2025; AJC-21927

Palbociclib is used for breast cancer treatment along with letrozole. Five major impurities were observed during the synthesis as well as in forced degradation studies, out of which, three impurities were identified during the penultimate step of palbociclib API synthesis. These three impurities were confirmed as palbociclib desacetyl impurity (PDA impurity), palbociclib desacetyl hydroxy impurity (PDH impurity) and palbociclib desacetyl hydroxyl methyl impurity (PDHM impurity). These impurities were formed due to the usage of palladium catalyst during the pre-final reaction between 1 and 2. The other two impurities were identified as N-oxides, which were formed due to the forced oxidative degradation studies of palbociclib. These two impurities were confirmed as palbociclib pyridine N-oxide and palbociclib piperazine N-oxide. All the five major impurities were identified, synthesized, characterized and confirmed by spectral analyses (^{1}H NMR, ^{13}C NMR and mass spectra). These impurities were potentially important as reference standards for the R&D scientists working in process development and formulation development of palbociclib.

Keywords: Impurities, Palbociclib, Pyridine N-oxide, Piperazine N-oxide.

INTRODUCTION

Palbociclib (IBRANCE®, Fig. 1) is an estrogen receptor (ER) positive drug and used for the treatment of breast cancer. Palbociclib is a CDK4 and CDK6 inhibitor developed by Pfizer against breast cancer [1,2]. In March 2017, the USFDA approved palbociclib for the treatment of harmone receptor positive and HER 2 (human epidermal growth factor receptor 2). The European Union approved palbociclib in November 2016 for the same treatment [3]. The maximum daily usage of palbociclib for cancer patients is 125 mg [4]. According to International Council for Harmonization (ICH) guidelines, the reporting threshold is 0.05% and the identification threshold is 0.10% for impurities in new drug substances for a maximum daily dose of < 2 g/day [5,6].

During the manufacturing of an active pharmaceutical ingredient (API), several undesirable products as well as unreacted starting materials were present in the final crude product [7]. Palbociclib is an anticancer compound and due to this, these impurities will impact the potency of the final product. According to ICHQ7, the manufacturers must maintain the impurities below the set limit. For this reason, the identification of impurities was very important to control the presence of impurities below 0.05% in the final substance.

Palbociclib was first manufactured by Pfizer in 2005. After this, several synthetic methods were reported for the synthesis of palbociclib in the literature [8-14]. Several impurities were reported by different researchers in the literature for palbociclib (Fig. 2) [15]. Most of the synthetic process involves the use of palladium catalysts during the synthesis of acetyl compound from bromo compound in pre-final step (**Scheme-I**).

Due to the use of palladium catalysts during the synthesis of palbociclib, several undesirable products were observed. The formation of three major impurities was identified during this step. These impurities were palbociclib desacetyl impurity (PDA impurity), palbociclib desacetyl hydroxy impurity (PDH impurity) and palbociclib desacetyl hydroxyl methyl impurity (PDHM impurity) (Fig. 3).

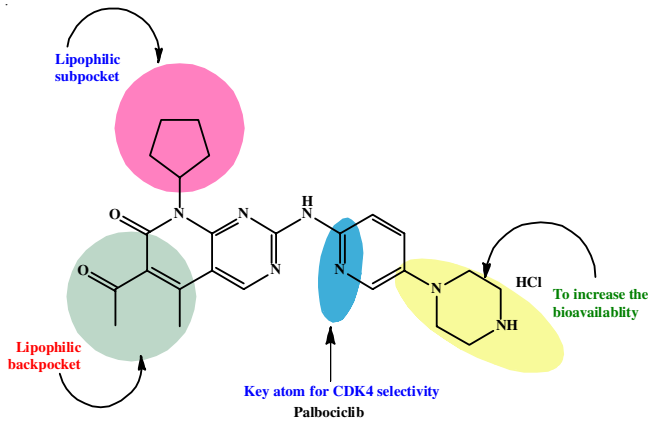


Fig. 1. Palbociclib hydrochloride

For the past few years, our focuss has been working on the identification and synthesis of impurities, which were forming during the development as well as during the stability conditions [16-20]. In the present work, three major impurities were identified during the final step of the palbociclib synthesis (PDA, PDH and PDHM impurities) and two N-oxides were identified in the oxidative stress degradation (Fig. 4). In this work, the synthesis of five novel impurities and their identification using different analytical methods are reported.

EXPERIMENTAL

No additional purification was conducted on the solvents and reagents that were purchased from commercial sources. At room temperature, ^{1}H NMR spectra were acquired in DMSO-d_6

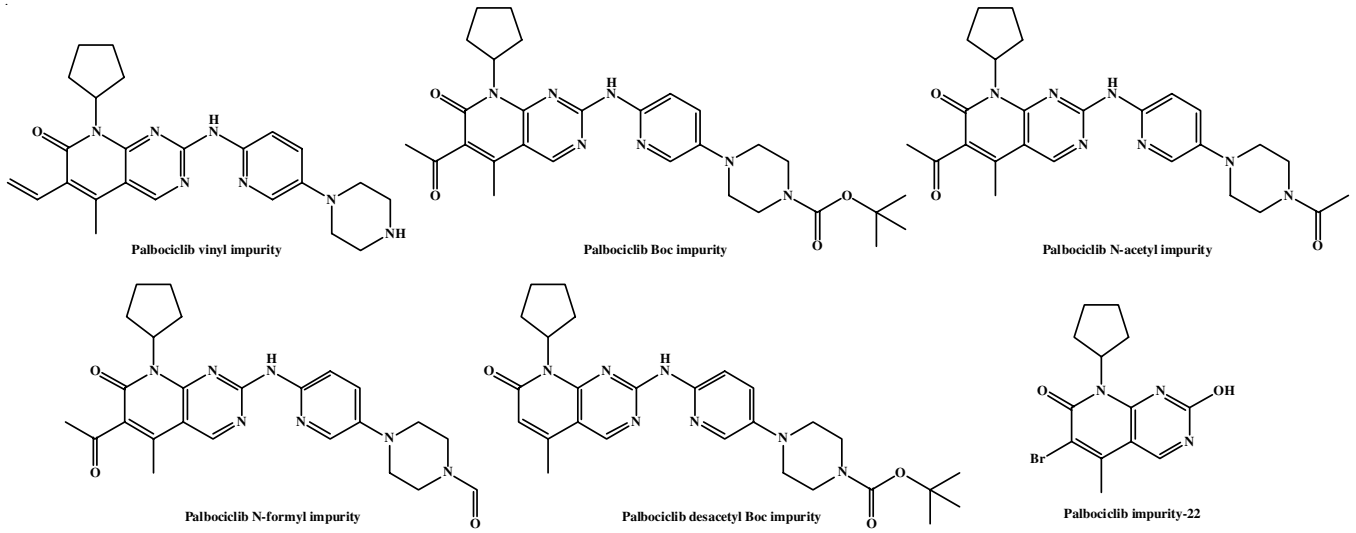
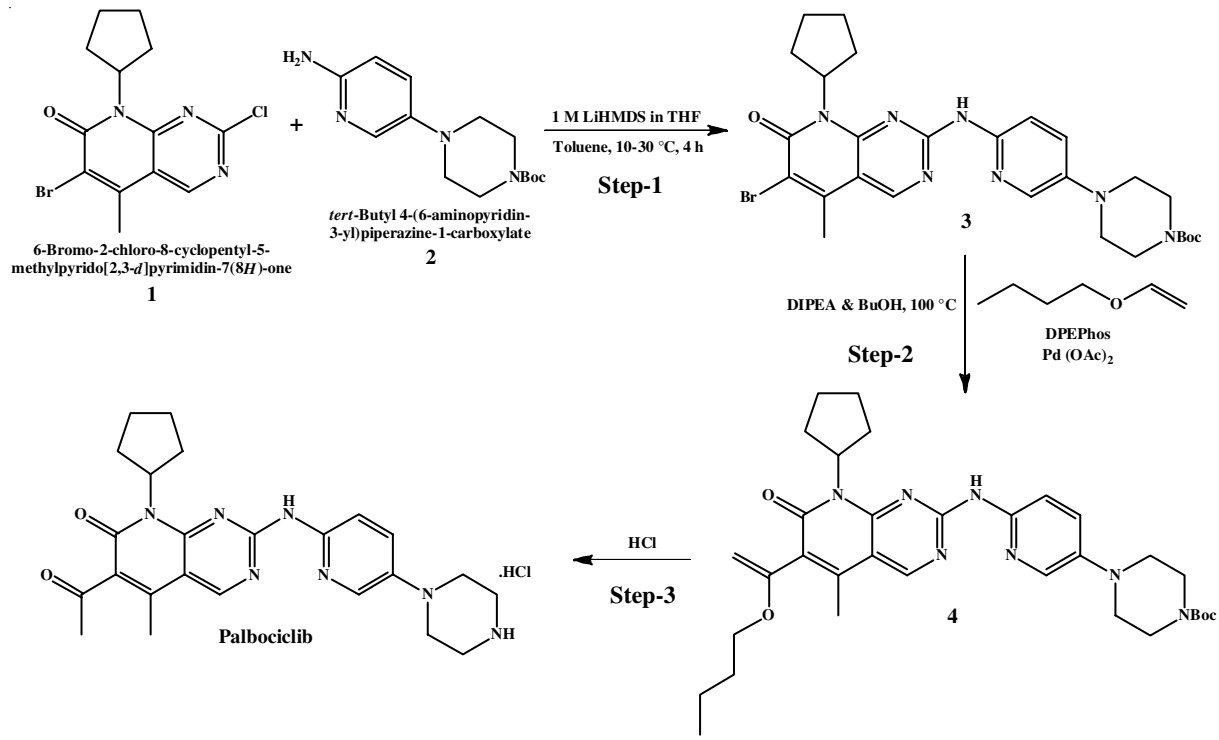


Fig. 2. Known process impurities of palbociclib HCl present in the literature



Scheme-I: Chemical synthesis of palbociclib

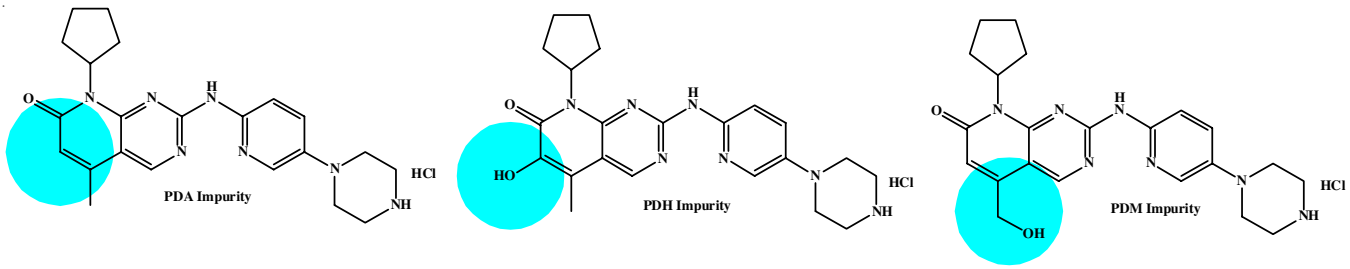


Fig. 3. Novel process impurities of palbociclib HCl

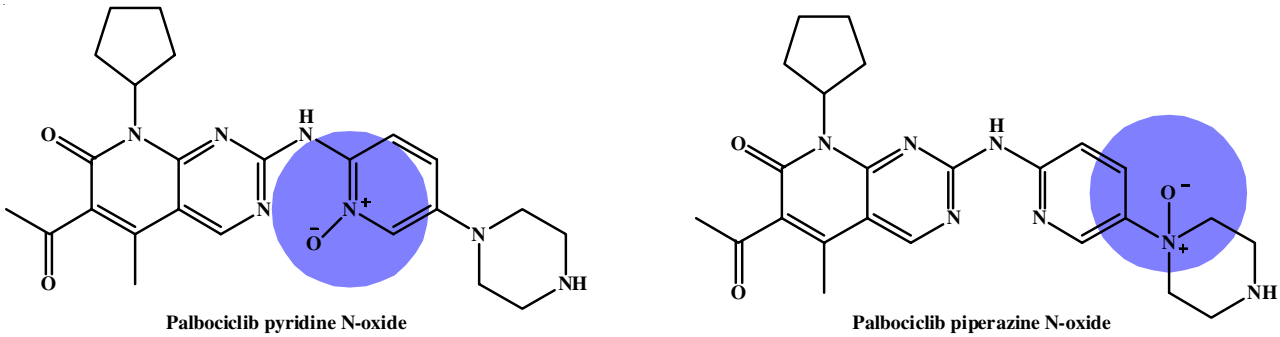


Fig. 4. Oxidative degradation impurities of palbociclib

using an internal standard of TMS on a Varian Mercury spectrometer plus 400 MHz. A Varian Mercury plus 100 MHz spectrometer in DMSO-d_6 was used to obtain ^{13}C NMR spectra at the ambient temperature. The best chromatographic behaviour for palbociclib and its impurities was observed while using the optimal chromatographic parameters. Merck silica gel $60F_{254}$ plates were used for TLC studies.

High performance liquid chromatography (analytical): The examination of palbociclib impurities was performed utilizing a Waters (LC2695) HPLC system and a PDA-2996 detector calibrated to 295 nm. The HPLC data was analyzed utilizing the Empower 2 software. The HPLC analysis utilized an EPIC C_{18} column of 250 mm × 4.6 mm with a particle size of 5 µm. Alpha consisted of 0.1% aqueous solution of trifluoroacetic acid, whereas beta comprised an acetonitrile solution. The following settings were programmed into the linear gradient program: $T_{min/B}$ (mL/min); $T_{0/10}$; $T_{15/90}$; $T_{20/90}$; $T_{21/10}$; $T_{25/10}$. The injection volume was 10 µL and the flow rate was set to 1.0 mL/min. The sample was produced with a diluent composed of acetonitrile and trifluoroacetic acid at an 8:2 ratio (Fig. 5).

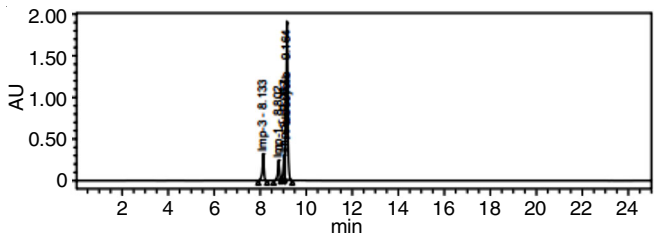


Fig. 5. HPLC chromatogram of crude palbociclib

Liquid chromatography-mass spectroscopy (LC-MS): An oxidative stress sample of palbociclib was examined utilizing the Waters 2695 ACQUITY LC-MS. A Luna C18 column (250 × 4.6 mm, 5 mm) was employed for chromatographic separation. The wavelength of UV detector was established at 245 nm. Mobile phase A consists of 0.1% trifluoroacetic acid in water, whereas mobile phase B comprises HPLC grade acetonitrile. The flow rate was established at 1.0 mL/min and the column oven temperature was configured to 25 ºC. The cone voltage was 30 volts and the capillary voltage was 3.5 kV. The source temperature was sustained at 120 ºC. The linear gradient protocol was established as follows: $T_{min/B}$ (%); $T_{0/10}$; $T_{15/90}$; $T_{20/90}$; $T_{21/10}$; $T_{25/10}$.

HPLC (preparative): Using the methods as described above, palbociclib impurities were removed from palbociclib samples subjected to the oxidative stress. The intended impurity peaks were isolated using a Waters 2545 preparative HPLC system that was outfitted with a Phenomenex Luna C18 column (50 × 250 µm, 10 µm) and a PDA2996 detector. A wavelength of 295 nm was determined. Data processing was carried out using the Mass Lynx program. The first mobile phase was an aqueous solution of 0.1% trifluoroacetic acid (TFA) and the second was a mixture of methanol and HPLC-grade acetonitrile at a ratio of 1:1. For 100 min, the flow rate was maintained at 35 mL/min. The following time intervals were used to develop the linear gradient protocol: $T_{0/05}$, $T_{2/20}$, $T_{4/40}$, $T_{6/45}$, $T_{20/45}$, $T_{25/45}$, $T_{30/50}$, $T_{38/50}$, $T_{43/60}$, $T_{50/60}$, $T_{70/70}$ and $T_{80/100}$. After neutralizing the impurities in the preparative HPLC fractions with $NaHCO_3$ solution, the organic layer was concentrated to yield pale-yellow solids containing piperazine N-oxide and pyridine N-oxide. The impurities were extracted with 100 mL of MeOH-DCM (1:9). Finally, the isolated samples were characterized for in-depth analysis.

Palbociclib impurities purification was done from the oxidative stress samples of palbociclib using the procedure mentioned below. Waters 2545 preparative HPLC system with Phenomenex Luna C18 column (50 × 250 mm, 10 µm) and PDA2996 detector was used to isolate the required impurity peaks. The

wavelength was set at 295 nm. To process the data Mass Lynx software was used. 0.1% aqueous TFA was used in mobile phase A and 1:1 mixture of HPLC grade acetonitrile and methanol was used in mobile phase B. The flow rate was maintained at 35 mL/min and the run time was 100 min. The linear gradient program was set as follows: $T_{min/B}$ (mL/min): $T_{0/05}$; $T_{2/20}$; $T_{4/40}$; $T_{6/45}$; $T_{20/45}$; $T_{25/45}$; $T_{30/50}$; $T_{38/50}$; $T_{43/60}$; $T_{50/60}$, $T_{70/70}$, $T_{80/100}$. Piperazine N-oxide and Pyridine N-oxide impurities obtained from the preparative HPLC fraction were neutralized with sodium bicarbonate solution, extracted with 100 mL of MeOH-DCM (1:9) and the organic layer was concentrated to get piperazine N-oxide and pyridine N-oxide impurities as pale-yellow solids. The isolated samples further used for its complete characterization.

Experimental procedure for the synthesis of palbociclib impurities

Experimental procedure for the synthesis of palbociclib API: Compound **1** (2 g, 0.003 mol) was dissolved in 40 mL of dry 1-butanol at 25-30 ºC. The suspension was placed under nitrogen, to this butyl vinyl ether (1.2 g, 0.012 mol), diisopropylethylamine (1.2 g, 0.01 mol), $Pd(OAc)_2$ (0.063 g, 0.0003 mol) and DPEPhos (0.189 g, 0.00035 mol) were added. The reaction mixture was heated at 90-100 ºC for 18 h. After completion, the reaction mixture was filtered, to the filtrate water (35 mL) was added slowly for 15 min. The suspension was cooled to 0-5 ºC, solid compound was filtered and washed with a mixture of methanol and water (4:1). The solid was dried at 65 ºC to obtain compound **2** (1.75 g, yield 85%), which was taken in dichloromethane (25 mL) and treated with 4 M HCl in 1,4-dioxane (5 mL) at 5-10 ºC. The reaction mixture was stirred at room temperature for 6-7 h. After completion, the reaction mixture was concentrated under reduced pressure to get crude compound (1.8 g). The crude compound was taken in 30 mL of water, solution was basified with 1 N NaOH (pH = 8-9) at 5-10 ºC, resultant solid was filtered and dried under vacuum for 5-6 h to afford 1.2 g (yield 82.2%) of palbociclib.

Synthesis of PDA impurity: (8-cyclopentyl-5-methyl-2-((5-(piperazin-1-yl)pyridin-2-yl)amino)pyrido[2,3-*d*]-pyrimidin-7(8*H*)-one hydrochloride): Compound **3** (5 g, 0.0085 mol) was dissolved in dry 1-butanol (50 mL) at 25-30 ºC under inert atmosphere. To this diisopropylethylamine (3.25 g, 0.025 mol), $Pd(OAc)_2$ (0.5 g, 0.002 mol), DPEPhos (0.5 g, 0.0009 mol) were added and degassed with nitrogen for 30 min. The reaction mixture was heated to 115-120 ºC and stirred at this temperature for 18 h. The mixture was cooled to room temperature, added 25 mL of 1-butanol and filtered. To the filtrate 75 mL of water was added during 20 min. The suspension was cooled to 0-5 ºC, resultant solid was filtered and washed with a 50 mL of water. The filtered cake was dried at 65 ºC for 2 h to get 4.9 g of crude compound **5**. The crude compound was purified by column chromatograph to afford 2.16 g of compound **5** (yield: 50%). Compound **5** (2 g) was taken in 25 mL of dichloromethane and treated with 5 mL of 4 M HCl in 1,4-dioxane at 5-10 ºC. The reaction mixture was stirred at room temperature for 5-6 h. The mixture was concentrated under reduced pressure to afford 1.5 g (93.75%) of PDA impurity. Pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.81 (1H, brs), 9.33 (2H, s), 8.87 (1H, s), 8.06 (1H, d, J = 1.6 Hz), 7.85 (2H, brs), 6.33 (1H, s), 5.78-5.86 (1H, m), 3.42-3.44 (4H, m), 3.25 (4H, m), 2.41 (3H, s), 2.19-2.24 (2H, m), 1.91-1.96 (2H, m), 1.77-1.79 (2H, m), 1.58-1.62 (2H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 162.2, 156.8, 155.7, 155.2, 148.7, 145.0, 141.9, 134.2, 119.4, 116.1, 108.7, 52.7, 45.0, 42.1, 27.6, 25.2 and 16.6. IR (KBr, ν_{max}, cm^{-1}): 3423, 2959, 1668; Exact mass: calculated ($C_{22}H_{27}N_7O$) 405.23, MS (ES+) found: 406.35 (MH+); HPLC purity: 98.92%.

Synthesis of (8-cyclopentyl-6-hydroxy-5-methyl-2-((5-(piperazin-1-yl)pyridin-2-yl)amino)pyrido[2,3-*d*]-pyrimidin-7(8*H*)-one) (PDH Impurity) and (8-cyclopentyl-5-(hydroxymethyl)-2-((5-(piperazin-1-yl)pyridin-2-yl)-amino)pyrido[2,3-d]pyrimidin-7(8*H*)-one hydrochloride) (PDHM impurity): Compound **3** (5 g, 0.0085 mol) was dissolved in dry 1-butanol (50 mL) at 25-30 ºC under inert atmosphere and then potassium acetate (5 g, 0.05 mol), $Pd(OAc)_2$ (0.05 g, 0.0002 mol) and DPEPhos (0.5 g, 0.0009 mol) were added at the same temperature. The reaction mixture was heated to 115-120 ºC and stirred at this temperature for 18 h. The mixture was cooled to room temperature and ethyl acetate (100 mL) was added and filtered. The filtrate was washed with water (50 mL) and the organic layer was separated and washed with brine solution (20 mL). The organic layer was dried over anhy-drous Na_2SO_4. The organic layer was evaporated to get 5.2 g of crude compound (contains compounds **6** and **7**) and then the crude compound was dissolved in dichloromethane (50 mL) followed by the addition of 4 M HCl in 1,4-dioxane (7 mL) at 5-10 ºC. The reaction mixture was stirred at room temperature for 5-7 h and concentrated under reduced pressure to afford 5 g of crude compound. Then the crude compound was dissolved in water (50 mL) followed by adjusting the pH to 8-9 using 1 N NaOH at 5-10 ºC. The resultant solid was filtered purified by silica gel (100-200 mesh) using column chromatography to afford 800 mg (yield 22.2%) of PDH impurity and 930 mg of PDHM impurity as residue. PDHM residue was taken in 5 mL of MDC, treated with 1 N HCl in diethyl ether (1 mL) at 0-5 ºC, stirred at 30 min and concentrated to afford 950 mg (26.3%) of PDHM impurity as pale-yellow solid.

PDH impurity: Pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.59 (1H, s), 8.75 (1H, s), 7.98-7.99 (1H, d, J = 4 Hz), 7.90-7.92 (1H, d, J = 8.0 Hz), 7.40-7.43 (1H, dd, J = 2.8 Hz & 2.8 Hz), 5.92-5.98 (1H, m), 3.02-3.04 (4H, m), 2.83-2.85 (4H, m), 2.27 (3H, s), 2.22-2.24 (2H, m), 1.93-1.94 (2H, m), 1.81-1.83 (2H, m), 1.62-1.65 (2H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 161.2, 159.2, 156.3, 154.5, 150.5, 145.1, 143.4, 140.2, 135.3, 124.9, 116.8, 113.8, 107.9, 53.1, 49.6, 48.5, 45.4, 27.8 and 25.3.; IR (KBr, ν_{max}, cm^{-1}): 3315, 2939, 1642, 1227; Exact mass: calculated ($C_{22}H_{27}N_7O_2$) 421.22, MS (ES+) found: 422.35 (MH^+); HPLC purity: 98.56%.

PDHM impurity: Pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.94 (1H, brs), 8.78 (1H, s), 8.05 (1H, s), 7.87-7.89 (1H, d, J = 8 Hz), 7.46-7.49 (1H, d, J = 12 Hz), 6.33 (1H, s), 5.79 (1H, m), 5.54 (1H, t), 4.70-4.71 (2H, d, J = 4 Hz), 3.26 (4H, m), 3.07 (4H, m), 2.22 (2H, m), 1.89 (2H, m), 1.72

(2H, m), 1.57 (2H, m); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 162.5, 157.3, 155.5, 155.2, 148.6, 144.5, 142.0, 131.78 128.52 115.4, 115.3, 106.0, 58.6, 52.7, 45.3, 42.2, 27.6 and 25.1; IR (KBr, ν_{max}, cm^{-1}): 3380, 2944, 1656, 1279; Exact mass: calculated ($C_{22}H_{27}N_7O_2$) 421.22, MS (ES+) found: 422.34 (MH+); HPLC purity: 95.4%.

Synthesis of palbociclib N-oxides

Experimental procedure for the synthesis of palbociclib N-oxide impurities: Palbociclib (5 g, 0.011 mol) was dissolved in MeCN (100 mL, 20 vol.) at 25-30 ºC under inert atmosphere. To this 30% H_2O_2 (3.1 mL, 0.0275 mol) was added at 0-5 ºC. The reaction mixture was stirred at 25-30 ºC for 72 h. About 10% of impurities were observed using TLC. Then the reaction mixture was filtered and purified by column chromatography using silica gel (3% methanol in DCM) to afford crude compound (contains pyridine N-oxide and piperazine N-oxide, Fig. 6). These two compounds were separated by using preparative HPLC to afford 500 mg of piperazine N-oxide and 220 mg of pyridine N-oxide.

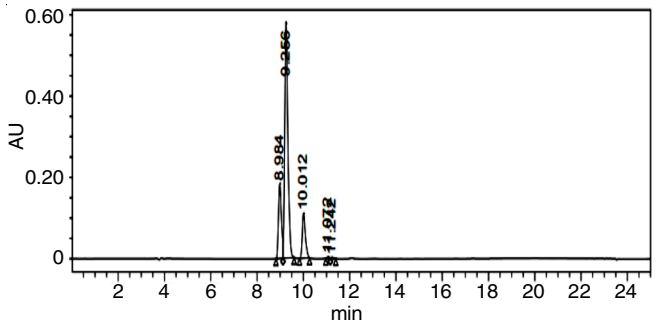


Fig. 6. HPLC chromatogram of forced oxidative degradation sample of palbociclib

Piperazine N-oxide (PDH): Pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.09 (1H, s), 9.10 (1H, s), 8.972-8.979 (1H, d, *J* = 2.8 Hz), 8.47-8.50 (1H, dd, *J* = 3.2 Hz & 3.2 Hz), 8.38-8.40 (1H, d, *J* = 9.6 Hz), 5.91-5.87 (1H, m), 4.71 (2H, br), 4.50-4.47 (2H, m), 3.78-3.76 (4H m), 2.44 (3H, s), 2.35 (3H, s), 2.26 (2H, m), 1.98-1.83 (4H, m) & 1.65-1.64 (2H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 202.4, 160.6, 157.9, 157.6, 154.8, 154.1, 141.6, 140.6, 138.9, 130.8, 113.2, 108.2, 62.0, 53.3, 38.1, 31.3, 27.6, 25.2 and 13.7; IR (KBr, ν_{max}, cm^{-1}): 3417, 2956, 1703, 1660, 1269; Exact mass: calculated ($C_{24}H_{29}N_7O_3$) 463.23, MS (ES+) found: 464.31 (M + 1) & (ES-) found: 462.25 (MH-); HPLC purity: 97.93%.

Pyridine N-oxide (PDM): Yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.83 (1H, s), 9.16 (1H brs), 9.08 (1H, s), 8.28-8.31 (*J* = 9.6 Hz, 1H, d), 8.25-8.26 (1H, d, *J* = 2.8 Hz), 7.34-7.37 (1H, dd, *J* = 2.4 Hz & 2.4 Hz), 5.85-5.80 (1H, m), 3.44-3.42 (4H, m), 3.22 (4H, br), 2.44 (3H, s), 2.34 (3H, s), 2.21-2.18 (2H, m), 1.99-1.91 (2H, m), 1.85-1.83 (2H, m) & 1.67-1.65 (2H, m); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 202.3, 160.55, 158.3, 156.9, 154.8, 142.5, 141.7, 136.9, 130.8, 125.8, 119.1, 108.3, 53.1, 44.8, 42.0, 31.3, 27.8, 25.4 and 13.8.; IR (KBr, ν_{max}, cm^{-1}): 3478, 2912, 1680, 1631, 1259; Exact mass: calculated ($C_{24}H_{29}N_7O_3$) 463.23, MS (ES+) found: 464.31 (MH+); HPLC purity: 95.13%.

RESULTS AND DISCUSSION

During the synthesis of palbociclib API, the formation of three major impurities was formed. To identify and confirm these impurities, the synthesis of palbociclib API using 1-piperazinecarboxylic acid, 4-[6-[(6-bromo-8-cyclopentyl-7,8-dihydro-5-methyl-7-oxopyrido[2,3-*d*]pyrimidin-2-yl)amino]-3-pyridinyl]-, 1,1-dimethylethyl ester **3** as a starting material was conducted. Compound **3** on reaction with butyl vinyl ether in presence of [Pd(OAc)]$_2$, DIPEA, DPEPhos yielded compound **4** (**Scheme-I**). Compound **4** was subjected to deprotection *in situ* using aqueous HCl forms palbociclib API. Step-2 and step-3 were *in situ* reactions without isolating compound **4**. In this reaction, the formation of three major impurities after LC-MS analysis (Fig. 7) was observed. These three impurities were palbociclib desacetyl impurity (PDA impurity), palbociclib desacetyl hydroxy impurity (PDH impurity) and palbociclib desacetyl hydroxyl methyl impurity (PDHM impurity).

Upon identifying these three impurities during the synthesis of palbociclib through LC-MS analysis, our objective is to synthesize each impurity in a pure form. PDA impurity was synthesized by the reaction of compound **3** with palladium acetate, DIPEA and DPEPhos in butanol at 115-120 ºC for 18 h. After completion of the starting material, the reaction mixture was isolated to get compound **5**, which was subjected to Boc deprotection using 4 M HCl in 1,4-dioxane gave PDA impurity in 50% yield.

In ^{1}H NMR, a singlet at δ 6.32 ppm which corresponds to the proton at H-5 confirms the formation of des-bromo compound. Similarly, in ^{13}C NMR, a peak at δ 119.4 ppm for C-5 in PDA impurity was observed. A slight change in the chemical shift of C-6 carbon towards up field from δ 162.1 ppm from δ 160.7 ppm due to the des-bromination. In mass spectrum, *m/z* value 406.35 in positive mode was observed, which confirms the formation of desbromo impurity (PDA impurity). From all the above data, the formation of PDA impurity was confirmed. This was further confirmed by spiking the pure PDA impurity with the peak isolated from the crude palbociclib.

PDH and PDHM impurities were synthesized by changing the base from DIPEA to K_2CO_3 using the same reaction condition that were used to prepare PDA impurity. PDH and PDHM Impurities were synthesized by the reaction of compound **3** with palladium acetate, potassium acetate, DPEPhos in butanol at 115-120 ºC for 18 h. After completion of the starting material, compounds **6** & **7** were isolated followed by Boc deprotection with 4 M HCl in 1,4-dioxane yields the PDH and PDHM impurities in about 20% yield. PDH and PDHM impurities were separated using column chromatography and confirmed by ^{1}H NMR, ^{13}C NMR and Mass.

In ^{1}H NMR, a slight change in the chemical shift of methyl protons of H-24 from δ 2.25 ppm in PDH impurity was observed when compared with δ 2.42 ppm in palbociclib shows the formation hydroxy group at H-5. Similarly, in ^{13}C NMR, a peak at δ 161.2 ppm for C-5 in PDH impurity was observed when compared with δ 124.5 ppm for C-5 in palbociclib due to the formation of -OH group at C-5 carbon, which confirms the formation of -OH group at C-5 position. In mass spectrum,

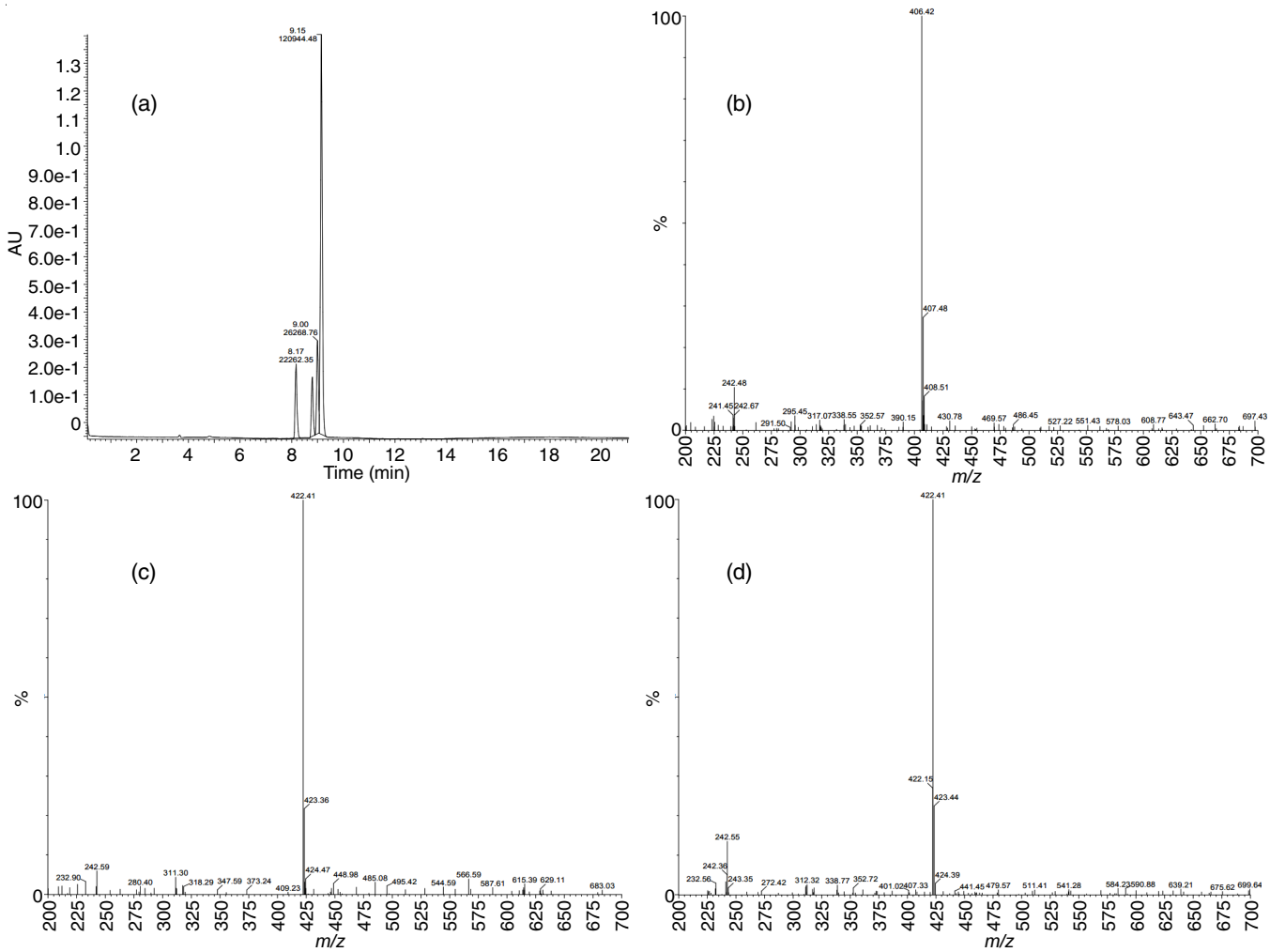


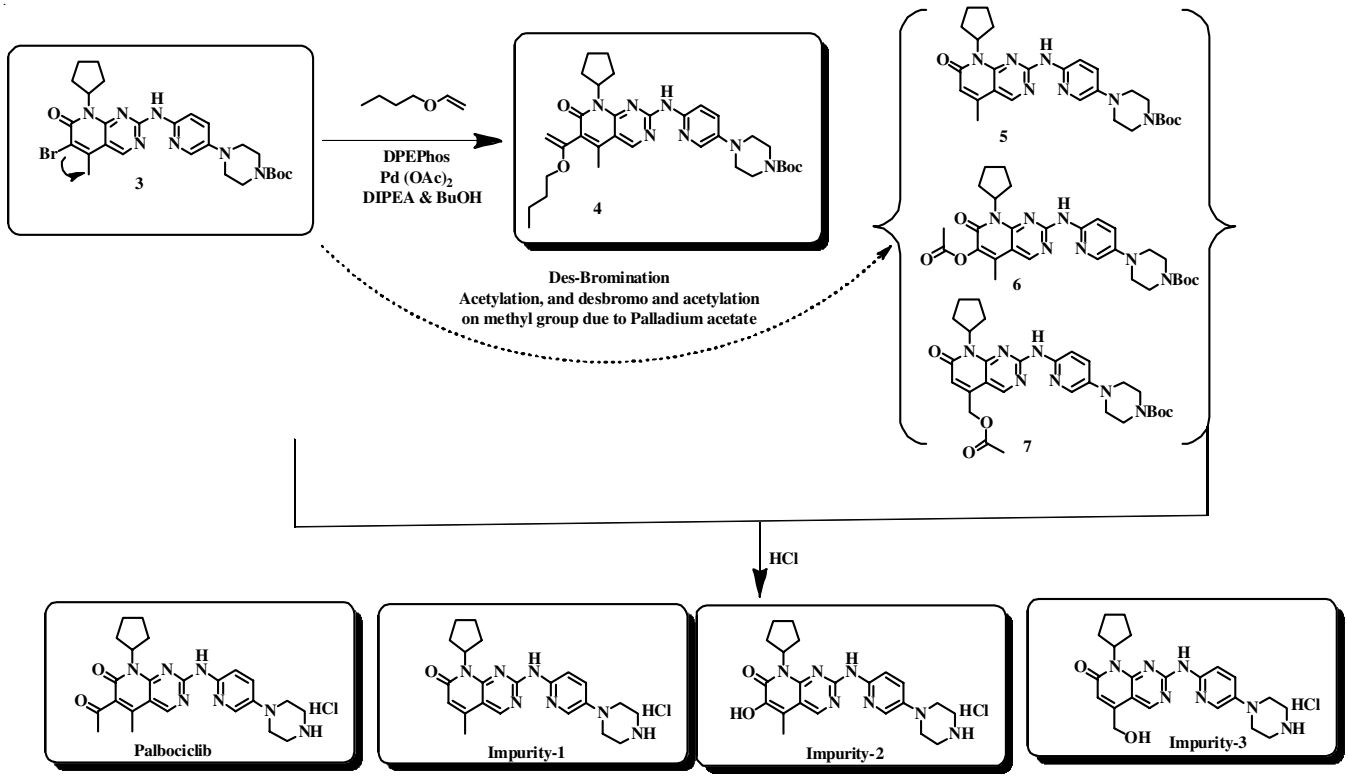
Fig. 7. LC-MS spectrum of (a) crude palbociclib, (b) mass spectrum of PDA impurity at 8.8 room temperature (c) PDHM impurity at 8.17 room temperature and (d) PDH impurity at 9.0 room temperature

the *m/z* value 422.35 in positive mode confirms the formation of hydroxy impurity (PDH impurity). This was further confirmed by spiking the pure PDH impurity with the peak isolated from the crude palbociclib.

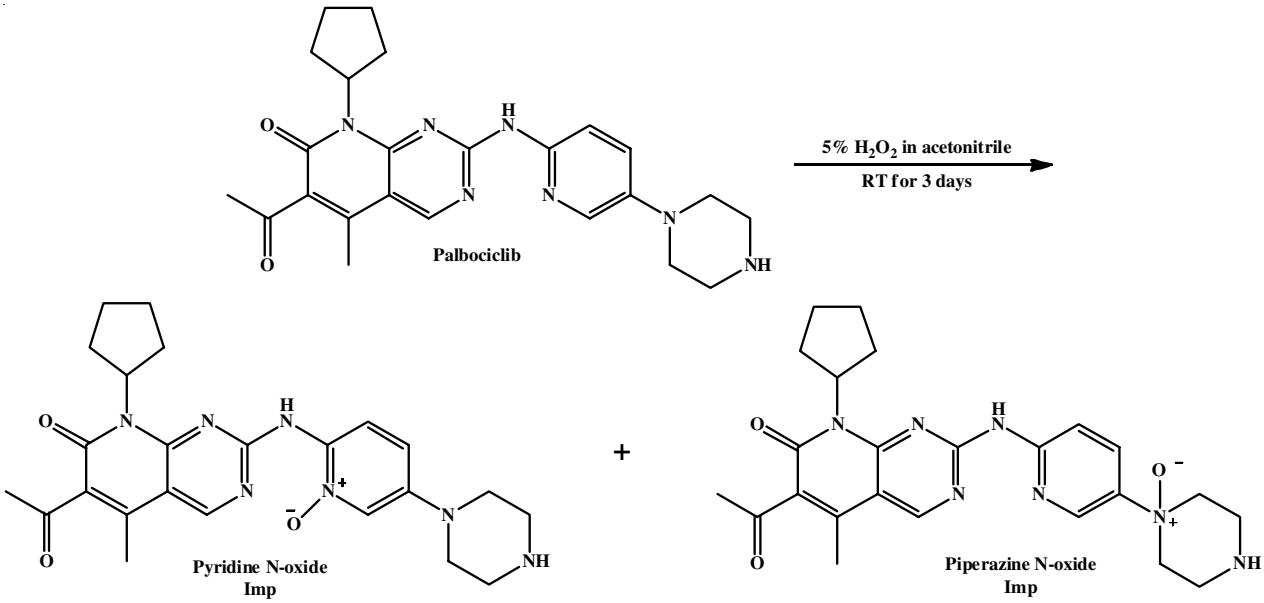
For PDHM impurity, In ^{1}H NMR, a doublet at δ 4.70 ppm corresponds to two protons at H-24 and a singlet at δ 5.54 ppm confirms the formation hydroxy group on methyl group. A singlet at δ 6.33 ppm confirms the -C-H proton at H-5. Similarly, in ^{13}C NMR, a peak at δ 115.4 ppm for C-5 in PDHM impurity was observed when compared with δ 124.5 ppm in palbociclib confirms desacetylation at C-5 and also δ 58.6 ppm for C-24 when compared with δ 13.5 ppm for C-24 in palbociclib, which further confirms the formation of hydroxy group at C-24 carbon. In mass spectrum, the *m/z* value 422.35 in positive mode confirms the formation of palbociclib desacetyl hydroxy methyl impurity. This was further confirmed by spiking the pure PDHM impurity with the peak isolated from the crude palbociclib.

Scheme-II shows the formation of three impurities from compound **3**. When compound **3** reacts with palladium acetate using DIPEA as base in 1-butanol majorly forms des-bromination product to get PDA impurity. The same compound **3**, on reaction with palladium acetate using potassium acetate as a base in 1-butanol, where the acetate anion reacts with bromide group followed by hydrolysis of acetate will form PDH impurity. PDHM impurity was formed by des-bromination followed by activation of methyl group using acetate anion through compound **7**.

When palbociclib was on the oxidative degradation, the formation of two impurities which matched the same mass in the mass spectrum. Thus, these two impurities were palbociclib pyridine N-oxide and piperazine N-oxides. The forced degradation of palbociclib was conducted to obtain pyridine N-oxide and piperazine N-oxides by utilizing various concentrations of H_2O_2 solution in acetonitrile over different time intervals at room temperature. Initially, this degradation was tried using 0.5%, 1% and 3% H_2O_2 solution in acetonitrile (**Scheme-III**) for 24 h-72 h. The formation of required impurity when used 0.5%, 1% and 3% H_2O_2 solution for 24 h was not observed. When the concentration of H_2O_2 solution was increased to 5% in acetonitrile for 72 h, around 10% of the degradation product was observed. The reaction mass was isolated and purified the crude compound using preparative HPLC to get the required N-oxides.



Scheme-II: Reaction pathway for the synthesis of PDA impurity, PDH impurity and PDHM impurity



Scheme-III: Synthetic scheme of plabociclib pyridine N-oxide and plabociclib piperidzine N-oxide

Palbociclib pyridine N-oxide was confirmed by using ^{1}H NMR, ^{13}C NMR and Mass. In ^{1}H NMR, the bridged -NH proton was shifted from δ 10.09 to δ 9.83 ppm and protons attached to N-oxide present on pyridine H-16, H-18 were shifted to downfield when compared with the same protons in palbociclib. Similarly in ^{13}C NMR, the carbons attached to N-oxide present on pyridine were shifted slightly to downfield due to the formation of N-oxide on pyridine. In mass spectrum, the *m/z* value 464.30 in positive mode was observed, which confirms the formation of pyridine N-oxide. From all the above data, it is evident that the isolated compound was palbociclib pyridine N-oxide.

In another impurty, piperizine N-oxide, based on ^{1}H NMR analysis, there was a chemical shift in H-19, H-20, H-22, H-23 protons to downfield corresponding to piperizine protons in palbociclib confirms the formation of piperizine N-oxide. In ^{13}C NMR shift of carbon values from δ 49.4 to 53.3 of C-19 and δ 44.8 to 53.3 of C-23 confirms the formation of piperizine N-oxide. In mass spectrum, the *m/z* value 464.31 in positive mode was observed and 462.25 in negative mode confirms

TABLE-1
^{1}H NMR CHEMICAL SHIFT VALUES OF PALBOCICLIB IMPURITIES

Carbon number	Palbociclib	PDA impurity	PDH impurity	PDM impurity	Pyridine N-oxide Imp	Piperazine N-oxide Imp
1	–	–	–	–		
2	8.95 (1H, s)	8.87 (1H, s)	8.75 (1H, s)	8.78 (1H, s)	9.03 (1H, s)	9.10 (1H, s)
3	–	–	–	–		
4	–	–	–	–		
5	–	6.32 (1H, s)	–	6.33 (1H, s)		
6	–	–	–	–		
7	–	–	–	–		
8	5.79-5.84 (1H, m)	5.78-5.86 (1H, m)	5.92-5.98 (1H, m)	5.79 (1H, m)	5.80-5.85 (1H, m)	5.86-5.91 (1H, m)
9a, 9b	2.22-2.23 (1H, m), 1.75-1.77 (1H, m)	2.19-2.27 (1H, m), 1.73-1.80 (1H, m)	2.25 (1H, br), 1.81-1.83 (1H, m)	2.22 (1H, m), 1.72 (1H, m)	2.18-2.21 (1H, m), 1.83-1.85 (1H, m)	2.27-2.32 (1H, m), 1.82 (1H, m)
10a, 10b	1.87 (1H, m), 1.56-1.59 (1H, m)	1.91-1.96 (1H, m), 1.57-1.63 (1H, m)	1.93 (1H, br), 1.62-1.63 (1H, m)	1.89 (1H, m), 1.57 (1H, m)	1.91-1.99 (1H, m), 1.64-1.67 (1H, m)	1.98 (1H, m), 1.63-1.64 (1H, m)
11a, 11b	1.87 (1H, m), 1.56-1.59 (1H, m)	1.91-1.96 (1H, m), 1.57-1.63 (1H, m)	1.93 (1H, br), 1.62-1.63 (1H, m)	1.89 (1H, m), 1.57 (1H, m)	1.91-1.99 (1H, m), 1.64-1.67 (1H, m)	1.98 (1H, m), 1.63-1.64 (1H, m)
12a, 12b	2.22-2.23 (1H, m), 1.75-1.77 (1H, m)	2.19-2.27 (1H, m), 1.73-1.80 (1H, m)	2.25 (1H, br), 1.81-1.83 (1H, m)	2.22 (1H, m), 1.72 (1H, m)	2.18-2.21 (1H, m), 1.83-1.85 (1H, m)	2.27-2.32 (1H, m), 1.82 (1H, m)
13	10.09 (Ar-NH, s)	10.81 (Ar-NH, s)	9.59 (Ar-NH, s)	9.94 (Ar-NH, brs)	9.83 (Ar-NH, s)	11.09 (Ar-NH, s)
14	–	–	–	–		
15	7.42-7.45 (1H, dd, *J* = 2.8 Hz)	7.85 (1H, br)	7.40-7.43 (1H, dd, *J* = 2.8 Hz)	7.49 (1H, d, *J* = 9.2 Hz)	7.34-7.37 (1H, dd, *J* = 2.4 Hz)	8.38-8.40 (1H, d, *J* = 9.6 Hz)
16	7.84 (1H, d, *J* = 9.2 Hz)	7.85 (1H, br)	7.92 (1H, d, *J* = 8.8 Hz)	7.89 (1H, d, *J* = 8.8 Hz)	8.25-8.26 (1H, d, *J* = 2.8 Hz)	8.47-8.50 (1H, dd, *J* = 3.2 Hz)
17	–	–	–	–		
18	8.03 (1H, d, *J* = 2.8 Hz)	8.06 (1H, d, *J* = 1.6 Hz)	7.99 (1H, d, *J* = 2.8 Hz)	8.05 (1H, br)	8.28-8.31 (1H, d, *J* = 9.6 Hz)	8.98-9.0 (1H, d, *J* = 2.8 Hz)
19	3.04-3.07 (2H, m)	3.42-3.44 (2H, br)	3.02-3.04 (2H, br)	3.25 (2H, br)	3.42-3.44 (2H, m)	4.46-4.71 (2H, m)
20	2.83-2.86 (2H, m)	3.25 (2H, br)	2.83-2.85 (2H, br)	3.07 (2H, br)	3.37-3.39 (2H, m)	3.74 (2H, m)
21	–	9.32 (NHHCl, br)	–		9.16 (2H, NHHCl, br)	9.8-10.02 (1H, NHHCl, br
22	2.83-2.86 (2H, m)	3.25 (2H, br)	2.83-2.85 (2H, br)	3.07 (2H, br)	3.37-3.39 (2H, m)	3.74 (2H, m)
23	3.04-3.07 (2H, m)	3.42-3.44 (2H, br)	3.02-3.04 (2H, br)	3.25 (2H, br)	3.42-3.44 (2H, m)	4.46-4.71 (2H, m)
24	2.42 (3H, s)	2.41 (3H, s)	2.25 (3H, s)	4.71 (2H, d, *J* = 4.4 Hz)	2.33 (3H, s)	2.33 (3H, s)
25	–	–	–	–		
26	2.30 (3H, s)	–	–	–	2.43 (3H, s)	2.44 (3H,s)
27	–	–	–	–		
28	–	–	–	5.54 (1H, t)		

TABLE-2
^{13}C NMR CHEMICAL SHIFT VALUES OF PALBOCICLIB IMPURITIES

Carbon number	Palbociclib	PDA impurity	PDH impurity	PDM impurity	Pyridine N-oxide Imp	Piperazine N-oxide Imp
1	158.5 (C)	156.8 (C)	156.3 (C)	157.3 (C)	158.3 (C)	157.9 (C)
2	158.2 (CH)	155.7 (CH)	154.5 (CH)	155.5 (CH)	156.9 (C)	157.6 (C)
3	106.4 (C)	108.7 (C)	107.9 (C)	106.0 (C)	108.3 (C)	108.1 (C)
4	142.0 (C)	145.0 (C)	140.2 (C)	142.0 (C)	141.6 (C)	141.6 (C)
5	124.5 (C)	119.4 (CH)	161.2 (C-Ph-OH)	115.4 (CH)	125.8 (C)	130.8 (C)
6	160.7 (C=O)	162.1 (C=O)	159.2 (C=O)	162.5 (C=O)	160.5 (C)	160.5 (C)
7	154.7 (C)	155.2 (C)	150.5 (C)	155.2 (C)	154.7 (C)	154.7 (C)
8	52.8 (CH)	52.7 (CH)	53.1 (CH)	52.7 (CH)	53.1 (C)	62.0 (C)
9	27.4 (CH_2)	27.6 (CH_2)	27.8 (CH_2)	27.6 (CH_2)	27.8 (C)	27.6 (C)
10	25.3 (CH_2)	25.2 (CH_2)	25.3 (CH_2)	25.1 (CH_2)	25.4 (C)	25.2 (C)
11	25.3 (CH_2)	25.2 (CH_2)	25.3 (CH_2)	25.1 (CH_2)	25.4 (C)	25.2 (C)
12	27.5 (CH_2)	27.6 (CH_2)	27.8 (CH_2)	27.6 (CH_2)	27.8 (C)	27.6 (C)
13	–	–	–	–		
14	144.0 (C)	148.8 (C)	145.1 (C)	148.6 (C)	142.5 (C)	154.1 (C)
15	115.2 (CH	116.1 (CH)	113.8 (CH)	115.3 (CH)	119.9 (C)	113.2 (C)
16	129.1 (CH)	123.7 (CH)	124.9 (CH)	128.5 (CH)	130.8 (C)	138.9 (C)
17	144.1 (C)	141.9 (C)	143.4 (C)	144.5 (C)	142.5 (C)	154.1 (C)
18	135.2 (CH)	134.3 (C)	135.3 (CH)	131.8 (CH)	136.9 (C)	140.6 (C)
19	49.4 (CH_2)	45.0 (CH_2)	48.5 (CH_2)	45.3 (CH_2)	44.8 (C)	53.3 (C)

20	45.4 (CH_2)	42.1 (CH_2)	45.4 (CH_2)	42.3 (CH_2)	42.0 (C)	38.1 (C)
21	–	–	–	–		
22	45.4 (CH_2)	42.1 (CH_2)	45.4 (CH_2)	42.3 (CH_2)	42.0 (C)	38.1 (C)
23	49.4 (CH_2)	45.0 (CH_2)	48.5 (CH_2)	45.3 (CH_2)	44.8 (C)	53.3 (C)
24	13.5 (CH_3)	16.7 (CH_3)	9.3 (CH_3)	58.6 (CH_2-OH)	13.8 (C)	13.7 (C)
25	202.4 (C=O)	–	–	–	202.3 (C)	202.3 (C)
26	31.2 (CH_3)	–	–	–	31.2 (C)	31.3 (C)
27	–	–	–	–		
28	–	–	–	–		

the formation of piperzine N-oxide. From all the above data, it confirms the formation of palbociclib piperizine N-oxide.

The ^{1}H NMR values and ^{13}C NMR values of the impurities compared with palbociclib were depicted in Tables 1 and 2.

Conclusion

In conclusion, five novel impurities of palbociclib were isolated, synthesized and confirmed using different analytical techniques. Three impurities were obtained during the manufacturing process of palbociclib API. These impurities were formed in the penultimate step due to the usage of palladium acetate in the manufacturing process. These three impurities were identified as palbociclib desacetyl (PDA impurity), palbociclib desacetyl hydroxy (PDH impurity) and palbociclib desacetyl hydroxyl methyl (PDHM impurity). Another two impurities were identified as palbociclib pyridine N-oxide and palbociclib piperazine N-oxide, which were formed during the oxidative degradation of palbociclib. All the above impurities were easily prepared in the laboratory and can be used as reference materials during the manufacturing process of palbociclib API and formulation.

ACKNOWLEDGEMENTS

The authors are thankful to SVAK Life Sciences and KL University for supporting to conduct this work.

CONFLICT OF INTEREST

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

REFERENCES

1. K.A. Cadoo, A. Gucalp and T.A. Traina, *Breast Cancer*, **6**, 123 (2014); https://doi.org/10.2147/BCTT.S46725
2. J. McCain, *P T.*, 40, 511 (2015).
3. R.S. Finn, M. Martin, H.S. Rugo, S. Jones, S.-A. Im, K. Gelmon, N. Harbeck, O.N. Lipatov, J.M. Walshe, S. Moulder, E. Gauthier, D.R. Lu, S. Randolph, V. Diéras and D.J. Slamon, *N. Engl. J. Med.*, **375**, 1925 (2016); https://doi.org/10.1056/NEJMoa1607303
4. K. Tamura, H. Mukai, Y. Naito, K. Yonemori, M. Kodaira, Y. Tanabe, N. Yamamoto, S. Osera, M. Sasaki, Y. Mori, S. Hashigaki, T. Nagasawa, Y. Umeyama and T. Yoshino, *Cancer Sci.*, **107**, 755 (2016); https://doi.org/10.1111/cas.12932
5. A. Teasdale, D. Elder, J. Harvey and S. Spanhaak, in eds.: A. Teasdale, D. Elder and R.W. Nims, Q2(R1) Validation of Analytical Procedures - Impurities in New Drug Substances and New Drug Products, In: ICH Quality Guidelines, Wiley (2017).
6. H.J. Bowles and K.L. Clarke, J. Adv. Pract. Oncol., 6, 577 (2015).
7. H.K. Chandrawanshi, K. Pilaniya, U. Pilaniya, P. Manchandani, P. Jain and N. Singh, *J. Adv. Pharm. Technol. Res.*, **1**, 302 (2010); https://doi.org/10.4103/0110-5558.72422
8. P.L. Toogood, P.J. Harvey, J.T. Repine, D.J. Sheehan, S.N. VanderWel, H. Zhou, P.R. Keller, D.J. McNamara, D. Sherry, T. Zhu, J. Brodfuehrer, C. Choi, M.R. Barvian and D.W. Fry, *J. Med. Chem.*, **48**, 2388 (2005); https://doi.org/10.1021/jm049354h
9. S.N. VanderWel, P.J. Harvey, D.J. McNamara, J.T. Repine, P.R. Keller, J. Quin, R.J. Booth, W.L. Elliott, E.M. Dobrusin, D.W. Fry and P.L. Toogood, *J. Med. Chem.*, **48**, 2371 (2005); https://doi.org/10.1021/jm049355+
10. S. Duan, D. Place, H.H. Perfect, N.D. Ide, M. Maloney, K. Sutherland, K.E. Price Wiglesworth, K. Wang, M. Olivier, F. Kong, K. Leeman, J. Blunt, J. Draper, M. McAuliffe, M. O'Sullivan and D. Lynch, *Org. Process Res. Dev.*, **20**, 1191 (2016); https://doi.org/10.1021/acs.oprd.6b00070
11. M.T. Maloney, B.P. Jones, M.A. Olivier, J. Magano, K. Wang, N.D. Ide, A.S. Palm, D.R. Bill, K.R. Leeman, K. Sutherland, J. Draper, A.M. Daly, J. Keane, D. Lynch, M. O'Brien and J. Tuohy, *Org. Process Res. Dev.*, **20**, 1203 (2016); https://doi.org/10.1021/acs.oprd.6b00069
12. B.P. Chekal, J. Ewers, S.M. Guinness, N.D. Ide, K.R. Leeman, R.J. Post, A.M. Rane, K. Sutherland, K. Wang, M. Webster, G.J. Withbroe, J. Draper, D. Lynch, M. McAuliffe and J. Keane, *Org. Process Res. Dev.*, **20**, 1217 (2016); https://doi.org/10.1021/acs.oprd.6b00071
13. S.-T. Li, J. Chen, Cl. Feng, W. Yang and M. Ji, *Chem. Pap.*, **73**, 3043 (2019); https://doi.org/10.1007/s11696-019-00841-7
14. B. Li, R. Li, P. Dorff, J.C. McWilliams, R.M. Guinn, S.M. Guinness, L. Han, K. Wang and S. Yu, *J. Org. Chem.*, **84**, 4846 (2019); https://doi.org/10.1021/acs.joc.8b02909
15. X. Ma, L. Liu, N. Cao, Q. Zou, K. Xiong and P. Ouyang, *J. AOAC Int.*, **99**, 638 (2016); https://doi.org/10.5740/jaoacint.15-0251
16. R.K. Sadineni, R.K. Rapolu, V.V.N.K.V.P. Raju, S. Srinivasu, S. Malladi and N. Mulakayala, *Chem. Pap.*, **75**, 1475 (2021); https://doi.org/10.1007/s11696-020-01402-z
17. R.K. Rapolu, S. Areveli, V.V.N.K.V.P. Raju, S. Navuluri, N. Chavali and N. Mulakayala, *ChemistrySelect*, **4**, 4422 (2019); https://doi.org/10.1002/slct.201803825
18. M.R. Gudisela, P. Bommu, S. Navuluri and N. Mulakayala, *ChemistrySelect*, **3**, 7152 (2018); https://doi.org/10.1002/slct.201800948
19. G. Vaddamanu, A. Goswami, N.R.S. Reddy, K.R.V. Kumar Reddy and N. Mulakayala, *ACS Omega*, **8**, 9583 (2023); https://doi.org/10.1021/acsomega.3c00100
20. G. Vaddamanu, A. Goswami, R.S.R. Nandipati, Y.T. Malireddy, V.K.R. Katam Reddy and N. Mulakayala, *ACS Omega*, **8**, 38494 (2023); https://doi.org/10.1021/acsomega.3c05325