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Identification, synthesis, and control of efinaconazole impurities

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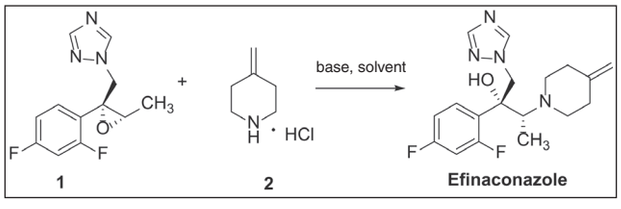
Impurities A–F were observed, identified, and confirmed during the efinaconazole production process. The possible formation pathways of the mentioned impurities were understood, and thereafter, a controlling strategy was established by locating the proper process parameters with the consideration of efficient cost and less waste as well. This impurity investigation is also essential for quality control of consistently delivering of qualified efinaconazole API.

1. Introduction

Efinaconazole (trade name Julia®), the first triazole drug for the treatment of onychomycosis, was approved by FDA in 2014 as a 10% topical solution (Patel and Dhillon 2013). It has potent antifungal activity against dermatophytes, *Candida* spps, and inhibits fungal lanosterol 14α-demethylase in the ergosterol biosynthesis pathway (Lipner and Scher 2015; Tosti and Elewski 2014). Although several synthetic routes for efinaconazole have been reported (Ogura et al. 1999; Pesti et al. 2009; Tamura et al. 2014), the ring opening reaction of epoxide **1** with piperidine hydrochloride **2** outlined in Scheme 1 was considered to be the most advantageous for the synthesis of efinaconazole and selected for process optimization on the base of our initial process research and suitability for scale-up practice.

The comprehensive identification and study of the impurities in drug substance is an essential and integral part of manufacturing process development, and is receiving more and more attention from the regulatory authorities. Among the latest guidance of FDA, MAPP 5017.2, states that ICH Q3A (R2) or Q3B (R2) qualification threshold is not sufficient for impurities with special toxicological, immunological, or clinical concerns (FDA, MAPP 5017.2). From the view of meeting with regulatory guidelines and consistently delivering a qualified product, an important goal in the process development is to evaluate and control the potential impurities. To date, the impurity profile and controlling strategy of producing efinaconazole have not yet been described. Herein we report the identification, formation, synthesis and strategy to control the level of these impurities within the acceptable criterions by the regulators.



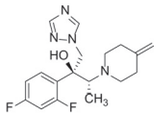
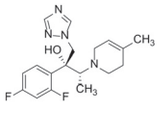
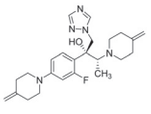
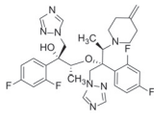
Scheme 1: Synthetic route of efinaconazole

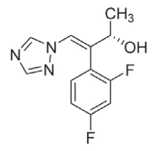
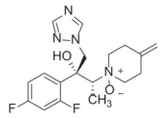
2. Investigations, results and discussion

2.1. Possible structures of unknown impurities

The final ring-opening reaction of epoxide **1** with 4–methylenepiperidine hydrochloride **2** is a common and easy transformation in view of chemistry. However, it did not appear reproducible and high-yielding as expected in our initial studies. Several impurities were observed in the laboratory batches of crude product and mother liquor by HPLC analysis. The molecular weights of impurities A–F (Table 1) were determined by liquid chromatography/mass spectrometry (LC/MS). Their definitive structures were later characterized and confirmed by ^{1}H NMR,^{13} C NMR, MS, and co-injection with the authentic compounds in HPLC. Impurities A–F can be classified into three categories based on the sources of formation, including carry-over from inputting materials (impurities A, B), side reactions (impurities C, D, E) and degradation (impurity F).

Table 1: Impurities A–F observed during process development

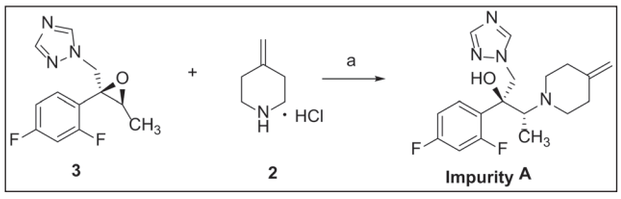
Name	Structure	Relative Retention Time (RRT) [a]
Impurity A		0.93
Impurity B		0.95
Impurity C		1.41
Impurity D		0.79

Name	Structure	Relative Retention Time (RRT) [a]
Impurity E		0.34
Impurity F		0.50

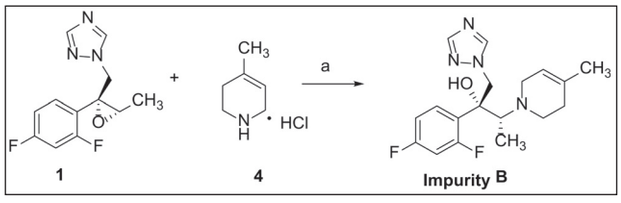
[a]Agilent Zorbax Eclipse Plus; column: C18 (3.5 µm, 100 mm × 4.6 mm); flow rate: 1.0 mL/min; and detection at 210 nm; injection vol: 20 µL; gradient elution from 83:17 A/B to 30:70 A/B over 50 min. Mobile phase A: 0.01 mol/L KH_2PO_4- K_3PO_4/MeOH, 92:8. Mobile phase B: acetonitrile. Run time: 55 min. Temperature: 30 °C. Efinaconazole retention time: 28.782 min.

2.2. Identification and plausible pathways of impurities

Two peaks (0.95 RRT and 0.96 RRT according to HPLC method) with the same MW as efinaconazole (*m/z* = 364) were observed through LC-MS. The two peaks were proposed to be the isomeric impurities of efinaconazole. In later studies, it became clear that there was a correlation between the content of impurities (**A** and **B**) and the impurity profile of compounds **1** and **2**. This discovery indicated that the two impurities could be traced back to compounds **1** and **2**. Subsequently, through the understanding of the process and impurity profile of inputting materials **1** and **2**, impurity A was attributed to impurity **3** present in compound **1**, as shown in Scheme 2. Similarly, impurity B was introduced from residual impurity **4** in compound **2**, as illustrated in Scheme 3.

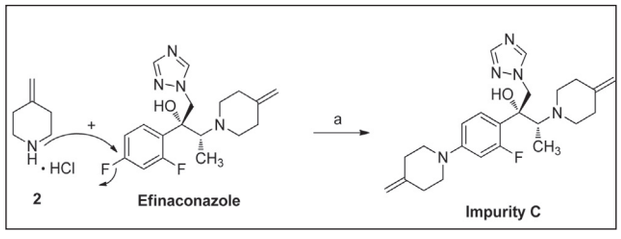


Scheme 2: Formation of Impurity A



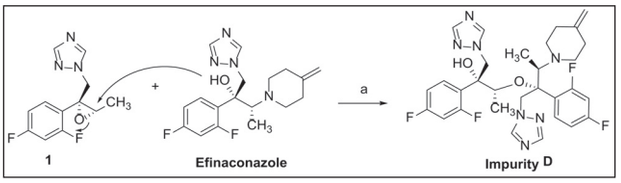
Scheme 3: Formation of impurity B

The MW of impurity C was identified as 425 by the LC-MS. It was shown that a fluorine atom was replaced by another molecule of 4-methylenepiperidine **2** (Scheme 4). It was also observed that the content of this impurity increased under the conditions of extending reaction time and charging of a large excess of 4-methylenepiperidine **2**.



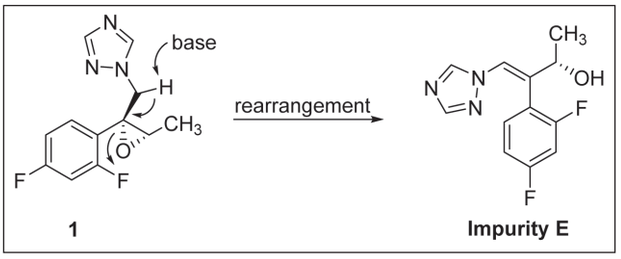
Scheme 4: Formation of Impurity C

Impurity D with the MW of 599 was determined by LC-MS, which suggested that it was probably an adduct of efinaconazole with epoxide **1** as shown in Scheme 5. Impurity D is an unexpected derivative of efinaconazole as hindered tertiary alcohols are generally regarded as weak nucleophiles. The structure of this impurity was confirmed by NMR and MS of the authentic sample later.



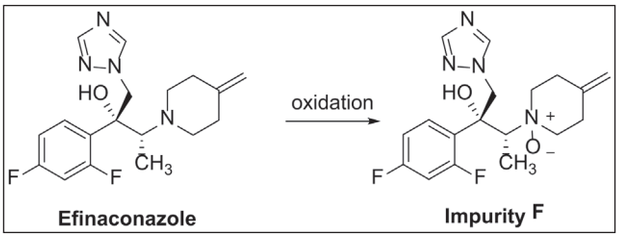
Scheme 5: Formation of Impurity D

The LC-MS analysis of the mother liquor showed another peak with the MW of 251. The structure of this impurity was assumed to be impurity E as shown in Scheme 6, which was a rearrangement product of epoxide **1** under strong alkaline conditions (Arredondo et al. 1993 and Ramachandran et al. 2013).



Scheme 6: Formation of Impurity E

Oxidative impurity F was also observed. It might be generated by oxidation of efinaconazole due to trace amount of oxygen present in the reaction system and long-time heating (16–20 h) (Scheme 7). It was also found that this impurity increased significantly under accelerated conditions (oxygen and temperature) during our later study.



Scheme 7: Formation of Impurity F

2.3. Synthesis of impurities

After preliminary identification of possible pathways for the formation of the aforementioned impurities, the structures were still required to be elucidated and confirmed. In addition, sufficient quantities of impurities were demanded for analytical methods development and validation. Thus, impurities A, B, C, E and F were synthesized. Sufficient quantity of impurity D was isolated from mother liquor. The structures of the synthesized compounds

were characterized and identified with NMR, ESI-MS, and HRMS analysis. After that, their UV absorbance and HPLC retention time of co-injection with crude samples of efinaconazole containing impurities A–F were tested. These results confirmed that the synthesized impurities were in good agreement with the postulated structures.

Impurity A was obtained by the reaction of compound **2** with diastereoisomer **3** in the presence of lithium hydroxide in acetonitrile followed by crystallization from 50% ethanol-water in good yield and with high purity (Scheme 2). Similarly, impurity B was synthesized with the same synthetic protocol for impurity A by the corresponding piperidine impurity **4** as shown in Scheme 3. Treatment of efinaconazole with excess of 4-methylenepiperidine in a sealed tube provided impurity C. Since impurity C was a viscous oil, its *p*-toluene sulfonate was prepared for handling convenience. Impurity D was isolated from the mother liquor of efinaconazole with flash column chromatography. Impurity E was synthesized following the modified procedure (Arredondo et al. 1993), in which epoxide **2** was subjected to sodium methoxide in methanol at reflux. Impurity F was prepared by oxidation of efinaconazole with *m*-CPBA in dichloromethane and purified with flash column chromatography.

2.4. Control of impurities

To ensure the quality and safety of the drug, the impurities must be controlled and reduced to the levels accepted by regulatory authorities (Ramirez et al. 2016). Additionally, the impurities that are always generated within the purging capability of the process and do not affect the quality of the final API should also be avoided or minimized in view of process optimization, by which will increase the yield, reduce the cost and the amount of waste.

Impurities A and B were originated by the corresponding impurities contained in compounds **1** and **2,** so their concentrations were significantly affected by the quality of the inputting materials. It was noted that impurity B, among all the impurities, was the most troublesome impurity. To investigate their purging capability and establish specifications of compounds **1** and **2**, multiple batches of inputting materials containing different levels of impurities **3** and **4** were evaluated in the course of process optimization. The results concluded that the level of piperidine impurity **4** in compound **2** should be lower than 0.15%; otherwise more than 0.10% impurity B in the final API would be produced. The content of impurities **3** in compound **1** should be less than 0.25%.

On account of the formation pathway of impurity C, it was expected that impurity C would be controlled by reducing the stoichiometry of compound **2** with respect to compound **1**. The level of impurity C was successfully decreased to 0.05% in reaction mixture when the equivalent of **2** was reduced to 1.2 equiv from initial 2.5 equiv. The content of impurity C in the final API was below the limit of detection by employing the established optimal reaction and work-up conditions.

The optimization study revealed that impurity D (0.07–0.16%) was always generated within the purging capability of the process and did not affect the quality of the API. After the reaction temperature was lowered to 65–70 °C from 81–83 °C (reflux in acetonitrile), this impurity level was decreased to below 0.02%.

Impurity E was a process impurity in the range of 8%–10% in reaction mixture. A lot of reaction conditions have been tried, but more than 2.5% of this impurity was still formed in the reaction. Luckily, impurity E could be removed in the crystallization stage due to the significant difference of structure feature with efinaconazole. On the course of optimizing crystallization conditions, we studied various solvent systems, of which the ethanol/water system was found to be the best choice on the base of impurities purging capability and recovery yield (Table 2). The level of impurity E was successfully reduced to below 0.02% in the final API.

Table 2: Screening results of the crystallization solvents[c]

Entry	Solvent (V/V) [a]	Impurity E [b] (%)	Efinaconazole [b] (%)	Yield (%)
1	MeOH-H_2O (2/2)	0.02	99.75	92.2
2	EtOH-H_2O (2.5/2.5)	0.01	99.96	95.6
3	*i*-PrOH-H_2O (2.5/2.5)	0.01	99.91	79.5
4	Acetone-H_2O (2/2)	0.03	99.73	85.2
5	Acetonitrile (3)	0.02	99.77	87.7
6	EtOAc/Heptane (8/1)	0.16	99.72	75.3

[a]The required volume for 1g of the crude API. [b]Purity was calculated from the HPLC area. [c]Conditions: crude efinaconazole (5 g scale).

The formation of impurity F was controlled by carrying out the coupling reaction under inert atmosphere and avoiding oxidative factors in work-up, crystallization and drying stages.

3. Experimental

3.1. Materials and reagents

HPLC grade acetonitrile was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). The silica gel (100–200 mesh) used for column chromatography was purchased from Qingdao Haiyang Chemical Co., Ltd China. DMSO-d_6 and $CDCl_3$ used in nuclear magnetic resonance spectroscopy (NMR) was purchased from Cambridge isotope laboratories, Inc. (Andover, MA, USA). Starting materials 1, 2 and reference standard (99.92%) of efinaconazole were obtained from Topharman Shanghai Co., Ltd. (Shanghai, China). All commercially available material and solvents were used directly without further purification.

3.2. High performance liquid chromatography (analytical)

Chromatographic separations were performed on HPLC system with Agilent 1260 separation module equipped with DAD detector with OpenLab pro data handling system [Agilent Corporation, USA]. The analysis was carried out on Agilent Zorbax Eclipse Plus; column, 100 mm long, 4.6 mm i.d., 3.5 µm particle size column. Mobile phase A was 0.01 mol/L KH_2PO_4-K_3PO_4/MeOH, 92:8. Mobile phase B was acetonitrile. Injection volume was 20µl, flow rate was 1.0 ml/min and column oven temperature was 30 °C. UV detection was carried out at 210 nm. Data acquisition time was 55 min. Pump was run in a gradient mode and the program was as follows: Time (min)/A (v/v): B (v/v); T0.01/83:17, T50/30:70.

3.3. LC-MS analysis

LC-MS analysis was carried out with an THERMO MSQ PLUS mass spectrometer coupled with THERMO UltiMate 3000 HPLC, ZORBAX Eclipse Plus C18 Papid Resolution column (100×4.6 mm, 3.5 m) and VWD detector. The capillary voltage was maintained at 3 KV, the drying gas temperature and pressure was kept at 300 °C and 80KPa respectively. High pure nitrogen was used as drying gas. Mobile phase A was 0.05%TFA/H2O. Mobile phase B was acetonitrile. Injection volume was 10 µl, flow rate was 1.0 ml/min and column oven temperature was 30 °C. UV detection was carried out at 210 nm. Data acquisition time was 25 min. Pump was run in a gradient mode and the program was as follows: Time (min)/A (v/v):B (v/v); T0.01/90:10, T25/30:70. Chromeleon 7 workstation software was employed for data acquisition and data processing. LC-MS spectra were acquired from m/z 100-800.

3.4. Mass spectroscopy

Mass spectral analysis were performed on a The ESI mass spectra were determined on a THERMO LTQ (Thermo Fisher Scientific Inc. USA). Detection of ions was performed in electrospray ionization, positive.

3.5. NMR spectroscopy

The ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) and NOE NMR analyses were performed using a Bruker spectrometer using deuterated dimethylsulfoxide (DMSO-d_6) or chloroform ($CDCl_3$) as a solvent and tetramethylsilane (TMS) as an internal standard at 25 °C. The ^{1}H chemical shift values were reported on the δ scale in ppm, relative to TMS (δ = 0.00 ppm) and in the ^{13}C NMR the chemical shift values were reported relative to DMSO-d_6 (δ = 39.50 ppm) or $CDCl_3$ (δ = 78.75 ppm).

3.6. High-resolution MS

Finnigan MAT-95/711 spectrometer (Thermo Fisher Scientific Inc. Barrington, IL).

3.7. Preparation of impurity A

To a mixture of compound **3** (3 g, 12 mmol) and compound **2** (3.2 g, 24 mmol) in CH_3CN (30 mL) was added LiOH·H_2O (1 g, 24 mmol) and the resulting reaction mixture was stirred at 80 °C for 15 h. Then the reaction mixture was concentrated under

reduced pressure, and the residue was dissolved in a solution of EtOH (6 mL) and H_2O (6 mL). When the resulting solution was cooled to 0–5 °C, the precipitated solid was filtered and dried in vacuum to give impurity A as off-white powder (2.5 g) in 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.00 (s, 1H), 7.76 (s, 1H), 7.45–7.40 (m, 1H), 6.76–6.70 (m, 2H), 4.99 (dd, *J* = 9.6, 1.2 Hz, 1H), 4.59 (s, 2H), 4.43 (d, *J* = 1.2 Hz, 1H), 3.12 (d, *J* = 2.4 Hz, 1H), 2.64–2.58 (m, 2H), 2.34–2.29 (m, 2H), 2.01–1.95 (m, 4H), 1.00 (d, *J* = 3.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm):164.80 (dd, *J* = 197.9, 10.2 Hz), 161.35 (dd, *J* = 195.3, 9.6 Hz), 152.74, 147.41, 145.47, 131.46 (dd, *J* = 7.4, 5.1 Hz), 126.99–120.86 (m), 112.49–112.30 (m), 109.31, 105.20–104.78 (m), 79.64 (d, *J* = 4.2 Hz), 66.87, 56.83 (d, *J* = 5.1 Hz), 53.83, 36.54, 10.01. MS (ESI): *m/z* = 349.2 $[M + H]^+$. HRMS (ESI): Calcd for $C_{18}H_{23}F_2N_4O$ $[M + H]^+$ 349.1834, Found 349.1831.

3.8. Preparation of impurity B

To a mixture of compound **1** (1 g, 4 mmol) and compound **4** (0.8 g, 6 mmol) in CH_3CN (10 mL) was added $LiOH{\cdot}H_2O$ (0.25 g, 6 mmol) and the reaction mixture was stirred at 80 °C for 18 h. The mixture was concentrated under vacuum. Then the residual was poured into water and extracted with dichloromethane. The organic phase was washed with brine, dried over sodium sulfate, and evaporated under reduced pressure to give the crude material. The crude material was purified by column chromatography on silica gel using *n*-heptane-EtOAc (3:1, v/v) as eluent to give impurity B (0.95 g) as a solid in 68% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.29 (s, 1H), 7.66 (s, 1H), 7.35–7.28 (m, 1H), 7.15–7.09 (m, 1H), 6.94–6.89 (m, 1H), 5.49 (s, 1H), 4.89 (s, 1H), 5.37 (s, 1H), 4.82–4.74 (m, 2H), 3.24–3.13 (m, 2H), 3.03–2.29 (m, 2H), 2.42–2.36 (m, 1H), 2.19–2.15 (m, 1H), 1.93–1.89 (m, 1H), 1.65 (s, 3H), 0.78 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 163.61 (dd, *J* = 199, 10 Hz), 159.65 (dd, *J* = 195.6, 9.8 Hz), 151.25, 144.49, 132.94, 130.89–130.77 (m), 124.75–124.62 (m), 119.62, 111.50–111.31 (m), 104.29 (dd, *J* = 22.6, 20.2 Hz), 77.74 (d, *J* = 3.7 Hz), 63.43, 55.85 (d, *J* = 6.6 Hz), 50.88 (d, *J* = 5.4 Hz), 46.10, 31.28, 22.88, 7.36 (d, *J* = 2 Hz). MS (ESI): *m/z* = 349.4 $[M + H]^+$. HRMS (ESI): Calcd for $C_{18}H_{23}F_2N_4O$ $[M + H]^+$ 349.1834, Found 349.1833.

3.9. Preparation of impurity C and p-toluene sulfonate

Efinaconazole (5 g, 14.3 mmol) was mixed with the free base of compound **2** (26.5 g, 273 mmol) and the resulting mixture was stirred in a sealed tube at 140 °C for 72 h. After the reaction mixture was cooled to 20–25 °C, dichloromethane (600 mL) and water (250 mL) were added, and the layers were separated. The separated organic phase was washed with water (250 mL), dried over Na_2SO_4 and concentrated under reduced pressure to give yellow oil. The oil was purified using column chromatography using CH_2Cl_2-MeOH (60:1, v/v) as eluent to afford the impurity **C** (2.8 g, 46% yield) as syrupy mass. The obtained impurity **C** (2.2 g, 5.17 mmol) and *p*-toluenesulfonic acid (0.93 g, 5.41 mmol) were dissolved in methanol (20 mL) and the mixture was stirred at room temperature for 2 h. Then methanol was evaporated under reduced pressure to give a foam. Ethyl acetate (5 mL) and hexane were added, and the precipitated solid was stirred at 20–30 °C for 1 h and filtered. The cake was washed with ethyl acetate-hexane (5 mL, 1:1, v/v) and dried at 40 °C under reduced pressure to afford impurity **C** *p*-toluene sulfonate (2.72 g) in 40.5% overall yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.79 (s, 1H), 8.34 (s, 1H), 8.05 (s, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.40 (m, 1H), 7.12 (d, *J* = 7.6 Hz, 2H), 6.83–6.71 (m, 3H), 5.02 (d, *J* = 14.8 Hz, 1H), 4.85–4.76 (m, 5H), 3.73 (d, *J* = 6.4 Hz, 1H), 3.52 (s, 6H), 3.14 (d, *J* = 10.0 Hz, 1H), 2.86 (d, *J* = 11.6 Hz, 2H), 2.64 (m, 1H), 2.43 (d, *J* = 14.4 Hz, 1H), 2.32–2.21 (m, 8H), 1.22 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 162.17, 160.25, 153.79 (d, *J* = 8.9 Hz), 153.03, 147.80, 147.37 (d, *J* = 13.6 Hz), 141.73, 139.61, 130.82 (d, *J* = 4.0 Hz), 129.95, 127.35, 116.49 (d, *J* = 0.9 Hz), 113.01, 112.31, 110.55, 103.90 (d, *J* = 22 Hz), 77.77, 69.49, 56.61, 51.50, 50.80, 34.98, 32.53 (d, *J* = 64 Hz), 22.66, 11.06. MS (ESI): *m/z* = 426.3. $[M + H]^+$. HRMS (ESI): Calcd for $C_{24}H_{33}FN_5O$ $[M + H]^+$ 426.2664, Found 426.2669.

3.10. Isolation of impurity D

Then impurity D was isolated from the recrystallization filtrate of efinaconazole and purified by flash chromatography on silica gel (CH_2Cl_2-MeOH, 20:1) as off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.38 (s, 1H), 8.96 (s, 1H), 8.43 (s, 1H), 7.78 (s, 1H), 7.40–7.24 (m, 3H), 7.08–6.01 (m, 3H), 6.90–6.88 (m, 1H), 6.02 (s 1H), 5.28 (d, *J* = 14.0 Hz, 1H), 5.20 (d, *J* = 7.2 Hz, 1H), 4.93 (d, *J* = 14.4 Hz, 1H), 4.84–4.80 (m, 1H), 4.66 (s, 2H), 4.51 (d, *J* = 14.4 Hz, 1H), 3.19–3.16 (m, 1H), 2.90 (brs, 2H), 2.47 (brs, 1H), 2.30–2.23 (m, 4H), 1.35 (d, *J* = 6.8 Hz, 3H), 0.76 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 164.24 (dd, *J* = 200.6, 9.8 Hz), 163.79 (dd, *J* = 198.6, 9.8 Hz), 160.05 (dd, *J* = 197.2, 9.3 Hz), 159.77 (dd, *J* = 196.0, 9.2 Hz), 151.64, 146.14, 145.06, 144.35, 142.29, 130.79–130.38 (m), 123.28–123.15 (m), 121.14 (d, *J* = 11.2 Hz), 112.29 (d, *J* = 16.4 Hz), 111.48 (d, *J* = 16.5 Hz), 107.97, 104.44–104.02 (m), 76.45 (d, *J* = 3.5 Hz), 64.60, 59.79–59.56 (m), 54.58, 45.13, 35.17, 30.91, 16.37, 8.52. ^{19}F NMR (376.5 MHz, $CDCl_3$) δ (ppm): –105.1, –108.1, –108.3, –110.0. MS (ESI): *m/z* = 600.44. $[M + H]^+$; HRMS (ESI): Calcd for $C_{30}H_{34}F_4N_7O_2$ $[M + H]^+$ 600.2705, Found 600.2712.

3.11. Preparation of impurity E

To a solution of epoxy compound **1** (3.5 g, 10 mmol) in methanol (20 mL) was added sodium methoxide (1.08 g, 20 mmol). The reaction mixture was heated and refluxed for about 6 h. After the complete conversion monitored by TLC, the reaction mixture was cooled and concentrated under reduced pressure. Then residual was poured into H_2O (20 mL) and was extracted with dichloromethane (50 mL). The separated organic phase was washed with brine, dried over Na_2SO_4 and then concentrated in vacuum to give the crude material. The crude material was then purified by column chromatography using as eluent to yield impurity **E** (1 g) as a pale yellow oil in 40% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.88 (s, 1H), 7.65 (s, 1H), 7.39 (d, *J* = 1.2 Hz, 1H), 7.21–7.15 (m, 1H), 6.98–6.88 (m, 2H), 4.70–4.67 (m, 1H), 2.59 (brs, 1H), 1.34 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 164.25 (dd, *J* = 198.5, 8.7 Hz), 160.81 (dd, *J* = 198.8, 10.0 Hz), 151.36, 142.39, 132.51, 131.43–131.32 (m), 123.17, 118.73–118.57 (m), 112.41–112.22 (m), 105.03–104.62 (m), 69.93, 21.94. MS (ESI): *m/z* = 252.2. $[M + H]^+$. HRMS (ESI): Calcd for $C_{12}H_{12}F_2N_3O$ $[M + H]^+$ 252.0943, found 252.0946.

3.12. Preparation of impurity F

To a solution of efinaconazole (2 g, 5.74 mmol) in dichloromethane (20 mL) was added 85% *m*-CPBA (1.75 g, 8.61 mmol) in six portions under 0–5 °C with cooling by the ice bath. The reaction mixture was stirred for 1h at room temperature. Then the pH of the reaction mixture was adjusted to 8–9 with 5% sodium bicarbonate solution. The phases were separated and aqueous phase was extracted with dichloromethane (20 mL). The combined organic phase was washed with brine, dried over Na_2SO_4 and then concentrated under reduced pressure to give the crude material. The crude material was purified by column chromatography using CH_2Cl_2-MeOH, (15:1, v/v) as eluent to obtain impurity **F** (1.44 g) as a pale yellow solid in 68.9% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.22 (s, 1H), 8.34 (s, 1H), 7.76–7.68 (m, 2H), 7.25–7.19 (m, 1H), 7.06–7.01 (m, 1H), 5.01 (d, *J* = 13.6 Hz, 1H), 4.73–4.69 (m, 2H), 4.64 (d, *J* = 14.0 Hz, 1H), 3.68 (q, *J* = 6.8 Hz, 1H), 3.59 (td, *J* = 12.8, 3.2 Hz, 1H), 3.11–3.08 (m, 1H), 3.02–2.90 (m, 2H), 2.81–2.74 (m, 1H), 2.67–2.65 (m, 1H), 2.10 (d, *J* = 13.6 Hz, 1H), 1.97 (d, *J* = 13.2 Hz, 1H), 1.40 (dd, *J* = 7.2, 3.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 163.98 (dd, *J* = 199.0, 9.8 Hz), 160.04 (dd, *J* = 195.7, 9.2 Hz), 150.94, 145.70, 140.52, 133.06–132.93 (m, 1H), 123.84–123.69 (m, 1H), 111.96–111.77 (m, 1H), 110.44, 104.43–104.01 (m, 1H), 78.46 (d, *J* = 3.6 Hz), 76.15, 67.61, 59.27, 57.37 (d, *J* = 7.5 Hz), 28.87 (d, *J* = 44.8 Hz), 12.97 (d, *J* = 5.9 Hz). MS (ESI): *m/z* = 365.27 $[M + H]^+$. HRMS (ESI): Calcd for $C_{18}H_{23}F_2N_4O_2$ $[M + H]^+$ 365.1784, Found 365.1790.

Conflicts of interest: None declared.

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