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# Synthesis and characterization of four process impurities in pazopanib

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*Received May 15, 2018, accepted June 20, 2018*

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*Pharmazie 73: 494-497 (2018)* *doi: 10.1691/ph.2018.8538*

Pazopanib (trade name Votrient®) is a potent and selective multi-targeted tyrosine kinase inhibitor that blocks tumor growth and inhibits angiogenesis. Based on a recently reported procedure, we herein report the first synthesis of four potential process impurities generated in the production of pazopanib. The structure of these impurities were synthesized and characterized by $^{1}H$ NMR, $^{13}C$ NMR and HRMS data. The possible formation mechanisms of these impurities were also elucidated. These findings should be useful for the quality control of pazopanib in manufacture.

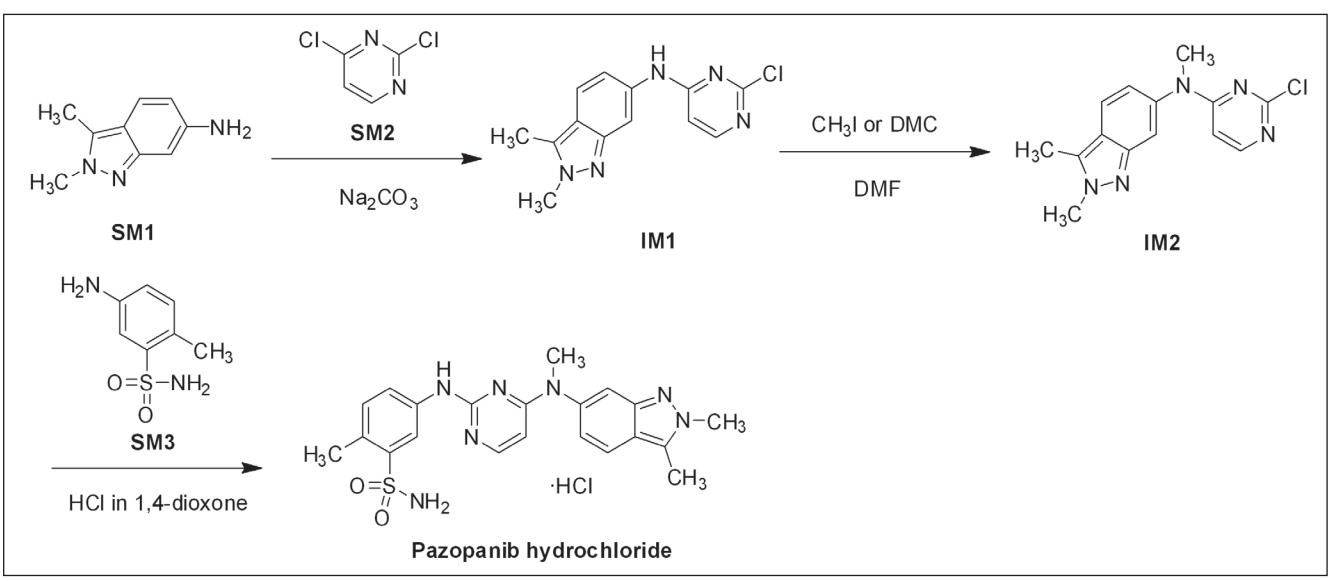
## 1. Introduction

Pazopanib, a novel multi-target inhibitor of vascular endothelial growth factor receptor (VEGFR)-1,2,3, platelet derived growth factor receptor (PDGFR) α/β, fibroblast growth factor receptor and the stem cell receptor/ c-Kit, can increase progression free survival in renal cell carcinoma and in soft tissue sarcoma compared to placebo (Goh et al. 2010; Sonpavde et al. 2008; Keisner et al. 2011). It was approved by U.S. Food and Drug Administration (FDA) for the treatment of metastatic renal cell carcinoma under the brand name of Votrient ® in 2009 (FDA Label 2009).

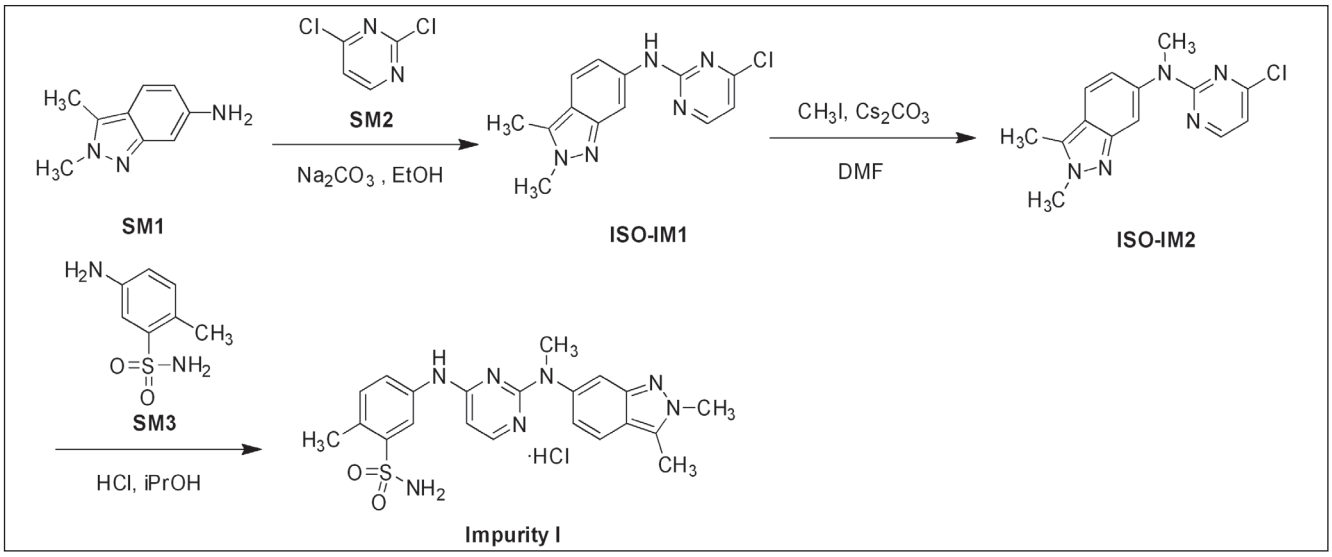
The detection, identification, quantification control of impurities especially process impurities originating in the active pharmaceutical ingredient (API) manufacturing process have become an important element of drug development (Gorog et al. 1997; ICH 2006). Thus, it is mandatory to characterize and synthesize the potential process impurities produced in the manufacture of pazopanib for the quality control purpose. Some potential process impurities with or without structures were consistently detected in amounts of 0.1% or above determined by HPLC assay during the preparation of pazopanib (Li et al. 2010). However, to the best of our knowledge, some of these impurities are not commercially available and no synthetic methods were reported. As a result, attempts were made to synthesize and then confirm these new related substances in this work.

## 2. Investigations, results and discussion

One of the most common synthetic method of pazopanib is summarized in Scheme 1 (Okaniwa et al. 2012; Elder et al. 2013). Condensation of the key starting material 2,3-dimethyl-6-amino-2H-indazole (**SM1**) with 2,4-dichloropyrimidine (**SM2**) in the presence of sodium carbonate afforded intermediate I (**IM1**), which was then methylated by $CH_3I$ or dimethylcarbonate (DMC) under mild conditions providing intermediate II, 2,3-dimethyl-N-(2-chloropyrimidin-4-yl)-N-methyl-2H-indazol-6-amine. (**IM2**). Nucleophilic substitution of **IM2** with 2-methyl-5-aminobenzene sulfonamide (**SM3**) resulted in the target compound pazopanib. Based on the process route and literature, four possible process impurities were proposed in the Table.

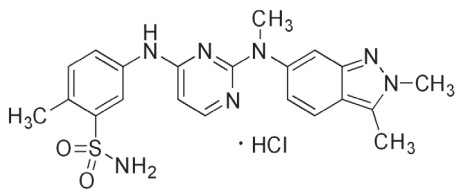
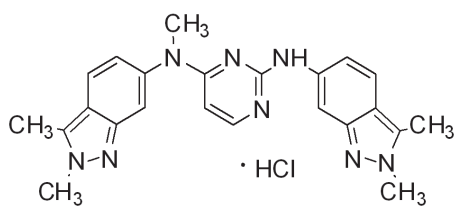
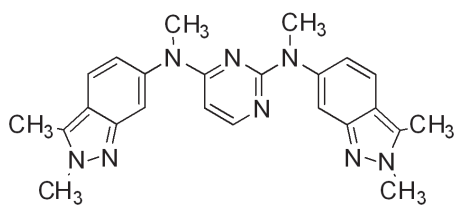
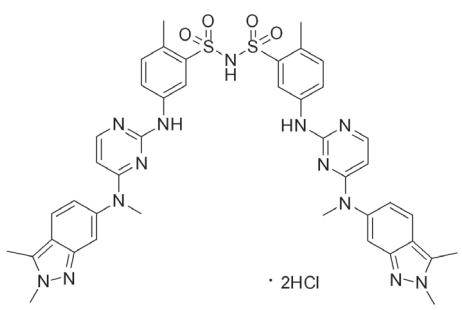
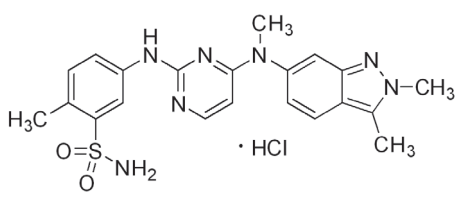



**Scheme 1:** Manufacturing process of pazopanib hydrochloride




**Scheme 2:** Synthesis of pazopanib impurity **I**

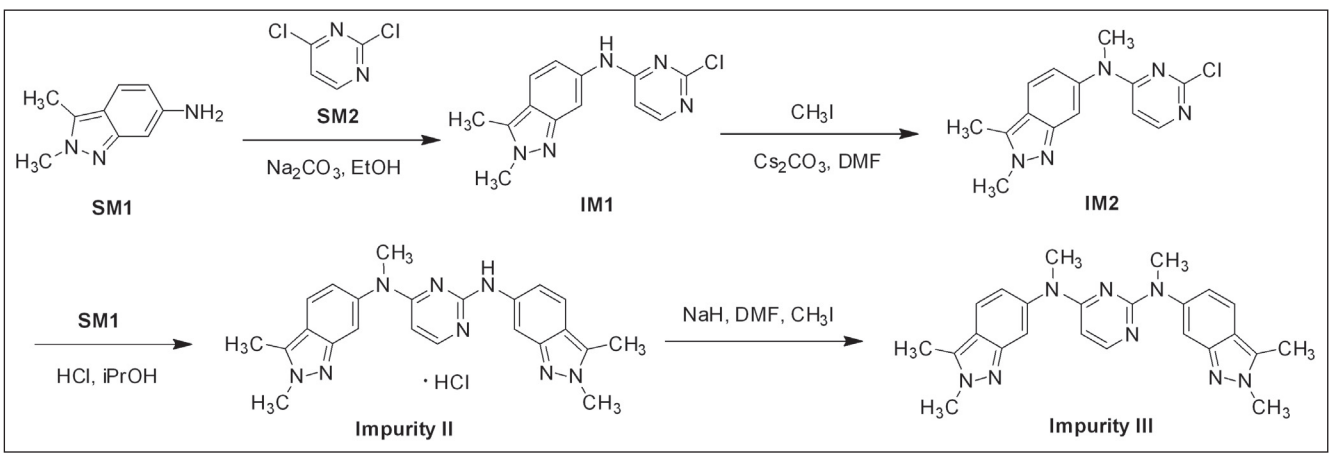
**Table: Possible structures of impurities I-IV**

| Name | m/z ([M+H]⁺) | Retention time (min) | Suggested structure |
|---|---|---|---|
| Impurity **I** | 438.1705 | 18.877 |  |
| Impurity **II** | 413.2193 | 21.641 |  |
| Impurity **III** | 427.2348 | 24.414 |  |
| Impurity **IV** **(CQA impurtiy)** | 858.3082 | 16.119 |  |
| API | | 20.487 |  |

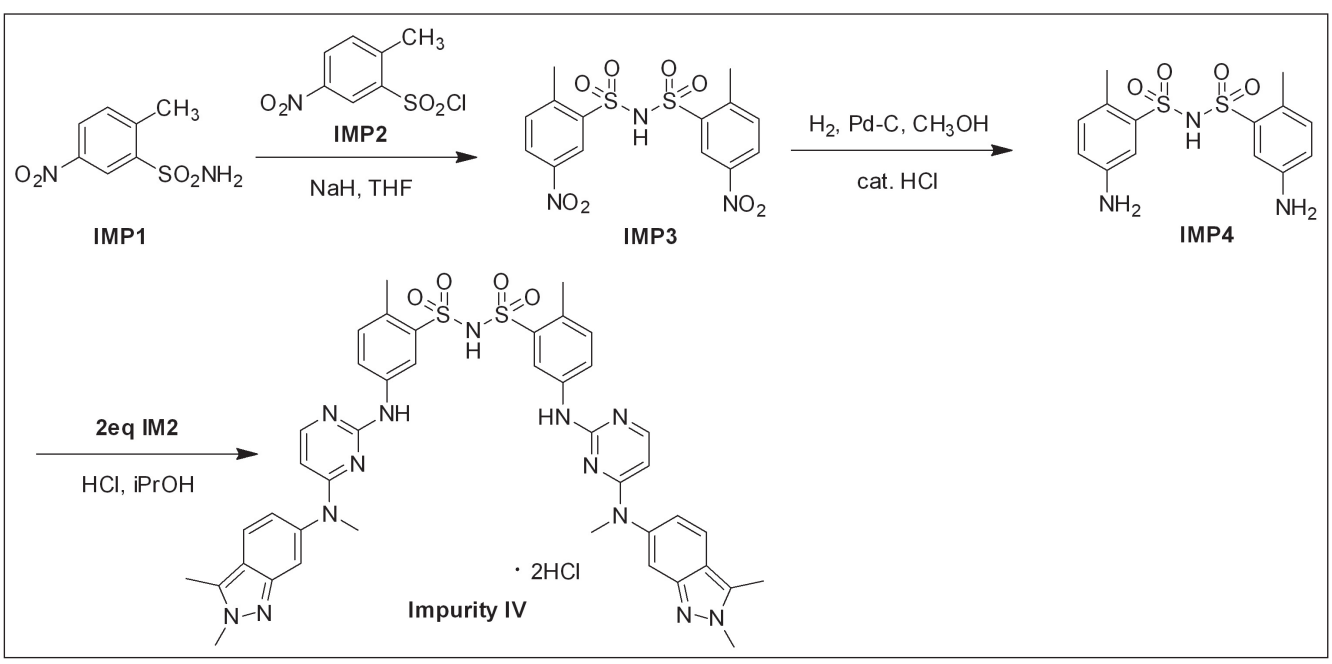
Impurity **I** was supposed to be a regioisomer of pazopanib. During the preparation of **IM1**, the two chlorine atoms of **SM2** showed different reactivities towards **SM1** in the presence of base. The 4 position is more prone to be attacked by nucleophile reagents under basic conditions. However, the 2 position can also respond to the nucleophile reagents under the same condition producing the minor product (detected at nearly 4% area level in the reaction mixture by HPLC). This impurity was thus supposed to be formed due to the amination competition at 2-Cl position versus 4-Cl position of **SM2**. Thus, in order to obtain impurity **I**, the isomer of **IM1** was firstly enriched and isolated from the mother liquor, subsequently reacted with $CH_3I$ and **SM3** afforded impurity **I** via the same route as pazopainib (Scheme 2).

On the other hand, in the manufacturing process of **IM1**, the pseudo dimer of **IM1** was also detected and mentioned in the literature (Li et al. 2010). It may be the reaction product of **SM1** and **IM1**. Small amounts of this impurity leave residue in the second step would react with $CH_3I$ to give N-monomethylation and N-dimethylation compound, which were proposed to be impurity **II** and impurity **III**, respectively. The synthetic method for impurity **II** and impurity **III** is shown in Scheme 3.

Impurity **IV**, referred to as the dimer of API, was detected in the final product and defined as a critical quality attribute (CQA) impurity as previously reported (Li et al. 2010). Plausible formation of impurity **IV** can be tracked back to the diamine impurity of **SM3**, a by-product formed in the preparation of **SM3**. However,




**Scheme 3:** Synthesis of pazopanib impurity **II** and impurity **III**




**Scheme 4:** Synthesis of pazopanib impurity **IV**

deserved to be mentioned, the literature revealed no more information about the dimer impurity, especially the structure. Therefore, according to the synthetic process of **SM3**, the possible structure and synthetic route for impurity **IV** is firstly proposed by us (Scheme 4; Yuan et al. 2017). Treatment of 2-methyl-5-nitrobenzenesulfonamide (**IMP1**) with 2-methyl-5-nitrobenzenesulfonyl chloride (**IMP2**) under mild conditions (NaH/THF) provided **IMP3**, $H_2$-Pd/C reduction of **IMP3** in $CH_3OH$ yielded the diamine impurity **IMP4**, which was further condensed with two molecules of **IM2** in isopropanol in the presence of HCl to give impurity **IV** (Scheme 4).

Furthermore, a novel HPLC analytical method was established for the detection of these impurities. The overlay of HPLC chromatograms of all impurity samples is given in the Fig. This HPLC method could separate all impurities and the retention time of each impurity is included in the Table.

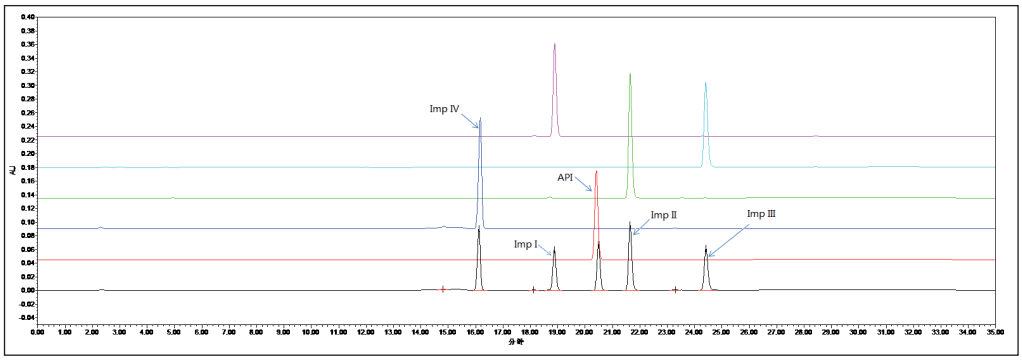



Fig.: Chromatograms of the HPLC method for all impurity samples

## 3. Experimental

### 3.1. General

All reagents were obtained from Adamas, tansoole etc and used without further purification unless otherwise noted. $^1$H NMR and $^{13}$C NMR spectra were obtained from a solution in DMSO-$d_6$ with TMS as internal standard using a Bruker spectrometer. MS spectra were obtained with a waters QDA instrument. HRMS spectra were obtained with a Thermo Fisher Scientific LTQ FTICR-MS.

### 3.2. HPLC method

A Waters 2695 liquid chromatography apparatus was used. The column was Agilent RP-C18(ZORBAX Ecipse Plus, 4.6mm×250mm i.d., 5μm particle size). The flow rate was 1 ml/min. The column temperature was 30 °C. The injection quantity was 20 μl. The mobile phase was employed as the gradient elution method. The conditions were as follows: Mobile phase A: methanol-deionized water (10:90), B: acetonitrile. Gradient: 20-56 % B in 20 min.

### 3.3. Synthesis of impurity I

*Step 1:* A solution of **SM1** (18.0 g, 0.11 mol), **SM2** (27.1g, 0.18 mol), $NaHCO_3$ (23.3g, 0.22 mol) in EtOH (360 mL) and THF (90 mL) was placed into a 1L three necked round-bottom flask. The resulting solution was warmed to 70 °C and stirred at this temperature for 4 h. The reaction was cooled to 0 °C for another 3 h, then filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel using dichloromethane/methanol as eluent to afford **ISO-IM1** (0.5 g, 1.6%) as a yellow solid. $^1$H NMR (500 MHz, DMSO-$d_6$) (δ, ppm): 9.98 (s, 1H), 8.45 (d, *J* = 5.1 Hz, 1H), 8.08 (s, 1H), 7.53 (d, *J* = 8.9 Hz, 1H), 7.11 (d, *J* = 8.9 Hz, 1H), 6.94 (d, *J* = 5.1Hz, 1H), 3.98 (s, 3H), 2.55 (s, 3H).
*Step 2:* The suspension of **ISO-IM1** (1g, 3.6 mmol), $CH_3I$ (0.76g, 5.4 mmol) and $Cs_2CO_3$ (2.34g, 7.2 mmol) in DMF (15 mL) was stirred for 2 h at room temperature. Then to the reaction mixture was added 100 mL $H_2O$, filtered and dried to afford **ISO-IM2** (0.9 g, 85.7%) as a yellow solid. $^1$H NMR (300 MHz, DMSO-$d_6$) (δ, ppm): 8.28 (d, *J* = 5.1 Hz, 1H), 7.62 (d, *J* = 8.8 Hz, 1H), 7.39 (s, 1H), 6.87 (d, *J* = 8.8 Hz, 1H), 6.80 (d, *J* = 5.1Hz, 1H), 4.03 (s, 3H), 3.47 (s, 3H), 2.59 (s, 3H).
*Step 3:* A mixture of **ISO-IM2** (0.7g, 2.4 mmol) and **SM3** (0.44 g, 2.4 mmol), conc HCl 0.5ml in isopropanol (50 mL) was stirred at reflux for 2 h. The mixture was cooled to room temperature and the resultant precipitate was collected by filtration and washed with isopropanol. The solid was dried to give impurity **I** (0.52 g, 45.2 %) as a off-white solid. $^1$H NMR (300 MHz, DMSO-$d_6$) (δ, ppm): 11.84 (s, 1H), 7.84 (d, *J* = 7.2 Hz, 1H), 7.73 - 7.75 (m, 2H), 7.67 (s, 1H), 7.40 - 7.42 (m, 2H), 6.98 (d, *J* = 8.7 Hz, 1H), 6.71 (d, *J* = 6.8 Hz, 1H), 4.10 (s, 3H), 3.58 (s, 3H), 2.67 (s, 3H), 2.53 (s, 3H). $^{13}$C NMR (600 MHz, DMSO-$d_6$) (δ, ppm): 160.8, 153.7, 147.2, 143.1, 142.6, 136.4, 133.0, 131.9, 124.2, 123.3, 120.8, 119.9, 119.1, 115.7, 99.6, 38.0, 19.7, 9.88; HRMS m/z calcd for $C_{21}H_{24}N_7O_2S$ $[M+H]^+$ 438.1707, found 438.1705.

### 3.4. Synthesis of impurity II

To a 100 mL round-bottom flask was charged **IM2** (1.1 g, 3.8 mmol), **SM1** (0.75 g, 3.8 mmol), 1 ml conc HCl and isopropanol (50 mL). The mixture was stirred at reflux for 4 h then cooled to room temperature and the resultant precipitate was collected by filtration and successfully washed with water and ethanol. Recrystallized from EtOH/$H_2O$ to yield impurity **II** (0.65 g, 41.6 %) as a brown solid. $^1$H NMR (600 MHz, DMSO-$d_6$) (δ, ppm): 10.77 (s, 1H), 7.94(s, 1H), 7.84 - 7.88 (m, 2H), 7.63 (d, *J* = 8.6 Hz, 1H), 7.59 (s, 1H), 7.06 (d, *J* = 8.7 Hz, 1H), 6.94 – 6.96 (m, 1H), 5.89(s, 1H), 4.08 (s, 3H), 4.02 (s, 3H), 3.58 (s, 3H), 2.65 (s, 3H), 2.58 (s, 3H). $^{13}$C NMR (600 MHz, DMSO-$d_6$) (δ, ppm): 162.6, 150.9, 146.2, 143.4, 142.7, 140.5, 137.6, 137.2, 134.2, 123.4, 122.2, 120.5, 119.0, 117.7, 117.6, 114.1, 103.6, 97.9, 40.1, 37.8, 37.1, 9.86, 9.81; HRMS m/z calcd for $C_{23}H_{25}N_8$ $[M+H]^+$ 427.2353, found 427.2348.

### 3.5. Synthesis of impurity III

A solution of impurity **II** (0.8 g, 1.9 mmol) in DMF (5 mL) was added to a suspension of NaH (60%, 0.15 g, 3.8 mmol) in DMF (5 mL). After stirring for 30 min at 0 °C, $CH_3I$ (0.32 g, 2.8 mmol) was slowly added to the mixture. Then the mixture was warmed to room temperature and stirred for another 1 h. After cooling, $H_2O$ (50 mL) was added, filtered, washed with 15 mL MeOH to afford impurity **III** (0.51 g, 61.7 %) as a white solid. $^1$H NMR (500 MHz, DMSO-$d_6$) (δ, ppm): 7.69 - 7.73 (m, 2H), 7.54 (d, *J* = 8.9 Hz, 1H), 7.38 (s, 1H), 7.34 (s, 1H), 6.99 (d, *J* = 8.8 Hz, 1H), 6.83 (d, *J* = 8.8 Hz, 1H), 5.65 (d, *J* = 5.8 Hz, 1H), 4.04 (s, 3H), 4.02 (s, 3H), 3.49 (s, 3H), 3.28 (s, 3H), 2.60 (s, 3H), 2.58 (s, 3H). $^{13}$C NMR (600 MHz, DMSO-$d_6$) (δ, ppm): 162.4, 161.6, 156.0, 147.6, 147.4, 144.1, 142.3, 132.5, 131.8, 122.0, 121.7, 120.1, 119.8, 119.6, 118.8, 114.0, 112.1, 95.8, 39.5, 38.6, 37.8, 37.6, 37.6, 9.87; HRMS m/z calcd for $C_{24}H_{27}N_8$ $[M+H]^+$ 427.2353, found 427.2348.

### 3.6. Synthesis of impurity IV

*Step 1:* A solution of **IMP1** (0.8 g, 3.7 mmol) in THF (20 mL) was added to a suspension of NaH (60%, 0.44 g, 11.1 mmol) in THF (20 mL). After stirring for 30 min at 0 °C, **IMP2** (0.87 g, 3.7 mmol) was slowly added into the mixture. Then the mixture was warmed to room temperature and stirred for 2 h. After reaction completion, the mixture was quenched with $H_2O$ 50 mL, extracted with 100 mL $CH_2Cl_2$ twice. The organic layer was washed with $H_2O$ (50 mL) and then brine (50 mL), dried over $Na_2SO_4$ (anhyd), filtered, and concentrated under reduced pressure. The residue was directly used in the next step without purification.
*Step 2:* The residue above was dissolved in 100 mL MeOH, then 10% Pd–C (0.2 g) and 5mL conc HCl was added, the mixture was stirred for 8 h at room temperature under $H_2$ atmosphere. After the pressure was leaked, the reaction mixture was filtered, concentrated under reduced pressure to afford **IMP4** as a yellow solid. The residue was directly used in the next step without purification. $^1$H NMR (600 MHz, DMSO-$d_6$) (δ, ppm): 7.64 (s, 2H), 7.28 (d, *J* = 7.8 Hz, 2H), 7.20 (d, *J* = 7.8 Hz, 2H), 2.43 (s, 6H). ESI-MS:356.2$[M+H]^+$.
*Step 3:* The residue above was dissolved in 50 mL iPrOH, followed by addition of **IM2** (2.13 g, 7.4 mmol) and 0.5 mL conc HCl. The mixture was stirred for 3 h at reflux temperature. After cooling, the reaction mixture was concentrated under reduced pressure. 0.16 g (overall yield 5.1%) compound was obtained as a yellow solid after purification by flash column chromatography ($CH_2Cl_2$/$CH_3OH$) on silica gel. $^1$H NMR (500 MHz, DMSO-$d_6$) (δ, ppm): 10.89 (brs, 2H), 8.12 – 8.22 (m, 2H), 7.86 (d, *J* = 8.7 Hz, 2H), 7.76 – 7.80 (m, 2H), 7.62 – 7.68 (m, 2H), 7.42 – 7.50 (m, 2H), 7.12-7.15(m, 2H), 6.97 (d, *J* = 8.7 Hz, 2H), 5.70 – 5.78 (m, 2H), 4.09 (s, 6H), 3.55 (s, 6H), 2.64 (s, 6H), 2.41 (s, 6H). $^{13}$C NMR (600 MHz, DMSO-$d_6$) (δ, ppm): 162.4, 151.0, 146.5, 143.9, 142.6, 140.4, 134.5, 133.8, 133.6, 131.9, 123.3, 121.7, 120.6, 120.2, 118.9, 114.6, 96.3, 37.9, 19.8, 9.9; HRMS m/z calcd for $C_{42}H_{44}N_{13}O_4S_2$ $[M+H]^+$ 858.3075, found 858.3082.

Acknowledgements: We appreciate the Fundamental and Advanced Research Projects of Chongqing City (No. cstc2017jcyjAX0228)

Conflicts of interest: None declared.

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