Supplementary Information

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1. Secondary mass spectra and fragment ion analysis of each impurity

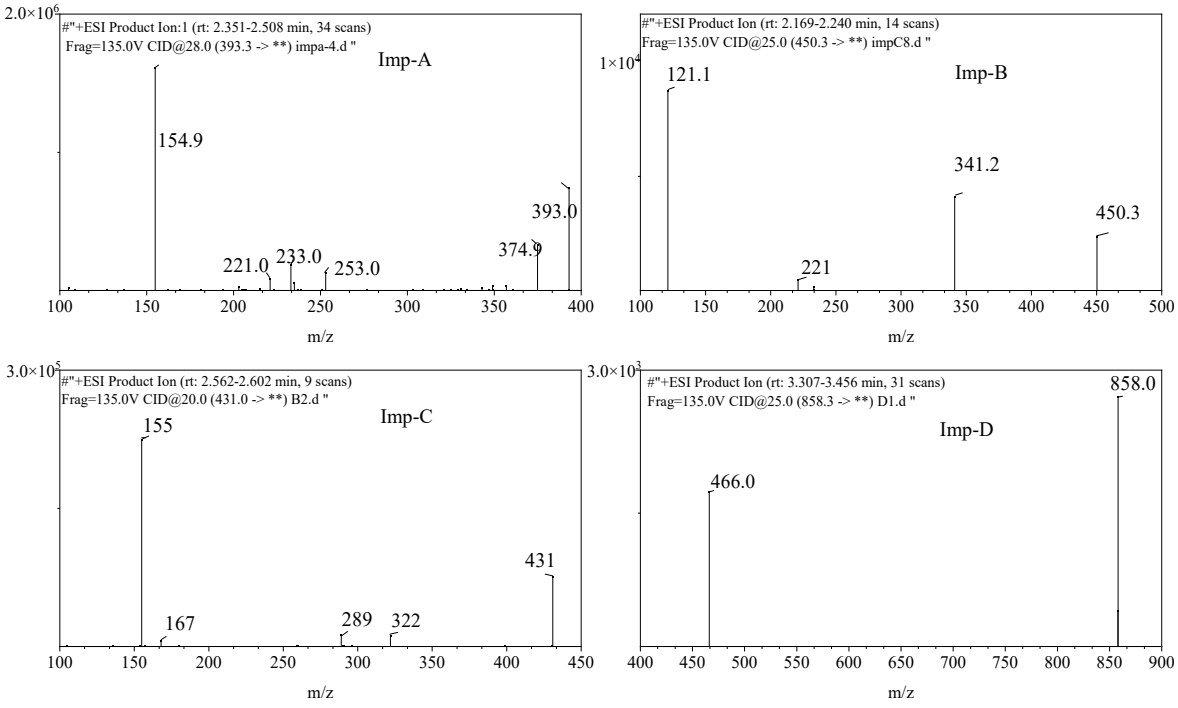


Figure S1. UPLC-MS/MS spectra of the four impurities

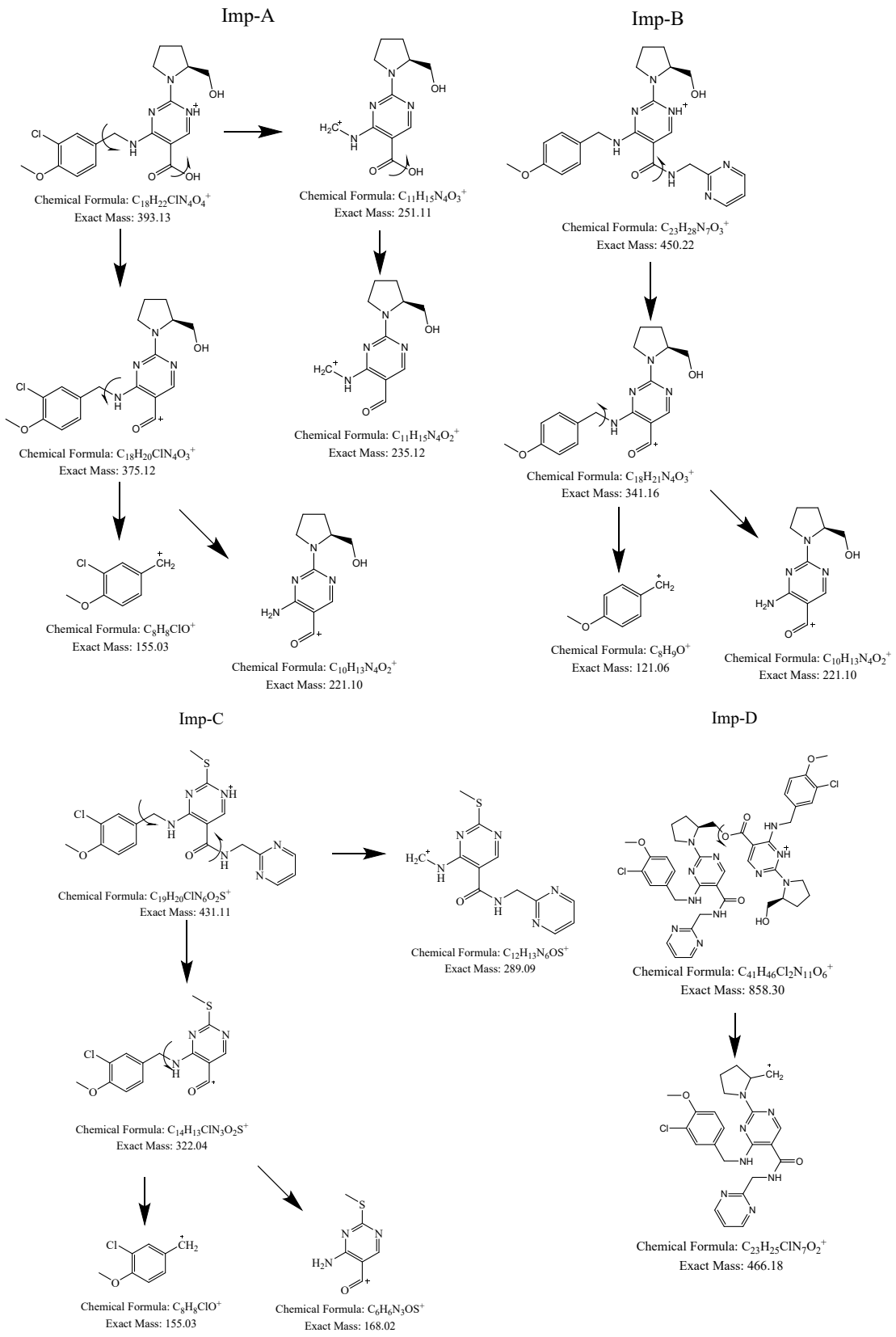


Figure S2. Structure inference of each impurity by LC-MS/MS

The mass spectrometry of avanafil intermediates

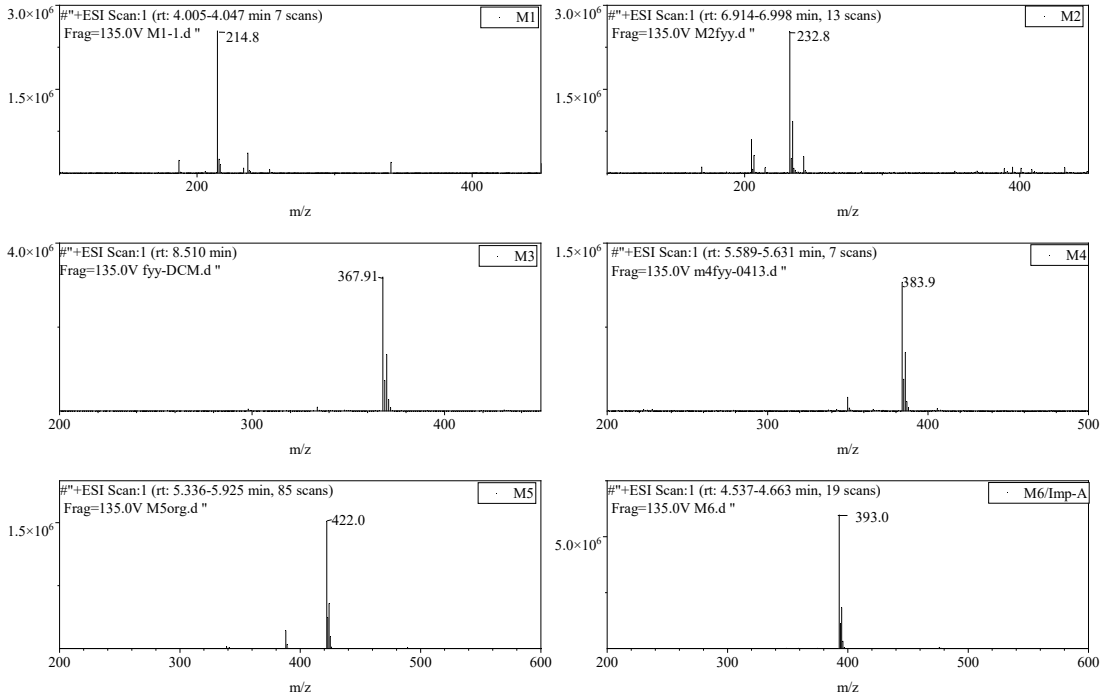


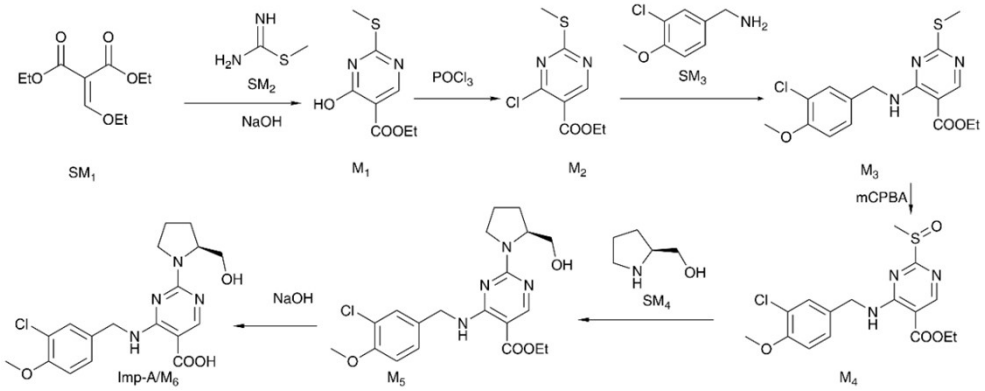
Figure S3. Mass spectrometry of avanafil intermediates.

The synthesis of avanafil followed Yamada's.[1]

1. Yamada. K *et al*. *U.S. Patent,* No. USOO6797709B2, 28 Sep, 2004.

2. Preparation of Imp A-D

3.1. Synthesis of Imp-A



Imp-A is the product of the penultimate step of the route to synthesize avanafil.

Colorless solid. ^{1}H NMR spectral data of Imp-A was consistent with reported data. [1]

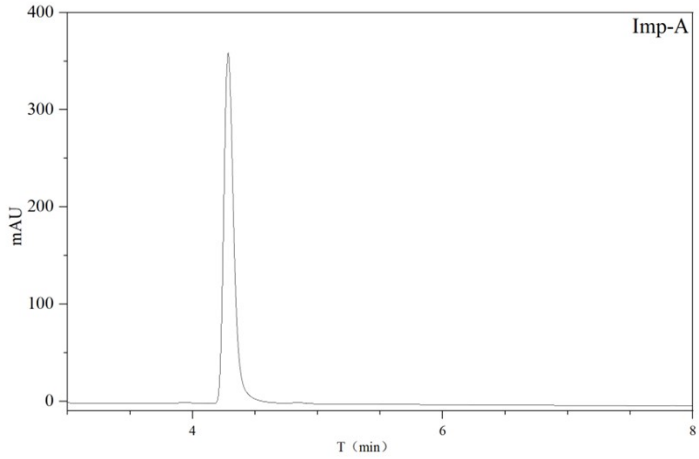
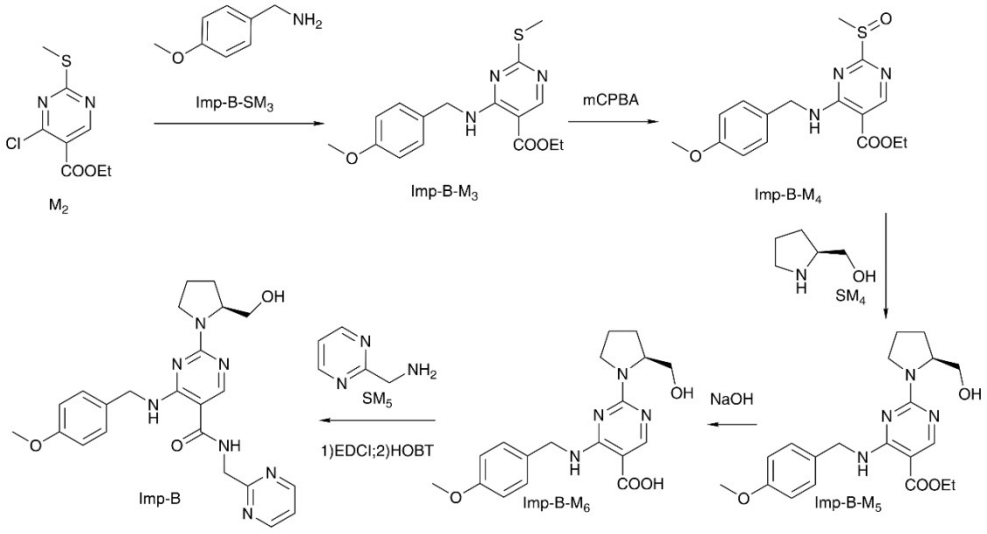


Figure S4. UPLC spectrum of Imp-A

3.2. Preparation of Imp-B



Ethyl 4-((4-methoxybenzyl) amino)-2-(methylthio) pyrimidine-5-carboxylate (Imp-B-M3)

Add Imp-B-SM3(13.45 g, 98.05 mmol), triethylamine（TEA）(19.84 g, 196.07 mmol) to the toluene solution of intermediate 2 (M2) (98.02 mmol), stir for 2 h, add 100 mL H_2O to the upper organic phase spot plate (petroleum ether: ethyl acetate(PE:EA) = 3:1), stir and then stand and partition twice, wash the organic phase with 5% formic acid solution again, aqueous phase pH = 5, partition the organic phase, concentrate at 50 ℃ under reduced pressure, precipitate a large amount of solid, add 20 mL ethanol slurry, filter, vacuum dry to obtain 11 g Imp-B-M3.

Ethyl 4-((4-methoxybenzyl) amino) -2-(methylsulfinyl) pyrimidine-5-carboxylate (Imp-B-M4)

Imp-B-M3(6.12 g, 18.5 mmol) was taken and added to dichloromethane (DCM) 50 mL, dissolved clear and then cooled down to 0-10 ℃. Then slowly drop in mCPBA (4.118 g, 20.3 mmol), control the reaction temperature at 0-10 ℃ and react for 2 h. The reaction was monitored by TLC (PE: EA = 2:1) and the reaction was complete. Finally, a dichloromethane solution of impurity B intermediate 4 (Imp-B-M4) was obtained.

Ethyl (S)-2-(2-(hydroxymethyl) pyrrolidin-1-yl)-4-((4-methoxybenzyl) amino) pyrimidine-5-carboxylate (Imp-B-M5)

L-prolinol (2.62 g, 25.9 mmol), TEA (2.61 g, 25.9 mmol) was added to the DCM of Imp-B-M4(18.5 mmol) and reacted for 1−1.5h. TLC (PE: EA = 1:1) was monitored, no raw material point. The reaction solution was added with 2% Na_2CO_3, stirred thoroughly and left to partition. The organic phase was washed once more with 100mL H_2O, and the organic phase was separated after standing and concentrated dry under reduced pressure at 45℃ to obtain the oily substance (Imp-B-M5).

(S)-2-(2-(hydroxymethyl) pyrrolidin-1-yl)-4-((4-methoxybenzyl) amino) pyrimidine-5-carboxylic acid (Imp-B-M6)

Add 5% NaOH 100mL to the oil, gradually increase the temperature to 100 ℃ reflux 8 h, TLC (dichloromethane: methanol (DCM: MeOH) = 10:1) monitoring, the reaction is complete. Adjust the pH to 6-7 with dilute hydrochloric acid, precipitated a large amount of white solid, filtered, dried at 45 ℃, to obtain intermediate 6 of impurity B (Imp-B-M6) 4.5 g.

(S)-2-(2-(hydroxymethyl) pyrrolidin-1-yl)-4-((4-methoxybenzyl) amino)-N-(pyrimidin-2-ylmethyl) pyrimidine-5-carboxamide (Imp-B)

DCM 15 mL, Imp-B-M6 (3 g, 8.4 mmol) was added in a single mouth flask and the system was cloudy. After adding EDCI(2.41 g, 12.56 mmol), HOBT(1.7g, 12.56 mmol) and TEA(2.56 g, 25.12mmol), the dissolution was clear, and finally SM5（1.59g, 10.88 mmol）. The reaction was found to be incomplete as monitored by TLC (DCM: MeOH = 8:1). The reaction solution was detected by UPLC, the raw material contained 25%, after reduced pressure distillation in addition to dichloromethane, Imp-B was obtained by a Semi-preparative liquid chromatography (see 2.4 for the procedure). Imp-B: light yellow solid, 500 mg, 97%.

The preparation of Imp-B has not been reported so far, but this structure is the same as the degradation product AV I reported by Patel *et al*. because they have the same fragment ions.[2] The mass spectra of all intermediates of Imp B are shown in Figure S4. The liquid phase diagram of

Imp-B is shown in Figure S5.

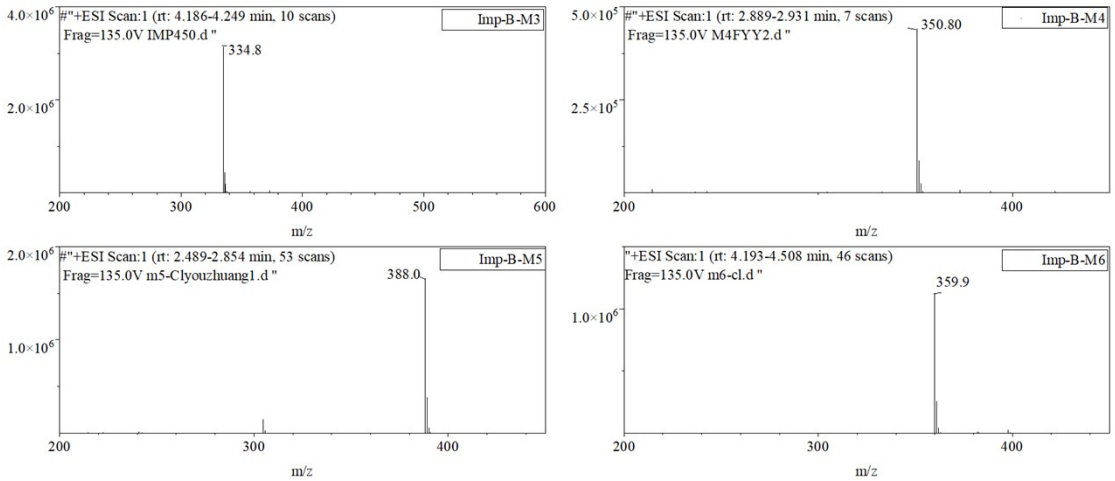


Figure S5. Mass spectrometry of Imp-B intermediates

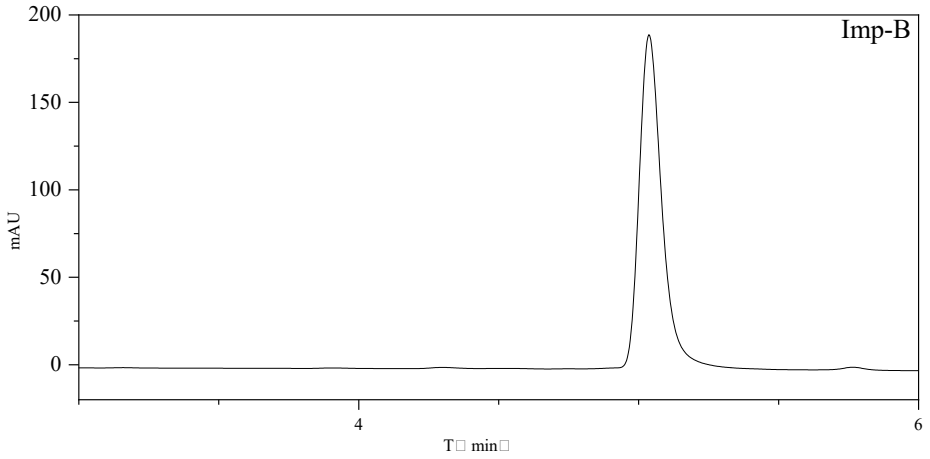
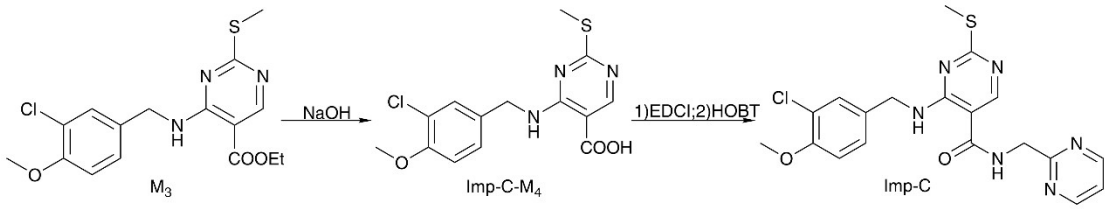


Figure S6. UPLC spectrum of Imp-B

2. P. Mital, K. Charmy and V. Vivek, *Arab. J. Chem.*, 2020, **13**, 6493–6509.

3.3. Synthesis of Imp-C



The synthetic route of impurity C is the same as that of Hui-Min Jiang, except for the reactant equivalents and solvent types in some steps. ^{1}H NMR spectral data of Imp-C was consistent with reported data.[3]

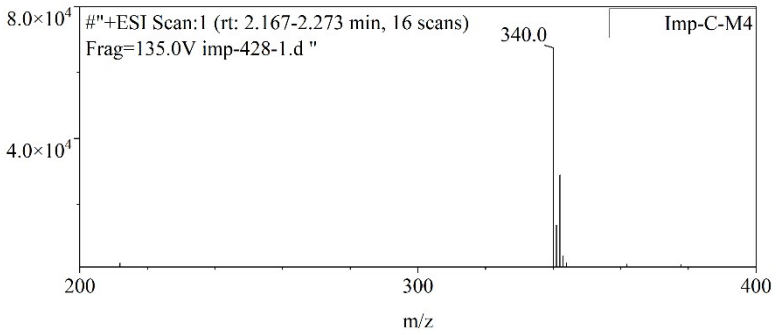


Figure S7. Mass spectrometry of Imp-C-M4. Imp-C-M4 is the only Intermediate of Imp-C.

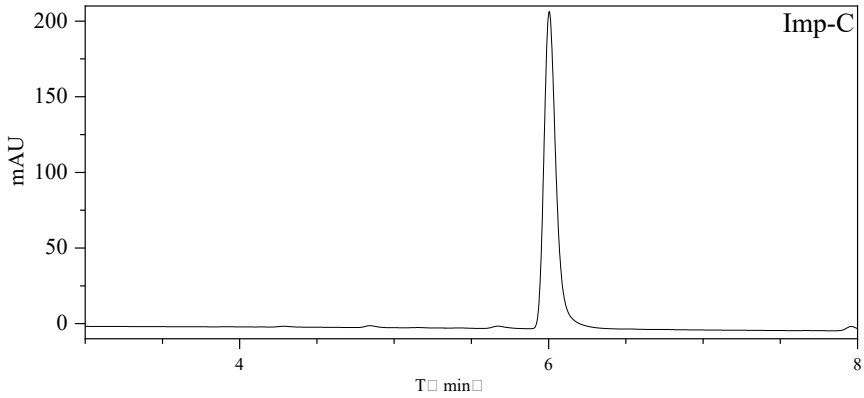
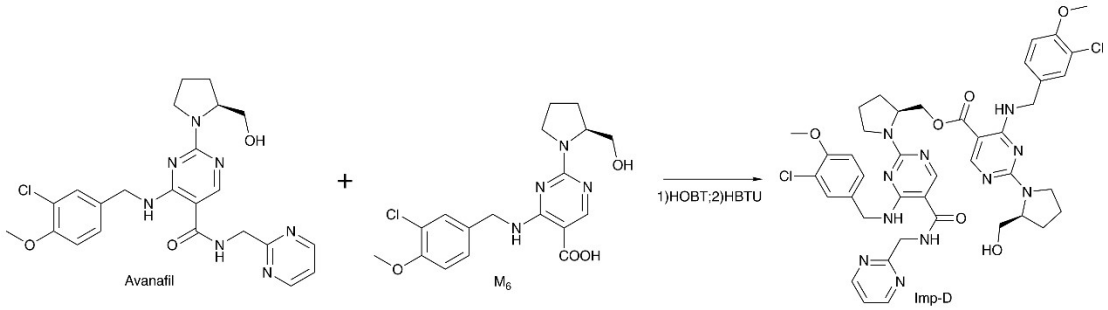


Figure S8. UPLC spectrum of impurity B

3. T. Sakamoto, Y. Koga, M. Hikota, K. Matsuki, M. Murakami, K. Kikkawa, K. Fujishige, J. Kotera, K. Omori, H. Morimoto, K. Yamada. *Bioorg. Med. Chem. Lett.*, 2014, **24**, 5460–5465

3.4. Synthesis of Imp-D



HBTU（5.9 g, 15.4 mmol）and M6 (4.5 g, 11.36 mmol)was added to the solvent dissolved in N,N-Dimethylacetamide(20mL) at room temperature. Then avanafil (4.96 g, 10.33 mmol) was

added and the reaction was carried out at 25 °C for 8 h. After that, the reaction solution was added dropwise to 200 mL of water, extracted with ethyl acetate, spun at 40-45 °C and separated by column chromatography using a Semi-preparative liquid chromatography (see 2.4 for the procedure).

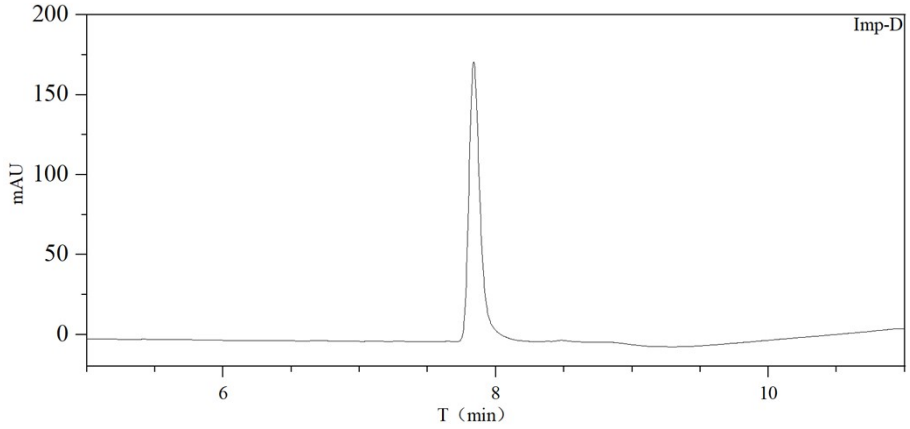


Figure S9. UPLC spectrum of Imp-D

3. NMR of avanafil and Imp A-D

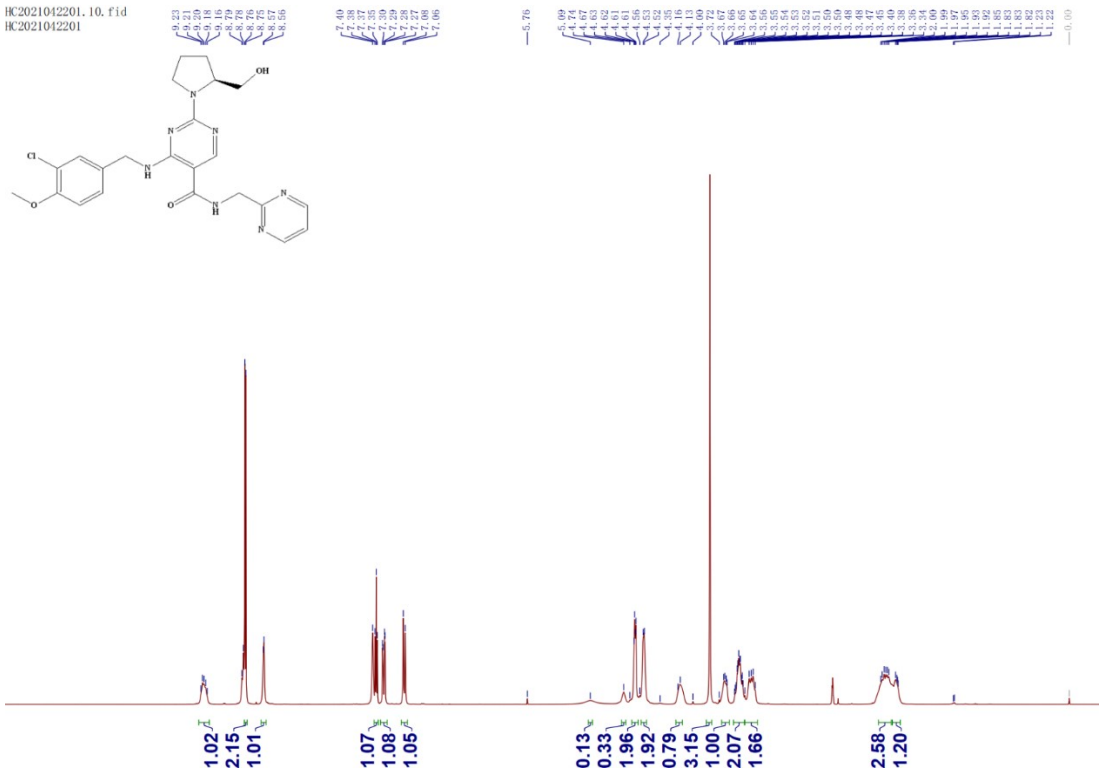


Figure S10. ^{1}H NMR spectra of avanafil (DMSO-d_6)

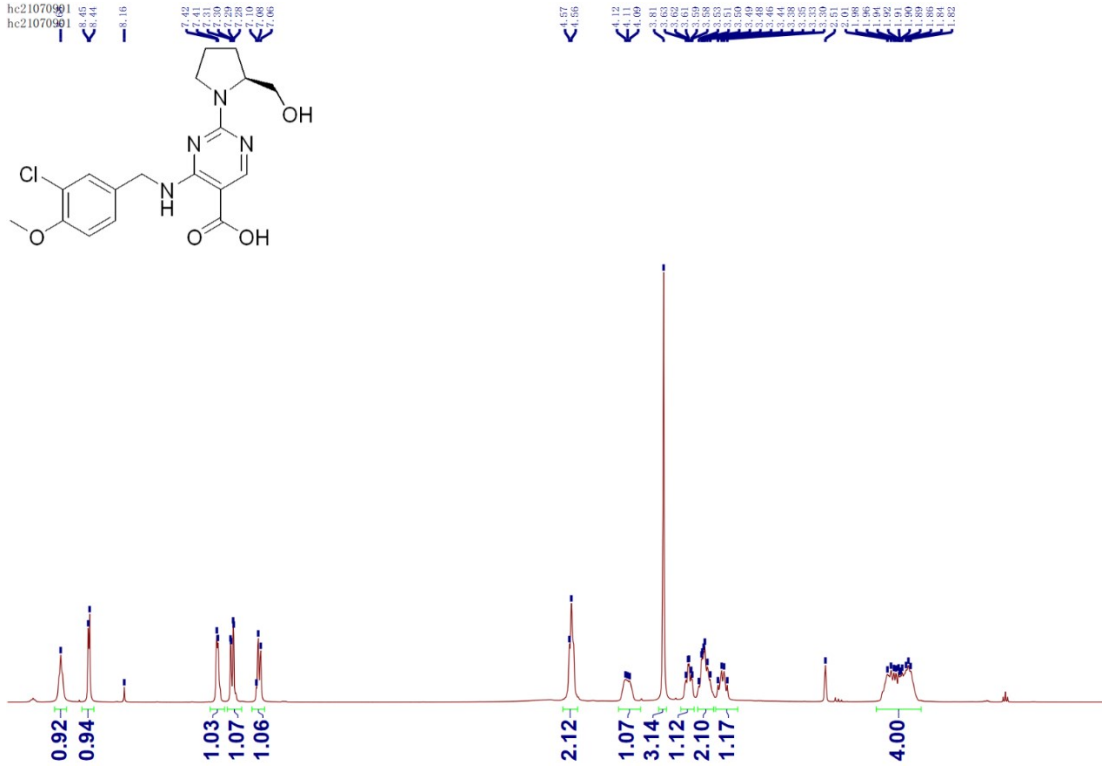


Figure S11. ^{1}H NMR spectra of Imp-A (DMSO-d_6)

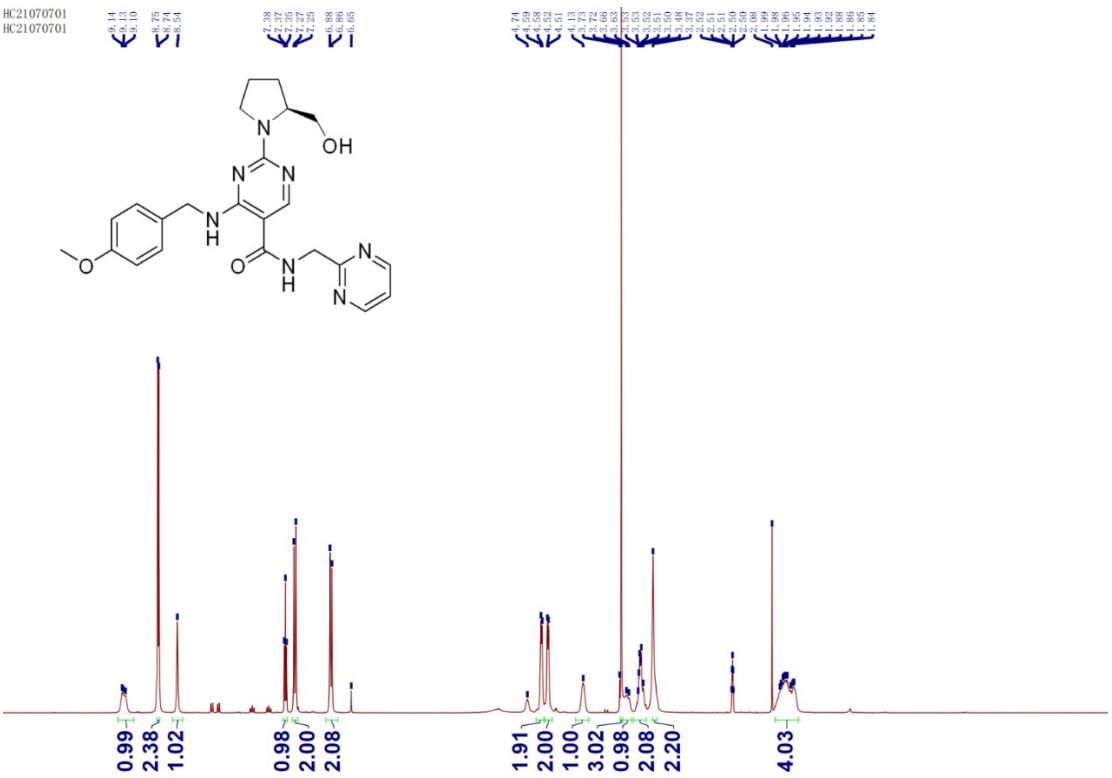


Figure S12. ^{1}H NMR spectra of Imp-B (DMSO-d_6)

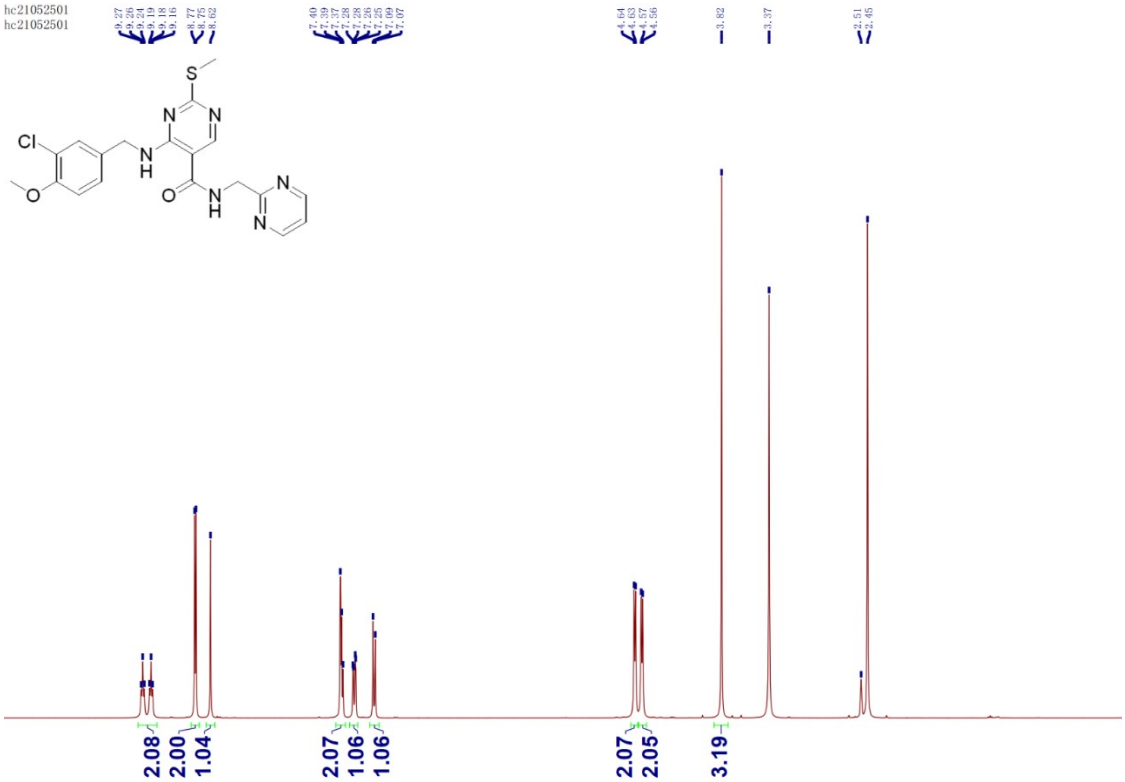


Figure S13. ^{1}H NMR spectra of Imp-C (DMSO-d_6)

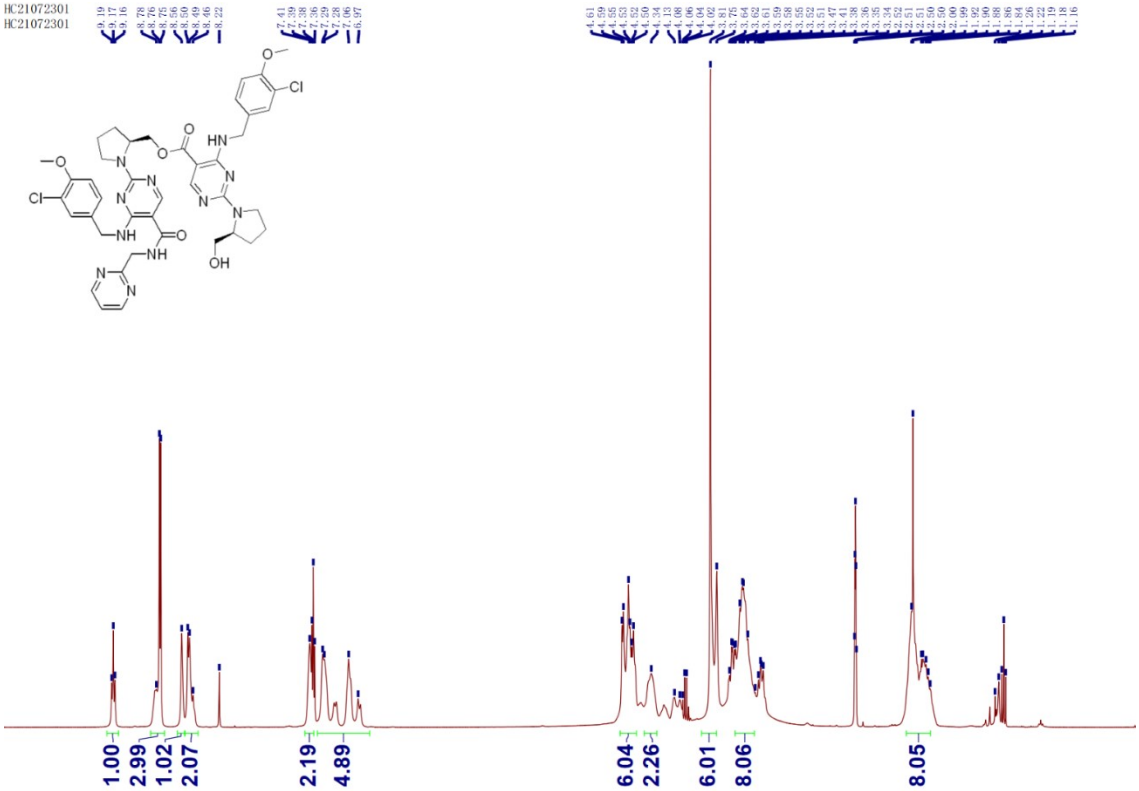


Figure S14. ^{1}H NMR spectra of Imp-D (DMSO-d_6)

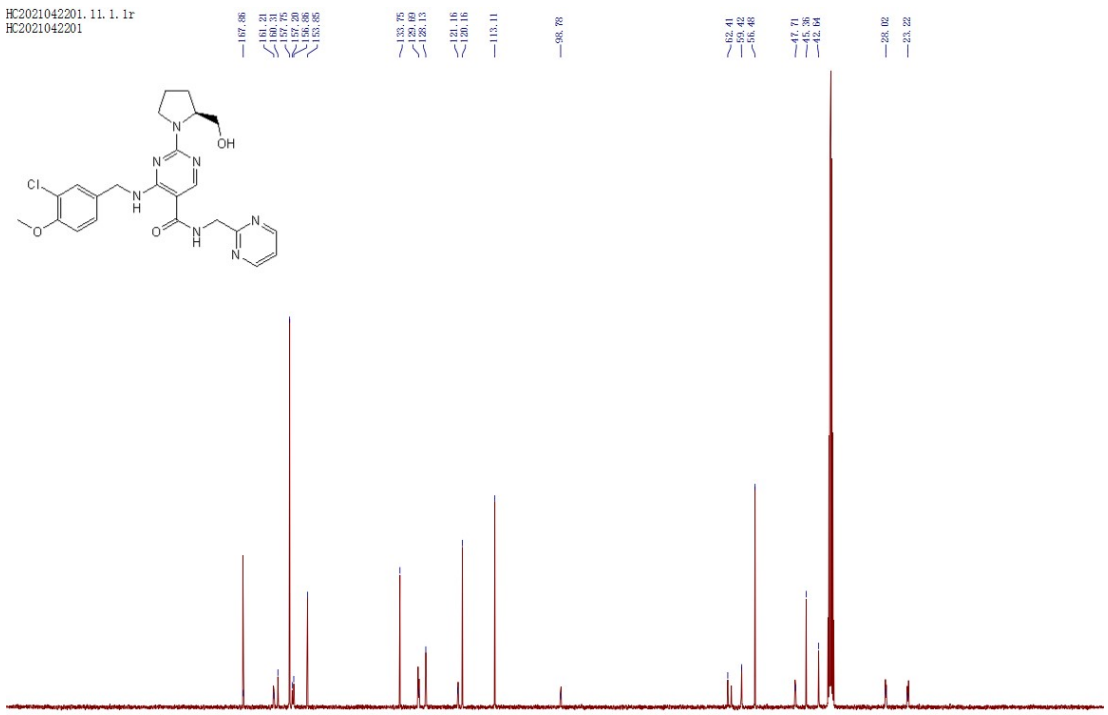


Figure S15. ^{13}C NMR spectra of avanafil (DMSO-d_6)

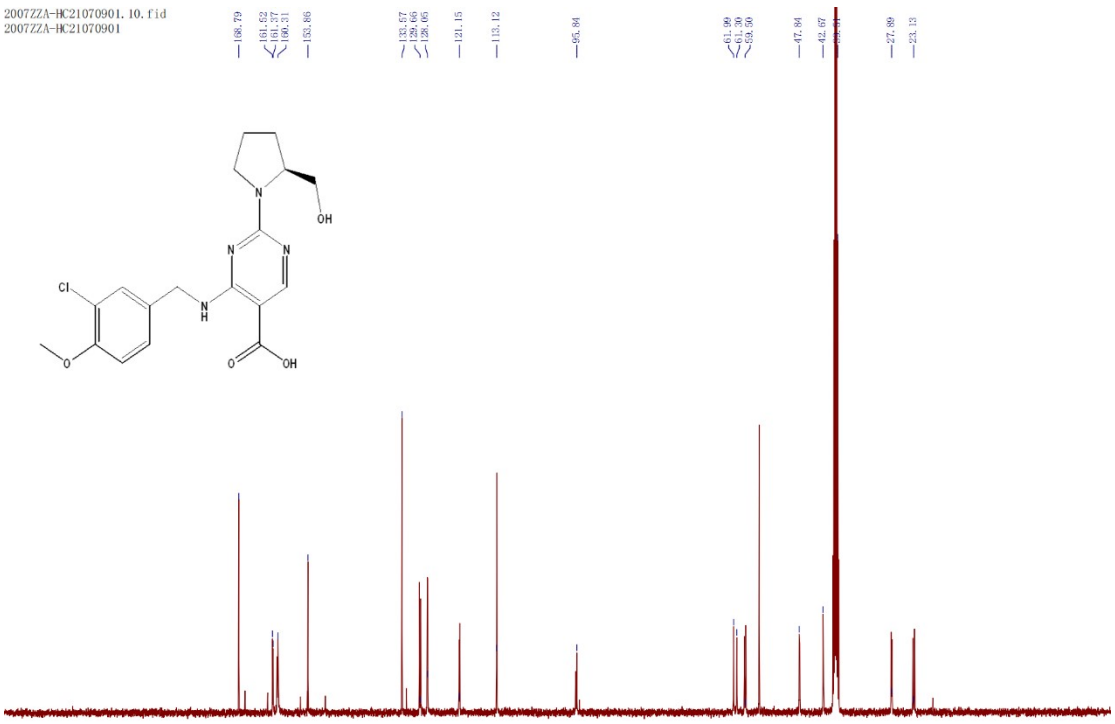


Figure S16. ^{13}C NMR spectra of Imp-A (DMSO-d_6)

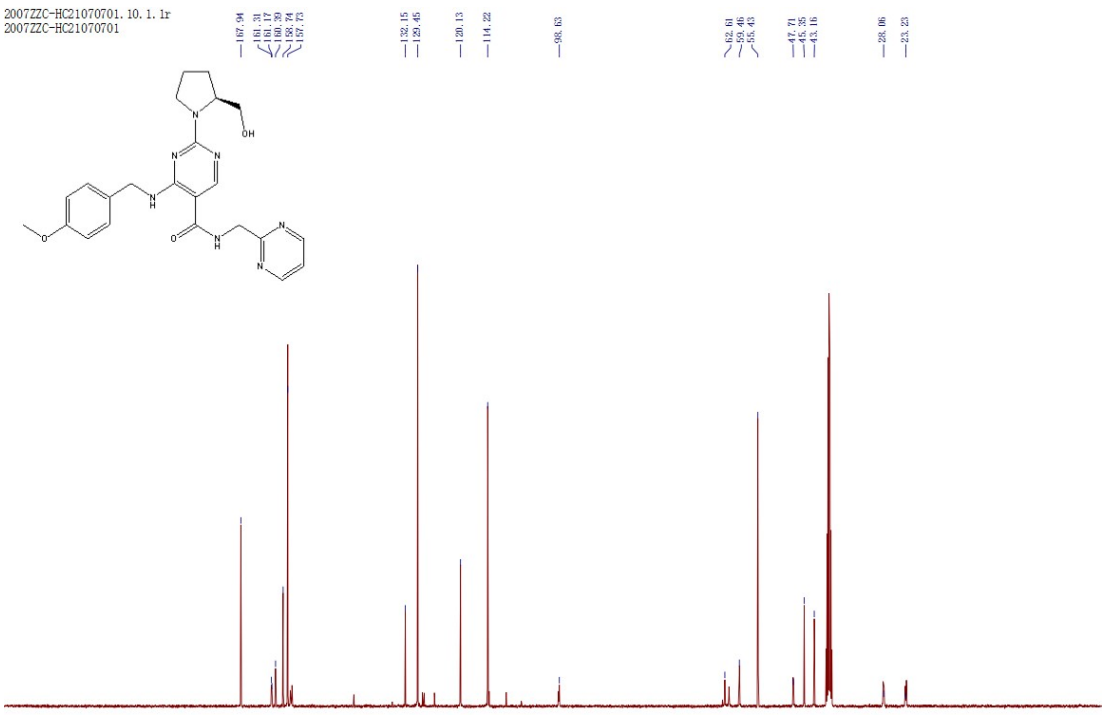


Figure S17. ^{13}C NMR spectra of Imp-B (DMSO-d_6)

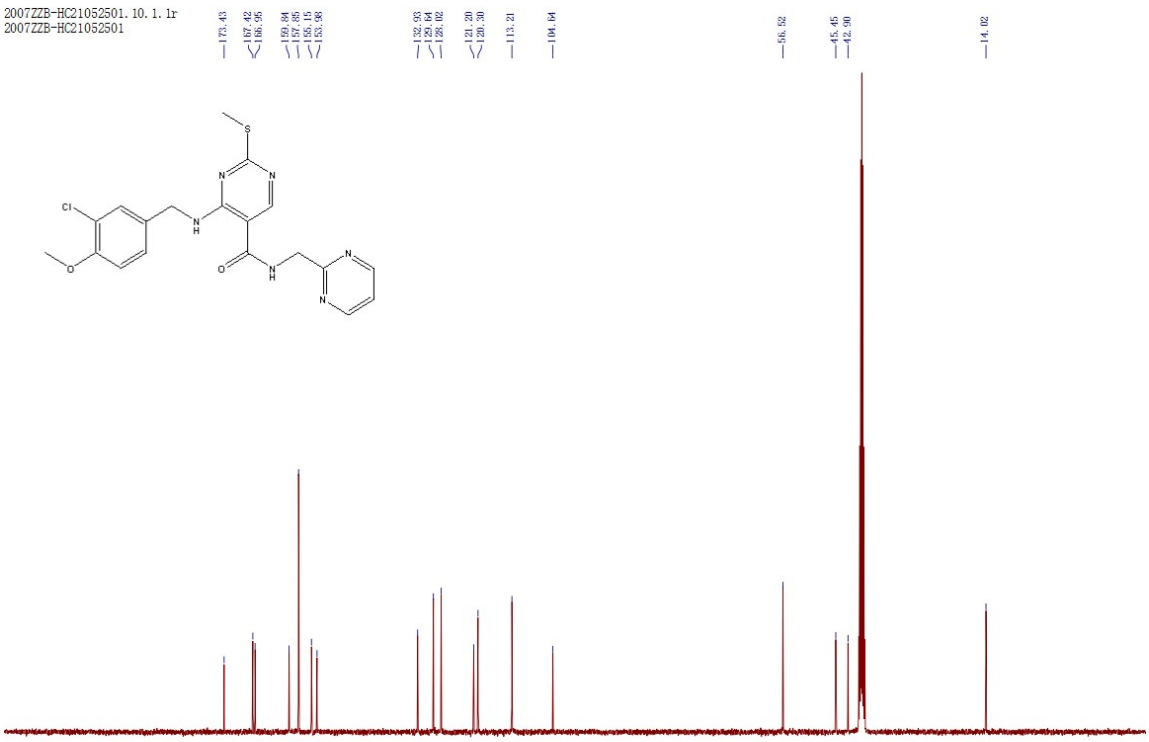


Figure S18. ^{13}C NMR spectra of Imp-C (DMSO-d_6)

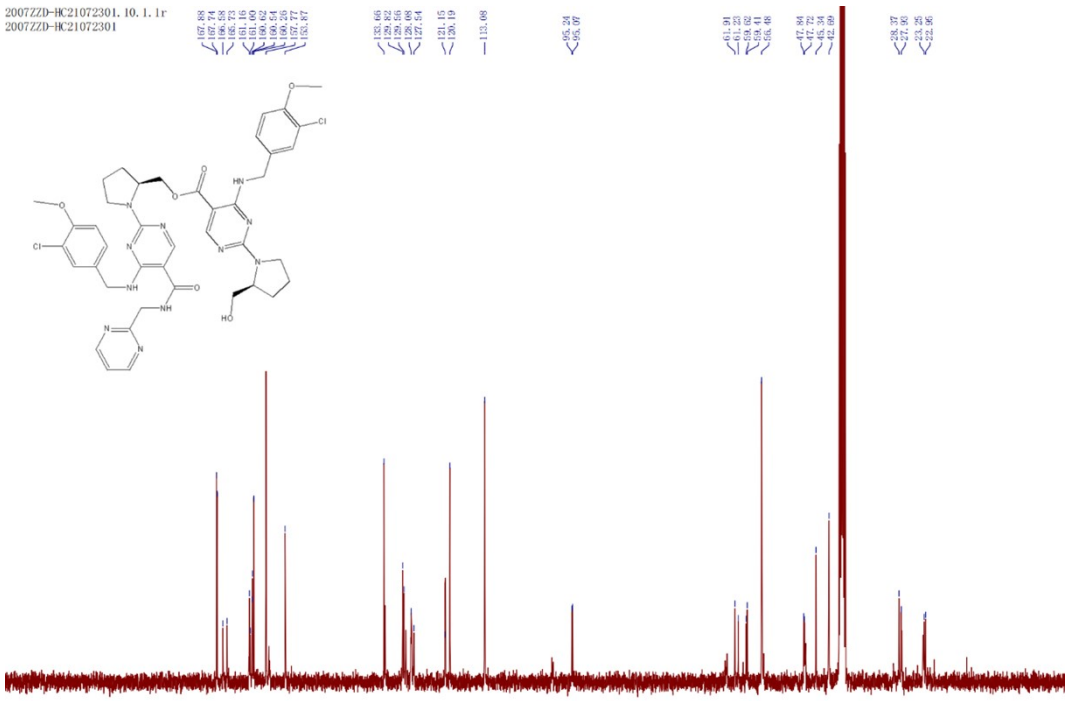


Figure S19. ^{13}C NMR spectra of Imp-D (DMSO-d_6)

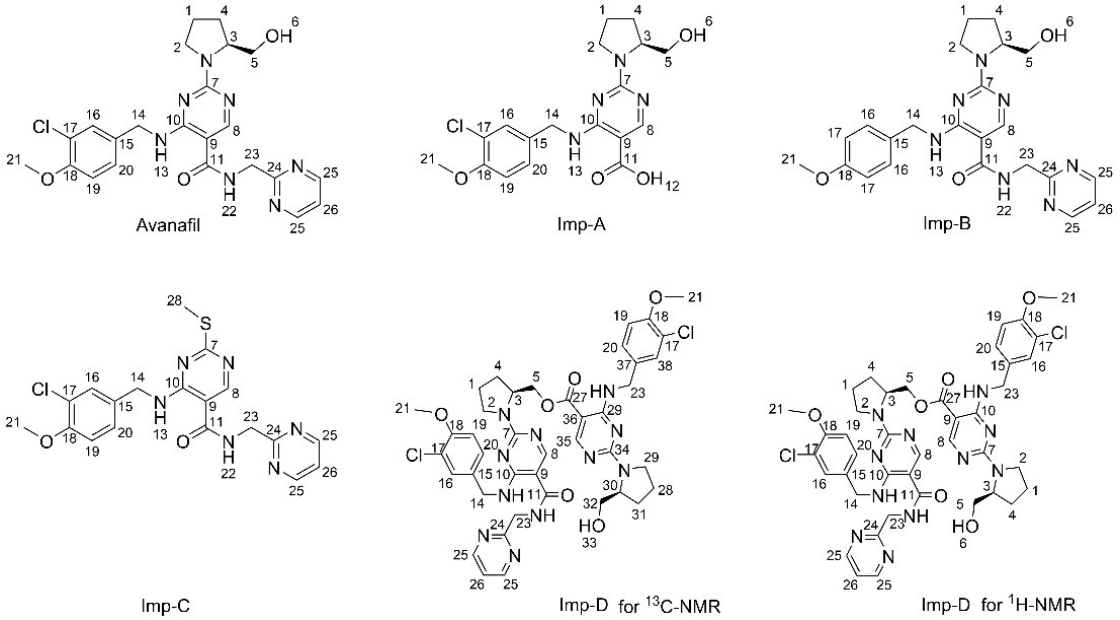


Figure S20. Atomic number for avanafil and its impurities

Table S1. ^{1}H-NMR assignments for avanafil and its impurities

Position	Avanafil	Imp-A	Imp-B	Imp-C	Imp-D
1	2.03–1.80 m, 4H	2.10–1.74 m, 4H	2.04 – 1.79 m, 4H	---	2.07–1.77 m, 8H
2	3.58–3.31 m, 4H	3.54–3.41 m, 4H	3.51 d, *J* = 11.4, 8.2, 5.6 Hz, 4H	---	3.64–3.36 m, 8H
3	3.65 dd, *J* = 10.1, 4.6 Hz, 1H	3.61 dt, *J* = 10.2, 5.2 Hz, 1H	3.65 d, *J* = 10.6 Hz, 1H	---	3.64–3.36 m, 8H
4	2.03–1.80 m, 4H	2.10–1.74 m, 4H	2.04 – 1.79 m, 4H	---	2.07–1.77 m, 8H
5	3.58–3.31 m, 4H	3.54–3.41 m, 4H	3.51 d, *J* = 11.4, 8.2, 5.6 Hz, 4H	---	3.64–3.36 m, 8H
8	8.56 d, *J* = 3.2 Hz, 1H	8.45 d, *J* = 4.7 Hz, 1H	8.54 s, 1H	8.62 s, 1H	8.56 s, 1H
12	---	8.68 s, 1H	---	---	---
13	9.20 dt, *J* = 13.8, 6.0 Hz, 1H	---	9.19–9.01 m, 1H	9.18 t, *J* = 5.9 Hz, 1H	9.17 t, *J*=6.0 Hz,1H
14	4.52 d, *J* = 5.4 Hz, 2H	4.57 d, *J* = 6.1 Hz, 2H	4.51 d, *J* = 5.0 Hz, 2H	4.57 d, *J* = 5.9 Hz, 2H	4.69–4.47 m, 6H
16	7.43-7.33 m, 2H	7.41 d, *J* = 3.7 Hz, 1H	---	---	7.39 dt, *J* = 14.2, 5.6 Hz, 2H
19	7.29 dd, *J* = 8.4, 2.2 Hz, 1H	7.29 dd, *J* = 8.4, 1.8 Hz, 1H	6.87 d, *J* = 8.6 Hz, 2H	7.27 dd, *J* = 8.4, 2.1 Hz, 1H	7.33 – 7.15 m, 2H
20	7.07 d, *J* = 8.5 Hz, 1H	7.08 t, *J* = 7.2 Hz, 1H	7.26 d, *J* = 8.6 Hz, 2H	7.08 d, *J* = 8.5 Hz, 1H	7.11 – 6.93 m, 2H
21	3.82 s, 3H	3.81 s, 3H	3.72 s, 3H	3.82 s, 3H	3.81 s, 6H
22	9.20 dt, *J* = 13.8, 6.0 Hz, 1H	---	9.19–9.01 m, 1H	9.18 t, *J* = 5.9 Hz, 1H	9.17 t, *J*=6.0 Hz,1H
23	4.62 dd, *J* = 6.0, 2.8 Hz, 2H	---	4.58 d, *J* = 5.7 Hz, 2H	4.63 d, *J* = 5.8 Hz, 2H	4.69–4.47 m, 6H
25	8.76 t, *J* = 5.6 Hz, 2H	---	8.75 d, *J* = 4.9 Hz, 2H	8.76 d, *J* = 4.9 Hz, 2H	8.75 t, *J*=7.8 Hz, 2H
26	7.43-7.33 m, 2H		7.37 t, *J* = 4.9 Hz, 1H	7.42–7.35 m, 2H	8.53–8.44 m, 2H
27	---	---	---	2.45 s, 3H	---

Table S2. ^{13}C-NMR assignments for avanafil and its impurities

Position	Avanafil	Imp-A	Imp-B	Imp-C	Imp-D
1	28.02	27.89	28.06	---	27.93
2	47.71	47.84	47.71	---	47.72
3	62.41	61.99	62.61	---	61.23
4	23.22	23.13	23.23	---	23.25
5	59.42	61.30	59.46	---	59.41
7	160.31	161.37	158.74	167.42	160.54
8	157.20	160.31	160.39	155.15	161.00
9	98.78	95.84	98.63	104.64	95.24
10	167.86	168.79	167.94	173.43	167.74
11	161.29	161.52	161.31	166.95	166.58
14	42.64	42.67	43.16	42.9	42.69
15	128.13	128.05	132.15	128.02	127.54
16	129.69	129.66	129.45	129.64	129.56
17	121.16	121.15	114.22	121.20	120.19
18	156.86	153.86	157.73	153.98	153.87
19	113.11	113.12	---	113.21	113.08
20	133.75	133.57	---	132.93	133.66
21	56.48	59.50	55.43	56.52	56.48
23	45.36	---	45.35	45.45	45.34
24	161.14	---	161.17	159.84	165.73
25	157.75	---	157.73	157.85	157.77
26	120.16	---	120.13	120.30	121.08
27	---	---	---	14.02	---
28	---	---	---	---	22.95

29	---	---	---	---	47.84
30	---	---	---	---	61.91
31	---	---	---	---	28.37
32	---	---	---	---	59.62
34	---	---	---	---	161.16
35	---	---	---	---	160.26
36	---	---	---	---	95.07
37	---	---	---	---	128.08
38	---	---	---	---	129.82