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Synthesis and characterization of related substances and metabolite of tadalafil, a PDE-5 inhibitor

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Abstract: Tadalafil (Cialis) is a potent and selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type-5 (PDE-5) and it is administered orally for the treatment of erectile dysfunction (ED). Two synthetic schemes were evaluated to study the impurity profile of tadalafil. Six impurities were detected in the bulk substance (prepared by two methods) at a level of 0.1-0.15%. Identification, synthesis, characterization of impurities (related substances) and metabolite and origin of their formation is described.

Keywords: Tadalafil; PDE-5 inhibitor; metabolite; related substances; two synthetic schemes.

1. Introduction

Tadalafil (**1**) is a selective PDE-5 inhibitor and it is indicated for the treatment of erectile dysfunction.[1] Additionally, tadalafil is 700-fold more potent for PDE-5 than for PDE-6 receptors, which is present in the retina, which would explain the higher case reports of visual adverse events associated with sildenafil. Tadalafil has the longest duration of action (~36 hours) among the current PDE-5 inhibitors, which allows a greater window for sexual activity. It is the only PDE-5 inhibitor whose activity is unaffected by meals. Tadalafil is predominantly metabolized by CYP3A[2] to a catechol metabolite.
The presence of impurities in an active pharmaceutical ingredient (API) can have a significant impact on quality and safety of the drug product. As per the guide lines recommended by ICH,[3] the acceptable level for a known and unknown related compound is less than 0.15 % and 0.10 % respectively. In order to meet the stringent regulatory requirements, the impurities present in the drug substance must be identified, characterized and eliminated. These impurities are required in pure form to understand the complete impurity profile and to check the analytical performance characterization.

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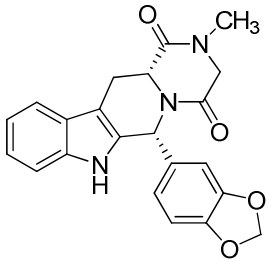
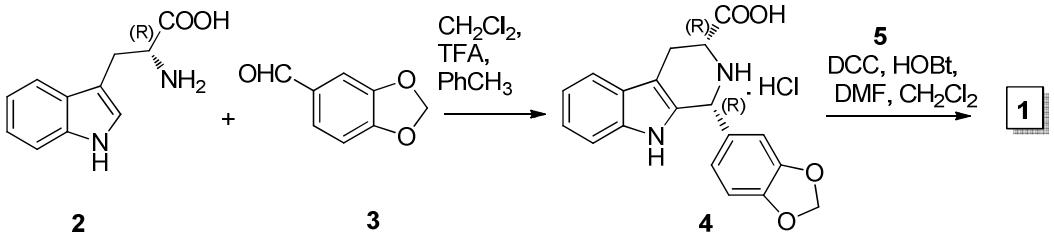


Tadalafil (**1**)

There are many routes reported for the synthesis of tadalafil **1** [2, 4-6] by employing Pictet-Spengler reaction and till now there are no reports in the literature describing the complete impurity profile of tadalafil. We have chosen two synthetic schemes for the process development, wherein one route reported by us[7] and the other one reported by Daugan group.[8,9] These two synthetic routes critically evaluated by means of quality, yield and to achieve an optimum process. As a part of this process, complete impurity profile in both the schemes has been studied. The present article describes the identification, synthesis and characterization of tadalafil impurities, metabolite and root cause of their formation.

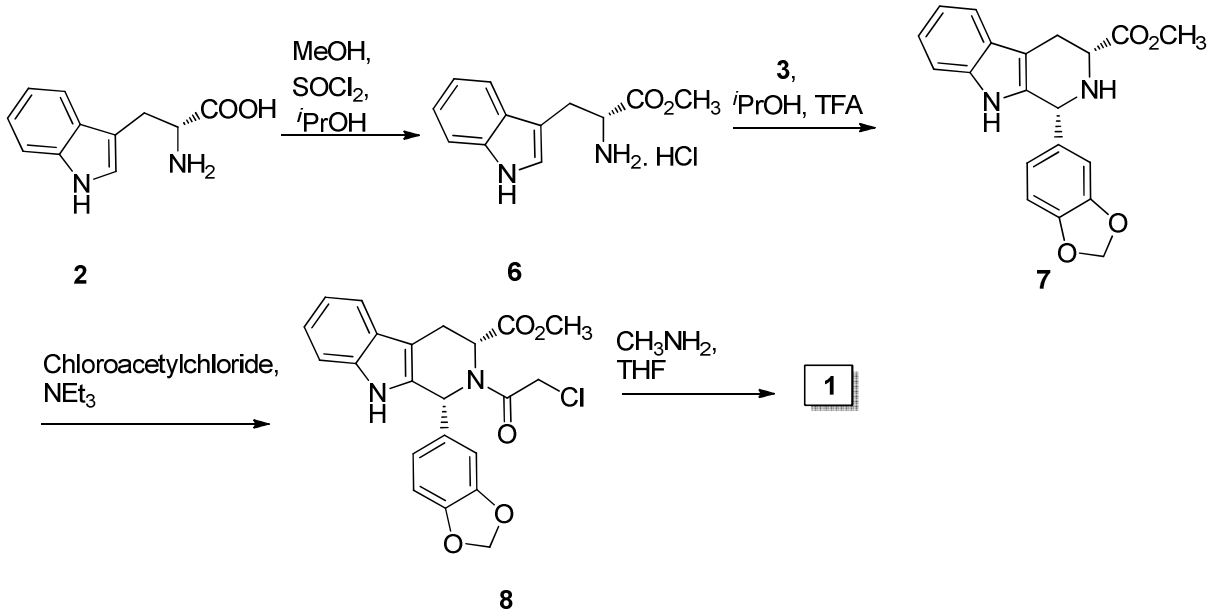
2. Results and discussion

The synthetic route reported by us involved the Pictet-Spengler reaction between **2** and **3** in presence of trifluoroacetic acid (TFA) yielded **4** which was coupled in a subsequent step with sarcosine ethyl ester hydrochloride **5** under DCC and HOBT in dimethylformamide conditions furnished tadalafil **1** (Scheme 1).[7]



Scheme 1. Synthesis of tadalafil **1** using sarcosine ethyl ester hydrochloride **5**

Daugan and co-workers[8,9] also utilized Pictet–Spengler reaction as key step wherein coupling between D-tryptophan methyl ester hydrochloride **6** and piperonal **3** under acidic conditions provided chiral pure **7**. *N*-Acylation of **7** with chloroacetyl chloride resulted in amide **8**, which upon treatment with methyl amine yielded tadalafil **1** (Scheme 2).



Scheme 2. Synthesis of tadalafil **1** using chloro acetyl chloride

Tadalafil was synthesized by following the above two synthetic routes to study the impurity profile. In this regard the final samples were analyzed in HPLC method and found six impurities. To identify the probable structures for these impurities LC-MS analysis was carried out. On the basis of LC-MS data and chemistry involved in the schemes, following tentative structures were proposed for impurities (Figure 1).

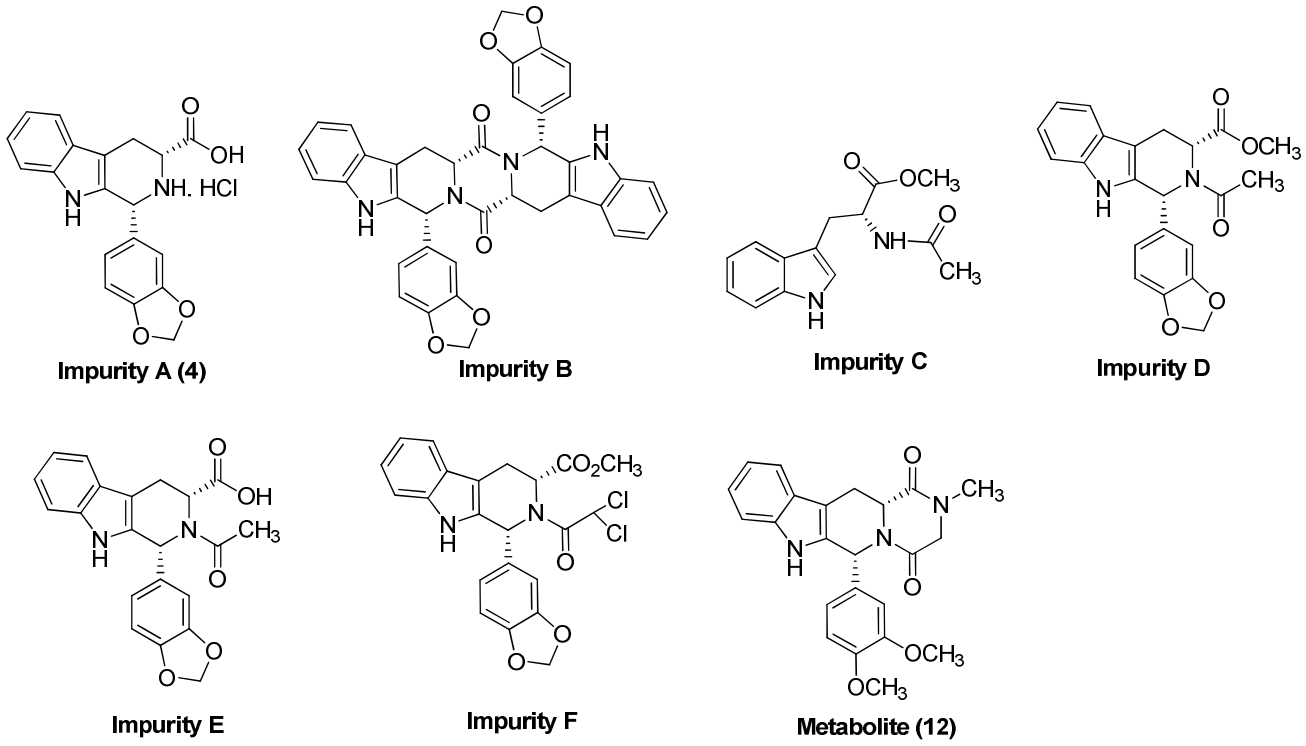


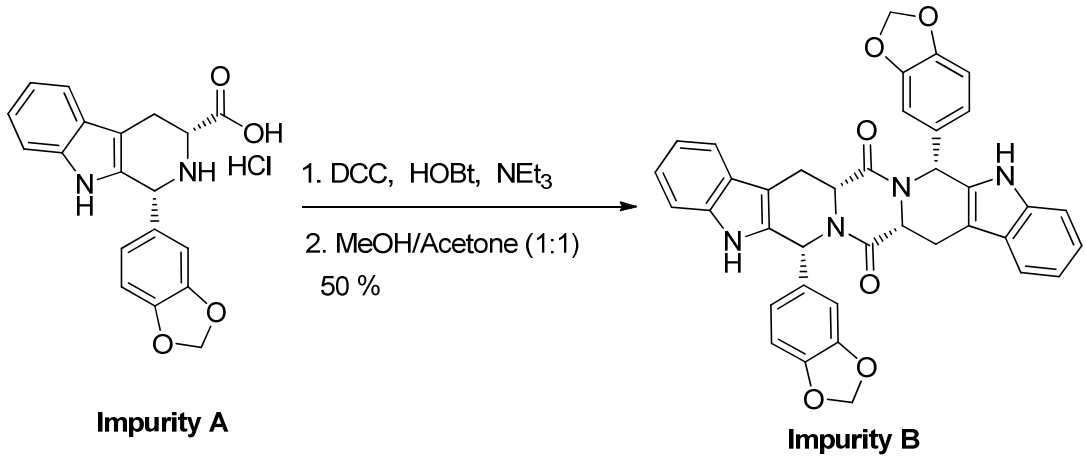
Figure 1. Structures of related substances (impurity A, impurity B, impurity C, impurity D, impurity E, impurity F and metabolite) of tadalafil

2.1. Impurity A (4)

Impurity A (**4**) is the process related impurity, which is identified in the final drug substance obtained via scheme 1. This impurity is one of the unreacted starting materials in the final step. The HPLC retention time and the spectral data (IR, mass, and ^{1}H NMR) of impurity A are consistent with β-carboline acid **4** reference sample.

2.2. Impurity B

Impurity B is the potential impurity identified during the synthesis of **1** by scheme 1. Impurity B is formed by dimerisation of compound **4** under final cyclization conditions. This impurity was independently synthesized by amide bond coupling between two molecules of **4** by using dicyclo hexyl carbodiimide (DCC), 1- hydroxy benzotriazole (HOBt) and triethylamine (Scheme 3).[7]



Scheme 3. Synthesis of **impurity B**

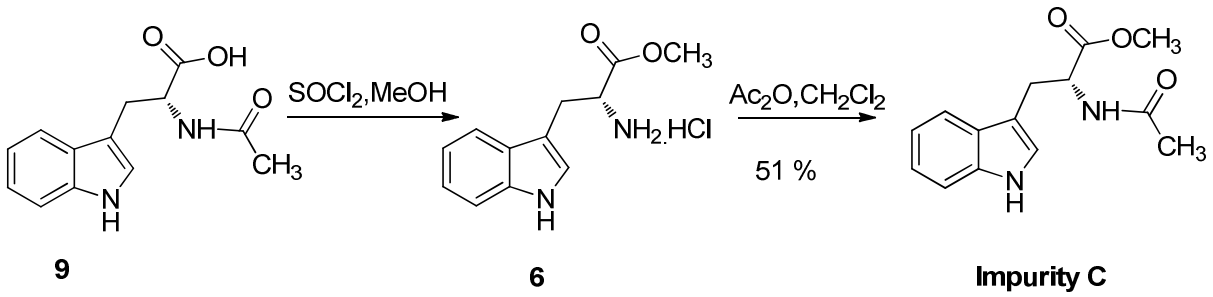
In LC–MS, the peak corresponding to the impurity B has shown a molecular weight 636 and proposed structure of impurity B illustrated that it is a symmetrical dimer of **4**. Hence this compound could be resulted from **4**. It must be mentioned that compound **4** processes both carboxylic acid and amino groups. Under these reaction conditions there exists a possibility for the intermolecular amidation between two molecules of compound **4** to result impurity B.

To confirm the above assumption, impurity B was prepared by exposure of compound **4** to DCC/HOBt reaction conditions. The obtained compound was characterized using various analytical tools as described.

Mass spectrum displayed a deprotonated molecular ion at *m/z* 635 as a base peak, which was in alignment with the predicted dimer structure. In the IR spectrum, stretching due to carbonyl group of amide functionality was observed at 1662 cm^{-1}. In addition, amine and acid OH stretchings observed in compound **4** were not observed in the impurity B. These observations were influenced us to conclude that the compound **4** was converted in to impurity B.

2.3. Impurity C

Compound **9** is the potential impurity in D-tryptophan **2**. Impurity C was obtained along with desired product **6** during the esterification of **2** with thionyl chloride in methanol (Scheme 2). Impurity C was independently from **6**, acetylation of **6** with acetic anhydride in dichloro methane (DCM) provided the desired impurity C in 51 % yield. (Scheme 4).



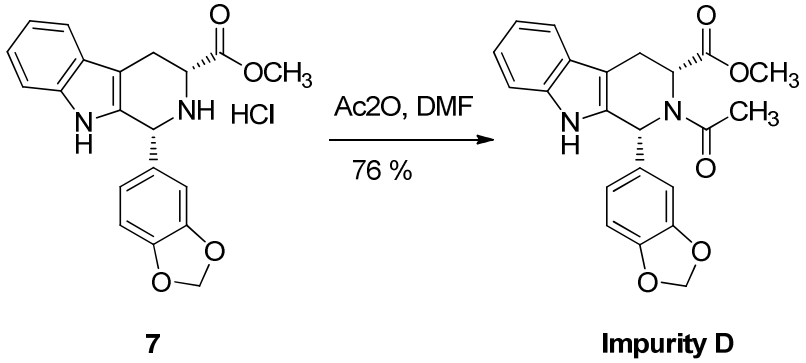
Scheme 4. Synthesis of **impurity C**

Mass spectrum of impurity C showed peaks at *m/z* 261.1 and *m/z* 283.1 corresponding to protonated molecular ion and sodium adduct. In the IR spectrum, bands at 1734 and 1663 cm^{-1} displayed stretching corresponding to amide and ester carbonyl groups.

^{1}H NMR spectrum two singlets at δ 3.57 and 1.81 corresponding to ester methyl and acetyl group respectively. A multiplet at δ 4.53–4.45 for alpha proton of amino acid, two doublets at δ 7.48 & 7.33 and two triplets at δ 7.06 & 6.98 corresponding to aromatic protons further supporting the structure of impurity C. In ^{13}C NMR spectrum two signals were observed at δ 172.6 and 169.4 corresponding to two carbonyl carbons. The above spectral data observations and synthetic path way confirms the proposed structure for impurity C. Detailed spectral data was provided in the experimental section

2.4. Impurity D

Impurity D is another potential impurity (Scheme 2) resulting from the contamination of chloroacetyl chloride with acetyl chloride. Impurity D was formed during the chloro acylation of compound 7. This impurity was independently synthesized by direct acetylation of compound 7 with acetic anhydride using triethylamine in dimethylformamide (Scheme 5).Chloroacetyl chloride was synthesized from chlorination of acetyl chloride.



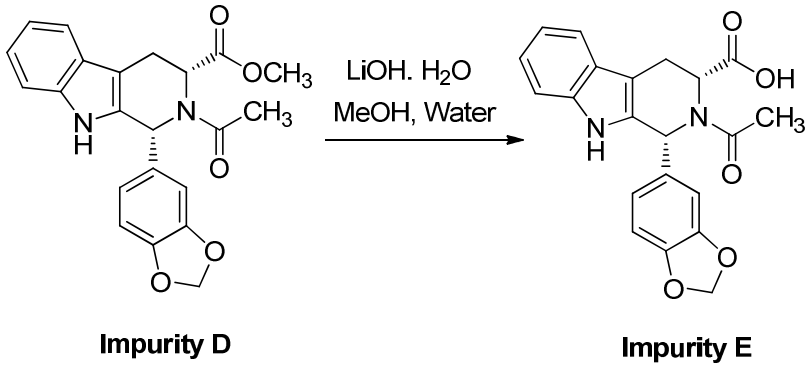
Scheme 5. Synthesis of **impurity D**

In LC–MS, the peak corresponding to the impurity D has shown a molecular ion at *m/z* 392, which is 42 amu more than compound **7** (*m/z* 350). This molecular weight is may be corresponding to the acetylated compound **7**. Since, the synthesis of tadalafil **1** (Scheme 2) involves chloroacetylation of compound **7** with chloroacetylchloride. There is a chance to present the acetyl chloride in chloroacetylchloride, because it is one of the impurity in chloro acetyl chloride. In the synthetic sequence acetyl chloride reacts with **7** and leads to the formation of impurity D. This impurity was independently synthesized by acetylating the compound **7** with acetic anhydride in DMF. Mass spectrum showed peaks corresponding to protonated molecular ion (M^+ + H), sodium adduct (M^+ + Na) at *m/z* 393 and 415 respectively. IR spectrum displayed stretching at 1736 cm^{-1} (Ester, C═O), additionally another peak was observed at 1639 cm^{-1} (Amide, C═O stretching).

A singlet equivalent to three protons was observed in the ^{1}H NMR spectrum at δ 2.32 in addition to the other signals, those were not observed in the compound **7**. In ^{13}C NMR spectrum two signals were observed at δ 170.9 and 170.5 corresponding to carbonyl carbons and another signal was observed at δ 21.2 corresponding to methyl group carbon. Where as in the compound **7** at δ 170.5 and 21.2 signals were not observed, indicating that the transformation into other compound. The above spectral data observations and synthetic path way confirms the proposed structure for impurity D. Detailed spectral data was provided in the experimental section.

2.5. Impurity E

Impurity E is the process related impurity which is identified in the penultimate step in scheme 2. This impurity might form from unreacted **2** present in compound **6** reacting with piperonal under acidic conditions followed by acetylation. Synthesis of impurity E commenced from impurity D. Alkaline hydrolysis of impurity D using lithium hydroxide monohydrate in aqueous methanol gave the desired impurity E in 72 % yield (Scheme 6). The obtained compound structure was confirmed using various analytical tools



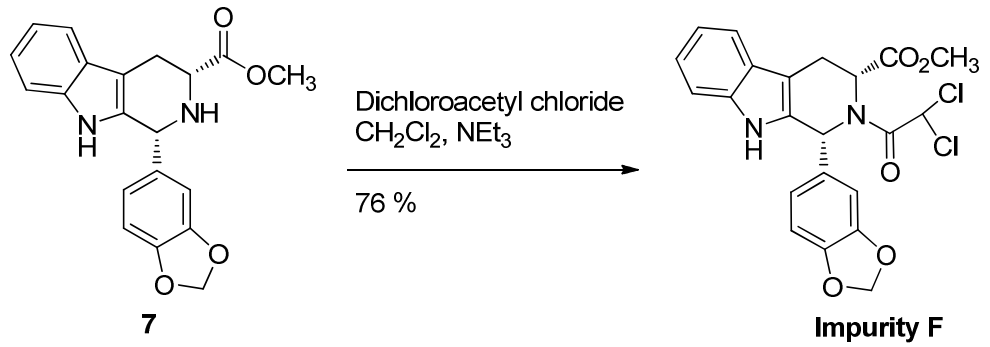
Scheme 6. Synthesis of **impurity E**

Deprotonated molecular ion (M^+– H) peak of impurity E was observed at *m/z* 377 in the mass spectrum. In IR spectrum acid C═O and amide C═O stretching appeared at 1728 cm^{-1} and 1610 cm^{-1}, respectively. Decrease in the frequency of carbonyl stretching from 1737 cm^{-1} (observed in impurity D as ester carbonyl stretching) to 1728 cm^{-1} indicating the formation of hydrolyzed product as acid C═O stretching appears at lower frequency than ester C═O stretching.

Absence of OCH_3 protons in the ^{1}H NMR spectrum clearly indicating the conversion of ester to acid and remaining protons were observed same as impurity D. In ^{13}C NMR also supporting the conclusion arrived from ^{1}H NMR data as it shows absence of OCH_3 carbon signal, which was observed at δ 53.2 in impurity D. The above spectral data observations were supporting the proposed structure for impurity E. Full characterization data was presented in the experimental section.

2.6. Impurity F

Impurity F raised due to small amount of dichloroacetyl chloride present in chloroacetyl chloride and reaction with **7** under conditions followed for tadalafil. This impurity independently synthesized by coupling of compound **7** with dichloroacetyl chloride using triethyl amine (TEA) in dichloromethane (DCM) (Scheme 7). Chloroacetyl chloride was synthesized from chlorination of acetyl chloride and excess vigorous chlorination to form dichloroacetyl chloride in chloroacetyl chloride.



Scheme 7. Synthesis of **impurity F**

Based on the LC–MS data and proposed structure, it can be concluded that this impurity will be formed due to the presence of dichloroacetylchloride in chloroacetylchloride. Mass spectrum of impurity F showed a molecular ion peak at *m/z* 460, which is 35 amu more than the compound **7** (*m/z* 426). The difference in the mass number is supporting the proposed structure. In the synthesis of chloroacetylchloride (chlorination of acetylchloride), there is a chance to form dichloroacetylchloride. Dichloroacetylchloride will react with compound **7** in a similar way as chloroacetylchloride (Scheme 2). Hence the formation of impurity F cannot be ruled out.

De protonated molecular ion (M^+– H) peak of impurity F was observed at *m/z* 459. IR spectrum displayed stretching at 1741 and 1668 cm^{-1} for two carbonyl groups.

^{1}H NMR spectrum revealed that the presence of characteristic singlet at δ 6.83 for dichloro attached proton and other protons appeared in the respective regions. In ^{13}C NMR spectrum signal corresponding to dichloro attached carbon observed at δ 66.0 further confirmed the structure of impurity F. Full characterization data was presented in the experimental section.

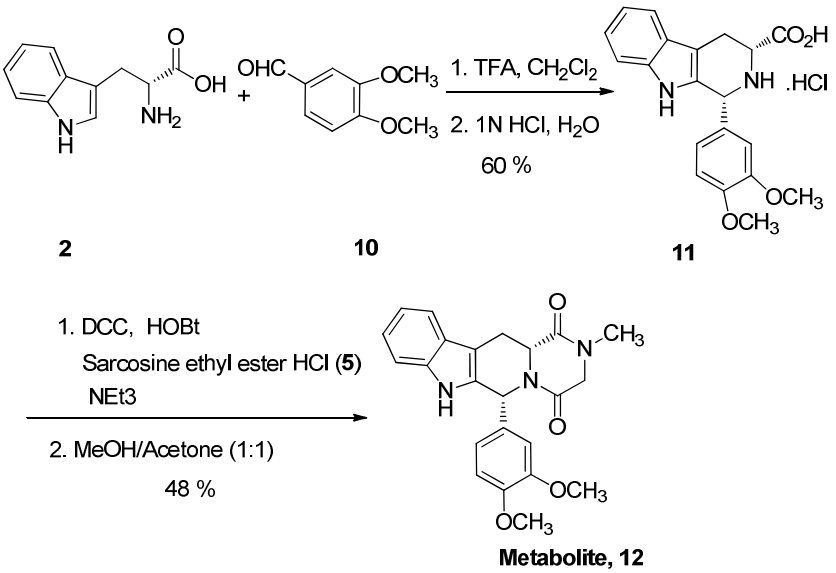
2.7. Metabolite 12

Compound **12** is one of the metabolites of tadalafil. Metabolite **12** was prepared by following the synthetic scheme 2, wherein 3, 4-dimethoxybenzaldehyde **10** was used instead of piperonal **3**. Preparation of compound **11** was accomplished by Pictet-Spengler cyclization of D-tryptophan **2** with 3, 4-dimethoxybenzaldehyde **10** in the presence of trifluoroacetic acid. Synthesis of metabolite **12** was achieved by coupling of compound **11** with sarcosine ethyl ester HCl under DCC/HOBt coupling conditions (Scheme 8). Characterization study was done using ^{1}H NMR,^{13}C NMR, mass and IR.

Mass spectral analysis showed protonated molecular ion (M^+ + H) at *m/z* 406 and sodium adduct (M^+ + Na) at m/z 428. IR spectrum exhibited NH stretching at 3329 cm^{-1} and the characteristic amide C═O stretching at 1660 cm^{-1}.

^{1}H NMR spectrum of compound **12** displayed two additional signals corresponding to NCH_3 and NCH_2 at δ 2.94 (s, 3H) and δ 3.55–3.35 (m, 2H), respectively apart from signals observed in compound **10**. In addition, the signal corresponding to OH of acid functionality of compound **10** observed at δ 10.18 was disappeared in compound **12**.

In ^{13}C NMR spectrum two peaks corresponding to amide carbonyl carbons were observed at δ 166.7 and NCH_2 & NCH_3 corresponding signals were present at δ 54.9 and 51.5. These spectral data observations concluded the conversion of compound **10** into compound **12**, there by confirm the proposed structure for compound **12**. Detailed spectral data was given in the experimental section.



Scheme 8. Synthesis of compound **12**

3. Conclusion

In conclusion, critical process impurities and origin of their formation were identified during the process development. All the impurities and metabolite were independently synthesized, characterized and confirmed by spectral analysis. However, from the knowledge about their root cause of formation, it is apparent that they should be controlled by setting appropriate specifications for their precursors.

4. Experimental

The ^{1}H and ^{13}C spectra were measured in $CDCl_3$ and DMSO-d_6 using 200, 400 and 500 MHz on a Varian Gemini (200 MHz), Mercury Plus (Varian 400 MHz) and Unity INOVA (Varian 500 MHz) FT–NMR spectrometer; the chemical shifts were reported in δ ppm. IR spectrum was recorded in the solid state as KBr dispersion using a Perkin-Elmer 1650 FT–IR spectrometer. Mass spectrum (70 eV) was recorded on a HP 5989A LC MS spectrometer. The HRMS analysis has been performed on the micromass LCT Premier XE mass spectrometer equipped with an ESI Lock spray source for accurate mass values (Water Corporation, Milford, MA, USA). Solvents and reagents were obtained from commercial sources and used without further purification.

(1R,3R)-1-(benzo[d][1,3]dioxol-5-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylic acid hydrochloride (Impurity A): Impurity A was synthesized as described in our previously paper.[7]
mp 215–220 ^{o}C, IR (KBr, ν_{max}, cm^{-1}): 3450, 3225, 2927, 1757, 1626, 1205, 1040, 753. ^{1}H NMR (200 MHz, DMSO-d_6), δ_H 10.79 (s, 1H), 10.20 (br, 2H), 7.55 (d, J = 7.0 Hz, 1H), 7.28 (d, J = 7.2 Hz, 1H), 7.19–6.93 (m, 5H), 6.09 (s, 2H), 5.83 (s, 1H), 4.58 (dd, J = 5.0, 11.0 Hz, 1H), 3.60–3.10 (m, 2H). ^{13}C NMR (50 MHz, DMSO-d_6), δ_C 169.7, 148.5, 147.2, 136.7, 128.9, 127.1, 125.5, 124.9, 122.0, 119.2, 118.2, 111.6, 110.2, 108.3, 106.7, 101.5, 57.5, 55.4, 22.2; MS**,** *m/z*: 337.1 [M^{+}+H].

Synthesis of impurity B: To a stirred solution of compound **4** (2 g, 0.006 mol) in DMF (20 mL) were added dicyclohexylcarbodiimide (1.5 g, 0.007 mol), 1-hydroxybenzotriazole (1.5 g, 0.007 mol) and followed by NEt_3 (2.0 g, 0.009 mol) at 25–35 °C. The resultant reaction mixture was heated to 50–55 °C. After stirring for 10 h at 50–55 °C, the mixture was allowed to cool to 10 °C and unwanted dicyclohexylurea (DCU) was filtered. A mixture of CH_2Cl_2/water (20 mL, 1:1) was added to the filtrate and washed with 8 % aqueous $NaHCO_3$ solution (8 mL). The organic layer was separated, and

aqueous layer was extracted with CH_2Cl_2 (2 x 10 mL). The combined organic layer was concentrated under reduced pressure, cooled to 0–5 °C and re-crystallized in a mixture of MeOH and acetone (20 mL, 1:1) to furnish impurity-B (1.8 g, 50 %). mp 205-210 °C, IR (KBr, ν_{max}, cm^{-1}): 3353, 3300, 2908, 2873, 1677, 1662, 1497, 1487, 1239, 1037, 744. ^{1}H NMR (400 MHz, DMSO-d_6), δ_H 11.25 (s, 2H), 7.55 (d, J = 7.6 Hz, 2H), 7.36 (d, J = 8.0 Hz, 2H), 7.08 (t, J = 7.2 Hz, 2H), 7.0 (t, J = 7.6 Hz, 2H), 6.87 (s, 2H), 6.78 (d, J = 8.0 Hz, 2H), 6.73 (d, J = 8.4 Hz, 2H), 6.43 (s, 2H), 5.95 (s, 4H), 4.65 (dd, J = 5.6, 11.2 Hz, 2H), 3.47 (dd, J = 5.6, 15.6 Hz, 2H), 3.40 (m, 1H), 3.24 (m, 1H), 2.95 (dd, J = 5.6, 15.6 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6), δ_C 169.2, 147.4, 146.2, 136.4, 135.8, 133.7, 125.6, 121.2, 118.9, 118.0, 111.3, 108.0, 106.1, 104.1, 101.0, 54.8, 54.0, 21.5. MS, *m/z*: 637 [M^++H] and 659 [M+Na]. Anal. Calcd for $C_{38}H_{28}N_4O_6$: C, 71.69; H, 4.43; N, 8.80, Found : C, 71.64; H, 4.49; N, 8.76.

(R)-Methyl 2-acetamido-3-(1H-indol-3-yl) propanoate (Impurity C): A mixture of **6** (25 g, 0.098 mol) in water (65 mL) and CH_2Cl_2 (100 mL) was added 8.0 % aqueous $NaHCO_3$ solution (110 mL), thereby stirred for 1 h. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (100 mL). The combined layer was concentrated under vacuum below 50 °C. Ac_2O (64 mL, 0.677 mol) was charged to reaction mass and the resultant reaction mixture was stirred at 25-35 °C for 6 h. The precipitated solid was filtered, washed with water (65 mL) and dried at 65-75 °C for 6 h to obtain 13.0 g (51 %) of title compound. mp 135-142 °C, IR (KBr, ν_{max}, cm^{-1}): 3404, 3319, 1734, 1663, 1524, 1437, 1223, 748. ^{1}H NMR (400 MHz, DMSO–d_6), δ_H 10.85 (s, 1H), 8.30 (d, J = 7.6 Hz, 1H), 7.48 (d, J = 7.6 Hz, 1H), 7.33 (d, J = 8.0 Hz, 1H), 7.14 (s, 1H), 7.06 (t, J = 7.2 Hz, 1H), 6.98 (t, J = 7.6 Hz, 1H), 4.53–4.45 (m, 1H), 3.57 (s, 3H), 3.19–2.99 (m, 2H), 1.81 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6), δ_C 172.6, 169.4, 136.1, 127.0, 123.6, 121.0, 118.4, 118.0, 111.4, 109.5, 53.1, 51.7, 27.1, 22.3; MS, *m/z*: 261.1 [M^++H] and 283.1 (M^++Na);

***(1R,3R)-methyl2-acetyl-1-(benzo[d][1,3]dioxol-5-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b] indole-3-carboxylate (Impurity D)*:** A mixture of **7** (2 g, 0.006 mol), Ac_2O (5 g, 0.005 mol) and DMF (10 mL) was heated to 50–55 °C, thereby stirred for 6 h. Subsequently, the obtained reaction mixture was quenched with water (50 mL) and extracted with EtOAc (2 x 50 mL). The organic layer was separated and concentrated under vacuum below 55 °C to furnish 1.7 g (75 %) of title compound. mp 215-220 °C, IR (KBr, ν_{max}, cm^{-1}): 3394, 2948, 1736, 1639, 1426, 1236, 1038, 745. ^{1}H NMR (400 MHz, CD_3OD), δ_H 7.52 (d, J = 7.6 Hz, 1H), 7.26 (d, J = 8.4 Hz, 1H), 7.10 (t, J = 6.8 Hz, 1H), 7.03 (t, J = 7.2 Hz, 1H), 6.93 (s, 1H), 6.76 (s, 1H), 6.65 (d, J = 8.0 Hz, 1H), 6.53 (d, J = 6.8 Hz, 1H), 5.88 (s, 2H), 5.13 (d, J = 6.4 Hz, 1H), 4.59 (s, 1H), 3.59 (d, J = 4.8 Hz, 1H), 3.12 (s, 3H), 3.06 (dd, J = 6.0, 14.8 Hz, 1H), 2.32 (s, 3H).^{13}C NMR (100 MHz, DMSO-d_6), δ_C 170.9, 170.5, 146.8, 146.4, 136.3, 134.1, 130.3, 125.9, 122.1, 121.4, 118.5, 118.0, 111.1, 109.0, 107.4, 106.4, 100.9, 52.7, 51.6, 49.8, 22.2, 21.2. MS, m/z: 393 [M^++H] and 415 [M^++Na]. HRMS (EI); *m/z* calcd. for (M+H) $C_{22}H_{20}N_2O_5$: 393.1450; found: 393.1457.

(1R,3R)-2-acetyl-1-(benzo[d][1,3]dioxol-5-yl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylic acid (Impurity E): Impurity-D (1 g, 0.002 mol) was added to a stirred solution of $LiOH.H_2O$ (0.2 g, 0.003 mol) in MeOH (6 mL) and water (1.5 mL). The reaction mixture was heated to 65–70 °C and stirred for 4 h at the same temperature. The reaction mixture was cooled to 25–30 °C and adjusted the pH to 1–2 with conc. HCl. The resulted reaction mixture was maintained at 25–30 °C for 2 h. Subsequently, the precipitated solid was filtered and washed with water (5 mL). Wet solid was dried under vacuum at 75 °C to yield the 0.7 g (72 %) of title compound. mp 235-241 °C, IR (KBr, ν_{max}, cm^{-1}): 3406, 2903, 1728, 1501, 1420, 1610, 1239, 1037, 749. ^{1}H NMR (400 MHz, DMSO-d_6), δ_H 10.75 (s, 1H), 7.51 (d, J = 7.5 Hz, 1H), 7.27 (d, J = 8.0 Hz, 1H), 7.08 (t, J = 7.0 Hz, 1H), 7.01 (t, J = 7.5 Hz, 1H), 6.74 (s, 1H), 6.73 (d, J = 8.0 Hz, 1H), 6.67 (s, 1H), 6.62 (d, J = 7.5 Hz, 1H), 5.95 (d, J = 12.5 Hz, 2H), 5.03 (d, J = 6.5 Hz, 1H), 3,59 (m, 1H), 2.97 (dd, J = 6.5 Hz, J = 15.5 Hz, 2H), 2.22 (s, 3H).^{13}C NMR (50 MHz, DMSO-d_6), δ_C 172.2, 170.5, 146.3, 136.3, 134.1, 130.9, 125.9, 122.6, 121.2, 118.4, 117.9, 111.1, 109.3, 107.3, 106.4, 100.7, 53.2, 50.4, 22.4, 21.2. MS, m/z: 377 [M^+–H]. HRMS (EI); *m/z* calcd. for (M–H) $C_{21}H_{18}N_2O_5$: 377.1137; found: 377.1144.

(1R,3R)-methyl-1-(benzo[d][1,3]dioxol-5-yl)-2-(2,2-dichloroacetyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylate (Impurity F): Dichloroacetyl chloride (2.0 g, 0.0068 mol) was added drop wise to a solution of **7** (2.0 g, 0.0057 mol) and NEt_3 (0.7 g, 0.0069 mol) in CH_2Cl_2 (20 mL) at 0-5 ^oC. The reaction mixture temperature was raised to 25-35 ^oC and stirred for 14-16 h. The reaction mixture was quenched with water, organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (10 mL). The combined layers were concentrated under vacuum below 50 °C to afford yellow foam, which was purified by flash chromatography by eluting with CH_2Cl_2 /MeOH (9:1) to provide 2.0 g (75.9 %) of title compound as a yellow color solid. mp 205-210 ^oC, IR (KBr, ν_{max}, cm^{-1}): 3390, 2903, 1741, 1668, 1487, 1440, 1238, 1038, 811, 745. ^1H NMR (400 MHz, $CDCl_3$), δ_H 7.74 (s, 1H), 7.60 (d, J = 7.2 Hz, 1H), 7.32–7.16 (m, 3H), 6.89 (s, 1H), 6.83 (s, 1H), 6.66 (s, 2H), 6.41 (s, 1H), 5.92 (s, 2H), 5.16 (d, J = 7.2 Hz, 1H), 3.71 (d AB q, J = 15.6 Hz, 1H), 3,26 (m, 1H), 3.27–3.20 (m, 4H). ^{13}C NMR (50 MHz, DMSO-d_6), δ_C 169.5, 163.8, 147.6, 136.3, 132.2, 129.1, 126.2, 123.1, 122.7, 119.9, 118.5, 111.0, 110.0, 107.7, 107.5, 101.1, 66.0, 53.6, 53.3, 52.4, 21.6. MS, *m/z*: 461 (M^++H). HRMS (EI); *m/z* calcd. for (M+H) $C_{22}H_{18}Cl_2N_2O_5$: 461.0671; found: 461.0666.

(6R,12aR)-2,3,6,7,12,12a-Hexahydro-2-methyl-6-(3,4-dimethoxyphenyl)-pyrazino [2',1':6,1]pyrido[3,4-b]indole-1,4-dione (metabolite 12):

Step-1: (1R,3R)-1-(3,4-dimethoxyphenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylic acid (11): Trifluoroacetic acid (6 mL, 0.08 mol) was added to a solution of D-tryptophan 2 (10 g, 0.05 mol) and 3, 4-dimethoxybenzaldehyde 10 (9.7 g, 0.06 mol) in CH_2Cl_2 (100 mL). The reaction mixture was refluxed for 7 h, allowed to cool to 35 °C. CH_2Cl_2 and MeOH (100 mL, 1:1) was added to the reaction mixture and washed with 8 % aqueous $NaHCO_3$ solution (50 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (50 mL). The combined organic layer was concentrated under reduced pressure and 1N HCl (150 mL) was added slowly at 25–35 °C. Thus, the resultant mixture was concentrated under vacuum below 65 °C. Acetone (50 mL) was added at 25–30 °C, after 2 h, the precipitated solid was filtered, washed with acetone (10 mL) and dried at 75 °C for 6 h to obtain 12.2 g (90 %) of title compound. mp 210-216 ^oC, IR (KBr, ν_{max}, cm^{-1}): 3454, 2938, 1763, 1521, 1256, 1151, 1022, 749. ^1H NMR (500 MHz, DMSO–d_6), δ_H 10.76 (s, 1H), 10.20 (br, 2H (corresponds to 2-NH protons), 7.55 (d, J = 7.5 Hz, 1H), 7.28 (d, J = 8.0 Hz, 1H), 7.15 (s, 1H), 7.14–7.01 (m, 4H), 5.84 (s, 1H), 4.60 (dd, J = 5.5, 11.5 Hz, 1H), 3.81 (s, 3H), 3.72 (s, 3H), 3.37 (dd, J = 5.0, 15.5 Hz, 1H), 3.29–3.23 (m, 1H). ^{13}C NMR (50 MHz, DMSO-d_6), δ_C 169.7, 150.0, 148.4, 136.7, 129.1, 125.6, 123.3, 121.9, 119.1, 118.1, 113.8, 111.6, 111.4, 106.4, 57.7, 55.6, 55.5, 55.4, 22.1. MS, *m/z*: 353 [M^++H] and 375 [M^++Na].

Step-2: (6R,12aR)-2,3,6,7,12,12a-Hexahydro-2-methyl-6-(3,4-dimethoxyphenyl)-pyrazino [2',1':6,1]pyrido[3,4-b]indole-1,4-dione (metabolite 12): To a solution of sarcosine ethyl ester hydrochloride 5 (1.3 g, 0.08 mol) in DMF (6 mL) was added NEt_3 (1 g, 0.01 mol) and stirred for 20 min at 25–30 °C. The separated solid (NEt_3.HCl) was filtered and washed with DMF (2 mL). To the filtrate, compound 6 (1 g, 0.03 mol), dicyclohexylcarbodiimide (0.7 g, 0.03 mol), 1-hydroxybenzotriazole (0.5 g, 0.04 mol) and NEt_3 (0.4 g, 0.04 mol) were added at 25–35 °C. Thereafter the reaction mixture was heated to 50–55 °C and stirred for 10 h. Subsequently, reaction mixture was cooled to 10 °C and unwanted dicyclohexylurea (DCU) was filtered. A mixture of CH_2Cl_2 and water (10 mL, 1:1) was added to the filtrate and washed with 8 % aqueous $NaHCO_3$ solution (8 mL). The organic layer was separated and aqueous layer was extracted with CH_2Cl_2 (2 x 5 mL). The combined organic layer was concentrated, the obtained residue was cooled to 0–5 °C and re-crystallized from the mixture of MeOH and acetone (3 mL, 1:1) to yield 0.5 g (50 %) of title compound. mp 165-170 ^oC, IR (KBr, ν_{max}, cm^{-1}): 3329, 2931, 1660, 1514, 1453, 1418, 1261, 1024, 744. ^1H NMR (400 MHz, DMSO–d_6), δ_H 11.10 (s, 1H), 7.54 (d, J = 7.6 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.06 (t, J = 6.8 Hz, 1H), 7.01 (s, 1H), 6.99 (t, J = 7.2 Hz, 1H), 6.80 (d, J = 8.0 Hz, 1H), 6.74 (d, J = 8.8 Hz, 1H), 6.19 (s, 1H), 4.41 (dd, J = 4.4, 11.2 Hz, 1H), 4.17 (d ABq, J = 16.8 Hz, 1H), 3.94 (d ABq, J = 16.8 Hz, 1H), 3.73 (s, 3H), 3.65 (s, 3H),3,50 (m, 2H) 3.05(s, 3H), 3,01(m, 1 H) 2.94 (s, 3H). ^{13}C NMR (50 MHz, DMSO-d_6), δ_C 166.7, 148.3, 147.7, 136.0, 135.5, 134.0, 125.7, 121.1, 118.7, 117.9, 117.5, 111.9, 111.2, 110.7, 104.5, 55.5, 54.9, 51.5, 33.3, 32.9, 24.4, 22.9. MS, *m/z*: 406 [M^++H] and 428 [M^++Na]. Anal. Calcd for $C_{23}H_{23}N_3O_4$: C, 68.13; H, 5.72; N, 10.36, Found : C, 68.10; H, 5.78; N, 10.40.

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References

[1] Rajfer, J.; Aronson, W. J.; Bush, P. A.; Dorey, F. J.; Ignarro, L. J. Nitric oxide as a mediator of relaxation of the corpus cavernosum in response to nonadrenergic, noncholinergic neurotransmission. *New Engl. J. Med.* **1992**, *326*, 90–94.
[2] Daugan, A. C. M. Tetracyclic derivatives, process of preparation and use. *US Patent*, *US5859006*, **1999**.
[3] ICH harmonized tripartite guideline, Impurities in New Drug Substances Q3A (R2), current step 4 version dated 25 October 2006. http://www.ich.org.
[4] Sorbera, L. A.; Martin, L.; Leeson, P. A.; Castaner, J. IC-351 - Treatment of erectile dysfunction - Treatment of female sexual dysfunction - Phosphodiesterase 5 inhibitor. *Drug. Future*, **2001**, 26, 15–19.
[5] Crasto, A. M.; Joshi, N. S. Process for the preparation of tadalafil. *WO Patent*, *WO2007110734*, **2007**.
[6] Xiao, S.; Shi, X. X.; Xing, J.; Yan, J. J.; Liu, S. L.; Lu, W. D. Synthesis of tadalafil (Cialis) from L-tryptophan. *Tetrahedron: Asymm.* **2009**, *20*, 2090–2096.
[7] Anumula, R. R.; Madivada, L. R.; Gilla, G.; Raju, W. N. K. P.; Kagga, M.; Reddy, P.P.; Bhattacharya, A.; Bandichhor, R. Alternative synthesis of Tadalafil: PDE5 Inhibitor. *Synth. Commun.* **2008**, *38*, 4265-4271.
[8] Daugan, A.; Grondin, P.; Ruault, C.; de Gouville, A. C. L.; Coste, H.; Kirilovsky, J.; Hyafil, F.; Labaudiniere, R. The discovery of tadalafil: A novel and highly selective PDE5 inhibitor. 1: 5,6,11,11a-tetrahydro-1H-imidazo[1 ',5 ': 1,6]pyrido[3,4-b]indole-1,3(2H)-dione analogues. *J. Med. Chem.* **2003**, *46*, 4525–4532.
[9] Daugan, A.; Grondin, P.; Ruault, C.; de Gouville, A. C. L.; Coste, H.; Linget, J. M.; Kirilovsky, J.; Hyafil, F.; Labaudiniere, R. The discovery of tadalafil: A novel and highly selective PDE5 inhibitor. 2: 2,3,6,7,12,12a-hexahydropyrazino[1 ',2 ': 1,6]pyrido[3,4-b]indole-1,4-dione analogues. *J. Med. Chem.* **2003**, *46*, 4533–4542.