CHAPTER-5
AN ALTERNATIVE SYNTHESIS OF TADALAFIL - PDE5 INHIBITOR IDENTIFICATION, SYNTHESIS AND CHARACTERIZATION OF IT`S RELATED SUBSTANCES
5.1 INTRODUCTION

Phosphodiesterase type 5 (PDE5), member of the super family of cyclic nucleotide hydrolyzing enzymes that specifically cleaves cyclic guanosine monophosphate (cGMP), is distributed throughout vascular smooth muscle tissue and to a lesser extent in the lung, kidney, and platelets. Inhibition of PDE5 as a therapeutic target has received considerable attention over the years, particularly for the treatment of cardiovascular diseases, e.g., angina, hypertension, and congestive heart failure.\(^1,2\) More recently, it was demonstrated that PDE5 plays a critical role in the mechanism of penile erection and that PDE5 inhibition would be of therapeutic utility in treating male erectile dysfunction (MED).\(^3\) Increased cGMP levels eventually cause a decrease in intracellular calcium concentration. Lower calcium concentration leads to relaxation of smooth muscle in the corpus cavernosum, resulting in increased arterial blood flow to the penis and ultimately erection. PDE5 inhibition blocks cGMP degradation, facilitates cGMP accumulation, induces adequate relaxation of corpus cavernosal smooth muscle and thus erection in patients with male erectile dysfunction (MED).

The incidence of MED\(^4\) increases dramatically with age. The large patient population, estimated to be 30 million men in the United States alone,\(^5\) combined with the success of Viagra (sildenafil)\(^6\) have provided strong stimuli for the discovery and development of additional PDE5
inhibitors, such as recently launched Levitra (vardenafil) and Cialis (tadalafil).

Despite the efficacy of sildenafil, clinically significant adverse effects have been noted, and it has been proposed that some of these side effects may be due to the lack of selectivity for PDE5. Tadalafil is similarly effective as sildenafil in the treatment of erectile dysfunction (ED). Tadalafil is recommended to sildenafil (50/100 mg), because tadalafil having longer duration action (more than 24 hr), than the sildenafil and vardenafil by men with ED. So usage of tadalafil is safe for men with erectile dysfunction for effective treatment.
## 5.2 LITERATURE REVIEW

The various synthetic methods for the preparation of tadalafil 1 are well documented. A few among them are as follows.

Daugan and co-workers developed a process, which involved the classical Pictet–Spengler reaction between D-tryptophan methyl ester hydrochloride 2 and piperonal 3 to furnish chirally pure 4. *N*-chloroacetylation of 4 with chloroacetylchloride resulted in amide 5, which upon treatment with methyl amine followed by cyclization furnished tadalafil 1 (scheme 5.1).\(^9\text{a}\)

![Scheme 5.1: Synthesis of tadalafil 1 using chloroacetylchloride](image)

Daugan has reported a process, which starts with acylation of compound 2 with piperonyloylchloride 6 to obtain the compound 7. This was further treated with Lawesson’s reagent to furnish thioamide 8.
Alkylation of 8 provided thioimidate 9, that underwent instantaneous cyclization to afford compound 10 with evolution of methyl mercapton. Reduction of 10 employing sodium borohydride afforded advanced intermediate 4. Which was taken to tadalafil 1 after few transformations (scheme 5.2).  

Scheme 5.2: Synthesis of tadalafil 1 via thioamide  

A design around synthetic route for tadalafil 1 was developed from chirally pure carboline 4, upon treatment with MeNH$_2$ resulted in 11. Chloroacetylation of amine 11 with chloroacetylchloride resulted in 12,
which was converted to 1 by intramolecular cyclization in the presence of 
n-BuLi (scheme 5.3).$^{13}$

![Chemical Diagram]

**Scheme 5.3: Synthesis of tadalafil 1**

Alternatively, tadalafil 1 was synthesized by converting the 
D-tryptophan methyl ester 2 (base) into D-tryptophan methyl amide 13, 
which was subjected to Pictet–Spengler type reaction with piperonal 3 to 
yield the known amide 11. Utilization of known reactions, the compound 
11 was transformed to tadalafil 1 (scheme 5.4).$^{13}$
Xiao et al.,\textsuperscript{14} synthesized tadalafil 1 from L-tryptophan methyl ester hydrochloride 14. This process involves condensation of 14 and piperonal 3 followed by cyclization under Pictet–Spengler reaction conditions. Reaction of compound 15 with benzyl chloroformate resulted 16. Thus, obtained compound 16 was subjected to several transformations to obtain the tadalafil 1 (scheme 5.5).
Scheme 5.5: Synthesis of tadalafil 1 from L-tryptophan methyl ester HCl
5.3 PRESENT WORK

5.3.1 Objective

To develop a concise, alternative and high yielding synthetic route for tadalafil involving Pictet-Spengler type reaction and DCC/HOBt mediated double amidation employing sarcosine ethyl ester hydrochloride. To study the complete impurity profile of tadalafil including identification, synthesis and characterization of potential related substances and potential metabolite of tadalafil 1.

5.3.2 Results and discussion

The synthetic procedures described for tadalafil 1 start from the naturally abundant chiral synthon D-tryptophan. The inherent chirality directs the newly created stereo center. Unlike many asymmetric synthetic methods where in the chirality inducing agent is removed after its application, this synthon bears the advantage that it is incorporated into the drug substance.

Methods described earlier for the construction of D-ring in tadalafil 1 utilized chloroacetylchloride as a source for the two carbon appendage. Our interest lay in the quest for an alternative two carbon tether other than that mentioned above.

In our endeavor, we started the synthesis with D-tryptophan 19. Making use of a known protocol namely, Pictet–Spengler reaction between 19 and 3 in the presence of trifluoroacetic acid afforded compound 20 with a mixture of diastereomers \([dr = 7 \text{ (cis): 3 (trans)}] \).
Going forward with a mixture of diastereomers was not recommended as it decreases the yield and increases the cost of the final product. Moreover the separation of the diastereomers or their related products formed in the latter stages would lead to complications in the process. Therefore, it was thought that purification at this stage would be an ideal way out. After examining various conditions such as solvents and temperature, these two diastereomers are found to be dynamic equilibrium and hydrochloride salt of cis-isomer is less soluble in aqueous media that shifts the equilibrium towards the conversion of trans to cis isomer. Subjecting the diastereomeric mixture to hydrochloride salt formation, followed by washing with water resulted in the isolation of diastereo pure 21 (90 % yield, 98 % purity). The next task was the build up of D-ring with the two carbon linker. Amidation of 21 with sarcosine ethyl ester hydrochloride followed by facile inter molecular cyclization under DCC/HOBt coupling conditions resulted in D-ring, hence completing the synthesis of tadalafil 1. The crude product thus obtained was 99 % pure and 55 % yield and contained impurities above the ICH limit of 0.1 %. So the crude product was recrystallized in mixture of methanol and acetone (1:1) to furnish pure tadalafil 1 of greater than 99.95 % purity and 85 % yield with all the impurities under control as per ICH guidelines. This synthesis was successfully implemented in plant on kilogram scale.
Scheme 5.6: Alternative synthesis of tadalafil 1 using sarcosine ethyl ester hydrochloride

Tadalafil obtained by alternative process was confirmed by spectral data of is in complete agreement with the reported data.

Mass spectral analysis showed protonated molecular ion (M⁺ + H) at m/z 390 and sodium adduct (M⁺ + Na) at m/z 412 (figure 5.2). FT-IR spectrum exhibited NH stretching at 3325 cm⁻¹, amide C=O stretching at 1677 and 1650 cm⁻¹ and characteristic C-O-C stretching at 1242 and 1040 cm⁻¹ (figure 5.3).
Figure 5.2: Mass spectrum of compound 1

Figure 5.3: IR spectrum of compound 1
**Figure 5.4:** $^1$H spectrum of compound 1

$^1$H NMR spectrum (figure 5.4) showed NCH2 and NCHCO protons appeared as doublets at $\delta$ 4.17 & $\delta$ 3.94 and a double doublet at $\delta$ 4.40 respectively. Benzylic proton appeared as a singlet at $\delta$ 6.13 and another singlet at $\delta$ 2.93 corresponds to N-CH3 group.

In $^{13}$C NMR spectrum two signals were observed at $\delta$ 166.8 and 166.5 corresponding to carbonyl carbons and another signal was observed at $\delta$ 32.8 corresponding to N-methyl group carbon (figure 5.5). The above spectral data observations and synthetic path way confirms the proposed structure for compound 1. Detailed spectral data was provided in the experimental section 5.5.2.2.
The developed process certain advantages over the reported processes those are mentioned below.

i) Does not involve the usage of toxic and lacrymatomic chloroacetyl chloride.

ii) Avoids column chromatography purification.

iii) Limits the usage of relatively expensive solvents.

Having completed the synthesis does not necessarily fulfill the product development cycle. The final product synthesized using the said process should meet the quality requirement as per ICH.

To have a better understanding of impurities formed in the synthetic sequence, synthesis and characterization of their structures, a systematic study was undertaken. Along with scheme 5.6, scheme 5.1
also taken up for complete impurity profile study, since scheme 5.1 is a widely used synthetic route for tadalafil as it is product patent (innovator) route.\textsuperscript{9} In this regard the product synthesized by following the scheme 5.1 and scheme 5.6 was analyzed by a simple high performance liquid chromatographic (HPLC) method and performed LC–MS analysis to know the mass numbers of unknown impurities as this would provide some insight on the probable structures. Based on the molecular weights and synthetic path ways, the following tentative structures (figure 5.2 and 5.3) were predicted for related substances observed in the tadalafil 1. Related substances 21 and 22 were observed in the final sample of tadalafil 1 synthesized by scheme 5.6 (figure 5.6)

![Potential related substances in tadalafil from scheme 5.6](image)

**Figure 5.6:** Potential related substances in tadalafil from scheme 5.6

Related substances 23, 24, 25 and 26 were observed in the final compound synthesized via synthetic scheme 5.1 (figure 5.7).
Figure 5.7: Potential related substances in tadalafil from scheme 5.1

The structure shown in the figure 5.8 is a potential metabolite of tadalafil. To the best of our knowledge synthetic process and spectral data of metabolite was not reported in the literature. In this regard we have taken up the synthesis and characterization of said metabolite 27.

Figure 5.8: Metabolite of tadalafil 27
5.3.2.1 Root cause of formation and synthesis of compound 4

Compound 4 is an intermediate of tadalafil 1 (scheme 5.1). Presence of unreacted compound 4 leads to the contamination of tadalafil 1. Presence of 4 was proved by co-injection analysis with an authentic sample. Compound 4 was independently prepared by following the known synthetic procedure (scheme 5.1 and experimental section 5.5.2.3) and confirmed by IR, mass and NMR spectral data (experimental section 5.5.2.3).

5.3.2.2 Root cause of formation and synthesis of compound 21

Compound 21 is a precursor in the synthesis of tadalafil 1 (scheme 5.6). Unreacted 21 may carry over to tadalafil 1. Presence of compound 21 was confirmed by co-injection analysis with an authentic sample. Condensation of D-tryptophan 19 and piperonal 3 followed by cyclization under Pictet-Spengler conditions provided compound 21 (scheme 5.6 and experimental section 5.5.2.1). Compound 21 was confirmed by mass, IR and NMR spectral data (experimental section 5.5.2.1).

5.3.2.3 Root cause of formation and synthesis of compound 22

The reagents for the D-ring construction of tadalafil 1 were DCC/HOBt. These reagents affect the amidation between carboxylic acid in 21 and the amino functionality of sarcosine ethyl ester hydrochloride to furnish tadalafil 1 (scheme 5.6).

In LC–MS, the peak corresponding to the compound 22 has shown a molecular weight 636 and proposed structure of 22 illustrated that it is a
symmetrical dimer of 21. Hence this compound could be resulted from 21. It must be mentioned that compound 21 processes both carboxylic acid and amino groups. Under these reaction conditions there exists a possibility for the intermolecular amidation between two molecules of 21 to result 22.

To confirm the above assumption, compound 22 was prepared by exposure of compound 21 to DCC/HOBt reaction conditions (scheme 5.7 and experimental section 5.5.2.4). The obtained compound was characterized using various analytical tools as described.

**Scheme 5.7:** Synthesis of compound 22 under DCC/HOBt coupling conditions

Mass spectrum (figure 5.9) 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 (figure 5.10), stretching due to carbonyl group of amide functionality was observed at 1662 cm\(^{-1}\). In addition, amine and acid OH stretchings observed in compound 21 were not observed in the
These observations were influenced us to conclude that the compound 21 was converted in to compound 22.

**Figure 5.9:** Mass spectrum of compound 22

**Figure 5.10:** IR spectrum of compound 22
Compound 22 being a symmetrical dimer of 21, the $^1$H and $^{13}$C NMR
(figure 5.11 and 5.12) were identical to the compound 21. No major differences were observed in the NMR. Based on these observations it was concluded that compound 22 was a dimer of 21. The complete spectral data was furnished in the experimental section 5.5.2.4.

5.3.2.4 Root cause of formation and synthesis of compound 23

In LC–MS, the peak corresponding to the compound 23 has shown a molecular ion at m/z 392, which is 42 amu more than compound 4 (m/z 350). This molecular weight is may be corresponding to the acetylated compound 23. Since, the synthesis of tadalafil 1 (scheme 5.1) involves chloroacetylation of compound 4 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 4 and leads to the formation of compound 23. This impurity was independently synthesized by acetylating the compound 4 with acetic anhydride in DMF (scheme 5.8 and experimental section 5.5.2.5) and confirmed by using IR, mass, $^1$H NMR and $^{13}$C NMR techniques.

**Scheme 5.8: Synthesis of compound 23**
Mass spectrum showed peaks corresponding to protonated molecular ion \((M^+ + H)\), sodium adduct \((M^+ + Na)\) at \(m/z\) 393 and 415 respectively (figure 5.13). IR spectrum displayed stretching at \(1736\, \text{cm}^{-1}\) (Ester, \(\text{C}═\text{O}\)), additionally another peak was observed at \(1639\, \text{cm}^{-1}\) (Amide, \(\text{C}═\text{O}\) stretching) (figure 5.14).

![Mass spectrum of compound 23](image)

**Figure 5.13:** Mass spectrum of compound 23

A singlet equivalent to three protons was observed in the \(^1\text{H}\) NMR spectrum (figure 5.15) at \(\delta\) 2.32 in addition to the other signals, those were not observed in the compound 4. In \(^{13}\text{C}\) NMR spectrum two signals were observed at \(\delta\) 170.9 and 170.5 corresponding to carbonyl carbons and another signal was observed at \(\delta\) 21.2 corresponding to methyl group carbon (figure 5.16). Where as in the compound 4 \(\delta\) 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 compound 23. Detailed spectral data was provided in the experimental section 5.5.2.5.
Figure 5.14: IR spectrum of compound 23

Figure 5.15: $^1$H NMR spectrum of compound 23
5.3.2.5 Root cause of formation and synthesis of compound 24

Presence of D-tryptophan 19 in compound 2 leads to the formation of compound 24 in the synthetic sequence during the preparation of tadalafil 1 (scheme 5.1).

Compound 24 is the process related impurity, which is identified in the penultimate step in scheme-5.1. This impurity might form from unreacted D-tryptophan 19 present in compound 2 reacting with piperonal 3 under acidic conditions followed by acetylation.

Compound 24 was efficiently synthesized by hydrolyzing compound 23 using lithium hydroxide mono hydrate (scheme 5.9 and experimental section 5.5.2.6). The obtained compound structure was confirmed using various analytical tools.
**Scheme 5.9:** Synthesis of compound 24

De protonated molecular ion (M– H) peak of compound 24 was observed at m/z 377 in the mass spectrum (figure 5.17). In IR spectrum acid C═O and amide C═O stretching appeared at 1728 cm⁻¹ and 1610 cm⁻¹, respectively (figure 5.18). Decrease in the frequency of carbonyl stretching from 1737 cm⁻¹ (observed in the compound 23 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.

![Mass spectrum of compound 24](image)

**Figure 5.17:** Mass spectrum of compound 24
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 compound 23 (figure 5.19).

In $^{13}$C NMR also supporting the conclusion arrived from $^1$H NMR data as it shows absence of OCH$_3$ carbon signal (figure 5.20), which was observed at $\delta$ 53.2 in compound 23. The above spectral data observations were supporting the proposed structure for compound 24. Full characterization data was presented in the experimental section 5.5.2.6.
Figure 5.19: $^1$H NMR spectrum of compound 24

Figure 5.20: $^{13}$C NMR spectrum of compound 24
5.3.2.6 Root cause of formation and synthesis of compound 25

N-Acetyl tryptophan is a potential impurity in D-tryptophan \(19\). Compound 25 will be formed along with desired product 2 during the esterification of tryptophan with thionyl chloride in methanol. Compound 2 is one of the starting materials for the synthesis of 1 (scheme 5.1). Compound 25 was independently synthesized from compound 2, acetylation of 2 with acetic anhydride provided the desired compound 25 in 51 % yield and confirmed by mass, IR and NMR spectral data (experimental section 5.5.2.7).

![Scheme 5.10: Synthesis of compound 25](image)

5.3.2.7 Root cause of formation and synthesis of compound 26

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 compound 26 showed a molecular ion peak at \(m/z\) 460, which is 35 amu more than the compound 5 \((m/z\) 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 4 in a similar way as chloroacetylchloride (scheme 5.1). Hence the formation of compound 26 cannot be ruled out. This impurity was independently synthesized by coupling compound 4 with dichloroacetylchloride in the presence of TEA in dichloromethane (scheme 5.11).

![Reaction Scheme 5.11: Synthesis of compound 26](image)

**Scheme 5.11**: Synthesis of compound 26

De protonated molecular ion (M− H) peak of compound 26 was observed at m/z 459 (figure 5.21). IR spectrum displayed stretching at 1741 and 1668 cm⁻¹ for two carbonyl groups (figure 5.22).

![Mass Spectrum Figure 5.21](image)

**Figure 5.21**: Mass spectrum of compound 26
Figure 5.22: IR spectrum of compound 26

Figure 5.23: $^1$H NMR spectrum of compound 26

$^1$H NMR spectrum revealed that the presence of characteristic singlet at $\delta$ 6.83 for dichloro attached proton (figure 5.23) and other protons appeared in the respective regions. In $^{13}$C NMR spectrum (figure 5.24) signal corresponding to dichloro attached carbon observed at $\delta$ 66.0
further confirmed the structure of compound 26. Full characterization data was presented in the experimental section 5.5.2.8.

![Chemical structure of compound 26](image)

**Figure 5.24:** $^{13}$C NMR spectrum of compound 26

### 5.3.2.8 Synthesis of compound 27

Compound 27 is one of the metabolite of tadalafil. Metabolite 27 was prepared by following the synthetic scheme 5.6, where in 3,4-dimethoxybenzaldehyde 28 was used instead of piperonal 3. Preparation of compound 29 was accomplished by Pictet-Spengler cyclization of D-tryptophan 19 with 3,4-dimethoxybenzaldehyde 28 in the presence of trifluoroacetic acid. Synthesis of metabolite 27 was achieved by coupling of compound 29 with sarcosine ethyl ester HCl under DCC/HOBt coupling conditions (scheme 5.12 and experimental section 5.5.2.9). Characterization study was done using $^1$H NMR, $^{13}$C NMR, mass and IR.
Scheme 5.12: Synthesis of compound 27

Mass spectral analysis showed protonated molecular ion (M⁺ + H) at m/z 406 and sodium adduct (M⁺ + Na) at m/z 428 (figure 5.25). IR spectrum exhibited NH stretching at 3329 cm⁻¹ and the characteristic amide C=O stretching at 1660 cm⁻¹ (figure 5.26).
$^1$H NMR spectrum of compound 27 displayed two additional signals corresponding to NCH$_3$ and NCH$_2$ at $\delta$ 2.94 (s, 3H) and $\delta$ 3.55–3.35 (m, 2H), respectively apart from signals observed in compound 28. In addition, the signal corresponding to OH of acid functionality of compound 28 observed at $\delta$ 10.18 was disappeared in compound 27 (figure 5.27).

![Figure 5.26: IR spectrum of compound 27](image)

In $^{13}$C NMR spectrum two peaks corresponding to amide carbonyl carbons were observed at $\delta$ 166.7 and NCH$_2$ & NCH$_3$ corresponding signals were present at $\delta$ 54.9 and 51.5 (figure 5.28). These spectral data observations concluded the conversion of compound 28 into compound 27, thereby confirm the proposed structure for compound 27. Detailed spectral data was given in the experimental section 5.5.2.9.
Figure 5.27: $^1$H NMR spectrum of compound 27

Figure 5.28: $^{13}$C NMR spectrum of compound 27
5.4 CONCLUSION

In conclusion, an alternative and robust synthetic route was developed for the preparation of tadalafil 1 and it is more compatible on commercial scale.

The possible potential impurities were identified in two commercially viable routes and probable root cause for their formation also discussed. All impurities were synthesized and characterized by using different spectroscopic techniques.

5.5 EXPERIMENTAL SECTION

5.5.1 High Performance Liquid Chromatography (HPLC)

Waters Alliance – 2695 separation module equipped with Waters – 2998 Photo Diode Array detector was used. Waters Symmetry shield RP-18, 250 X 4.6 mm, 5μm particle size column was used to perform the analysis. The mobile phase used was, mobile phase- A: Degassed mixture of buffer (1.38g of Sodium di hydrogen phosphate monohydrate in 1000 ml of Milli-Q water, pH adjusted to 2.5 with dilute ortho phosphoric acid) and acetonitrile in the ratio of 800:200 v/v, and mobile phase-B: Degassed mixture of milli-Q water and acetonitrile in the ratio of 200:800 v/v. The following gradient was used (time (min) / % mobile phase - B v/v: 0/10, 5/10, 40/90, 50/90, 55/10, 60/10). The detection was carried out at 220 nm. Sample was dissolved in diluent (milli-Q water : acetonitrile in the ratio of 1:1 v/v). The injection load is 5μl and column temperature was maintained at 27° C. The data was recorded using
waters empower software and this HPLC method is able to detect all the impurities.

### 5.5.2 Process description

#### 5.5.2.1 (1R,3R)-1,2,3,4-Tetrahydro-1-(3,4-methylene dioxyphenyl)-9H-pyrido[3,4-b]indole-3-carboxylic acid hydrochloride (21)

![Chemical structure](image)

Trifluoroacetic acid (9.21 Kg, 80.77 mol) was added to a solution of D-tryptophan 19 (10.0 kg, 48.96 mol) and piperonal 3 (8.8 kg, 58.61 mol) in dichloromethane (100 L) and heated to 40 °C. After stirring for 10 h at 40 °C, the mixture was allowed to cool down to 35 °C and a mixture of dichloromethane and methanol (50 L, 1:1) was added. The resultant reaction mixture was quenched with 8% sodium bicarbonate solution (50 L). The organic layer was separated and the aqueous layer was extracted with dichloromethane. The combined organic layers were concentrated to afford 20. To a suspension of 20 in water, 1N hydrochloric acid (150 L) was added slowly. After stirring for 15 h at 55 °C, toluene (30 L) was added and the reaction mass was cooled to 15-20 °C. After 30 min, the precipitated solid was filtered, washed with toluene.
and dried at 75 °C for 6 h to afford 16.6 kg (90 %) of title compound 21 with 99.61 % purity by HPLC.

**Mp:** 215–220 °C.

**IR (KBr, cm⁻¹):** 3450 (OH), 3225 (NH), 2927 (Ali, CH), 1757 (C=O, acid), 1205 (C-O-C), 1040 (C-O-C).

**¹H NMR (200 MHz, DMSO-d₆):** \( \delta_H 10.79 \) (s, 1H), 10.20 (brs, 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).

**¹³C NMR (50 MHz, DMSO-d₆):** \( \delta_C 169.7, 148.5, 147.2, 136.7, 128.9, 127.1, 125.5, 124.9, 122.0, 119.2, 118.2, 111.6, 110.2, 108.3, 106.7, 101.5, 57.5, 55.4, 22.2. \)

**M/S (m/z):** 337.1 [M+ + H].

5.5.2.2  (6R,12aR)-2,3,6,7,12,12a-Hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl)-yrazino[2′,1′:6,1]pyrido[3,4b]indole-1,4-dione (1)

![Chemical Structure](image)

Triethylamine (10 kg, 98.82 mol) was added to a solution of dimethyl formamide (65 L) and sarcosine ethyl ester hydro chloride (13.2 kg, 85.93 mol), then the mixture was stirred at 25-30 °C for 20 min. The separated
solid (triethylamine hydrochloride) was filtered and washed with dimethyl formamide (15 L). To the obtained filtrate, **21** (10 kg, 29.76 mol), dicyclohexylcarbodiimide (6.6 kg, 31.98 mol), triethylamine (4.1 kg, 40.52 mol) and 1-hydroxybenzotriazole (5.4 kg, 39.96 mol) were added at 25-35 °C, then reaction mass was heated to 50-55 °C. After stirring for 10 h at 50-55 °C, the mixture was allowed to cooled down to 10 °C and filtered the unwanted dicyclohexylurea (DCU). A mixture of dichloromethane/water (100 L, 1:1) was added to the filtrate and quenched with 8 % sodium bicarbonate solution (80 L). The organic layer was separated and aqueous layer was extracted with dichloromethane. The combined organic layer was concentrated and, cooled to 0-5 °C. The precipitated solid was recrystallized from a mixture of methanol and acetone (1:1) to afford 5.5 kg (52.6 %) of compound **1** with 99.9 % purity. **Mp**: 303–306 °C.

**IR (KBr, cm⁻¹)**: 3325 (NH), 2904 (Ali, CH), 1677 (C═O, amide), 1650 (C═O, amide), 1242 (C-O-C), 1040 (C-O-C).

**¹H NMR (400 MHz, DMSO-d₆)**: δH 11.0 (s, 1H), 7.54 (d, J = 7.4 Hz, 1H), 7.29 (d, J = 8.0 Hz, 1H), 7.07 (t, J = 7.8 Hz, 1H), 7.03 (t, J = 7.8 Hz, 1H), 6.86 (s, 1H), 6.79 (s, 2H), 6.13 (s, 1H), 5.92 (s, 2H), 4.40 (dd, J = 4.6, 11.6 Hz, 1H), 4.17 (d, J = 16.8 Hz, 1H), 3.94 (d, J = 16.8 Hz, 1H), 3.50 (dd, J = 4.6, 16.0 Hz, 1H), 3.0-2.95 (m, 1H), 2.93 (s, 3H).
\[^{13}\text{C NMR (100 MHz, DMSO-}d_6\text{): } \delta_c 166.8, 166.5, 147.0, 146.0, 136.9, 136.2, 133.9, 125.7, 121.2, 119.3, 118.8, 118.0, 111.3, 107.9, 107.0, 104.7, 100.8, 55.5, 55.3, 51.4, 32.8, 23.1.\]

\[^{\text{M/S (m/z): } 390.5 (M^+ + H).}\]

5.5.2.3 (1R,3R)-1,2,3,4-Tetrahydro-1-(3,4-methylene dioxyphenyl)-9H-pyrido[3,4-\text{b}]indole-3-carboxylic acid methyl ester HCl (4)

A solution of compound 2 (50 g, 0.19 mol), chloroform (200 mL) and water (50 mL) was cooled to 0-5 °C. Reaction mass was neutralized (pH = 6.5-7.5) with ammonium hydroxide solution (50 mL). Organic layer was separated and aqueous layer extracted with chloroform (50 mL). Piperonal 3 (29.5 g, 0.19 mol) was added to the combined organic layer and cooled to 0-5 °C. Trifluoroacetic acid (30 mL, 0.38 mol) was added to the resultant reaction mixture and heated to reflux. After stirring for 24 h at reflux, the mixture was allowed to cool down to 35 °C and quenched with 8 % sodium bicarbonate solution (1000 mL). The organic layer was separated and the aqueous layer was washed with water (3 × 100 mL). The organic layer was concentrated under reduced pressure at below 60 °C to afford 4. To a suspension of 4 in water (500 mL), hydrochloric acid
(37.4 mL) was added slowly at 40-45 °C and stirred for 34-36 h at same temperature. Reaction mixture was cooled to 0-10 °C and the precipitated solid was filtered after 3 h, washed with water (50 mL) and dried at 55-60 °C for 3 h to afford 53.1 g (60 %) of title compound 4 with 98 % HPLC purity.

**Mp:** 205–210 °C

**IR (KBr, cm⁻¹):** 3598 (NH), 3303 (NH), 2921 (Ali, CH), 1741 (C=O, ester), 1251 (C-O-C), 1032 (C-O-C).

**¹H NMR (200 MHz, DMSO-d₆):** δH 10.8 (s, 1H), 7.57 (d, J = 7.4 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H), 7.22-7.0 (m, 4H), 6.10 (s, 2H), 5.80 (s, 1H), 4.80-4.60 (m, 1H), 3.80 (s, 3H), 3.45-3.05 (m, 2H).

**¹³C NMR (50 MHz, DMSO-d₆):** δC 168.5, 148.4, 147.1, 136.7, 128.9, 127.1, 125.5, 125.0, 122.0, 119.1, 118.2, 111.2, 110.0, 108.2, 106.3, 101.5, 57.5, 55.1, 52.9, 22.0.

**M/S (m/z):** 351 (M⁺ + H).

### 5.5.2.4 Synthesis of compound 22

![Synthesis of compound 22](image-url)
To a solution of amine 21 (2 g, 0.006 mol) in dimethylformamide (20 mL) were added dicyclohexylcarbodiimide (1.5 g, 0.007 mol), 1-hydroxybenzotriazole (1.5 g, 0.011 mol) and triethylamine (2.0 g, 0.020 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 dichloromethane/water (20 mL, 1:1) was added to the filtrate and quenched with 8 % aqueous sodium bicarbonate solution (8 mL). The organic layer was separated and aqueous layer was extracted with dichloromethane (2 × 10 mL). The combined organic layer was concentrated on rotary evaporator, cooled to 0-5 °C and recrystallized in mixture of methanol and acetone (20 mL, 1:1) to afford 1.89 g (50 %) of compound 22 with 97.7 % purity by HPLC.

Mp: 205-210 °C.

**IR (KBr, cm⁻¹):** 3353 (NH), 3300 (NH), 2908 (Ali, CH), 1677 (C=O, amide), 1662 (C=O, amide), 1239 (C-O-C), 1037 (C-O-C).

**¹H NMR (400 MHz, DMSO-ᵈᵉ):** δH 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), 2.95 (dd, J = 5.6, 15.6 Hz, 2H).
\textbf{\(^{13}\text{C NMR (100 MHz, DMSO-}d_6\text{):} \delta_{\text{C}} 169.3, 147.5, 146.3, 136.4, 136.0, 133.8, 125.6, 121.3, 119.0, 118.1, 111.4, 108.1, 106.1, 104.2, 101.0, 54.9, 54.1, 21.6.}

\textbf{M/S (}m/z\text{):} 635 [M\text{– H}].

\textbf{5.5.2.5 \textit{(1R,3R)-Methyl 2-acetyl-1-[benzo[d][1,3]dioxol-5-yl]-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylate (23)}}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{structure.png}
\caption{Structure of \textit{(1R,3R)-Methyl 2-acetyl-1-[benzo[d][1,3]dioxol-5-yl]-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylate (23)}}
\end{figure}

A mixture of 4 (2 g, 0.006 mol), acetic anhydride (5 g, 0.049 mol) and dimethylformamide (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 product was extracted with ethyl acetate (2 × 50 mL). Total organic layer was concentrated under vacuum below 55 °C to furnish 1.7 g (76 %) of title compound 23 with 97.46 % purity by HPLC.

\textbf{Mp:} 215–220 °C.

\textbf{IR (KBr, cm\textsuperscript{-1}):} 3394 (NH), 2948 (Ali, CH), 1736 (C=O, ester), 1639 (C=O, amide), 1236 (C-O-C), 1038 (C-O-C).

\textbf{\(^1\text{H NMR (400 MHz, CD\textsubscript{3}OD):} \delta_{\text{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,
5.13 (d, J = 6.4 Hz, 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-$d_6$): $\delta_C$ 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.

M/S ($m/z$): 393 [M$^+$ + H] and 415 [M$^+$ + Na].

5.5.2.6 (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 (24)

![Chemical Structure Image]

Compound 23 (1.0 g, 0.002 mol) was charged to a solution of lithium hydroxide mono hydrate (0.2 g, 0.005 mol), methanol (6 mL) and water (1.5 mL). Thus, obtained reaction mixture was heated to 65-70 °C. After stirring for 4 h at 65-70 °C, temperature of the reaction mixture was bring down to 25-30 °C and adjusted the pH to 1-2 with conc HCl. The resulted reaction mixture was maintained for 2 h at 25-30 °C. Subsequently, the precipitated solid was filtered and washed with water (5 mL). Wet solid was dried under vacuum at 70-75 °C to afford the 0.7 g (72 %) of title compound 24 with 94.90 % purity by HPLC.

Mp: 235–241 °C.
IR (KBr, cm\(^{-1}\)): 3406 (NH), 2903 (Ali, CH), 1728 (C\(=\)O, acid), 1610 (C\(=\)O, amide), 1239 (C-O-C), 1037 (C-O-C).

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\)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), 2.97 (dd, \(J = 6.5\) Hz, \(J = 15.5\) Hz, 2H), 2.22 (s, 3H).

\(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\)C 172.2, 170.5, 146.4, 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.

M/S (m/z): 377 [M\(^-\) – H].

5.5.2.7 \((R)\)-Methyl 2-acetamido-3-(1H-indol-3-yl) propanoate (25)

To a mixture of 2 (25 g, 0.098 mol) in water (65 mL) and dichloromethane (100 mL) was added 8.0 % aqueous sodium bicarbonate solution (110 mL), thereby stirred for 1 h. The organic layer was separated, and the aqueous layer was extracted with dichloromethane (100 mL). The combined layer was concentrated under vacuum below 50 °C. Acetic anhydride (64 mL, 0.688 mol) was charged to residue 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 25 with 99.9 % purity by HPLC.

**Mp:** 135-142 °C.

**IR (KBr, cm⁻¹):** 3404 (NH), 3319 (NH), 1734 (C=O, ester), 1663, (C=O, amide).

**¹H NMR (400 MHz, DMSO-d₆):** δH 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).

**¹³C NMR (100 MHz, DMSO-d₆):** δC 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.

**M/S (m/z):** 261.1 (M⁺ + H) and 283.1 (M⁺ + Na).

5.5.2.8 (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 (26)

Dichloroacetylchloride (2.0 g, 0.014 mol) was added drop wise to a solution of 4 (2.0 g, 0.006 mol) and triethylamine (0.7 g, 0.007 mol) in dichloromethane (20 mL) at 0-5 °C. The reaction mixture temperature was raised to 25-35 °C and stirred for 14-16 h. The reaction mixture was
quenched with water, organic layer was separated and the aqueous layer was extracted with dichloromethane (10 mL). The combined layer was concentrated under vacuum below 50 °C to afford yellow foam, which was purified by flash chromatography by eluting with dichloromethane/MeOH (9:1) to provide 2.0 g (76 %) of title compound 26 as a yellow color solid with 94.9 % purity by HPLC.

**Mp:** 205-210 °C.

**IR (KBr, cm⁻¹):** 3390 (NH), 1741 (C=O, ester), 1668 (C=O, amide), 1238 (C-O-C), 1038 (C-O-C).

**¹H NMR (400 MHz, CDCl₃):** δH 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), 3.27-3.20 (m, 4H).

**¹³C NMR (100 MHz, DMSO-δ₆):** δC 169.5, 163.8, 147.6, 147.5, 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.

**M/S (m/z):** 459.0 (M⁻ - H).
5.5.2.9 (6R,12aR)-2,3,6,7,12,12a-Hexahydro-2-methyl-6-(3,4-dimethoxyphenyl)-pyrazino[2′,1′:6,1]pyrido[3,4b]indole-1,4-dione (27)

5.5.2.9.1 (1R,3R)-1-(3,4-dimethoxyphenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylic acid (28)

Trifluoroacetic acid (6 mL, 0.08 mol) was added to a solution of D-tryptophan 19 (10 g, 0.05 mol) and 3, 4-dimethoxybenzaldehyde 29 (9.7 g, 0.06 mol) in dichloromethane (100 mL). The reaction mixture was refluxed for 7 h, allowed to cool to 35 °C. Dichloromethane and methanol (100 mL, 1:1) was added to the reaction mixture and washed with 8% aqueous sodium bicarbonate solution (50 mL). The organic layer was separated and the aqueous layer was extracted with dichloromethane (50 mL). The combined organic layer was concentrated under reduced pressure and 1N hydrochloric acid (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 28 with 98.1% purity by HPLC.
Mp: 210-216 °C.

IR (KBr, cm⁻¹): 3454 (NH), 1256 (C=O), 1022 (C=O).

¹H NMR (500 MHz, DMSO–d₆): δH 10.76 (s, 1H), 10.20 (br, 1H), 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).

¹³C NMR (50 MHz, DMSO–d₆): δC 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, 55.4, 22.1.

M/S (m/z): 353 [M⁺ + H] and 375 [M⁺ + Na].

5.5.2.9.2 Compound 27

To a solution of sarcosine ethyl ester hydrochloride (1.3 g, 0.008 mol) in dimethylformamide (6 mL) was added triethylamine (1.0 g, 0.010 mol) and stirred for 20 min at 25-30 °C. The separated solid (triethylamine hydrochloride) was filtered and washed with dimethylformamide (2 mL). To the obtained filtrate, compound 2, dicyclohexylcarbodiimide (0.7 g, 0.003 mol), 1-hydroxybenzotriazole (0.5 g, 0.004 mol) and triethylamine (0.4 g, 0.004 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 solid (DCU) was filtered. A mixture of dichloromethane and water (10 mL, 1:1) was added to the filtrate and quenched with 8% aqueous sodium bicarbonate solution (8 mL). The organic layer was separated and aqueous layer was
extracted with dichloromethane (2 × 5 mL). The total organic layer was concentrated at below 45 °C and cooled to 0-5 °C and recrystallized from the mixture of methanol and acetone (3 mL, 1:1) to yield 0.5 g (48 %) of title compound 27 with 98.7 % purity by HPLC.

**Mp:** 165-170 °C.

**IR (KBr, cm⁻¹):** 3329 (NH), 1660 (C=O, amide), 1261 (C-O-C), 1024 (C-O-C).

**¹H NMR (400 MHz, DMSO–d₆):** δH 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), 3.55-3.35 (m, 2H), 2.94 (s, 3H).

**¹³C NMR (100 MHz, DMSO–d₆):** δC 166.7, 166.6, 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.

**M/S (m/z):** 406 [M⁺ + H] and 428 [M⁺ + Na].

### 5.6 REFERENCES


12. Daugan, A. C. M. *US 5859006*, **1999**.
