CHAPTER-II

SYNTHESIS OF INDOLE-N-ACETIC ACID

DERIVATIVES AS CASPASE-3 INHIBITORS
INTRODUCTION

The interest and development in indole chemistry was started in mid-nineteenth century. Indoles are one of the most important nitrogen containing heterocyclic molecules, found extensively in biological system which play vital role in biochemical process. They were mainly related with essential amino acid, tryptophan (Trp). The important members of the family were the plant hormone indole-3-acetic acid and the animal hormone, melatonin.

The indole ring system is found in many natural products, pharmaceutical agents and polymer material. The interesting chemical properties and importance of indole have inspired organic and medicinal chemists to design and synthesize a variety of indole derivatives. Many indole derivatives were reported to exhibit antibacterial, antifungal, antimicrobial, anti HIV, antioxidant, antiviral, antidepressant, anticonvulsant, antituberculosis, antithrombotic, anticancer, anti-inflammatory and analgesic activities. Various indole thiazoles have been synthesized and reported for their CNS depressant, anti-inflammatory and anticancer activities.

Indole and its derivatives have great importance in clinical chemistry. Examples of indole based drugs approved for clinical use include Indomethacin (I), Fendosal (II), Sumatriptan (III), Besipirdine (IV), Zolmitriptan (V), Naratriptan (VI), Pindolol (VII), Indolmycin (VIII), Adrenochrome (IX), Indigo carmine, etc. The medicinal and
biochemical properties of indole had inspired us to explore indole derivatives as novel caspase-3 inhibitors.

**Figure-1**

![Chemical structures of various compounds](image)

**PRESENT WORK**

In our search for potent and novel caspase-3 inhibitors we have undertaken a) structural modification of various known inhibitors by changing the position of attachment of main core to the warhead (b)
their biological evaluation (c) deriving Structure Activity Relationships. Based on the promising caspase-3 activity reported for IDN-1965 (X) and compound XI\(^{28a}\) (Figure-2), we became interested to study the indole derivatives. In literature 5 and 2 substituted indole-N-acetic acids were reported\(^{30}\) but not widely used in drug discovery, hence investigation of indole-N-acetic acid derivatives were taken up for the study as caspase-3 inhibitors.

**Figure-2**

![Chemical structures](image)

Initially we have used the pharmacophore present in IDN-1965 and synthesized compound (10)\(^{28b}\) (Figure-2), and has shown an IC\(_{50}\) = 0.19 µM which encouraged exploring different R and R' groups. Based on different R' groups (as reported in chapter-I compounds I-VIII) the study has been divided into 3 different diversities (Figure-3). For all the three diversities substitutions on indole ring (R) are same as 5-bromo, chloro, fluoro, methoxy and 2-methyl. Where as incase of diversity-I, R' is fluoromethyl ketone while in diversity-II, R' is 2,3,5,6-
tetrafluorophenoxy methyl ketone and in case of diversity-III, \(R'\) is 2,6-difluorophenoxy methyl ketone as shown below.

**Figure-3**

![Chemical Structure](image)

**Diversity-I**
- \(R = 5\text{-}\text{Br, Cl, F, OCH}_3, 2\text{-CH}_3\)
- \(R' = F\)

**Diversity-II**
- \(R = 5\text{-}\text{Br, Cl, F, OCH}_3, 2\text{-CH}_3\)
- \(R'\) is shown in the diagram

**Diversity-III**
- \(R = 5\text{-}\text{Br, Cl, F, OCH}_3, 2\text{-CH}_3\)
- \(R'\) is shown in the diagram

In the present study designed and synthesized 15 novel compounds with various \(R\) and \(R'\) groups (fluoromethyl ketone derivatives, 2,3,5,6-tetrafluorophenoxy derivatives and 2,6-difluorophenoxy derivatives of indole-N-acetic acid) and tested against caspase-3 activity. The potencies of these compounds are discussed in detail in latter part of this chapter (2.5).

**MOLECULAR MODELING STUDIES**

To get some insight to the binding mode of indole-N-acetic acid derivatives, compounds 10b and 10d were docked into the catalytic domain of caspase-3 using a covalent constraint employing Gold 5.1 software through a covalent bond between the fluoromethyl carbon and the sulphur atom of the Cys285 thiol group of the enzyme as
shown in **Figure-4** and **Figure-5**. Each pose was ranked according to its chem scoring function result.

**Figure-4** explains that the biaryl moiety might occupy a predominantly hydrophobic cavity suggesting that hydrophobic groups in this region might be better tolerated. The carboxylic acid and amide carbonyl were observed to be orienting towards a polar cavity with the carboxylic acid engaged in salt-bridge interaction with Arg179 (**Figure-5**). This probably indicates that replacement of the carboxylic acid might not be a good idea. It was also observed that having a hydrophobic group at R (**Figure-5**) might be better because this can engage in vdW contact with Phe381 and orient the biaryl portion favorably to induce $\pi$-stacking interaction with Trp340. From **Figure-4**, we further observed that there can be some space available in vicinity of the fluoromethyl for compound profile optimization. For example, replacement of fluoromethyl with bulky substituents (**Figure-6**) didn’t affect the potency as observed in compound 20a,b,c in same series.
Figure-4: Proposed binding modes of 2 compounds in caspase-3 catalytic domain (PDB ID: 1RHJ). Pale cyan and green: hydrophobic regions; red, orange and deep blue: polar regions
Figure-5: Proposed binding modes of indole-N-acetic acid series of compounds in caspase-3 catalytic domain (PDB ID: 1RHJ) revealing the interactions observed: (a) hydrogen bonding interactions with Arg179, His237, Gln283, Ser339 and Arg341; (b) vdW contact with Phe381 when R = -Cl; (c) π-stacking interaction with Trp340 when R = -Cl
Figure-6: Proposed binding mode of a compound having F replaced with a bulkier group demonstrating region for compound optimization. The compound with tetrafluorophenoxy attachment exhibited better ADME properties without any loss in potency.
2.1 SYNTHESIS OF DIVERSITY-I

2.1.1 Synthesis of tert-butyl 3-amino-5-fluoro-4-hydroxy pentanoate

Synthesis of key intermediate 4 was carried out using commercially available 3-nitro propionic acid (1). Esterification using silver carbonate and tert-butyl bromide obtained intermediate 2. 2-Fluoroethanol was subjected to Swern oxidation, which was followed by the addition of the nitro derivative 2 without isolation of the intermediate 2-fluoroacetaldehyde. The nitroalcohol 3 is resulted from one-pot oxidation/condensation reaction. Further hydrogenation of 3 over Raney-Nickel gave amino alcohol 429.

Scheme-1

Reagents and conditions: (i) Ag₂CO₃, tert-butyl bromide, THF, 0-25 °C, 60 h; (ii) 2-fluoroethanol, (COCl)₂, DMSO, Et₃N, CH₂Cl₂, -78 °C –rt, 2 h; (iii) Raney Ni, H₂, MeOH, rt, 15 h.

2.1.2 Synthesis of Indole-N-acetic acid

Indole-N-acetic acids have been prepared by employing commercially available substituted indoles 5a-e. Compounds 7a-e were synthesized using literature method30 by alkylation of 5a-e with
ethyl bromoacetate to provide the compounds 6a-e, which on basic hydrolysis gives the desired indole-N-acetic acids 7a-e, **Scheme-2**.

**Scheme-2**

Reagents and conditions: (i) Ethyl bromoacetate, K$_2$CO$_3$, DMF, rt, 15 h; (ii) LiOH.H$_2$O, THF-H$_2$O (1:1), rt, 2 h.

2.1.3 Synthesis of 3-(2-(1H-indol-1-yl) acetamido)-5-fluoro-4-oxopentanoic acid

The intermediate amides 8a-e have been prepared by coupling of key intermediate 4 and 7a-e in the presence of EDCI and HOBr. Further, these intermediates upon oxidation with Dess-martin periodinane in dry DCM give the keto compounds 9a-e. These compounds on hydrolysis with TFA/anisole afford the desired caspase-3 inhibitors 10a-e, **Scheme-3**.
**Scheme-3**

Reagents and conditions: (i) EDCI, HOBt, DIPEA, DMF, rt, 15 h; (ii) Dess-Martin periodinane, CH2Cl2, rt, 15 h; (iii) CF3COOH, anisole, CH2Cl2, rt, 2 h.

2.2 SYNTHESIS OF DIVERSITY-II

2.2.1 Synthesis of (3S)-tert-butyl 5-(2,3,5,6-tetrafluorophenoxy)-3-amino-4-hydroxypentanoate

Synthesis of intermediate 14 has been carried out by using commercially available Cbz L-aspartic acid tert-butyl ester 11. Treating compound 11 with isobutyl chloroformate (IBCF) and N-methyl morpholine followed quenching with excess of diazomethane resulted in compound 12, which upon subjected in situ to a 1:1 mixture of AcOH and 45% aqueous hydrobromic acid (HBr) gave intermediate 14 as reported by literature method31. This intermediate
on etherification with 2,3,5,6-tetrafluorophenol using dry K$_2$CO$_3$ followed by reduction with sodium borohydride and deprotection of Cbz group with 10% Pd/C provide the key intermediate 17.

**Scheme-4**

Reagents and conditions: (i) IBCF, NMM, THF, -78 °C, 1 h; (ii) diazomethane in ether, 15 min; (iii) aq HBr-AcOH (1:1), 30 min; (iv) 2,3,5,6-tetrafluorophenol, K$_2$CO$_3$, acetone, rt, 1 h; (v) NaBH$_4$, MeOH-THF, 0 °C, 1 h; (vi) 10% Pd/C, H$_2$, EtOH, rt, 4 h.

2.2.2 Synthesis of (S)-5-(2,3,5,6-tetrafluorophenoxy)-3-(2-(1H-indol-1-yl) acetamido)-4-oxopentanoic acid

The intermediate amides 18a-e have been prepared by coupling of key intermediate 17 and 7a-e in the presence of EDCI and HOBt. Further, these intermediates upon oxidation with Dess-martin periodinane in dry DCM give the keto compounds 19a-e. These compounds on hydrolysis with TFA/anisole afford the desired caspase-3 inhibitors 20a-e, **Scheme-5**.
Reagents and conditions: (i) EDCI, HOBr, DIPEA, DMF, rt, 15 h; (ii) Dess-Martin periodinane, CH$_2$Cl$_2$, rt, 15 h; (iii) CF$_3$COOH, anisole, CH$_2$Cl$_2$, rt, 2-3 h.

2.3 SYNTHESIS OF DIVERSITY-III

2.3.1 Synthesis of (3S)-tert-butyl 5-(2,6-difluorophenoxy)-3-amino-4-hydroxypentanoate

Synthesis of intermediate 14 has been carried out as reported in 2.2.1. This intermediate 14 upon etherification with 2,6-difluorophenol using dry K$_2$CO$_3$ followed by reduction with sodium borohydride and deprotection of Cbz group with 10% Pd/C provide the key intermediate 23, Scheme-6.
Reagents and conditions: (i) 2,6-difluorophenol, K₂CO₃, acetone, rt, 1 h; (ii) NaBH₄, MeOH-THF, 0 °C, 1 h; (iii) 10% Pd/C, H₂, EtOH, rt, 4 h.

2.3.2 Synthesis of (S)-5-(2,6-tetrafluorophenoxy)-3-(2-(1H-indol-1-yl) acetamido)-4-oxopentanoic acid

The intermediate amides 24a-e have been prepared by coupling of key intermediate 23 and 7a-e in the presence of EDCI and HOBT. Further, these intermediates upon oxidation with Dess-martin periodinane in dry DCM give the keto compounds 25a-e. These compounds on hydrolysis with TFA/anisole afford the desired caspase-3 inhibitors 26a-e, Scheme-7.
**Reagents and conditions:** (i) EDCI, HOBT, DIPEA, DMF, rt, 15 h; (ii) Dess-Martin periodinane, CH₂Cl₂, rt, 15 h; (iii) CF₃COOH, anisole, CH₂Cl₂, rt, 2 h.

**2.4 SPECTRAL DATA DISCUSSION**

The compounds **8a-e, 18a-e** and **24a-e** obtained as an intimate mixture and were characterized directly without separation. The mixture was found to contain two compounds in a ratio of 8:2 by ¹H NMR spectrum analysis. The intense region δ 7.7 (d, 1H, H-7), 2.7-2.6 (dd, 1H, CH₃COOᵗBu) and 2.45-2.35 (dd, 1H, CH₂COOᵗBu) characterize the major constituent and the less intense signals at δ 7.74 (d, H-7) and 2.55-2.50 (m, CH₃COOᵗBu) characterize the minor constituent. The mass spectrum for both the peaks have shown same
mass in the form of m/z (M⁺+1-tBu), which suggested that the product is a mixture of two isomers.

When compounds 8a-e, 18a-e and 24a-e are oxidized with Dess-Martin periodinane, the keto compounds 9a-e, 19a-e and 25a-e are obtained. The absence of signal at δ 4.0-3.9 (m, 1H, -CHOH) in the above compounds and presence of peak at δ 5.0 (m, 1H, -NHCH) confirmed the transformation. The two proton signals at δ 3.5-2.8 are assigned to CH₂COOtBu. The mass spectrum have shown mass in the form of m/z (M⁺+1-tBu), which confirmed the formation of required product.

On acidic hydrolysis of 9a-e, 19a-e and 25a-e with TFA, the final compounds 10a-e, 20a-e and 26a-e are obtained and the signal at δ 1.1 (tert-butyl protons, 9H) disappears and peak at δ 10-12 appears indicating the formation of carboxylic acid. The infrared (IR) spectrum of all final compounds is similar exhibiting the presence of carboxyl acid (3300-3320 cm⁻¹) and carbonyl (1660-1746 cm⁻¹) groups. The mass spectrum have shown mass in the form of m/z (M⁺-1), which confirmed the formation of required product.
2.5 BIOLOGICAL ACTIVITY (In vitro Caspase-3 assay)

Fluorescence enzyme assays detecting the activity of the compounds of formula 10a-e, 20a-e and 26a-e utilizing the recombinant CPP32 enzyme and substrate (Acetyl-Asp-Glu-Val-Asp-7-amido-4-methylcoumarin) is performed essentially according to Nicholson et al. (Nature, 376: 37-43, 1995) in 96 well microtiter plate format. The reaction mixture contains buffer (25 mM HEPES, 50 mM KCl, 0.1% CHAPAS, 1 mM β-mercapto ethanol, pH 7.5), 5 µL of CPP32 (1 unit/µL concentration) in buffer, 5 µL compound of formula 10a-e or buffer (control) and 100 µL of 10 µM substrate at 30°C in duplicate.

The enzyme and the compound of formula 10a-e, 20a-e and 26a-e are allowed to preincubate in the microtiter plate wells for 10 min at 30°C prior to the addition of substrate to initiate the reaction and then the mixture was incubated for 30 min. Fluorescent AMC product formation was monitored by measuring the fluorescence emission at 460 nm using an excitation wavelength of 360 nm. The fluorescence change in duplicate (control) wells is averaged and the mean values are plotted as a function of inhibitor concentration to determine the inhibitor concentration producing 50% inhibition (IC$_{50}$). The reference compound for this assay was IDN-6556 and the values are denoted in table-I.
Table-I: Evaluation of caspase-3 activity of synthesized compounds

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>R'</th>
<th>IC$_{50}$ (µM)</th>
</tr>
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<tbody>
<tr>
<td>10a</td>
<td>5-F</td>
<td>F</td>
<td>1.12</td>
</tr>
<tr>
<td>10b</td>
<td>5-Cl</td>
<td>F</td>
<td>0.35</td>
</tr>
<tr>
<td>10c</td>
<td>5-Br</td>
<td>F</td>
<td>0.58</td>
</tr>
<tr>
<td>10d</td>
<td>5-OCH$_3$</td>
<td>F</td>
<td>1.29</td>
</tr>
<tr>
<td>10e</td>
<td>2-CH$_3$</td>
<td>F</td>
<td>1.23</td>
</tr>
<tr>
<td>20a</td>
<td>5-F</td>
<td>2,3,5,6-tetrafluorophenoxy</td>
<td>0.84</td>
</tr>
<tr>
<td>20b</td>
<td>5-Cl</td>
<td>2,3,5,6-tetrafluorophenoxy</td>
<td>0.64</td>
</tr>
<tr>
<td>20c</td>
<td>5-Br</td>
<td>2,3,5,6-tetrafluorophenoxy</td>
<td>0.98</td>
</tr>
<tr>
<td>20d</td>
<td>5-OCH$_3$</td>
<td>2,3,5,6-tetrafluorophenoxy</td>
<td>1.11</td>
</tr>
<tr>
<td>20e</td>
<td>2-CH$_3$</td>
<td>2,3,5,6-tetrafluorophenoxy</td>
<td>0.82</td>
</tr>
<tr>
<td>26a</td>
<td>5-F</td>
<td>2,6-difluorophenoxy</td>
<td>37% @ 10 µM</td>
</tr>
<tr>
<td>26b</td>
<td>5-Cl</td>
<td>2,6-difluorophenoxy</td>
<td>20.8</td>
</tr>
<tr>
<td>26c</td>
<td>5-Br</td>
<td>2,6-difluorophenoxy</td>
<td>1.2</td>
</tr>
<tr>
<td>26d</td>
<td>5-OCH$_3$</td>
<td>2,6-difluorophenoxy</td>
<td>18% @ 10 µM</td>
</tr>
<tr>
<td>26e</td>
<td>2-CH$_3$</td>
<td>2,6-difluorophenoxy</td>
<td>2.5</td>
</tr>
<tr>
<td>IDN-6556</td>
<td></td>
<td></td>
<td>0.06</td>
</tr>
</tbody>
</table>

Values are IC$_{50}$ (µM) expressed as the mean of two replicate determinations.
The novel aspartyl fluoromethyl ketoamides have been synthesized that exhibited significant caspase-3 activity. Some of these compounds 10b and 10c have shown nanomolar potency against caspase-3 with an IC$_{50}$ of 0.35 µM and 0.58 µM respectively. Interestingly, halogens 5-chloro and 5-bromo substitutions on indole are more potent than the corresponding 5-fluoro substitution. 5-Methoxy and 2-methyl substitutions showed similar activity as 5-fluoro substituent against caspase-3.

Further explored other side chain like in IDN-6556 to improve the in vitro activity against caspase-3. Some of the compounds 20a, 20b and 20e have shown similar activity as in fluoromethyl ketoamides. Here, 5-chloro substitution was more favored than 5-fluoro, followed by 5-bromo substitution. 2-Methyl substitution in this case was equally potent as 5-fluoro substitution, and 5-methoxy substitution which was similar to 5-bromo substitution indicating that size of the atom was important for activity.

Similarly another side chain was explored as reported in chapter-I, compounds (V-VIII), i.e. 2,6-difluorophenoxymethyl ketone to evaluate in vitro activity against caspase-3. Some of the compounds 26c and 26e have shown low micro molar activity against caspase-3. In this case 5-bromo substitution was more favored than 5-chloro, followed by 5-fluoro. 2-Methyl substitution in this case was equally potent as 5-bromo substitution and 5-methoxy was least active against caspase-3 activity. Hence in terms of comparative studies with
two warheads (2,3,5,6-tetrafluorophenoxy methyl ketone and 2,6-difluorophenoxy methyl ketone) the number of fluorine atoms present on the warhead greatly influenced the inhibition activity of caspase-3 with different indole derivatives explored.

**CONCLUSION**

The novel indole aspartyl ketone derivatives were synthesized and evaluated their activity as caspase-3 inhibitors. The preliminary SAR reveals that the activity of this series can be optimized by using fluoromethyl ketone or 2,3,5,6-tetrafluorophenoxy methyl ketone as the warhead where as 2,6-difluorophenoxy methyl ketone has not shown considerable activity against all the indole derivatives. The order of *in vitro* activity against caspase-3 follows the order of fluoromethyl keto amides > 2,3,5,6-tetrafluorophenoxy methyl ketoamides > 2,6-difluorophenoxy methyl ketoamides for the tested compounds.
2.6 EXPERIMENTAL

2.1.1 3-Nitro propionic acid tert-butyl ester (2): To a stirred solution of 3-nitro propionic acid (5 g, 42 mmol) in dry THF (300 mL) at 0 °C was added Ag$_2$CO$_3$ (46 g, 168 mmol) followed by tert-butyl bromide (19 mL, 168 mmol) and stirred at room temperature for 60 h until TLC indicated completion of the reaction. The reaction mixture was filtered and filtrate was evaporated in vacuum at low temperature to afford 2 as pale yellow oil (6.8 g, 92%). Molecular formula: C$_7$H$_{13}$NO$_4$ (m/z: 175); LCMS: m/z = 176.1 (M$^+$+1); $^1$H NMR (DMSO-d$_6$, 300 MHz): δ 4.70 (t, 2H), 3.85 (t, 2H), 1.45 (s, 9H, tBu).

5-Fluoro-4-hydroxy-3-nitro pentanoic acid tert-butyl ester (3): Dry DCM (180 mL) was cooled to −78 °C and oxalyl chloride (2.4 mL, 27.3 mmol) was added drop wise followed by DMSO (3.76 mL, 53 mmol) and stirred for 5 min. 2-Fluoroethanol (1.33 mL, 23 mmol) was added to the reaction mixture followed by triethylamine (18 mL, 5 vol) at same temperature. The reaction mixture was brought to room temperature and 2 (3.6 g, 20.6 mmol) was added and stirred for 1 h until TLC indicated completion of the reaction. The reaction mixture was diluted with DCM (100 mL) and washed with water and saturated brine. The combined organic layers were dried over anhydrous Na$_2$SO$_4$ and evaporated in vacuum to get crude product which was purified by silica gel column chromatography (20% EtOAc-hexane) to afford 3 as pale yellow oil (2.8 g, 57%). Molecular formula: C$_9$H$_{16}$FNO$_5$ (m/z: 237); LCMS: m/z = 238.1 (M$^+$+1); $^1$H NMR (CDCl$_3$, 300 MHz): δ 4.90 (m, 1H),
4.60 (m, 3H), 3.20 (m, 1H), 3.0 (m, 1H), 2.80 (m, 1H), 1.45 (s, 9H, tBu).

3-Amino-5-fluoro-4-hydroxy pentanoic acid tert-butyl ester (4):

![Chemical Structure]

To a stirred solution of 3 (2.8 g, 11.8 mmol) in methanol (35 mL) was added raney nickel (~3 g) and stirred under hydrogen balloon for 15 h at room temperature until TLC indicated that reaction was complete. The reaction mixture was filtered over celite bed and filtrate was evaporated in vacuum to afford 4 as yellow gum (2.3 g, 94%). Molecular formula: C₉H₁₈FNO₃ (m/z: 207); LCMS: m/z = 208.1 (M⁺+1); ¹H NMR (CDCl₃, 300 MHz): δ 4.60 (m, 1H), 4.40 (dd, J = 15.4 Hz, J = 6.1 Hz, 2H), 3.30 (m, 1H), 3.0 (m, 1H), 2.60 (m, 1H), 2.40 (m, 1H), 2.30 (brs, 2H), 1.45 (s, 9H, tBu).

2.1.2 (5-Bromo indol-1-yl)-acetic acid ethyl ester (6c): To a stirred solution of 5-bromo indole (1 g, 5.1 mmol) in DMF (10 mL) was added K₂CO₃ (2.1 g, 15.3 mmol) followed by drop wise addition of methyl bromoacetate (0.73 mL, 7.6 mmol) and stirred at room temperature for 15 h until TLC indicated that reaction was complete. The reaction mixture was poured in ice water and extracted with ethyl acetate (2×25 mL). The combined organic layers were dried over anhydrous Na₂SO₄ and evaporated in vacuum to afford 6c as yellow oil (1.3 g, 95%). Molecular formula: C₁₂H₁₂BrNO₂ (m/z: 282); LCMS: m/z = 283.9 (M⁺+2); ¹H NMR (CDCl₃, 300 MHz): δ 7.75 (d, J = 3 Hz 1H), 7.30
(dd, $J = 12.1$ Hz, $J = 3.1$ Hz, 1H), 7.15-7.05 (m, 2H), 6.62 (d, $J = 6.1$ Hz, 1H), 4.85 (s, 2H), 3.75 (s, 3H).

**5-Bromo indol-1-yl)-acetic acid (7c):** The compound 6c (1.3 g, 4.85 mmol) dissolved in THF-water (1:1) mixture and added lithium hydroxide (0.3 g, 7.2 mmol) at 0 °C and stirred at room temperature for 2 h until TLC indicated that reaction was complete. The reaction mixture was carefully acidified with 1N HCl and extracted with ethyl acetate (2×25 mL). The combined organic layers were dried over anhydrous Na$_2$SO$_4$ and evaporated in vacuum to afford 7c as yellow solid (1.1 g, 89%). Molecular formula: C$_{10}$H$_8$BrNO$_2$ (m/z: 254); LCMS: m/z = 252.08 (M$^+$-2); $^1$H NMR (DMSO-d$_6$, 300 MHz): δ 13.0 (brs, 1H), 7.75 (d, $J = 0.3$ Hz, 1H), 7.40 (m, 2H), 7.25 (dd, $J = 12.1$ Hz, $J = 3.0$ Hz, 1H), 6.45 (d, $J = 6.2$ Hz, 1H), 5.0 (s, 2H).

**5-Fluoro indol-1-yl)-acetic acid ethyl ester (6a):** The compound 6a has been prepared according to the method described for the compound 6c employing the compound 5-fluoro indole (1 g, 7.4 mmol) and methyl bromoacetate (1.05 mL, 11 mmol) to afford 6a as pale brown oil (1.5 g, 98%). Molecular formula: C$_{12}$H$_{12}$FNO$_2$ (m/z: 221); LCMS: m/z = 222.09 (M$^+$+1); $^1$H NMR (CDCl$_3$, 300 MHz): δ 7.35-7.20 (dd, $J = 11.8$ Hz, $J = 3.0$ Hz, 1H), 7.20 (dd, $J = 12.1$ Hz, $J = 3.0$ Hz, 1H), 7.15 (d, $J = 6.3$ Hz, 1H), 7.0 (m, 1H), 6.50 (d, $J = 6.2$ Hz, 1H).

**5-Fluoro indol-1-yl)-acetic acid (7a):** The compound 7a has been prepared according to the method described for the compound 7c employing the compound 6a (1.5 g, 7.2 mmol) and lithium hydroxide
(0.45 g, 10.8 mmol) to afford 7a as pale yellow solid (1.3 g, 93%). Molecular formula: C\textsubscript{10}H\textsubscript{8}FNO\textsubscript{2} (m/z: 193); LCMS: m/z = 191.9 (M\textsuperscript{-}1); \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}, 300 MHz): δ 13.0 (brs, 1H), 7.80 (d, J = 6.3 Hz, 1H), 7.40 (m, 2H), 7.35 (dd, J = 12.0 Hz, J = 3.0 Hz, 1H), 6.50 (d, J = 6.5 Hz, 1H), 5.0 (s, 2H).

(5-Chloro indol-1-yl)-acetic acid ethyl ester (6b): The compound 6b has been prepared according to the method described for the compound 6c employing the compound 5-chloro indole (1.5 g, 9.9 mmol) and methyl bromoacetate (1.4 mL, 14.8 mmol) to afford 6b as pale brown oil (2.1 g, 95%). Molecular formula: C\textsubscript{12}H\textsubscript{12}ClNO\textsubscript{2} (m/z: 237); LCMS: m/z = 238.6 (M\textsuperscript{+}1); \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 300 MHz): δ 7.60 (d, J = 6.8 Hz, 1H), 7.15 (m, 2H), 7.10 (d, J = 6.1 Hz, 1H), 6.60 (d, J = 8.7 Hz, 1H).

(5-Chloro indol-1-yl)-acetic acid (7b): The compound 7b has been prepared according to the method described for the compound 7c employing the compound 6b (2.1 g, 9.4 mmol) and lithium hydroxide (0.59 g, 14.1 mmol) to afford 7b as pale yellow solid (1.8 g, 92%). Molecular formula: C\textsubscript{10}H\textsubscript{8}ClNO\textsubscript{2} (m/z: 209); LCMS: m/z = 208.1 (M\textsuperscript{-}1); \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}, 300 MHz): δ 12.9 (brs, 1H), 7.75 (d, 1H), 7.30 (m, 2H), 7.20 (dd, 1H), 6.45 (d, 1H), 5.0 (s, 2H).

(5-Methoxy indol-1-yl)-acetic acid ethyl ester (6d): The compound 6d has been prepared according to the method described for the compound 6c employing the compound 5-methoxy indole (2.5 g, 17 mmol) and methyl bromoacetate (2.4 mL, 25.5 mmol) to afford 6d as
yellow oil (3.6 g, 97%). Molecular formula: C_{13}H_{15}NO_3 (m/z: 233); LCMS: m/z = 234.2 (M^+1); \(^1\)H NMR (CDCl_3, 300 MHz): δ 7.20 (d, J = 6.7 Hz, 1H), 7.10 (d, J = 7.3 Hz, 1H), 7.05 (d, J = 6.0 Hz, 1H), 6.90 (dd, J = 11.3 Hz, J = 3.3 Hz, 1H), 6.55 (d, J = 8.3 Hz, 1H).

**(5-Methoxy indol-1-yl)-acetic acid (7d):** The compound 7d has been prepared according to the method described for the compound 7c employing the compound 6d (3.6 g, 16.4 mmol) and lithium hydroxide (1.03 g, 24.6 mmol) to afford 7d as cream colour solid (2.6 g, 77%). Molecular formula: C_{11}H_{11}NO_3 (m/z: 205); LCMS: m/z = 204.2 (M^+1); \(^1\)H NMR (DMSO-d_6, 300 MHz): δ 13.0-12.80 (brs, 1H), 7.30-7.25 (m, 2H), 7.05 (d, J = 6.3 Hz, 1H), 6.75 (dd, J = 8.3 Hz, J = 2.3 Hz, 1H), 6.35 (d, J = 6.4 Hz, 1H), 4.95 (s, 2H), 3.80 (s, 3H).

**(2-Methyl indol-1-yl)-acetic acid ethyl ester (6e):** The compound 6e has been prepared according to the method described for the compound 6c employing the compound 2-methyl indole (2.5 g, 19 mmol) and methyl bromoacetate (2.7 mL, 28.6 mmol) to afford 6e as yellow oil (3.5 g, 91%). Molecular formula: C_{13}H_{15}NO_2 (m/z: 217); LCMS: m/z = 218.3 (M^+1); \(^1\)H NMR (CDCl_3, 300 MHz): δ 7.50 (d, J = 6.1 Hz, 1H), 7.25 (d, J = 6.8 Hz, 1H), 7.10 (m, 1H), 6.40 (s, 1H), 6.15 (d, J = 8.3 Hz, 1H).

**(2-Methyl indol-1-yl)-acetic acid (7e):** The compound 7e has been prepared according to the method described for the compound 7c employing the compound 6e (3.5 g, 17.2 mmol) and lithium hydroxide (1.08 g, 25.8 mmol) to afford 7e as tan colour solid (1.7 g, 52%).
Molecular formula: C_{11}H_{11}NO_{2} (m/z: 189); LCMS: m/z = 188.2 (M^-1);

^1H NMR (DMSO-d$_6$, 300 MHz): δ 13.0 (brs, 1H), 7.40 (d, J = 6.6 Hz, 1H), 7.30 (d, J = 7.3 Hz, 1H), 7.0 (m, 2H), 6.25 (s, 1H), 4.95 (s, 2H), 2.30 (s, 3H).

2.1.3 tert-Butyl 5-fluoro-3-(2-(5-fluoro-1H-indol-1-yl) acetamido)-4-hydroxypentanoate (8a): To a stirred solution of (5-fluoro indol-1-yl)-acetic acid (150 mg, 0.7 mmol) in DMF (5 mL) was added DIPEA (0.26 mL, 1.5 mmol), HOBt (114 mg, 0.84 mmol), 4 (193 mg, 0.93 mmol) and cooled to 0 °C. Then added EDCI (161 mg, 0.84 mmol) and stirred at room temperature until TLC indicated that reaction was complete. The reaction mixture was poured in ice water and extracted with ethyl acetate (2×25 mL). The combined organic layers were dried over anhydrous Na$_2$SO$_4$ and evaporated in vacuum to afford 8a as pale yellow solid (270 mg, 92%). Molecular formula: C_{19}H_{24}F$_2$N$_2$O$_4$ (m/z: 382); LCMS: m/z = 327.1 (M^+1-tBu).

**tert-Butyl 5-fluoro-3-(2-(5-fluoro-1H-indol-1-yl) acetamido)-4-oxopentanoate (9a):** To a stirred solution of Dess-Martin periodinane (599 mg, 1.4 mmol) in dry DCM (10 mL) at 0 °C was added 8a (270 mg, 0.7 mmol) in dry DCM (5 mL) and stirred at same temperature for 90 min until TLC indicated that reaction was complete. The reaction mixture was quenched with 1 g of Na$_2$S$_2$O$_3$ dissolved in 5 mL of 10% NaHCO$_3$ solution and extracted with DCM (2×10 mL). The combined organic layers were dried over anhydrous Na$_2$SO$_4$ and evaporated in vacuum to get crude product which was purified by silica gel column
chromatography (0.4% methanol-DCM) to afford 9a as cream colour solid (140 mg, 52%). Molecular formula: C\textsubscript{19}H\textsubscript{22}F\textsubscript{2}N\textsubscript{2}O\textsubscript{4} (m/z: 380); LCMS: m/z = 379.1 (M\textsuperscript{+}-1); \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 300 MHz): \(\delta\) 7.35-7.25 (dd, \(J = 12.1\) Hz, \(J = 3.2\) Hz, 1H), 7.20 (m, 1H), 7.15 (d, \(J = 4.5\) Hz, 1H), 7.0 (m, 1H), 6.60 (d, \(J = 3.3\) Hz, 1H), 6.25 (d, \(J = 7.5\) Hz, 1H), 5.0 (s, 1H), 4.90-4.80 (m, 4H), 2.90 (dd, \(J = 15.4\) Hz, \(J = 6.1\) Hz, 1H), 2.60 (dd, \(J = 15.5\) Hz, \(J = 5.5\) Hz, 1H), 1.20 (s, 9H, tBu).

5-Fluoro-3-(2-(5-fluoro-1H-indol-1-yl) acetamido)-4-oxopentanoic acid (10a):

The compound 9a (65 mg, 0.17 mmol) dissolved in dry DCM (5 mL) and added TFA (0.5 mL) followed by anisole (0.2 mL) and stirred at room temperature for 1 h until TLC indicated that reaction was complete. The reaction mixture was evaporated in vacuum to afford cream colour solid which was purified by preparatory HPLC to afford 10a as cream colour solid (18 mg, 33%). Molecular formula: C\textsubscript{15}H\textsubscript{14}F\textsubscript{2}N\textsubscript{2}O\textsubscript{4} (m/z: 324); LCMS: m/z = 323.1 (M\textsuperscript{+}-1); m.p. 60-62 °C; \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}, 300 MHz): \(\delta\) 8.70 (d, \(J = 9.1\) Hz, 1H, -NH), 7.40 (d, \(J = 6.5\) Hz, 1H), 7.40-7.28 (m, 2H), 7.0-6.90 (m, 1H), 6.45 (d, \(J = 6.5\) Hz, 1H), 5.20-4.90 (m, 4H), 4.65 (q, 1H), 2.80-2.70 (m, 2H, CH\textsubscript{2}COOH); \textsuperscript{13}C NMR (CD\textsubscript{3}OD, 400 MHz): \(\delta\) 174.27, 170.84, 160.6,
158.28, 134.68, 131.99, 130.73 (m), 111.15 (m), 110.69, 106.36 (m), 103, 82.03, 53.07, 51.26, 35.13; HPLC purity: 97%.

**tert-Butyl 3-(2-(5-chloro-1H-indol-1-yl) acetamido)-5-fluoro-4-hydroxypentanoate (8b):** The compound 8b has been prepared according to the method described for the compound 8a employing the compound 7b (160 mg, 0.8 mmol) and 4 (193 mg, 0.9 mmol) to afford 8b as pale yellow sticky solid (300 mg, 98%). Molecular formula: C$_{19}$H$_{24}$ClFN$_2$O$_4$ (m/z: 398); LCMS: m/z = 343.0 (M$^+$+1-tBu).

**tert-Butyl 3-(2-(5-chloro-1H-indol-1-yl) acetamido)-5-fluoro-4-oxopentanoate (9b):** The compound 9b has been prepared according to the method described for the compound 9a employing the compound 8b (300 mg, 0.7 mmol) and Dess-Martin periodinane (383 mg, 0.9 mmol) to afford the crude product which was purified by silica gel column chromatography (0.4% methanol-DCM) to afford 9b as cream colour solid (190 mg, 64%). Molecular formula: C$_{19}$H$_{22}$ClFN$_2$O$_4$ (m/z: 396); LCMS: m/z = 395.1 (M$^+$-1); $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.62 (s, 1H), 7.2 (m, 2H), 7.12 (d, $J = 6.3$ Hz, 1H), 6.60 (d, $J = 6.5$ Hz, 1H), 6.25 (d, $J = 9.1$ Hz, 1H, -NH), 5.0 (s, 1H), 4.90-4.80 (m, 4H), 2.90 (dd, $J = 17.1$ Hz, $J = 5.2$ Hz, 1H, Asp), 2.60 (dd, $J = 17.1$ Hz, $J = 5.4$ Hz, 1H, Asp), 1.25 (s, 9H, tBu).
3-(2-(5-Chloro-1H-indol-1-yl) acetamido)-5-fluoro-4-oxopentanoic acid (10b):

The compound 10b has been prepared according to the method described for the compound 10a employing the compound 9b (100 mg, 0.2 mmol) and TFA (0.7 mL) to afford the crude product which was purified by preparatory HPLC to afford 10b as cream colour solid (50 mg, 58%). Molecular formula: C_{15}H_{14}ClFN_2O_4 (m/z: 340); LCMS: m/z = 340.9 (M^+1); m.p. 77-79 °C; IR (KBr): 3287, 2929, 1744, 1698, 1670, 1469, 1303, 1235 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 12.60 (brs, 1H), 8.85-8.70 (brs, 1H, -NH), 7.60 (d, J = 0.3 Hz, 1H), 7.40 (m, 2H), 7.10 (d, J = 8.1 Hz, 1H), 6.45 (d, J = 6.4 Hz, 1H), 5.35-5.0 (m, 2H), 4.90 (s, 2H), 4.65 (m, 1H), 2.80-2.70 (m, 2H, CH_2COOH); ^13C NMR (CD_3OD, 300 MHz): δ 174.3, 170.68, 136.53, 131.78, 131.39, 126.56, 122.93, 121.07, 111.86, 111.64, 102.7, 82, 51.39, 49.92, 35.11; HPLC purity: 97%.

tert-Butyl 3-(2-(5-bromo-1H-indol-1-yl) acetamido)-5-fluoro-4-hydroxypentanoate (8c): The compound 8c has been prepared according to the method described for the compound 8a employing the compound 7c (195 mg, 0.7 mmol) and 4 (193 mg, 0.9 mmol) to afford 8c as cream colour solid (300 mg, 88%). Molecular formula: C_{19}H_{24}BrFN_2O_4 (m/z: 443). LCMS: m/z = 369.0 (M^+-2-OtBu).
tert-Butyl 3-(2-(5-bromo-1H-indol-1-yl) acetamido)-5-fluoro-4-oxopentanoate (9c): The compound 9c has been prepared according to the method described for the compound 9a employing the compound 8c (300 mg, 0.67 mmol) and Dess-Martin periodinane (430 mg, 1 mmol) to afford the crude product which was purified by silica gel column chromatography (0.4% methanol-DCM) to afford 9c as cream colour solid (190 mg, 64%). Molecular formula: C_{19}H_{22}BrFN_{2}O_{4} (m/z: 441); LCMS: m/z = 440.9 (M⁺-2); ¹H NMR (CDCl₃, 300 MHz): δ 7.80 (d, J = 0.3 Hz, 1H), 7.35 (d, J = 8.1 Hz, 1H), 7.20-7.15 (d, J = 9.1 Hz, 1H), 7.10 (d, J = 6.4 Hz, 1H), 6.6 (d, J = 6.3 Hz, 1H), 6.28 (d, J = 9.2 Hz, 1H, -NH), 5.0 (s, 1H), 4.90-4.80 (m, 4H), 2.90 (dd, J = 17.1 Hz, J = 5.1 Hz, 1H, Asp), 2.6 (dd, J = 17.1 Hz, J = 5.4 Hz, 1H, Asp), 1.20 (s, 9H, tBu).

3-(2-(5-Bromo-1H-indol-1-yl) acetamido)-5-fluoro-4-oxopentanoic acid (10c):

The compound 10c has been prepared according to the method described for the compound 10a employing the compound 9c (125 mg, 0.3 mmol) and TFA (0.6 mL) to afford the crude product which was purified by preparatory HPLC to afford 10c as cream colour solid (37 mg, 34%). Molecular formula: C_{15}H_{14}BrFN_{2}O_{4} (m/z: 385); LCMS: m/z = 383.0 (M⁺-2); m.p. 99-101 °C; ¹H NMR (DMSO-d₆, 300 MHz): δ
8.80 (d, \( J = 8.7 \) Hz, 1H, -NH), 7.75 (d, \( J = 0.5 \) Hz, 1H), 7.40-7.30 (m, 2H), 7.25-7.20 (d, \( J = 8.1 \) Hz, 1H), 6.45 (d, \( J = 6.1 \) Hz, 1H), 5.20-5.0 (m, 2H), 4.90 (s, 2H), 4.65 (m, 1H), 2.80-2.70 (m, 2H, \( \text{CH}_2\text{COOH} \)); \(^{13}\)C NMR (CD\(_3\)OD, 400 MHz): \( \delta \) 174.36, 170.65, 136.75, 132.02, 131.5, 125.64, 125.33, 124.28, 113.9, 112.24 (m), 102.64, 82, 53, 51.26, 35.12; HPLC purity: 93%.

tert-Butyl 5-fluoro-4-hydroxy-3-(2-(5-methoxy-1H-indol-1-yl)acetamido) pentanoate (8d): The compound 8d has been prepared according to the method described for the compound 8a employing the compound 7d (157 mg, 0.7 mmol) and 4 (193 mg, 0.9 mmol) to afford 8d as pale yellow sticky solid (220 mg, 73%). Molecular formula: C\(_{20}\)H\(_{27}\)FN\(_2\)O\(_5\) (m/z: 394); LCMS: m/z = 339.1 (M\(^+\)+1-tBu).

tert-Butyl 5-fluoro-3-(2-(5-methoxy-1H-indol-1-yl) acetamido)-4-oxopentanoate (9d): The compound 9d has been prepared according to the method described for the compound 9a employing the compound 8d (220 mg, 0.6 mmol) and Dess-Martin periodinan (284 mg, 0.7 mmol) to afford the crude product which was purified by silica gel column chromatography (0.4% methanol-DCM) to afford 9d as cream colour solid (95 mg, 43%). Molecular formula: C\(_{20}\)H\(_{25}\)FN\(_2\)O\(_5\) (m/z: 392); LCMS: m/z = 337.0 (M\(^+\)+1-tBu); \(^1\)H NMR (CDCl\(_3\), 300 MHz): \( \delta \) 7.15 (d, \( J = 6.2 \) Hz, 1H), 7.10 (d, \( J = 3.1 \) Hz, 1H), 7.05 (d, \( J = 3.1 \) Hz, 1H), 6.90 (dd, \( J = 12.1 \) Hz, \( J = 3.5 \) Hz, 1H), 6.55 (d, \( J = 3.4 \) Hz, 1H), 6.25 (d, \( J = 8.5 \) Hz, 1H), 5.0 (s, 1H), 4.90-4.80 (m, 4H), 3.85 (s,
3H), 2.90 (dd, \( J = 17.1 \text{ Hz} \), \( J = 5.2 \text{ Hz} \), 1H, Asp), 2.65 (dd, \( J = 17 \text{ Hz} \), \( J = 5.4 \text{ Hz} \), 1H, Asp), 1.25 (s, 9H, tBu).

5-Fluoro-3-(2-(5-methoxy-1H-indol-1-yl)acetamido)-4-oxopentanoic acid (10d):

The compound 10d has been prepared according to the method described for the compound 10a employing the compound 9d (95 mg, 0.2 mmol) and TFA (0.5 mL) to afford the crude product which was purified by preparatory HPLC to afford 10d as pale brown solid (12 mg, 15%). Molecular formula: C_{16}H_{17}FN_{2}O_{5} (m/z: 336); LCMS: m/z = 335.1 (M+1); m.p. 96-98 °C; IR (KBr): 3307, 2938, 1789, 1744, 1667, 1486, 1333, 1242 cm\(^{-1}\); \(^1\)H NMR (DMSO-d\(_6\), 300 MHz): δ 8.75-8.60 (brs, 1H), 7.30 (m, 2H), 7.05 (d, \( J = 3.2 \text{ Hz} \), 1H), 6.85-6.70 (dd, \( J = 12.1 \text{ Hz}, J = 3.3 \text{ Hz}, 1H)\), 6.35 (d, \( J = 3.1 \text{ Hz}, 1H)\), 5.30-5.0 (m, 2H), 4.85 (s, 2H), 4.60 (m, 1H), 3.75 (s, 3H, OCH\(_3\)), 2.80-2.70 (m, 2H, CH\(_2\)COOH).

tert-Butyl 5-fluoro-4-hydroxy-3-(2-(2-methyl-1H-indol-1-yl)acetamido) pentanoate (8e): The compound 8e has been prepared according to the method described for the compound 8a employing the compound 7e (145 mg, 0.7 mmol) and 4 (193 mg, 0.9 mmol) to afford 8e as cream colour solid (240 mg, 82%). Molecular formula: C_{20}H_{27}FN_{2}O_{4} (m/z: 378); LCMS: m/z = 323.1 (M+1-tBu).
**tert-Butyl 5-fluoro-3-(2-(2-methyl-1H-indol-1-yl) acetamido)-4-oxopentanoate (9e):** The compound 9e has been prepared according to the method described for the compound 9a employing the compound 8e (240 mg, 0.6 mmol) and Dess-Martin periodinane (323 mg, 0.7 mmol) to afford the crude product which was purified by silica gel column chromatography (0.4% methanol-DCM) to afford 9e as cream colour solid (80 mg, 34%). Molecular formula: C$_{20}$H$_{25}$FN$_2$O$_4$ (m/z: 376); LCMS: m/z = 377.2 (M$^+$+1); $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.55 (d, $J$ = 6.5 Hz, 1H), 7.20-7.10 (m, 3H), 6.40 (s, 1H), 6.15 (d, $J$ = 8.5 Hz, 1H), 4.95 (d, $J$ = 0.6 Hz, 1H), 4.90-4.75 (m, 4H), 2.90 (dd, $J$ = 17.1 Hz, $J$ = 5.2 Hz, 1H, Asp), 2.65 (dd, $J$ = 17.1 Hz, $J$ = 5.4 Hz, 1H, Asp), 2.40 (s, 3H), 1.20 (s, 9H, tBu).

**5-Fluoro-3-(2-(2-methyl-1H-indol-1-yl) acetamido)-4-oxopentanoic acid (10e):**

![Chemical Structure](image)

The compound 10e has been prepared according to the method described for the compound 10a employing the compound 9e (80 mg, 0.2 mmol) and TFA (0.5 mL) to afford the crude product which was purified by preparatory HPLC to afford 10e as pale brown solid (10 mg, 15%). Molecular formula: C$_{16}$H$_{17}$FN$_2$O$_4$ (m/z: 320); LCMS: m/z = 319.1 (M$^+$-1); m.p. 82-84 °C; IR (KBr): 3295, 2930, 1785, 1746, 1667, 1465, 1401, 1213 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$, 300 MHz): $\delta$ 8.70 (d, $J$ =
9.1 Hz, 1H), 7.45-7.40 (d, J = 6.3 Hz, 1H), 7.30 (d, J = 6.5 Hz, 1H),
7.15-6.90 (m, 3H), 6.20 (s, 1H), 5.20-5.0 (m, 2H), 4.85 (s, 2H), 4.65
(m, 1H), 2.85-2.70 (m, 2H, CH₂COOH), 2.35 (s, 3H, CH₃); HPLC purity:
93%.

2.2.1 3-Benzzyloxycarbonylamino-5-bromo-4-oxo-pentanoic acid
tert-butyl ester (14): To a stirred solution of Cbz-L-aspartic acid tert-
butyl ester (5 g, 15.4 mmol) in dry THF (50 mL) at -78 °C was added
isobutyl chloroformate (2.61 mL, 20.1 mmol) followed by N-methyl
morpholine (2.39 mL, 21.5 mmol) and stirred at 0 °C for 1 h until TLC
indicated completion of the reaction. The reaction mixture was again
cooled to -40 °C and added diazomethane solution in ether (120 mL)
(generated from 13 g of NMU and 50 g of KOH dissolved in 80 mL of
water) and stirred for 15 min until TLC indicated completion of the
reaction. Excess diazomethane was removed by bubbling argon gas
and quenched the reaction mixture with aq HBr/AcOH (1:1) (20 mL)
at 0 °C and stirred until TLC indicated completion of the reaction. The
reaction mixture was diluted with ethyl acetate (100 mL) and washed
with 10% NaHCO₃ solution and saturated brine. The combined
organic layers were dried over anhydrous Na₂SO₄ and evaporated in
vacuum to get crude product which was purified by silica gel column
chromatography (10% EtOAc-hexane) to afford 14 as colourless oil
(6.1 g, 99%). Molecular formula: C₁₇H₂₂BrNO₅ (m/z: 400); LCMS: m/z
= 345.9 (M⁺+2-tBu); ¹H NMR (CDCl₃, 300 MHz): δ 7.40 (m, 5H), 5.90
(d, J = 9.8 Hz, 1H, -NH), 5.15 (s, 2H), 4.75 (m, 1H), 4.20 (s, 2H), 3.0
(dd, J = 17.0 Hz, J = 5.2 Hz, 1H, Asp), 2.75 (dd, J = 17.0 Hz, J = 5.4 Hz, 1H, Asp), 1.45 (s, 9H, tBu).

3-Benzzyloxycarbonylamino-4-oxo-5-(2,3,5,6-tetrafluoro phenoxy)-pentanoic acid tert-butyl ester (15): To a stirred solution of 2,3,5,6-tetrafluoro phenol (1.5 g, 8.2 mmol) in dry acetone (20 mL) was added dry K$_2$CO$_3$ (3.1 g, 22.5 mmol) and stirred at room temperature for 15 min. Compound 14 (3 g, 7.5 mmol) in acetone (5 mL) was added and stirred at room temperature until TLC indicated completion of the reaction. The reaction mixture was evaporated in vacuum and the residue was taken in ethyl acetate (100 mL) and washed with water and saturated brine. The combined organic layers were dried over anhydrous Na$_2$SO$_4$ and evaporated in vacuum to get crude product which was purified by silica gel column chromatography (3-5% EtOAc-hexane) to afford 15 as white solid (3.1 g, 85%). Molecular formula: C$_{23}$H$_{23}$F$_4$NO$_6$ (m/z: 485); LCMS: m/z = 429.9 (M$^+$+1-tBu).

3-Benzzyloxycarbonylamino-4-hydroxy-5-(2,3,5,6-tetrafluoro phenoxy)-pentanoic acid tert-butyl ester (16): To a stirred solution of 15 (3.1 g, 6.4 mmol) in methanol-THF (1:1) at 0 °C was added NaBH$_4$ (241 mg, 6.4 mmol) and stirred at same temperature until TLC indicated completion of the reaction. The reaction mixture was poured over cold 1N HCl and extracted with ethyl acetate (2×50 mL). The combined organic layers were dried over anhydrous Na$_2$SO$_4$ and evaporated in vacuum to get crude product which was purified by silica gel column chromatography (8-12% EtOAc-hexane) to afford 16
as white solid (2.5 g, 80%). Molecular formula: C_{23}H_{25}F_{4}NO_{6} (m/z: 487); LCMS: m/z = 388.1 (M^+1-COOtBu).

(3S)-tert-Butyl-3-amino-4-hydroxy-5-(2,3,5,6-tetrafluoro phenoxy) pentanoate (17):

To a stirred solution of 16 (2.5 g, 5.1 mmol) in ethanol (60 mL) was added 10% Pd/C (360 mg) under argon atmosphere and stirred the reaction mixture under hydrogen balloon for 4 h until TLC indicated that reaction was complete. The reaction mixture was filtered over celite bed and filtrate was evaporated in vacuum to afford 17 as ash colour solid (1.7 g, 94%). Molecular formula: C_{15}H_{19}F_{4}NO_{4} (m/z: 353); LCMS: m/z = 354.1 (M^+1); ^1H NMR (CDCl₃, 300 MHz): δ 6.80 (m, 1H), 4.40-4.20 (m, 2H), 3.85 (m, 1H), 3.45 (m, 1H), 2.60 (m, 1H), 2.35 (m, 1H), 1.45 (s, 9H, tBu).

2.2.2 (3S)-tert-Butyl 3-(2-(5-fluoro-1H-indol-1-yl) acetamido)-4-hydroxy-5-(2,3,5,6-tetrafluorophenoxy) pentanoate (18a): The compound 18a has been prepared according to the method described for the compound 8a employing the compound 7a (100 mg, 0.5 mmol) and 17 (201 mg, 0.6 mmol) to afford 18a as pale yellow solid (290 mg, 100%). Molecular formula: C_{25}H_{25}F_{5}N_{2}O_{5} (M/Z: 528); LCMS: m/z = 529.0 (M^+1).
(S)-tert-Butyl 3-(2-(5-fluoro-1H-indol-1-yl) acetamido)-4-oxo-5-(2,3,5,6-tetrafluorophenoxy) pentanoate (19a): The compound 19a has been prepared according to the method described for the compound 9a employing the compound 18a (290 mg, 0.5 mmol) and Dess-Martin periodinane (279 mg, 0.6 mmol) to afford the crude product which was purified by silica gel column chromatography (0.4% methanol-DCM) to afford 19a as cream colour solid (175 mg, 61%). Molecular formula: C_{25}H_{23}F_{5}N_{2}O_{5} (M/Z: 526); LCMS: m/z = 471.0 (M\textsuperscript{+}+1-\text{tBu}); \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 300 MHz): δ 7.30 (dd, \textit{J} = 12.1 Hz, \textit{J} = 3 Hz, 1H), 7.20 (m, 1H), 7.15 (d, \textit{J} = 4.5 Hz, 1H), 7.0 (m, 1H), 6.85 (m, 1H), 6.65 (d, \textit{J} = 3.6 Hz, 1H), 6.30 (d, \textit{J} = 7.5 Hz, 1H), 4.92-4.82 (m, 5H), 2.98-2.88 (dd, \textit{J} = 15.1 Hz, \textit{J} = 6.2 Hz, 1H), 2.60-2.50 (dd, \textit{J} = 15.3 Hz, \textit{J} = 5.5 Hz, 1H), 1.25 (s, 9H, \text{tBu}).

(S)-3-(2-(5-Fluoro-1H-indol-1-yl) acetamido)-4-oxo-5-(2,3,5,6-tetrafluorophenoxy) pentanoic acid (20a):

The compound 20a has been prepared according to the method described for the compound 10a employing the compound 19a (100 mg, 0.2 mmol) and TFA (0.5 mL) to afford the crude product which was purified by preparatory HPLC to afford 20a as white solid (38 mg, 43%). Molecular formula: C\textsubscript{21}H\textsubscript{15}F\textsubscript{5}N\textsubscript{2}O\textsubscript{5} (M/Z: 470); LCMS: m/z =
73

471.1 (M+1); m.p. 60-63 °C; IR (KBr): 3314, 2952, 1740, 1704, 1666, 1513, 1488, 1232 cm⁻¹; ¹H NMR (DMSO-d₆, 300 MHz): δ 8.75 (d, J = 9.1 Hz, 1H), 7.65-7.55 (m, 1H), 7.45-7.25 (m, 3H), 6.95 (t, 1H), 6.45 (d, J = 6.3 Hz, 1H), 5.20-5.0 (m, 2H), 4.95 (s, 2H), 4.70 (m, 1H), 2.80-2.70 (d, J = 9.2 Hz, 2H, CH₂COOH); ¹³C NMR (CD₃OD, 400 MHz): δ 174.6, 170.8, 160.57, 158.2, 148.9, 146.59, 143.2, 140.7, 138.6, 134.6, 131.9, 131.7, 130.6, 111.2 (m), 106.4, 106.2, 103, 101 (m), 76.2, 52.9, 50.4, 35.69; HPLC purity: 97%.

(3S)-tert-Butyl 3-(2-(5-chloro-1H-indol-1-yl) acetamido)-4-hydroxy-5-(2,3,5,6-tetrafluorophenoxy) pentanoate (18b): The compound 18b has been prepared according to the method described for the compound 8a employing the compound 7b (108 mg, 0.5 mmol) and 17 (201 mg, 0.6 mmol) to afford 18b as pale yellow solid (290 mg, 100%). Molecular formula: C₂₅H₂₅ClF₄N₂O₅ (m/z: 544); LCMS: m/z = 489.0 (M⁺+1-OTBu).

(S)-tert-Butyl 3-(2-(5-chloro-1H-indol-1-yl) acetamido)-4-oxo-5-(2,3,5,6-tetrafluorophenoxy) pentanoate (19b): The compound 19b has been prepared according to the method described for the compound 9a employing the compound 18b (290 mg, 0.5 mmol) and Dess-Martin periodinane (270 mg, 0.6 mmol) to afford the crude product which was purified by silica gel column chromatography (0.4% methanol-DCM) to afford 19b as cream colour solid (160 mg, 55%). Molecular formula: C₂₅H₂₃ClF₄N₂O₅ (m/z: 542); LCMS: m/z = 487.1 (M⁺+1-OTBu); ¹H NMR (CDCl₃, 300 MHz): δ 7.61 (t, 1H), 7.26 (d, J
= 0.3 Hz, 1H), 7.19 (s, 1H), 7.13 (d, J = 3.1 Hz, 1H), 6.85-6.75 (m, 1H), 6.60 (d, J = 3.3 Hz, 1H), 6.35 (d, J = 8.4 Hz, 1H, -NH), 4.92-4.84 (m, 5H), 2.98-2.88 (dd, J = 17.1 Hz, J = 4.5 Hz, 1H, Asp), 2.60-2.50 (dd, J = 17.1 Hz, J = 5.4 Hz, 1H, Asp), 1.25 (s, 9H, tBu).

(S)-3-(2-(5-Chloro-1H-indol-1-yl) acetamido)-4-oxo-5-(2,3,5,6-tetrafluorophenoxy) pentanoic acid (20b):

The compound 20b has been prepared according to the method described for the compound 10a employing the compound 19b (160 mg, 0.3 mmol) and TFA (1 mL) to afford the crude product which was purified by preparatory HPLC to afford 20b as white solid (85 mg, 59%). Molecular formula: C_{21}H_{15}ClF_{4}N_{2}O_{5} (m/z: 486); LCMS: m/z = 485.2 (M⁺-1); m.p. 188-191 °C; IR (KBr): 3318, 2950, 1746, 1704, 1662, 1517, 1489, 1290 cm⁻¹; ¹H NMR (DMSO-d₆, 300 MHz): δ 8.75 (d, J = 7.5 Hz, 1H, -NH), 7.65-7.55 (m, 2H), 7.45-7.35 (m, 2H), 7.05 (dd, J = 8.7 Hz, J = 1.8 Hz, 1H), 6.42 (d, J = 3.2 Hz, 1H), 5.20 (d, J = 17.1 Hz, 1H), 5.05 (d, J = 17.1 Hz, 1H), 4.95 (d, J = 3 Hz, 2H), 4.50 (m, 1H), 2.65-2.55 (dd, J = 16.8 Hz, J = 6.1 Hz, 1H, CH₂COOH), 2.55-2.40 (m, 1H, CH₂COOH); ¹³C NMR (CD₃OD, 400 MHz): δ 176.8, 176.2, 148.9, 146.6, 143.2, 140.7, 136.4, 131.7, 131.57, 131.36, 126.5,
122.8, 121, 111.6, 102.7, 102, 100.9 (m), 76.8, 54.1, 50.3, 38; HPLC purity: 97%.

(3S)-**tert**-Butyl 3-(2-(5-bromo-1H-indol-1-yl) acetamido)-4-hydroxy-5-(2,3,5,6-tetrafluorophenoxy) pentanoate (**18c**): The compound **18c** has been prepared according to the method described for the compound **8a** employing the compound **7c** (131 mg, 0.5 mmol) and **17** (201 mg, 0.6 mmol) to afford **18c** as pale yellow solid (298 mg, 100%). Molecular formula: C$_{25}$H$_{25}$BrF$_4$N$_2$O$_5$ (m/z: 589); $^1$H NMR (CDCl$_3$, 300 MHz): δ 7.70 (d, $J$ = 0.3 Hz, 1H), 7.25 (dd, $J$ = 11.8 Hz, $J$ = 3.0 Hz, 1H), 7.15-7.05 (m, 2H), 6.95-6.80 (m, 1H), 6.55 (d, $J$ = 6.2 Hz, 1H), 6.25 (d, $J$ = 6.5 Hz, 1H), 4.80 (s, 2H), 4.30 (m, 1H), 4.15-3.90 (m, 3H), 3.05 (m, 1H), 2.70-2.55 (dd, $J$ = 15.5 Hz, $J$ = 5.6 Hz, 1H, Asp), 2.45-2.35 (dd, $J$ = 15.8 Hz, $J$ = 5.5 Hz, 1H, Asp), 1.30 (s, 9H, tBu).

(S)-**tert**-Butyl 3-(2-(5-bromo-1H-indol-1-yl) acetamido)-4-oxo-5-(2,3,5,6-tetrafluorophenoxy) pentanoate (**19c**): The compound **19c** has been prepared according to the method described for the compound **9a** employing the compound **18c** (298 mg, 0.5 mmol) and Dess-Martin periodinane (257 mg, 0.6 mmol) to afford the crude product which was purified by silica gel column chromatography (0.4% methanol-DCM) to afford **19c** as colourless sticky solid (212 mg, 72%). Molecular formula: C$_{25}$H$_{23}$BrF$_4$N$_2$O$_5$ (m/z: 586); LCMS: m/z = 588.0 (M$^+$+2); $^1$H NMR (CDCl$_3$, 300 MHz): δ 7.80 (d, $J$ = 0.3 Hz, 1H), 7.30 (d, $J$ = 8.1 Hz, 1H), 7.20-7.15 (d, $J$ = 9.2 Hz, 1H), 7.15 (d, $J$ = 6.1 Hz, 1H), 6.80 (m, 1H), 6.65 (d, $J$ = 6.5 Hz, 1H), 6.30 (d, $J$ = 9.2 Hz, 1H,
The compound 20c has been prepared according to the method described for the compound 10a employing the compound 19c (160 mg, 0.3 mmol) and TFA (1 mL) to afford the crude product which was purified by preparatory HPLC to afford 20c as white solid (9 mg, 6%). Molecular formula: C_{21}H_{15}BrF_{4}N_{2}O_{5} (m/z: 531); LCMS: m/z = 531.0 (M⁺-2); m.p. 199-201 °C; IR (KBr): 3318, 2950, 1745, 1704, 1662, 1517, 1489, 1290 cm⁻¹; ¹H NMR (DMSO-d₆, 300 MHz): δ 12.80-12.45 (brs, 1H), 8.80 (s, 1H), 7.75 (d, J = 0.5 Hz, 1H), 7.70-7.55 (m, 1H), 7.40-7.30 (m, 2H), 7.25-7.15 (d, J = 8.1 Hz, 1H), 6.45 (d, J = 6.2 Hz, 1H), 5.40-5.15 (m, 2H), 4.95 (s, 2H), 4.70 (m, 1H), 2.80-2.70 (m, 2H, CH₂COOH); HPLC purity: 91%.

(3S)-3-(2-(5-Bromo-1H-indol-1-yl) acetamido)-4-oxo-5-(2,3,5,6-tetrafluorophenoxy) pentanoic acid (20c):

(3S)-tert-Butyl 4-hydroxy-3-(2-(5-methoxy-1H-indol-1-yl) acetamido)-5-(2,3,5,6-tetrafluorophenoxy) pentanoate (18d): The compound 18d has been prepared according to the method described for the compound 8a employing the compound 7d (106 mg, 0.5 mmol) and 17 (201 mg, 0.6 mmol) to afford 18d as pale yellow solid (282 mg,
100%). Molecular formula: C_{26}H_{28}F_{4}N_{2}O_{6} (m/z: 540); LCMS: m/z = 485.1 (M+1-tBu).

(S)-**tert-Butyl 3-(2-(5-methoxy-1H-indol-1-yl) acetamido)-4-oxo-5-(2,3,5,6-tetrafluorophenoxy) pentanoate** (19d): The compound **19d** has been prepared according to the method described for the compound **9a** employing the compound **18d** (282 mg, 0.5 mmol) and Dess-Martin periodinane (243 mg, 0.6 mmol) to afford the crude product which was purified by silica gel column chromatography (0.3% methanol-DCM) to afford **19d** as cream colour solid (160 mg, 57%). Molecular formula: C_{26}H_{26}F_{4}N_{2}O_{6} (m/z: 538); LCMS: m/z = 538.1 (M^+1); ^1H NMR (CDCl_3, 300 MHz): δ 7.15 (d, J = 6.5 Hz, 1H), 7.10 (d, J = 3.1 Hz, 1H), 7.05 (d, J = 3.3 Hz, 1H), 6.90-6.75 (m, 2H), 6.55 (d, J = 3.1 Hz, 1H), 6.25 (d, J = 8.5 Hz, 1H), 4.90-4.75 (m, 5H), 3.82 (s, 3H), 2.98-2.88 (dd, J = 17.1 Hz, J = 4.5 Hz, 1H, Asp), 2.60-2.55 (dd, J = 17.1 Hz, J = 5.4 Hz, 1H, Asp), 1.25 (s, 9H, tBu).

(S)-**3-(2-(5-Methoxy-1H-indol-1-yl) acetamido)-4-oxo-5-(2,3,5,6-tetrafluorophenoxy) pentanoic acid** (20d):

The compound **20d** has been prepared according to the method described for the compound **10a** employing the compound **19d** (160 mg, 0.3 mmol) and TFA (1 mL) to afford the crude product which was
purified by preparatory HPLC to afford 20d as brown solid (58 mg, 42%). Molecular formula: C_{22}H_{18}F_{4}N_{2}O_{6} (m/z: 482); LCMS: m/z = 483.3 (M^+1); m.p. 58-62 °C; IR (KBr): 3305, 2950, 1740, 1704, 1661, 1518, 1490, 1243 cm^{-1}; ^1H NMR (DMSO-d_{6}, 300 MHz): δ 8.70 (d, J = 8.5 Hz, 1H), 7.65-7.55 (m, 1H), 7.30-7.20 (m, 2H), 7.05 (d, J = 3.1 Hz, 1H), 6.70 (dd, J = 12.1 Hz, J = 3.2 Hz, 1H), 6.35 (d, J = 3.1 Hz, 1H), 5.30-5.05 (m, 2H), 4.85 (s, 2H), 4.70 (m, 1H), 3.75 (s, 3H, OCH₃), 2.80-2.70 (m, 2H, CH₂COOH); HP LC purity: 97%.

(S)-**tert-**Butyl 4-hydroxy-3-(2-(2-methyl-1H-indol-1-yl)acetamido)-5-(2,3,5,6-tetrafluorophenoxy) pentanoate (18e): The compound 18e has been prepared according to the method described for the compound 8a employing the compound 7e (98 mg, 0.5 mmol) and 17 (201 mg, 0.6 mmol) to afford 18e as pale yellow solid (276 mg, 100%). Molecular formula: C_{26}H_{28}F_{4}N_{2}O_{5} (m/z: 524). LCMS: m/z = 469.1 (M^+1-4Bu).

(R)-**tert-**Butyl 3-(2-(2-methyl-1H-indol-1-yl)acetamido)-4-oxo-5-(2,3,5,6-tetrafluorophenoxy) pentanoate (19e): The compound 19e has been prepared according to the method described for the compound 9a employing the compound 18e (276 mg, 0.5 mmol) and Dess-Martin periodinane (245 mg, 0.6 mmol) to afford the crude product which was purified by silica gel column chromatography (0.3% methanol-DCM) to afford 19e as cream colour solid (100 mg, 36%). Molecular formula: C_{26}H_{26}F_{4}N_{2}O_{5} (m/z: 522); LCMS: m/z = 523.2 (M^+1); ^1H NMR (CDCl₃, 300 MHz): δ 7.55 (d, J = 6.5 Hz, 1H),
The compound **20e** has been prepared according to the method described for the compound **10a** employing the compound **19e** (100 mg, 0.29 mmol) and TFA (0.6 mL) to afford the crude product which was purified by preparatory HPLC to afford **20e** as brown solid (46 mg, 52%). Molecular formula: C_{22}H_{18}F_{4}N_{2}O_{5} (m/z: 466); LCMS: m/z = 467.0 (M+1); m.p. 74-77 °C; IR (KBr): 3299, 2953, 1742, 1702, 1670, 1513, 1490, 1174 cm\(^{-1}\); \(^1\)H NMR (DMSO-d\(_6\), 300 MHz): \(\delta\) 8.70 (d, \(J = 9.2\) Hz, 1H, -NH), 7.65-7.55 (m, 1H), 7.40 (d, \(J = 6.3\) Hz, 1H), 7.30 (d, \(J = 6.2\) Hz, 1H), 7.05-6.95 (m, 2H), 6.20 (s, 1H), 5.35-5.0 (m, 2H), 4.85 (s, 2H), 4.70 (m, 1H), 2.80-2.70 (m, 2H, CH\(_2\)COOH), 2.35 (s, 3H, CH\(_3\)); \(^{13}\)C NMR (CD\(_3\)OD, 300 MHz): \(\delta\) 174.19, 171.27, 170.8, 149.31, 146.05, 143.44, 140.17, 138.76, 138.1, 129.81, 121.88, 120.7, 120.38, 109.62, 101.92, 100.93 (m), 76.25, 52.46, 46.75, 35.42, 12.42; HPLC purity: 95%.
2.3.1 3-Benzylxycarbonylamino-5-(2,6-difluoro-phenoxy)-4-oxo-pentanoic acid tert-butyl ester (21): To a stirred solution of 2,6-difluoro phenol (1.31 g, 9 mmol) in dry acetone (20 mL) was added dry K$_2$CO$_3$ (3.1 g, 22.5 mmol) and stirred at room temperature for 15 min. Compound 14 (3 g, 7.5 mmol) in acetone (5 mL) was added and stirred at room temperature until TLC indicated completion of the reaction. The reaction mixture was evaporated in vacuum and the residue was taken in ethyl acetate (100 mL) and washed with water and saturated brine. The combined organic layers were dried over anhydrous Na$_2$SO$_4$ and evaporated in vacuum to afford 21 as pale yellow oil (2.9 g, 86%). Molecular formula: C$_{23}$H$_{25}$F$_2$NO$_6$ (m/z: 449); LCMS: m/z = 350.1 (M$^+$+1-COOtBu).

3-Benzylxycarbonylamino-5-(2,6-difluoro-phenoxy)-4-hydroxy-pentanoic acid tert-butyl ester (22): To a stirred solution of 21 (2.9 g, 6.4 mmol) in methanol-THF (1:1) at 0 °C was added NaBH$_4$ (0.24 g, 6.4 mmol) and stirred at same temperature until TLC indicated completion of the reaction. The reaction mixture was poured over cold 1N HCl and extracted with ethyl acetate (2×50 mL). The combined organic layers were dried over anhydrous Na$_2$SO$_4$ and evaporated in vacuum to get crude product which was purified by silica gel column chromatography (8-12% EtOAc-hexane) to afford 22 as white solid (2.6 g, 89%). Molecular formula: C$_{23}$H$_{27}$F$_2$NO$_6$ (m/z: 451); LCMS: m/z = 352.1 (M$^+$+1-COOtBu).
(3S)-**tert-**Butyl-3-amino-5-(2,6-difluorophenoxy)-4-hydroxy pentanoate (23):

To a stirred solution of **22** (2.6 g, 5.7 mmol) in ethanol (40 mL) was added 10% Pd/C (300 mg) under argon atmosphere and stirred the reaction mixture under hydrogen balloon for 4 h until TLC indicated that reaction was complete. The reaction mixture was filtered over celite bed and filtrate was evaporated in vacuum to afford **23** as ash colour solid (1.7 g, 93%). Molecular formula: C_{15}H_{21}F_{2}NO_{4} (m/z: 317); LCMS: m/z = 318.1 (M^{+}+1); \(^1\)H NMR (DMSO-d_{6}, 300 MHz): \(\delta\) 7.20-7.05 (m, 3H), 5.10 (d, \(J = 8.9\) Hz, 1H), 4.25-4.0 (m, 2H), 3.60-3.50 (m, 1H), 3.15-3.0 (m, 1H), 2.15-2.05 (m, 1H), 1.60-1.50 (brs, 2H), 1.45 (s, 9H, tBu).

2.3.2 (3S)-**tert-**Butyl 5-(2,6-difluorophenoxy)-3-(2-(5-fluoro-1H-indol-1-yl) acetamido)-4-hydroxypentanoate (24a): The compound **24a** has been prepared according to the method described for the compound **8a** employing the compound **7a** (100 mg, 0.5 mmol) and **23** (180 mg, 0.6 mmol) to afford **24a** (300 mg, 100%). Molecular formula: C_{25}H_{27}F_{3}N_{2}O_{5} (m/z: 492); LCMS: m/z = 419.1 (M^{+}+1-OtBu).

(3S)-**tert-**Butyl 5-(2,6-difluorophenoxy)-3-(2-(5-fluoro-1H-indol-1-yl) acetamido)-4-oxopentanoate (25a): The compound **25a** has been prepared according to the method described for the compound **9a**
employing the compound 24a (300 mg, 0.6 mmol) and Dess-Martin periodinane (284 mg, 0.7 mmol) to afford the crude product which was purified by silica gel column chromatography (0.4% methanol-DCM) to afford 25a as cream colour solid (120 mg, 40%). Molecular formula: C_{25}H_{25}F_{3}N_{2}O_{5} (m/z: 490); LCMS: m/z = 435.1 (M^{+}+tBu); ^{1}H NMR (CDCl_{3}, 300 MHz): δ 7.30 (dd, J = 12.1 Hz, J = 3.2 Hz, 1H), 7.20 (m, 1H), 7.15 (d, J = 5.3 Hz, 1H), 7.04-6.85 (m, 4H), 6.60 (d, J = 3.1 Hz, 1H), 6.35 (d, J = 8.5 Hz, 1H, -NH), 5.0 (m, 1H), 4.85 (s, 2H), 4.75 (s, 2H), 3.10-3.0 (dd, J = 17.1 Hz, J = 4.5 Hz, 1H, Asp), 2.70-2.60 (dd, J = 17.1 Hz, J = 5.4 Hz, 1H, Asp), 1.25 (s, 9H, tBu).

(S)-5-(2,6-Difluorophenoxy)-3-(2-(5-fluoro-1H-indol-1-yl)acetamido)-4-oxopentanoic acid (26a):

The compound 26a has been prepared according to the method described for the compound 10a employing the compound 25a (120 mg, 0.2 mmol) and TFA (1 mL) to afford the crude product which was purified by preparatory HPLC to afford 26a as cream colour solid (64 mg, 60%). Molecular formula: C_{21}H_{17}F_{3}N_{2}O_{5} (m/z: 434); LCMS: m/z = 435.1 (M^{+}+1); m.p. 174-177 °C; IR (KBr): 3315, 2966, 1732, 1698, 1661, 1592, 1491, 1297 cm^{-1}; ^{1}H NMR (DMSO-d_{6}, 300 MHz): δ 13.50-12.50 (brs, 1H, -COOH), 8.70 (d, J = 9.1 Hz, 1H, -NH), 7.45 (d, J = 8.2
Hz, 1H), 7.38-7.28 (m, 2H), 7.15-7.05 (m, 3H), 6.90 (t, 1H), 6.42 (d, \( J = 6.1 \) Hz, 1H), 5.05-4.80 (m, 4H), 4.80-4.70 (m, 1H), 2.75-2.60 (m, 2H, \( \text{CH}_2\text{COOH} \)); \(^{13}\text{C}\) NMR (CD\(_3\)OD, 400 MHz): \( \delta \) 174.39, 170.8, 160.5, 158.2, 155.6, 134.59, 131.9, 131.6, 130.5, 124.9, 113.4, 113.15, 111.38 (m), 110.9, 106.4, 106.2, 103, 76.14, 51.9, 50.1, 35.4; HPLC purity: 95%.

(3S)-tert-Butyl 3-(2-(5-chloro-1H-indol-1-yl) acetamido)-5-(2,6-difluorophenoxy)-4-hydroxypentanoate (24b): The compound 24b has been prepared according to the method described for the compound 8a employing the compound 7b (108 mg, 0.5 mmol) and 23 (180 mg, 0.6 mmol) to afford 24b (300 mg, 100%). Molecular formula: C\(_{25}\)H\(_{27}\)ClF\(_2\)N\(_2\)O\(_5\) (m/z: 508); LCMS: m/z = 435.1 (M\(^+\)+1-OtBu).

(S)-tert-Butyl 3-(2-(5-chloro-1H-indol-1-yl) acetamido)-5-(2,6-difluorophenoxy)-4-oxopentanoate (25b): The compound 25b has been prepared according to the method described for the compound 9a employing the compound 24b (300 mg, 0.6 mmol) and Dess-Martin periodinane (274 mg, 0.65 mmol) to afford the crude product which was purified by silica gel column chromatography (0.3% methanol-DCM) to afford 25b as cream colour solid (150 mg, 50%). Molecular formula: C\(_{25}\)H\(_{25}\)ClF\(_2\)N\(_2\)O\(_5\) (m/z: 506); LCMS: m/z = 507.1 (M\(^+\)+1); \(^1\text{H}\) NMR (CDCl\(_3\), 300 MHz): \( \delta \) 7.60 (d, \( J = 0.3 \) Hz, 1H), 7.20-7.10 (m, 2H), 7.04-6.85 (m, 4H), 6.60 (d, \( J = 3.3 \) Hz, 1H), 6.35 (d, \( J = 8.4 \) Hz, 1H, -NH), 5.0 (m, 1H), 4.85 (s, 2H), 4.75 (s, 2H), 3.10-3.0 (dd, \( J = 15.6 \) Hz,
\[ J = 6.1 \text{ Hz}, \text{1H, Asp}\), \[ 2.75-2.60 \text{ (dd, } J = 15.5 \text{ Hz, } J = 5.5 \text{ Hz, 1H, Asp)}, \]

1.20 (s, 9H, \text{tBu}).

\[ (S\)-3-(2-(5-Chloro-1H-indol-1-yl)acetamido)-5-(2,6-difluoro phenoxo)-4-oxopentanoic acid (26b):

\[ \text{The compound} \text{ 26b has been prepared according to the method described for the compound 10a employing the compound 25b (150 mg, 0.2 mmol) and TFA (0.7 mL) to afford the crude product which was purified by preparatory HPLC to afford 26b as cream colour solid (90 mg, 67%). Molecular formula: C}_{21}\text{H}_{17}\text{ClF}_{2}\text{N}_{2}\text{O}_{5} \text{ (m/z: 450); LCMS: m/z = 451.1 (M}^{+1}); m.p. 169-172 ^\circ \text{C; IR (KBr): 3307, 2945, 1736, 1658, 1594, 1499, 1475, 1292 cm}^{-1}; ^1\text{H NMR (DMSO-d}_6, 300 MHz): } \delta \text{ 8.75 (d, } J = 8.4 \text{ Hz, 1H, -NH)}, 7.60 \text{ (s, 1H), 7.45-7.35 (m, 2H), 7.16-7.05 (m, 4H), 6.45 (d, } J = 3.2 \text{ Hz, 1H), 5.05-4.85 (m, 4H), 4.70 (m, 1H), 2.75-2.70 (m, 2H, CH}_2\text{COOH); } ^{13}\text{C NMR (CD}_3\text{OD, 300 MHz): } \delta \text{ 173, 169.2, 157, 153.7, 135, 130.3, 129.9, 125, 123.4, 121.5, 119.6, 119.48, 112, 111.8, 110.3, 110.1, 101.3, 74.8, 51, 48.9, 34.49; HPLC purity: 97%.

\[ (3\text{S})\text{-tert-Butyl } \text{3-(2-(5-bromo-1H-indol-1-yl) acetamido)-5-(2,6-difluorophenoxy)-4-hydroxypentanoate (24c): The compound 24c has been prepared according to the method described for the} \]
compound 8a employing the compound 7c (131 mg, 0.5 mmol) and 23 (180 mg, 0.6 mmol) to afford 24c (340 mg, 100%). Molecular formula: C_{25}H_{27}BrF_{2}N_{2}O_{5} (m/z: 553); LCMS: m/z = 479.0 (M+2-tBu-OH).

(S)-tert-Butyl 3-(2-(5-bromo-1H-indol-1-yl) acetamido)-5-(2,6-difluorophenoxy)-4-oxopentanoate (25c): The compound 25c has been prepared according to the method described for the compound 9a employing the compound 24c (340 mg, 0.6 mmol) and Dess-Martin periodinane (286 mg, 0.7 mmol) to afford the crude product which was purified by silica gel column chromatography (0.4% methanol-DCM) to afford 25c as cream colour solid (110 mg, 32%). Molecular formula: C_{25}H_{25}BrF_{2}N_{2}O_{5} (m/z: 551); LCMS: m/z = 497.0 (M^+2-tBu); \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 300 MHz): δ 7.78 (d, J = 0.3 Hz, 1H), 7.30 (d, J = 8.1 Hz, 1H), 7.15 (d, J = 9 Hz, 1H), 7.10 (d, J = 6 Hz, 1H), 7.05-6.85 (m, 3H), 6.58 (d, J = 6.2 Hz, 1H), 6.35 (d, J = 9.1 Hz, 1H, -NH), 5.0 (m, 1H), 4.85 (s, 2H), 4.75 (s, 2H), 3.10-3.0 (dd, J = 15.1 Hz, J = 6.2 Hz, 1H), 2.75-2.60 (dd, J = 15.5 Hz, J = 5.5 Hz, 1H), 1.25 (s, 9H, tBu).

(S)-3-(2-(5-Bromo-1H-indol-1-yl)acetamido)-5-(2,6-difluorophenoxy)-4-oxopentanoic acid (26c):

![Chemical structure of 26c](image)

The compound 26c has been prepared according to the method described for the compound 10a employing the compound 25c (130
mg, 0.2 mmol) and TFA (0.7 mL) to afford the crude product which was purified by preparatory HPLC to afford **26c** as cream colour solid (49 mg, 42%). Molecular formula: C$_{21}$H$_{17}$BrF$_2$N$_2$O$_5$ (m/z: 495); LCMS: m/z = 497.0 (M$^+$+2); m.p. 166-171 °C; IR (KBr): 3306, 2938, 1737, 1652, 1592, 1498, 1474, 1293 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$, 300 MHz): δ 8.75 (d, $J$ = 8.2 Hz, 1H, -NH), 7.72 (d, $J$ = 0.5 Hz, 1H), 7.40-7.30 (m, 2H), 7.20-7.08 (m, 4H), 6.45-6.40 (d, $J$ = 6.3 Hz, 1H), 5.05-4.80 (m, 4H), 4.70 (m, 1H), 2.75-2.60 (m, 2H, CH$_2$COOH); $^{13}$C NMR (CD$_3$OD, 300 MHz): δ 173.3, 169, 157, 153.8, 135.29, 130.6, 129.9, 124.1, 123.47, 123.35, 122.8, 122.6, 112.5, 112, 111.89, 11.79, 110.6, 101.37, 74.9, 51.4, 48.9, 34.47; HPLC purity: 94%.

**(3S)-tert-Butyl 5-(2,6-difluorophenoxy)-4-hydroxy-3-(2-(5-methoxy-1H-indol-1-yl) acetamido) pentanoate (24d):** The compound **24d** has been prepared according to the method described for the compound **8a** employing the compound **7d** (106 mg, 0.5 mmol) and **23** (180 mg, 0.6 mmol) to afford **24d** (300mg, 100%). Molecular formula: C$_{26}$H$_{30}$F$_2$N$_2$O$_6$ (m/z: 504); LCMS: m/z = 449.1 (M$^+$+1-tBu).

**(S)-tert-Butyl 5-(2,6-difluorophenoxy)-3-(2-(5-methoxy-1H-indol-1-yl) acetamido)-4-oxopentanoate (25d):** The compound **25d** has been prepared according to the method described for the compound **9a** employing the compound **24d** (300 mg, 0.6 mmol) and Dess-Martin periodinane (277 mg, 0.65 mmol) to afford the crude product which was purified by silica gel column chromatography (0.3% methanol-DCM) to afford **25d** as cream colour solid (180 mg, 61%). Molecular
The compound 26d has been prepared according to the method described for the compound 10a employing the compound 25d (160 mg, 0.3 mmol) and TFA (1 mL) to afford the crude product which was purified by preparatory HPLC to afford 26d as brown solid (13 mg, 9%). Molecular formula: C_{22}H_{20}F_{2}N_{2}O_{6} (m/z: 446); LCMS: m/z = 447.1 (M^+1-tBu); m.p. 70-71 °C; \(^1\)H NMR (DMSO-d_6, 300 MHz): δ 8.70-8.60 (brs, 1H), 7.35-7.20 (m, 2H), 7.15-7.10 (m, 3H), 7.05 (d, J = 3.2 Hz, 1H), 6.70 (dd, J = 12.1 Hz, J = 3.3 Hz, 1H), 6.35 (d, J = 3.3 Hz, 1H), 5.10-4.80 (m, 4H), 4.70 (m, 1H), 3.75 (s, 3H, OCH_3), 2.75-2.65 (m, 2H, CH_2COOH).

(3S)-\textit{tert}-Butyl 5-(2,6-difluorophenoxy)-4-hydroxy-3-(2-(5-methoxy-1H-indol-1-yl) acetamido)pentanoate (24e): The compound 24e has
been prepared according to the method described for the compound 8a employing the compound 7e (98 mg, 0.5 mmol) and 23 (180 mg, 0.6 mmol) to afford 24e (280 mg, 100%). Molecular formula: C_{26}H_{30}F_{2}N_{2}O_{5} (m/z: 488); LCMS: m/z = 433.1 (M+1-tBu).

(S)-tert-Butyl 5-(2,6-difluorophenoxy)-3-(2-(2-methyl-1H-indol-1-yl)acetamido)-4-oxopentanoate (25e): The compound 25e has been prepared according to the method described for the compound 9a employing the compound 24e (280 mg, 0.6 mmol) and Dess-Martin periodinane (267 mg, 0.6 mmol) to afford the crude product which was purified by silica gel column chromatography (0.4% methanol-DCM) to afford 25e as cream colour solid (80 mg, 29%). Molecular formula: C_{26}H_{28}F_{2}N_{2}O_{5} (m/z: 486); LCMS: m/z = 431.0 (M+1-tBu); \(^1\)H NMR (CDCl\(_3\), 300 MHz): δ 7.52 (d, J = 6.2 Hz, 1H), 7.25-7.0 (m, 3H), 6.9-6.85 (m, 3H), 6.36 (s, 1H), 6.25 (d, J = 8.5 Hz, 1H, -NH), 5.0 (m, 1H), 4.80 (s, 2H), 4.70 (d, J = 0.5 Hz, 2H), 3.0 (dd, J = 15.6 Hz, J = 6.1 Hz, 1H, Asp), 2.65 (dd, J = 15.4 Hz, J = 5.5 Hz, 1H, Asp), 2.40 (s, 3H, -CH\(_3\)), 1.25 (s, 9H, tBu).

(S)-5-(2,6-Difluorophenoxy)-3-(2-(2-methyl-1H-indol-1-yl)acetamido)-4-oxopentanoic acid (26e):
The compound **26e** has been prepared according to the method described for the compound **10a** employing the compound **25e** (80 mg, 0.2 mmol) and TFA (0.5 mL) to afford the crude product which was purified by preparatory HPLC to afford **26e** as brown solid (36 mg, 51%). Molecular formula: C_{22}H_{20}F_{2}N_{2}O_{5} (m/z: 430); LCMS: m/z = 431.0 (M^+1); m.p. 82-86 °C; IR (KBr): 3315, 2959, 1732, 1704, 1658, 1593, 1499, 1475, 1296 cm\(^{-1}\); \(^1\)H NMR (DMSO-d\(_6\), 300 MHz): \(\delta\) 8.70 (d, \(J = 9.2\) Hz, 1H, -NH), 7.40 (d, \(J = 6.3\) Hz, 1H), 7.30 (d, \(J = 6.1\) Hz, 1H), 7.15-7.05 (m, 3H), 7.0-6.95 (m, 2H), 6.20 (s, 1H), 5.10-4.80 (m, 4H), 4.75 (m, 1H), 2.75-2.65 (m, 2H, CH\(_2\)COOH), 2.35 (s, 3H, CH\(_3\)); \(^{13}\)C NMR (CD\(_3\)OD, 400 MHz): \(\delta\) 174.45, 171.27, 158.07, 155.61, 132.8, 129.88, 124.78, 121.99, 120.75, 120.51, 113.43 (m), 109.64, 101.99, 76.19, 51.8, 46.7, 35.45, 11.2.
[Image of an NMR spectrum with chemical structure and parameters]
**Peak List for "TWC of DAD Spectral Data: from Sample 1 (IN1080-060) of Data30010910.wiff"**

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Mass spectrum of 10c
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**LCMS spectrum of 18a**
**AURIGENE DISCOVERY TECHNOLOGIES LTD**

**LC/MS REPORT**

**Data file:** C:\CHEM32\1\DATA\0902090000016.D  **Vial No.:** P1-8-01

**Injection Date:** 09/02/2009  **Injection vol.:** 3µL

**Sample Name:** IN1060-073  **Acq Method:** ATLANTIS.M

**Method info:**
- **Column:** AG/C18-5-001
- **Mobile Phase:** A=0.1% Formic Acid B=0.1% FA in 900ml ACN+100ml H2O
- **Flow:** 0.8ml/min, Temp: 30.0°C
- **Gradient (Time/%):** 0/70, 1.0/70, 1.5/95, 2.5/95, 3.0/70, 5.0/70

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**MSD1 SPC, time=3.421 of C:\CHEM32\1\DATA\0902090000016.D MM-ES, Pos. Scan, Frag. 50**

**MASS spectrum of 19a**
$^{1}H$ NMR spectrum of 20a

Pulse Sequence: 62961
Solvent: DMso
Temp: 27.0 °C, 295.1 K

Data: 100,000 scans 100 MHz
Acquire Time: 1.9usec
(fluorine 390, deuterium 3.9usec)

OBSERVED RESONANCE: 5.9-10.7 ppm
DATA PROCESSING: Total time 0 min, 26 sec

![Chemical Structure]
Peaks List for "TWC of DAD Spectral Data: from Sample 1 (IN1060-092P) of Data27020925.wiff"

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MASS spectrum of 20a
$^{1}$H NMR spectrum of 19b
MASS spectrum of 19b
$^1$H NMR spectrum of 20b
TIC of Q1: from Sample 1 (IN1958-163) of Data16091022.wiff (Turbo Spray) Max. 6.6x10^6 cps.

Q1: 1.616 to 1.750 min from Sample 1 (IN1958-163) of Data16091022.wiff (Turbo Spray), subtr...

TWC of DAD Spectral Data: from Sample 1 (IN1958-163) of Data16091022.wiff

MASS spectrum of 20b
Pulses Sequence: e2pul
Solvent: DMSO
Temp. 23.0 C / 295.1 K
File: IN1060-09SP
INOVA-600 "tealal"
Relax. delay 1.000 sec
Pulses 42.9 degrees
Acq. time 1.997 sec
Width 6000.0 Hz
15 repetitions
OBSERVE R1, 289.9690514 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FT size 32768
Total time 0 min, 48 sec

$^1$H NMR spectrum of 20e
**Mass Spectrum of 20e**

*Sample ID: 100  
Sample Name: IN1060-095P  
Acq. File: Data20020916.wiff  
Polarity/Scan Type: Positive Q1 ND  
Acq. Date: Saturday, February 28, 2009*

![Mass Spectrum Diagram]
**AURIGENE DISCOVERY TECHNOLOGIES LTD**

**LC/MS REPORT**

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**Method info**

- Column: Ag/C18-5-001
- Mobile Phase: A=0.1% Formic Acid B=0.1% FA in 900ml ACN+100ml H2O
- Flow: 0.8ml/min, Temp: 30.0°C
- Gradient (Time/%B): 0/70, 1.0/70, 1.5/95, 2.5/95, 3.0/70, 5.0/70

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**DAD1 C, Sig=210.4 Ref=off (2302009000008.D - 2302009000001.D)**

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**MASS spectrum of 25c**
Pulse Sequence: se2pul
Solvent: DMSO
Temp. 22.0 °C / 295.1 K
File: IM1598-004
INNOVA-600 "teslal"
Relax. delay 1.000 sec
Pulse 42.3 degrees
Acq. time 1.997 sec
Width 6006.0 Hz
12 repetitions
OBSERVE H1, 299.9690492 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FF size 2048
Total time 0 min, 36 sec

$^1$H NMR spectrum of 26c
MASS spectrum of 26c
AURIGENE DISCOVERY TECHNOLOGIES LTD

LC/MS REPORT

Data file: C:\CHM321\DATA\230209000007.D  Vial No.: P1-A-04
Injection Date: 23/02/2003  Injection vol.: 3uL
Sample Name: IN1060-090  Acq Method: ATLANTIS.M

Method info:
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Gradient (Time/%) : 0/70, 1.0/70, 1.5/95, 2.5/95, 3.0/70, 5.0/70.

Peak  RT  Area  Area %
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2  12.126  3.881e+001  1.847  
3  13.226  1.800e+003  85.674  
4  13.369  8.397e+001  3.996  
5  13.895  5.351e+002  2.546  
6  14.181  1.030e+002  4.903  

MSSD TIC, MS File: C:\CHM321\DATA\230209000007.D  MM-ES, Pos, Scan, Frag: 50

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MSSD SPC, file=4.235 of C:\CHM321\DATA\230209000007.D  MM-ES, Pos, Scan, Frag: 50

MASS spectrum of 25e
**TIC of +Q1 from Sample 1 (IN1060-100) of Data13030949.wiff (Turbo Spray)**

Max. 1.1e8 cp

**+Q1: 1.349 to 1.848 min from Sample 1 (IN1060-100) of Data13030949.wiff (Turbo Spray), sub...**

Max. 2.5e6 cp

**TIC of DAD Spectral Data: from Sample 1 (IN1060-100) of Data13030949.wiff**

Max. 1.4e4 mAU

**Peak List for "TIC of DAD Spectral Data: from Sample 1 (IN1060-100) of Data13030949.wiff"**

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**MASS spectrum of 26e**