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Computer based design, synthesis and biological evaluation of novel indole derivatives as HCV NS3-4A serine protease inhibitors

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ABSTRACT

A series of novel indoles were designed and their molecular modeling simulation study including fitting to a 3D pharmacophore model using CATALYST program and their docking into the NS3 active site was examined as HCV NS3 protease inhibitor. Several compounds showed significant high simulation docking score and fit values. The designed compounds were synthesized and biologically evaluated in vitro using an NS3 protease binding assay, where compounds **10a–k** showed significant inhibitory activity (⩾67% inhibition at 100 μg/mL). Of these, compounds **10c** and **10f** demonstrated potent HCV NS3 protease inhibitors with IC_{50} values of 15 and 13 μM, respectively. Enantio-selective Michael addition of an indole derivative in the presence of catalytic amount of $AlCl_3$ and quinine at room temperature afforded the adduct **7e** in excellent yield with 73% ee. The product was converted into **10l**, which showed lower activity than the mixture of the corresponding diastereoisomers.

1. Introduction

Hepatitis C virus (HCV) was identified in 1989 (Choo et al.) as the etiological agent for non-A, non-B hepatitis, which is a lethal single positive-stranded RNA virus.[1] An estimated 200 million cases of HCV infection exist worldwide.[2] Of those infected, over 85% will develop chronic hepatitis, and 20% of the chronic infections progress to liver cirrhosis and hepatocellular carcinoma.[3]

Presently, there is no vaccine for HCV and there is no broadly effective therapy for all genotypes of HCV.[4] The current approved therapy with interferon-α (IFN-α) is only partially effective and treatment is accompanied by undesirable side effects.[5] Furthermore, many of those who begin therapy with the current standard (IFN-α) with ribavirin do not achieve viral clearance and the sustained virological response (SVR) rates were limited (below 50%), thereby resulting in limited patient compliance.[6] Thus, it is highly desirable to develop effective chemotherapeutic agents for treatment of HCV-infected patients. The viral RNA genome encodes a polyprotein that includes two proteases; the NS2-3 protease mediates a single cleavage at the NS2/NS3 junction, whereas the NS3-4A protease cleaves at four downstream sites in the polyprotein. NS3-4A is characterized as a serine protease with a chymotrypsin-like fold.[7] The catalytic activity of NS3 is augmented by formation of complex with the viral NS4A protein, and heterodimeric NS3-NS4A is believed to be the physiologically relevant form of the enzyme,[8,9] and plays a pivotal role in replication of the HCV virus.[10] Inhibition of this enzyme has proven effective in reducing viral loads in humans, and considerable efforts by different research groups have been directed toward development of HCV NS3 protease inhibitors.[11] There have been several studies of the three-dimensional structure of HCV protease, including the holo-enzyme and inhibitor complexes. There are now several released crystal structure of the HCV protease in the PDB. The binding modes of inhibitors have been elucidated in multiple structure of inhibitor/protease complexes.[12] Harper and co-workers[13] proposed a structure–activity relationship (SAR) and disclosed enzyme bound crystal structure for indoline based peptidomimetic replacements for the N-terminal amino acid of **1** (Fig. 1).

They found that the heterocyclic scaffolds, which replace the N-terminal amino acid and capping group in peptides inhibitors, could (i) retain the double hydrogen bonding interaction with Ala157 observed in the enzyme bound crystal structure of peptide based inhibitors[14]; (ii) orient a p2-p1amino acid sequence as an active site 'anchor'; (iii) direct pendant functionality to engage the P3/S2 side chain interaction; and (iv) function as a bridge to auxiliary binding regions, such as those accessed by the N-terminal capping group in the peptide series. Our current investigation is based on optimization of lead compound by molecular modeling studies (pharmacophores model and docking), using the enzyme bound crystal structure of indoline based peptidomimetic inhibitor, which involve the synthesis of new indole derivatives by the replacement of the indoline ring of the lead compound **2** with indole. (i) The nitrogen of indole ring system makes H-bond donor with Ala157 and also can accommodate the requisite lipophilic interaction with S4,S6[13]; (ii) additionally, from the molecular mod-

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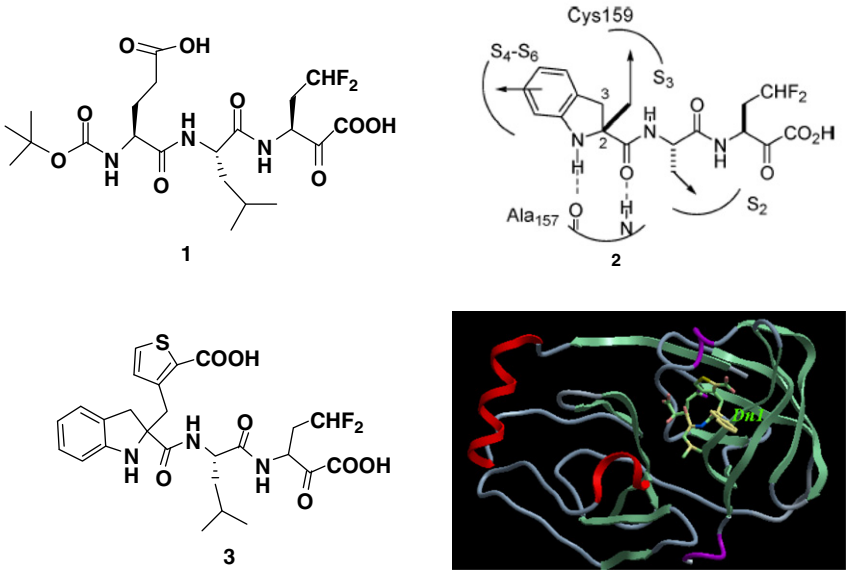


Figure 1. NS3/4A protease inhibitors and the crystal structure of compound 3 with the NS3/4A complex.

eling studies it was found that aryl substitution on side chain increases the hydrophobic interaction with Cys 159 more than aliphatic substitution; (iii) amidic carbonyl group side chain makes H-bond with Gly 137 and Ser 139; and (iv) amino acid residue interacts with lys 136 S2.

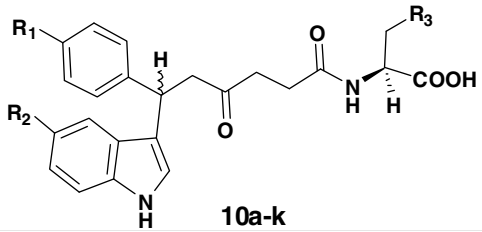
In this project, molecular modeling simulation studies were performed in order to predict the biological activity of the proposed compounds. Firstly, the hypothesis generation was performed by the manual technique using CATALYST software.[15] This method is applicable when the 3D structure of the bioactive conformation is known. The main idea of this approach is to identify the ideal hypothesis, which represents the geometry of the active sites as a collection of functional groups in space. The generated HCV NS3 protease inhibitors hypothesis was subjected to simulation compare/fit studies, using the best fit algorithm, with the conformational model of a test set of the proposed compounds (**10a**–**k**). Secondly, Docking Study Using Molsoft ICM software was also performed, where the crystal structure of inhibitor (**3**)/NS3 protease complex[13] was obtained from protein data bank website (pdb). This regularized protein complex structure was used in determination of the active site that is mentioned in the literature. Docking process was carried out for the test set of compounds (**10a**–**k**) using the compound energy as scoring function.[16] Retrospectively, we were pleased to note that the docking results were consistent with the hypothesis fit values discussed below (Table 1). The designed compounds which showed high fit values and docking scores were synthesized following the sequence outlined in Schemes 1, 2, predicting that they would have a potent HCV NS3 serine protease inhibitors.

2. Results and discussion

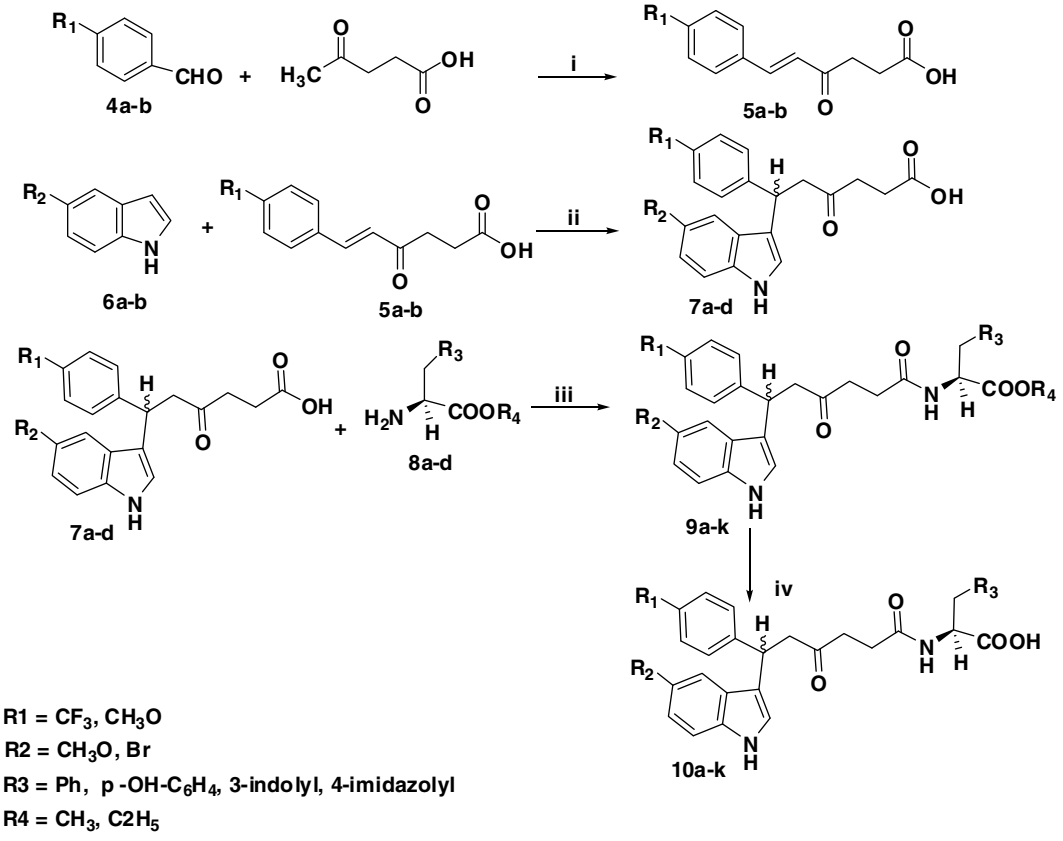
2.1. Synthesis

For the synthesis of the target compounds **10a**–**k**, the following straightforward pathway was pursued: This involved conden-

Table 1
Best fit and docking conformer for each compound in the test set (**10a**–**k**) mapped with generated hypothesis and active site of HCV protease



Entry	R_1	R_2	R_3	Compound	Fit value	Docking value (kcal/mol)
1	CH_3O	CH_3O	*p*-OH–C_6H_4	**10a**	4.65	−86.16
2	CH_3O	CH_3O	3-Indolyl	**10b**	4.61	−79.15
3	CH_3O	CH_3O	Phenyl	**10c**	4.71	−89.96
4	CF_3	CH_3O	*p*-OH–C_6H_4	**10d**	4.64	−90.48
5	CF_3	CH_3O	3-Indolyl	**10e**	4.59	−88.71
6	CF_3	CH_3O	Phenyl	**10f**	4.69	−91.67
7	CH_3O	Br	*p*-OH–C_6H_4	**10g**	4.41	−79.91
8	CH_3O	Br	3-Indolyl	**10h**	4.53	−76.11
9	CF_3	Br	*p*-OH–C_6H_4	**10i**	4.64	−84.13
10	CH_3O	CH_3O	4-Imidazolyl	**10j**	4.57	−86.97
11	CF_3	CH_3O	4-Imidazolyl	**10k**	4.67	−88.43



Scheme 1. Reagents: (i) pipridine, acetic acid, benzene; (ii) dry ethanol, I_2 ; (iii) EDAC, 1-hydroxybenzotriazol hydrate, triethylamine, DMF; (iv) THF, ethanol, LiOH, H_2O.

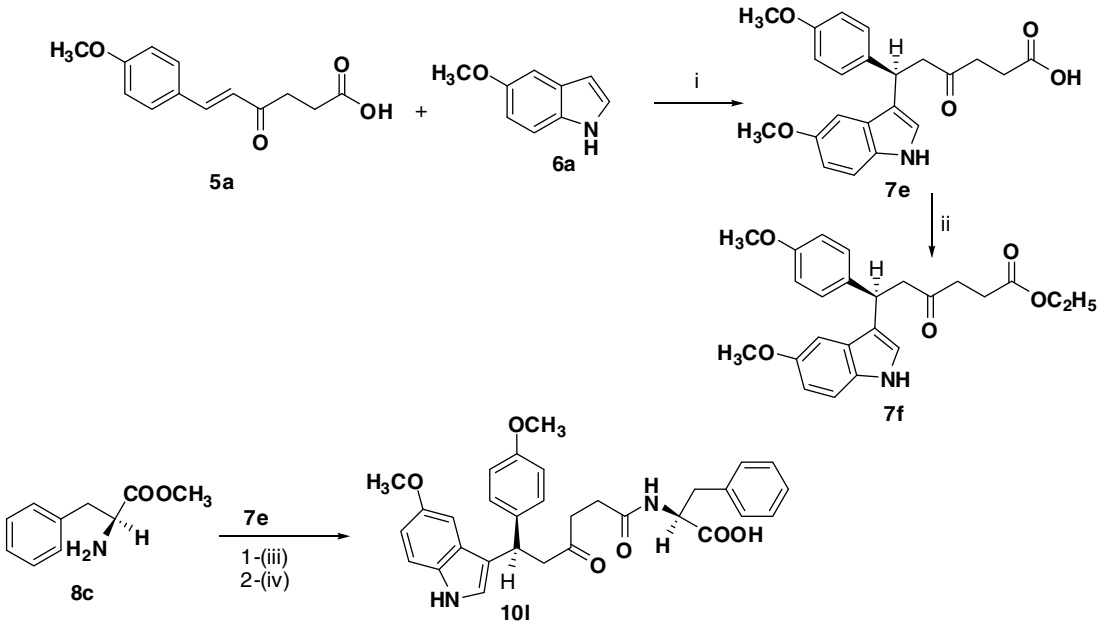
sation of appropriate aldehydes **4a**–**b** and levulenic acid using catalytic amounts of piperidine and acetic acid in benzene under reflux, with azeotropic removal of water using a Dean-Stark trap to give hexenoic acid derivatives **5a**–**b**.[17] Under these conditions, the enamine formed at the α-methyl group of levulenic acid adds to the aldehyde carbon followed by dehydration to afford the arylidine keto acid derivatives **5a**–**b**. ^{1}H NMR shows the formation of *E*-isomers where $J = 16.15$ Hz. The arylidene derivatives **5a**–**b** undergo Michael addition with indole derivatives **6a**–**b** in the presence of catalytic amount of molecular I_2[18] at room temperature to afford the corresponding adducts **7a**–**d**. The attack on the indole nucleus occurred exclusively at the 3-position. N-alkylation products have not been observed. Condensation reactions of acidic derivatives **7a**–**d** with amino acid esters **8a**–**d** using EDAC gave the corresponding ester derivatives **9a**–**k** as a diastereoisomeric mixture, which was considered as a prodrug.[19] The obtained esters **9a**–**k** were mildly hydrolyzed to the target carboxylic acids **10a**–**k** using lithium hydroxide at room temperature.[20]

The above mentioned method for addition of indole to α,β-unsaturated ketones using molecular I_2 affords **7a**–**d** in racemic mixture.[18] The asymmetric version of this reaction can provide a very useful approach to synthesis of single diastereoisomer of the target compounds. Herein, we achieve effective asymmetric synthesis for compound **7e** (Scheme 2) through the reaction of indole **6a** with α,β-unsaturated ketone **5a** using quinine and $AlCl_3$ as a Lewis acid in chloroform to afford **7e** with $[\alpha]_D^{21}$ +16.51 (*c* 0.76%, methanol). The optical purity = 73% ee. was determined by HPLC using chiral column Daicel chiralpak AD (0.46 cm × 25 cm) eluting with hexane/*i*-PrOH = 95:5. The optically active intermediate **7e** was then transformed into **10l**, $[\alpha]_D^{22}$ −11.83 (*c* 0.79%, methanol).

2.2. Molecular modeling

2.2.1. Generation of HCV NS3 protease inhibitors hypothesis

In this research, the bioactive conformer 3 was extracted from the crystal structure of 3 with the NS3/NS4 complex published by Harper et al. from 1w3c 'PDB' file using Cerus2 software. CATALYST software was used for manual generation of hypothesis. The validated hypothesis encompassed five features, namely hydrogen-bond acceptor (A), hydrogen-bond donor (D), two hydrophobic (H1 and H2), and negative ionizable (N) (Fig. 2). Recently, Wei et al.[21] reported a Hypogen-derived hypothesis generated from a set of HCV NS3 protease inhibitors. Such a reported CATALYST/HypoGen hypothesis consisted of five pharmacophore features, namely hydrophobic, hydrophobic aromatic, hydrogen-bond donor, hydrogen-bond acceptor, and negative ionizable. However, the dimensions between the features in these Hypogen hypotheses were not published. Herein, we report a new five-feature hypothesis (Fig. 2) that could be generated from the bioactive conformer **3** in order to qualitatively prioritize the biological activity of the test set of HCV NS3 protease inhibitors. Such a hypothesis has the same types of features such as the reported Hypogen hypothesis. Additionally, we recorded the constraint angles and distances between the different features of our hypothesis (see Supporting Information). Crucially, we exploited our pharmacophore model to design a small number of novel and potent HCV NS3 protease inhibitors, and these were evaluated in vitro. The structures of the test set of the target indoles were built using the CATALYST software, and their conformational models were generated in the energy range of 20 kcal/mol above the estimated global energy minimum. The fitting of the tested compound was performed using Best fit during the compare/fit process.[15] Different mappings for all the conformers of each compound of the test set



Scheme 2. Reagents: (i) quinine, $AlCl_3$, $CHCl_3$; (ii) dry ethanol, H_2SO_4; (iii) EDAC, 1-hydroxybenzotriazol hydrate, triethylamine, DMF, (iv) THF, ethanol, LiOH, H_2O.

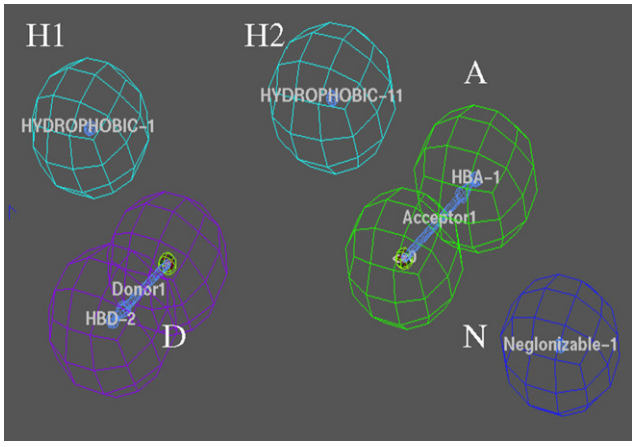


Figure 2. Hypothesis of HCV NS3 protease inhibitors.

to the hypothesis were visualized (Fig. 3) and the fit values of the best-fitting conformers were found (Table 1).

2.2.2. Docking Study Using Molsoft ICM software

This technique considered as direct molecular modeling, where the 3D structure of the enzyme was known and crystal structure of inhibitor **3** with the NS3/4A protease complex was used in determination of the active site, after regularization of protein and global optimization of side chains,[22] from the attached residues to the ligand that are mentioned in the literature (Fig. 4a). In the flexible-ligand-rigid enzyme docking, the enzyme was represented by six potential energy maps, namely electrostatic, hydrogen bond, hydrophobic, and three van der Waals. Interactive docking using Mol table ligand was carried out for all the conformers of each compound of the test set (**10a**–**k**) to the selected active site of HCV protease. Each docked compound was assigned a score according to its fit in the ligand binding pocket (LBP) (Table 1). These molecular modeling simulation studies revealed that compounds **10a**, **10c**, **10d**, and **10f** could be considered as promising candidates, due to their high fit values as well as their high docking scores. Accordingly, we synthesized these compounds and examined their biological activity.

2.3. HCV protease assay

A HCV protease binding assay was carried out by competitive displacement of the binding of peptide substrate (Ac-Asp-Glu-Asp(EDANS)-Glu-Glu-Abu-ψ), the fluorescence intensities were measured, and the inhibition percentages were calculated (see Supporting Information).[19] The experiments were performed on the candidate compounds as shown in Table 2. Over 67% inhibitory

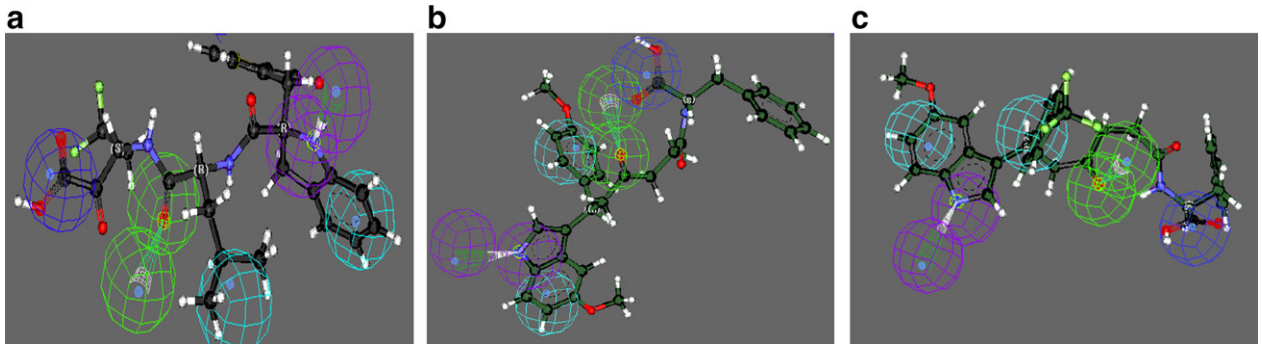


Figure 3. Mapping of (a) compound **3**, (b) compound **10c**, and (c) compound **10f** with the generated HCV NS3 protease inhibitors hypothesis.

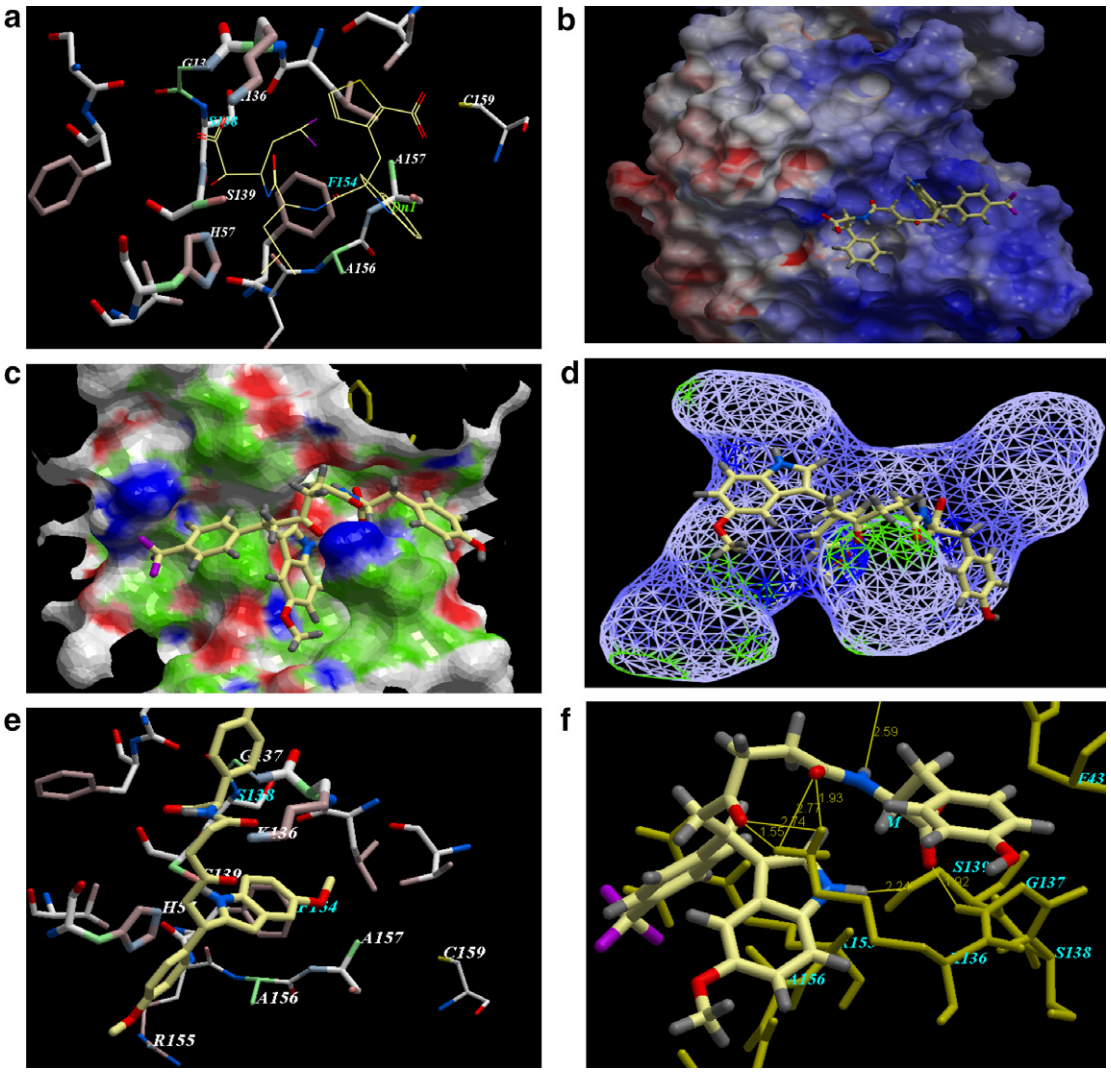


Figure 4. Docking of (a) compound **3**, (b) **10f**, (c) compound **10a**, (d) compound **10b**, and (e) **10c**, and (f) compound **10d** to different representations for Y-shaped active site of HCV NS3/4A protease enzyme.

Table 2
Inhibitory effects of compounds **10a–k** against hepatitis C virus (HCV) serine protease

Compound	HCV-PR inhibition (%) at100 μg/Ml	HCV-PR inhibition (%) at 50 μg/mL	HCV-PR inhibition (%) at 10 μg/mL
10a	78.8 ± 1	69.9 ± 2.7	29.6 ± 6
10b	67.4 ± 0.9	54.4 ± 5.8	—
10c	85.7 ± 0.2	84.4 ± 0.5	43.7 ± 3.5
10d	84.3 ± 0.9	57.1 ± 2.5	35.2 ± 4.3
10e	76.9 ± 0.7	51.2 ± 1.5	—
10f	84.9 ± 0.1	67.2 ± 0.8	48.5 ± 2.8
10g	68.7 ± 6.6	41.5 ± 8	—
10h	72.6 ± 0.1	54.8 ± 0.4	—
10i	82.9 ± 2.3	54.05 ± 1.1	19.1 ± 4
10j	75.7 ± 3	59.2 ± 8	22.4 ± 3
10k	77.6 ± 1	62.5 ± 7	23.5 ± 5

effects on the HCV-PR activity were demonstrated by all tested compounds at 100 μg/mL. Of these, four compounds exhibited significant inhibitory activity (⩾83% inhibition), namely compounds **10c**, **10e**, **10f**, and **10i** (Table 2). For compounds **10b**, **10e**, and **10h**, 25 μg/mL concentration was carried out and their inhibition % were 22.4 ± 2.1, 37.5 ± 0.7, and 43.3 ± 1, respectively. The study showed that compounds **10c** and **10f** were the most potent; and their 50% inhibitory concentrations (IC_{50}) are 15 and 13, respectively (Table 3). This promising result encourages us to enantio-selective synthesis for (**10l**) to improve its inhibitory effect on serine protease. But compound **10l** showed a lower activity than the mixture of the corresponding diastereoisomers, this result could be explained by the aid of molecular docking, where it was found that the docking score of the (*R*,*S*) isomer of compound **10l** was −81.5 k cal/mol, while the other isomer (*S*,*S*) of compound **10l** was 91.4 k cal/mol (Fig. 5). This suggests that enantio-selective synthesis of (**7e**) resulted in the (*R*) isomer, and consequently we prepared the (*R*,*S*) isomer of compound **10l**.

The low docking value of (*R*,*S*) isomer of compound **10l** could be attributed to the lack of formation of hydrogen bond between the (NH) group of the indole ring system and ser 139 in the binding

Table 3
The IC_{50} values of the target compounds **10a–k**

Compound	IC_{50} (μg/mL)	Compound	IC_{50} (μg/mL)
10a	27.2	**10g**	63.3
10b	44.8	**10h**	44.1
10c	15	**10i**	39.2
10d	39	**10j**	31
10e	47.9	**10k**	30.5
10f	13	**10l**	36.5

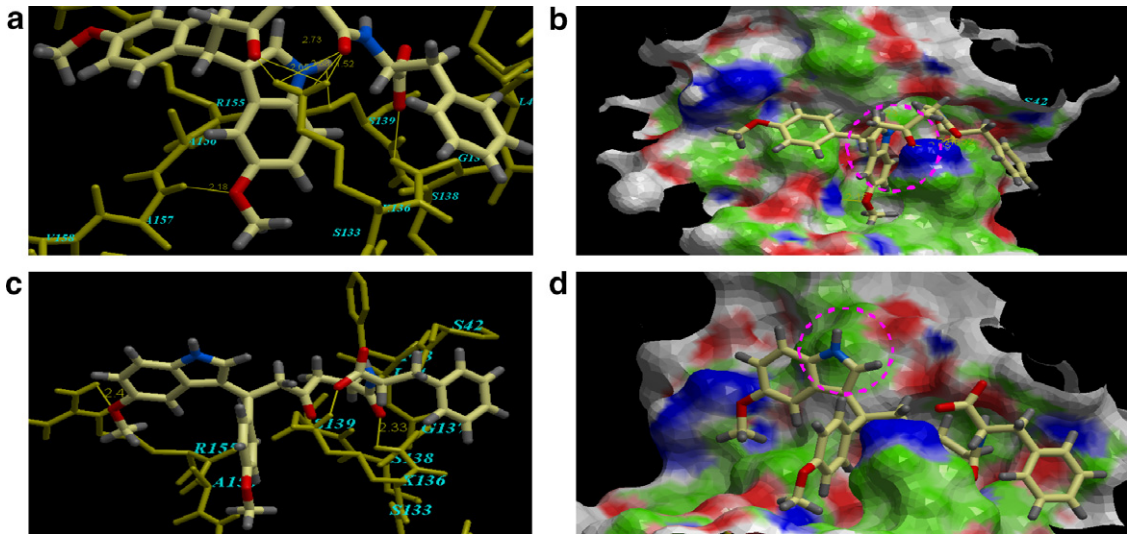
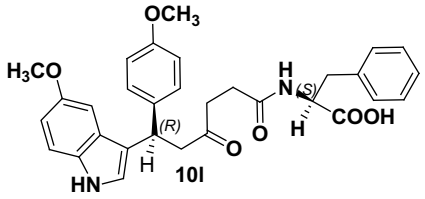


Figure 5. Docking of (a and b) (*S*,*S*) isomer of compound **10l** (c and d) (*R*,*S*) isomer of compound **10l** to active site of HCV NS3/4A protease enzyme. Hydrogen-bond donor (red), hydrogen-bond acceptor (blue), and hydrophobic (green).

site, in this conformation, while the (*S*,*S*) isomer could do (as shown in the Fig. 5).



3. Conclusion

The strategy used in this study has demonstrated that molecular modeling of inhibitor bound to NS3/4A protease structures proved to be a valuable tool in the design of a new series of potent NS3 protease inhibitor. Compounds **10a**, **10c**, **10d**, and **10f** showed high fit values and high docking scores. The synthesized compounds **10a**–**k** were biologically evaluated in vitro using HCV protease binding assay, and the result was consistent with molecular modeling study. Compounds **10c** and **10f** were the most potent; this suggests enantio-selective synthesis of **10l**, but compound **10l** showed a lower activity than the mixture of the corresponding diastereoisomer.

4. Experimental section

4.1. Chemical Experiments

All reagents and solvents were purchased from Aldrich, Wako and Tokyo Chemical Industry (TCI) and used as received. TLC was performed on silica gel 60 Partisil K6F plates (Whatman) Melting points are uncorrected and were measured on a Yanaco apparatus. The IR spectra were recorded on a JASCO FT/IR-4100 spectrometer using KBr disk.LC-MS (ESI^+) spectra were recorded with a Shimadzu LCMS-2010EV spectroscopy or on National Laboratory Center University of Hoshi, Tokyo. ^1H NMR spectral data were obtained from a Bruker 400 or vaian-500 spectrometer; TMS was used as an internal standard and solvent peak was used as an internal standard for ^{13}CNMR. The elemental analysis was performed with Yanano CHN corder MT-5 element analyzer.

4.2. General procedure for the synthesis of (5*E*)-6-(substituted)-4-oxohex-5-enoic acids (5a,5b)

Both the respective aldehyde (30 mmol) and levulinic acid (30 mmol) were dissolved in benzene (100 mL) containing acetic acid (3 mL) and piperidine (1 mL). The solution was heated under reflux using Dean-Stark water trap under argon until the theoretical amount of water had been collected (~6 h) and TLC analysis (7% methanol/$CHCl_3$) indicated disappearance of the starting material. The solvent was evaporated in vacuo and after cooling the solid product was washed twice with 10 mL of diethyl ether and then twice with 15 mL of 2 M HCl, dried and recrystallized from the appropriate solvent (given below).

4.3. (*E*)-6-[4-Methoxyphenyl]-4-oxohex-5-enoic acid (5a)

Pal yellow crystals (recrystallized from benzene); Yield: 81%; mp: 131 °C (reported 132 °C); IR ν (neat, cm^{-1}): 2934, 2838, 1736, 1709, 1646, 1599, 1571, 1422, 1306, 1254; ^1H NMR ($CDCl_3$) δ ppm 2.74 (t, J = 7.2, 2H), 3.01 (t, J = 7.2, 2H), 3.85 (s, 3H) 6.63–6.67 (d, J = 16.13, 1H), 6.93 (d, J = 8.3, 2H), 7.55–7.59 (d, J = 16.13, 1H); ^{13}C NMR (500 MHz, $CDCl_3$) δ ppm 27.86, 35.31, 55.31, 124.88, 125.93, 125.96, 127.67, 128.45, 131.66, 131.93, 132.19, 132.44, 137.77, 141.12, 178.64, 197.44; MS (EI^+): m/z: 234.2 $[M^{\cdot}]^+$.

4.4. (*E*)-6-[4-(Trifluoromethyl)phenyl]-4-oxohex-5-enoic acid (5b)

Yellow crystals (recrystallized from toluene); Yield: 78%; mp: 147–184 °C; IR ν (neat, cm^{-1}): 3443–2700 (br), 3045, 2927, 1712, 1661, 1615, 1577, 1415, 1323, 1206, 1166; ^1H NMR (400 MHz, $CDCl_3$) δ ppm 2.76 (t, J = 6.52 Hz, 2H), 3.03 (t, J = 6.52 Hz, 2H), 6.84 (d, J = 16.22 H, 1H), 7.72 (s, 4H) ; ^{13}C NMR (500 MHz, $CDCl_3$) δ ppm 27.87, 35.32, 122.71, 124.89, 125.93, 125.96, 127.67, 128.45, 131.66, 13193, 132.2, 132.45, 137.78, 141.12, 178.64, 197.44; MS (EI^+): m/z: 273.2 $[M^{\cdot} H]^+$.

4.5. General procedure for the synthesis of 6-[5-substituted-1*H*-indol-3-yl]-6-(4-substituted phenyl)-4-oxohexanoic acid (7a–d)

A mixture of respective substituted indole **6a**–**b**(3 mmol), **5a,b** (3 mmol), I2 (0.3 mmol) in dry ethanol (6 mL) was stirred in an

open vessel at room temperature (~6 h) and TLC analysis (7% methanol/$CHCl_3$) indicated disappearance of the indole derivative. After standing 2 h the reaction mixture was evaporated in vacuo,dissolved in ethyl acetate and wash by 2 M HCl, sodium bisulfide and water. The organic layer was dried over sodium sulfate anhydrous and evaporated under vacuum. The crude mixture recrystallized from the appropriate solvent (given below).

4.6. 6-(5-Methoxy-1*H*-indol-3-yl)-6-(4-methoxyphenyl)-4-oxohexanoic acid (7a)

Light yellow crystals (recrystallized from aceton/*n*-hexane); Yield: 89%; mp: 104–105 °C; IR ν (neat, cm^{-1}): 3397, 2994, 2832, 1707, 1609, 1510, 1484, 1299, 1248, 1030; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.30–2.33 (t, J = 7.15 Hz, 2H), 2.67–2.74 (m, 2H), 3.07–3.25 (m, 2H), 3.66 (s, 3H), 3.67 (s, 3H), 4.56–4.60 (t, J = 7.57 H, 1H), 6.67 (dd, J = 8.73, 2.43 H 1H), 6.78–6.80 (m, 3H), 6.93 (d, J = 2.12 H, 1H), 7.2 (d, J = 8.65 H, 3H), 7.95 (s, 1H); ^{13}C NMR (500 MHz, $CDCl_3$) δ ppm 27.59, 37.42, 37.48, 49.46, 55.18, 55.81, 101.55, 111.81, 112.08, 113.86, 118.58, 122.06, 126.91, 128.32, 128.60, 131.80, 135.90, 153.73, 158.03, 177.89, 207.76; MS (EI$^+$): m/z: 382.1 [M$^\cdot$ H]$^+$.

4.7. 6-(5-Methoxy-1*H*-indol-3-yl)-4-oxo-6-[4- (trifluoromethyl)-phenyl]hexanoic acid(7b)

Light yellow crystals (recrystallized from isopropanol); Yield: 88%; mp: 118–119 °C; IR ν (neat, cm^{-1}): 3403, 2994, 2933, 2830, 1712, 1619, 1484, 1416, 1325, 1217, 1165; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.34 (t, J = 6.52 H, 2H), 2.57–2.79 (m, 2H), 3.1-3.25 (m, 2H), 3.66 (s, 3H), 4.75 (t, J = 7.45 H, 1H), 6.68 (dd, J = 8.75, 2.38 H 1H), 6.83 (d, J = 2.27 H, 1H), 7.19 (d, J = 8.74 H, 1H), 7.26 (d, J = 2.29 H, 1H), 7.57 (s, 4H), 10.76 (s, 1H), 12.1(s, 1H); ^{13}C NMR (500 MHz, $CDCl_3$) δ ppm 27.57, 37.42, 37.48, 49.46, 55.18, 101.55, 111.81, 112.08, 113.86, 118.58, 122.06, 126.91, 128.32, 128.60, 131.80, 135.90, 153.73, 158.03, 177.89, 207.76; MS (EI$^+$): m/z: 420.1 [M$^\cdot$ H]$^+$.

4.8. 6-(5-Bromo-1*H*-indol-3-yl)-6-(4-methoxyphenyl)-4-oxohexanoic acid (7c)

Yellow crystals (recrystallized from isopropanol); Yield: 81%; mp: 142–143 °C; IR ν (neat, cm^{-1}): 3371, 3005, 2955, 2840, 1706, 1609, 1510, 1248, 1219; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.32 (t, J = 6.68 H, 2H), 2.51–2.89 (m, 2H), 3.1-3.25 (m, 2H), 3.68 (s, 3H), 4.62 (t, J = 7.51 H, 1H), 6.79 (d, J = 8.58 H 2H), 7.12 (d, J = 8.58 H, 1H), 7.22 (d, J = 8.58 H, 2H), 7.31 (d, J = 7.41 H, 1H), 7.48 (s, 1H), 11.06 (s, 1H), 12.04 (br, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm 27.50, 37.46, 49.46, 55.20, 55.81, 101.50, 111.81, 112.06, 113.85, 118.58, 122.05, 126.91, 128.60, 131.77, 135.90, 153.71, 158.02, 176.90, 207.85; MS (EI$^+$): m/z: 431.1 [M$^\cdot$ H]$^+$.

4.9. 6-(5-Bromo-1*H*-indol-3-yl)-4-oxo-6-[4-(trifluoromethyl)phenyl]hexanoic acid (7d)

Dark yellow crystals (recrystallized from isopropanol); Yield: 78 mp: 147–148 °C;%; IR ν (neat, cm^{-1}): 3403, 2926, 1709, 1618, 1459, 1324, 1218, 1164, 1122, 1067, 771; ^{1}H NMR (400 MHz, *DMSO*-d_6)δ ppm 2.32 (t, J = 7.12 H, 2H), 2.58-2.68 (m, 2H), 3.07-3.13 (m, 2H), 4.59–4.63 (t, J = 7.44 H, 1H), 6.79 (d, J = 8.69 H 2H), 7.12 (dd, J = 8.57, 1.76 H 1H), 7.22 (d, J = 8.69 H, 2H), 7.27 (d, J = 8.74 H, 1H), 7.32 (d, J = 2.12 H, 1H), 7.48 (s, 1H), 11.06 (s, 1H), 12.14 (s, 1H); ^{13}C NMR (500 MHz, $CDCl_3$)δ ppm 27.59, 37.42, 37.49, 48.55, 101.53, 111.81, 112.08, 113.86, 118.58, 122.06, 126.91, 128.32, 128.60, 131.80, 135.90, 153.73, 158.03, 177.89, 207.45; MS (EI$^+$): m/z: 467 [M$^\cdot$]$^+$, 469 [M$^\cdot$+2]$^+$.

4.10. 6-[5-Methoxy-1*H*-indol-3-yl]-6-[4-methoxyphenyl]-4-oxohexanoic acid (7e)

To cooled solution of quinine (0.4 mmol) in choroform, $AlCl_3$ (0.3 mmol) was added portionwise. The ice bath was removed after 10 min and warmed to room temperature over 1 h. Compound **5a** (1 mmol) was added to resulting mixture, followed by addition of compound **6a** (1 mmol). The reaction mixture was stirred at room temperature for overnight, then poured carfully into a solution of 1 N HCl. The organic layer was separated, and the aqueous layer was extracted with chlorofom (3×25 mL). The combined organic layer was dried over sodium sulfate anhydrous and evaporated under vacuum to provide compound 7e.

71% yield as brigh yellow solid. mp: 124–125 °C; ^{1}H NMR (400 MHz, DMSO-d_6)δ ppm 2.30–2.33 (t, J = 7.15 H, 2H), 2.67–2.74 (m, 2H), 3.07–3.25 (m, 2H), 3.66 (s, 3H), 3.67 (s, 3H), 4.56–4.60 (t, J = 7.57 H, 1H), 6.67(dd, J = 8.73, 2.43 H 1H), 6.78–6.80 (m, 3H), 6.93 (d, J = 2.12 H, 1H), 7.2 (d, J = 8.65 H, 3H), 7.95 (s, 1H); MS (EI$^+$): m/z: 382.1 [M$^\cdot$ H]$^+$; $[\alpha]_D^{21}$ +16.51 (c 0.76%, methanol). and optical purity (ee%)= 73%.

4.11. Ethyl 6-[5-methoxy-1*H*-indol-3-yl]-6-(4-methoxyphenyl)-4-oxohexanoate (7f)

To a solution of 7a (15.5 mmol) in absolut ethanol (150 mL) was added concentrated H_2SO4(2 mL), and the mixture was refluxed for 6 h. The reaction mixture was cooled to room temperature and concentrated. The residue was taken in cold water, solid sodium bicarbonate was added until the solution become basic, and the layer was extracted with diethyl ether (2×100 mL). the combiend ether layer was dried over sodium sulfate anhydrous and evaporated under vacuum. The crude product was purified by silica gel column chromatography, eluting by chloroform:methanol = 10: 0.5 (v/v) afford compound 7f yellow oil.

Yield 78%, ^{1}H NMR (400 MHz, $CDCl_3$)δ ppm 1.23 (t, J = 7.15 H, 3H), 2.51 (m, 2H), 2.67 (m, 2H), 3.11–3.28 (m, 2H), 3.74 (s, 3H), 3.75 (s, 3H), 4.75 (t, J = 7.57 H, 1H), 6.79 (m, 4H), 6.81 (d, J = 2.12 H, 1H), 7.21 (d, J = 8.65 H, 3H), 7.87 (s, 1H) ; MS (EI$^+$): m/z: 410.1 [M$^\cdot$ H]$^+$.

4.12. General procedure for the synthesis of alkyl 2-[6-(5-substituted-1*H*-indol-3-yl)-6-(4-substitutedphenyl)-4-oxo- hexanamido] propanoate (9a–k)

A mixture of respective carboxylic acid derivative (**7a–d**) (31 mmol), amino acid ester derivative (**8a–d**) (34.9 mmol) and 1-hydroxybenzotriazole hydrate (38.5 mmol) were dissolved in dimethylformamide (60 mL). The resulting solution was then placed in a water bath at 0 °C and treated with triethylamine (14 mL), followed by stirring for 10 min. To the resulting mixture 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (38.5 mmol) was added. After removing the water bath, the mixture was stirred for 18 h at room temperature. The reaction was diluted in water (200 mL), extracted with ethyl acetate, dried over sodium sulfate anhydrous, concentrated under reduced pressure and purified by silica gel column chromatography, eluting by chloroform:methanol = 10:1 (v/v) to obtain the product.

4.13. (2*S*) Ethyl 3-(4-hydroxyphenyl)-2-[6-(5-methoxy-1*H*-indol-3-yl)-6-(4-methoxyphenyl)-4- oxo hexanamido] propanoate (9a)

Yield 81%, mp 83–84 °C; IR ν (neat, cm^{-1}): 3353, 2994, 2833, 1733, 1717, 1657, 1612, 1511, 1484, 1442, 1246, 1214, 1175, 1030; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.08 (t, J = 7.09, H, 3H), 2.23 (t, J = 7.1 H, 2H), 2.60 (m, 2H), 2.7–2.84 (m, 2H) 3.05–

3.11 (m, 2H), 3.66 (s, 3H), 3.67 (s, 3H), 3.99 (q, J = 7.03, H, 2H), 4.28 (dd, J = 13.6, 8.28 H, 1H), 4.57 (t, J = 7.57, H, 1H), 6.63–6.65 (m, 4H), 6.78 (d, J = 7.98 H, 2H), 6.97 (d, J = 7.92 H, 2H), 7.16 (d, J = 2.26 H, 1H), 7.1 (s, 1H), 7.22 (d, J = 8.58 H, 2H), 8.24 (d, J = 7.63 H, 1H), 9.22 (s, 1H), 10.65 (s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6)δ ppm 13.9, 28.66, 30.74, 36.41, 37.48, 48.54, 54.01, 54.88, 55.28, 60.26, 100.88, 110.69,111.85, 113.43, 114.95, 117.82, 122.26, 126.57, 127.09, 128.46, 129.97, 131.54, 136.96, 152.70, 155.95, 157.28, 162.26, 171.26,171.68, 207.79; MS (EI$^+$): m/z: 572.2 [M$^-$]$^+$.

4.14. (2*S*) Methyl 3-(1*H*-indol-3-yl)-2-[6-(5-methoxy-1*H*-indol-3-yl)-6-(4-methoxyphenyl)-4- oxo hexanamido] propanoate (9b)

Yield 76%, mp 95–96 °C; IR v (neat, cm^{-1}): 3344 (br), 3055, 2952, 2834, 1738, 1716, 1610, 1510, 1484, 1457, 1439, 1247, 1213, 1175, 748; ^{1}H NMR (400 MHz, DMSO-d_6)δ ppm 2.28 (t, J = 7.2 H, 2H), 2.65–2.86 (m, 2H), 3.0–3.07 (m, 2H), 3.09–3.12 (m, 2H), 3.54 (s, 3H), 3.661 (s, 3H), 3.67 (s, 3H), 4.4 (dd, J = 13.6, 8.3 H, 1H), 4.58 (t, J = 7.53, H, 1H), 6.67 (dd, J = 8.68, 2.16 H, 1H), 6.79 (d, J = 8.71 H, 2H), 6.94–7.0 (t, J = 1A1, H, 2H), 7.06 (t, J = 7.51, H, 2H), 7.15 (d, j = 8.73 H, 3H), 7.22 (d, J = 8.40 H, 2H), 7.33 (d, J = 8.02 H, 1H), 7.47 (d, J = 7.78 H, 1H), 7.95 (s, 1H), 8.29 (d, J = 7.14 H, 1H), 10.65 (s, 1H), 10.85 (s, 1H);^{13}C NMR (500 MHz, *CDCl*$_3$)δ ppm 27.46, 29.71, 36.46, 37.32, 37.99,49.51, 52.33, 52.82, 52.86, 55.20, 101.59, 109.75, 109.82, 111.26, 1182, 112.09 113.86, 118.49, 118.81, 119.59, 121.96, 122.03, 122.10, 122.14, 123.06, 126.99, 127.57, 128.61, 131.76, 136.06, 153.77, 158.01, 171.51, 172.31, 208.37; MS (EI$^+$): m/z: 582.3.

4.15. (2*S*) Methyl 2-[6-(5-methoxy-1*H*-indol-3yl)-6-(4-methoxyphenyl)-4-oxohexanamido]-3- phenyl propanoate (9c)

Yield 84%, mp 90–91 °C; IR v (neat, cm^{-1}): 3298, 3030, 2952, 1743, 1709, 1660, 1510, 1484, 1455, 1247, 1215, 1175, 1031, 753; ^{1}H NMR (400 MHz, DMSO-d_6)δ ppm 2.23 (t, J = 7.3 H, 2H), 2.75 (m, 2H), 2.97 (m, 2H), 3.11 (m, 2H), 3.56(s, 1H), 3.66 (s, 3H), 3.67 (s, 3H), 4.37 (dd, J = 13.6, 8.28 H, 1H), 4.56 (t, J = 7.38, H, 1H), 6.65–6.67 (dd, J = 8.74, 2.28 H, 1H), 6.79 (d, J = 8.53 H, 2H), 7.16–7.25 (m, 10H), 7.95 (s, 1H), 8.32 (d, J = 8.12 H, 2H), 10.65 (s, 1H); ^{13}C NMR (500 MHz, *CDCl*$_3$)δ ppm 29.62, 36.05, 37.45, 38.08, 49.53, 52.27, 53.11, 54.16, 55.19, 55.83, 101.57, 111.76, 112.17, 113.86, 118.87, 121.94, 126.98, 127.10, 127.9, 128.56, 128.64, 129.08, 129.28, 129.58, 131.77, 135.82, 136.01, 153.81, 158.04, 171.46, 171.97, 208.34; MS (EI$^+$): m/z: 542.2 [M$^-$]$^+$.

4.16. (2*S*) Ethyl 3-(4-hydroxyphenyl)-2-[6-(5-methoxy-1*H*-indol-3yl)-4-oxo- 6-(4- trifluoromethyl) phenyl)hexanamido] propanoate (9d)

Yield 85%, mp 70–71 °C; IR v (neat, cm^{-1}): 3327 (br), 2930, 1732, 1707, 1660, 1616, 1515, 1485, 1324, 1214, 1165, 1120, 1067; ^{1}H NMR (400 MHz, DMSO-d_6)δ ppm 1.07 (t, J = 7.15 H, 3H), 2.26 (t, J = 7.1 H, 2H), 2.59–2.63 (m, 2H), 2.78–2.84 (m, 2H), 3.24 (m, 2H), 3.66 (s, 3H), 3.98–4.0 (dd, J = 13.94, 6.85 H, 2H), 4.29 (dd, J = 14.50, 7.02 H, 1H), 4.73 (t, J = 7.46, H, 1H), 6.64 (d, J = 7.77 H, 2H), 6.67 (dd, J = 8.92, 2.28 H, 1H), 6.83 (d, J = 2.19 H, 1H), 6.97 (d, J = 7.23 Hz, 2H), 7.19 (d, J = 8.75 H, 1H), 7.24 (s, 1H), 7.57 (d, J = 2.13 H, 2H),7.95 (s, 1H), 8.24 (d, J = 7.54 H, 1H), 9.21 (s, 1H), 10.75 (s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6)ppm 13.9, 28.66, 30.74, 36.07, 36.19, 37.55, 48.55, 54.14, 45.89, 110.80, 113.33, 113.56, 114.83, 117.93, 120.87, 123.36, 127.90, 128.12, 128.34, 130.02, 136.74, 155.75, 157.38, 170.26, 173.68, 207.72; MS (EI$^+$): m/z: 610.2 [M$^-$]$^+$.

4.17. (2*S*) Methyl 3-(1*H*-indol-3-yl)-2-[6-(5-methoxy-1*H*-indol-3yl)-4-oxo-6-(4- (trifluoromethyl) phenyl)hexanamido] propanoate (9e)

Yield 81%, mp 89–90 °C; IR v (neat, cm^{-1}): 3403 (br), 3051, 3005, 2952, 1739, 1712, 1661, 1619, 1582, 1485, 1457, 1438, 1324, 1214, 1165, 1120; ^{1}H NMR (400 MHz, DMSO-d_6)ppm 2.26–2.29 (t, J = 7. 2 Hz, 2H), 2.63–2.73 (m, 2H), 2.96–3.00 (m, 2H), 3.11–3.23 (m, 2H), 3.53 (s, 3H), 3.66 (s, 3H), 4.46 (dd, J = 14.05, 7.15 H, 1H), 4.73 (t, J = 7.46, H, 1H), 6.68 (dd, J = 8.74, 2.33 H, 1H), 6.82 (d, J = 2.07 H, 1H), 6.97 (t,J = 6.28 Hz, 1H), 7.11 (s, 1H), 7.19, (d, J = 8.73 H, 1H), 7.25 (t, J = 7.49 H, 1H), 7.33 (d, J = 8.04 H, 2H), 7.46 (d, J = 7.83 H, 2H), 7.56 (s, 4H) , 8.29 (d, J = 7.46 H, 1H), 10.75 (s, 1H), 10.85 (s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm , 28.85, 36.07, 36.19, 37.55, 48.55, 54.14, 54.89, 101.59, 109.75, 111.26, 111.82, 112.09, 113.86, 118.49, 118.81, 119.59, 121.96, 122.03, 122.10, 122.14, 123.06, 126.99, 127.57, 129.61, 131.76, 136.06, 153.77, 158.01, 171.51, 172.31, 207.71; MS (EI$^+$): m/z: 619.2 [M$^-$]$^+$.

4.18. (2*S*) Methyl 2-[6-(5-methoxy-1*H*-indol-3yl)-4-oxo-6-(4-trifluoromethyl)phenyl)hexanamido] -3-phenylpropanoate (9f)

Yield 89%, mp 107–108 °C; IR v (neat, cm^{-1}): 3352 (br), 2938, 2831, 1733, 1716, 1657, 1618, 1581, 1516, 1483, 1324, 1213, 1165, 1121, 1067; ^{1}H NMR (400 MHz, DMSO-d_6)δ ppm 2.25 (t, J = 7.2 H, 2H), 2.61–2.73 (m, 2H), 2.84–2.87 (m, 2H), 3.21–3.24 (m, 2H), 3.55 (s, 3H), 3.66 (s, 3H), 4.41 (dd, J = 14.38–7.03, H, 1H), 4.72 (t, J = 7.41, H, 1H), 6.68 (dd, J = 8.73, 2.04 H, 1H), 6.83 (d, J = 2.39 H, 1H), 7.17–7.25 (m, 5H), 7.57 (s, 4H), 8.32 (d, J = 7.58 H, 1H), 10.75 (s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6)δ ppm 28.9, 30.2, 36.9, 36.99, 37.5, 47.73, 48.55, 54.14, 54.89, 100.6, 110.93, 112.25, 116.68, 122.86, 123.29, 124.91, 125.44, 126.16, 126.33, 126.44, 126.58, 127.99, 128.39, 129.12, 129.25, 129.83, 131.5 137.69, 150.03, 152.89, 170.90, 173.44, 207.81; MS (EI$^+$): m/z: 580.2 [M$^-$]$^+$.

4.19. (2*S*) Ethyl 2-[6-(5-bromo-1*H*-indol-3yl) -6-(4-methoxyphenyl)-4-oxohexanamido]-3-(4-hydroxyphenyl)propanoate (9g)

Yield 73%, mp 104–105 °C; IR v (neat, cm^{-1}): 3323, 2984, 2845, 1733, 1712, 1657, 1612, 1551, 1484, 1266, 1214, 1175, 771; ^{1}H NMR (400 MHz, DMSO-d_6)δppm 1.1 (t, J = 7.09, H, 3H), 2.23 (t, J = 7.1 H, 2H), 2.6 (m, 2H), 2.7–2.84 (m, 2H) 3.05–3.11 (m, 2H), 3.6 (s, 3H), 3.67 (s, 3H), 3.99–4.0 (q, J = 7.03, H, 2H), 4.28 (dd, J = 13.6, 8.28 H, 1H), 4.59 (t, J = 7.57, H, 1H), 6.64 (d, J = 8.15 H, 2H), 6.79 (d, J = 8.51 Hz, 2H), 6.98 (d, J = 8.15 H, 2H), 7.21 (d, J = 8.32 H, 2H), 7.27 (d, J = 8.57 H, 2H), 7.3 (d, J = 7.63 H, 1H), 7.33 (s, 1H), 8.08 (d, J = 8.01 H, 1H), 9.19 (s, 1H),10.65 (s, 1H); ^{13}C NMR (500 MHz, *CDCl*$_3$) δ ppm 18.52, 29.62, 37.45, 37.83, 49.53, 52.27, 53.21, 54.16, 54.19, 101.57, 111.76, 112.17, 113.86, 118.87, 121.94, 126.98, 127.10, 127.90, 128.56, 128.64, 129.08, 129.28, 129.58, 131.77, 135.82, 136.01, 153.81, 158.04, 171.46, 171.97, 208.34; MS (EI$^+$): m/z: 620.1 [M$^-$]$^+$;622.1[M$^-$+2]$^+$.

4.20. (2*S*) Methyl 2-[6-(5-bromo-1*H*-indol-3yl)-6-(4-methoxyphenyl)-4-oxohexanamido]-3-(1*H*- indol-3yl)propanoate (9h)

Yield 86%, mp 204–205 °C; IR v (neat, cm^{-1}):3265, 2980, 2959, 1740, 1714, 1660, 1610, 1511, 1460, 1367, 1249, 1218, 1177, 1000, 885; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.32 (m, 2H), 2.67 (m, 2H), 3.09 (m, 2H) 3.25 (m, 2H), 3.66 (d, J = 3.44, 3H), 4.85 (m, 2H), 6.12 (t, J = 7.23 H, 1H) 6.74 (m, 1H), 6.8 (d, J = 7.63 H, 1H), 7.1 (d, J = 2.45 H, 1H), 7.17 (d, J = 7.7 4 Hz, 1H), 7.17–7.21(m, 2H), 7.28 (m, 2H), 7.38 (d, J = 8.58 H, 2H), 7.49 (d, J = 8.59 H, 2H), 8.01(br, 1H), 8.10 (br, 1H); ^{13}C NMR (500 MHz, *CDCl*$_3$)δ ppm 27.46, 29.71, 37.38, 37.99, 49.51, 52.33, 52.82, 55.20, 101.59, 109.75, 109.83, 111.26, 111.82, 112.09, 113.86, 118.49, 118.81, 119.59,

121.96, 122.03, 122.10, 122.14, 123.06, 126.99, 127.57, 128.61, 131.76, 136.06, 153.77, 157.37, 170.87, 173.41, 207.71; MS (EI^+): m/z: 629.1 $[M^-]^+$.

4.21. (2*S*) Ethyl2- [6-(5-bromo-1*H*-indol-3yl)-4-oxo-6-(4-trifluoromethyl)phenyl)hexanamido]- 3-(4-hydroxy phenyl) propanoate (9i)

Yield 85%, mp 141–142 °C; IR ν (neat, cm^{-1}): 3284 (br), 2930, 1731, 1716, 1661, 1514, 1452, 1376, 1324, 1217, 1164, 1122, 1067; ^1H NMR (400 MHz, DMSO-d_6)δ ppm 1.11 (t, J = 7.15Hz, 3H), 2.26 (t, J = 7.1 H, 2H), 2.65 (m, 2H), 2.83 (m, 2H), 3.24 (m, 2H), 3.98–4.01 (dd, J = 13.94, 6.85 H, 2H), 4.30 (dd, J = 14.50, 7.02 H, 1H), 4.74 (t, J = 7.46, H, 1H), 6.64 (d, J = 7.77 Hz, 2H), 6.67 (dd, J = 8.92, 2.28 H, 1H), 6.83 (d, J = 2.19 H, 1H), 6.97 (d, J = 7.23 H, 2H), 7.19 (d, J = 8.75 Hz, 1H), 7.24 (s, 1H), 7.57 (d, J = 2.13 H, 2H),7.95 (s, 1H), 8.24 (d, J = 7.54 H, 1H), 9.21 (s, 1H), 10.75 (s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm 13.91, 28.66, 30.74, 36..07, 36.19, 37.55, 48.55, 54.14, 54.89, 111.05, 113.44, 114.75, 116.77, 120.65, 122.52, 123.24, 123.57, 123.81, 125.03, 125.06, 125.40, 126.47, 126.72, 127.95, 128.36, 130.03, 134.97, 149.78, 155.74, 157.37, 170.26, 173.68, 207.71; MS (EI^+): m/z: 685.1 $[M^-]^+$, 660.1$[M^-+2]^+$.

4.22. (2*S*) Methyl 3-(1*H*-imidazol-4-yl)-2-(6-(5-methoxy-1*H*-indol-3yl)-6-(4-metoxyphenyl)-4- oxo hexanamido)propanoate (9j)

Yield 79%, mp 97–98 °C; IR ν (neat, cm^{-1}): 3346 (br), 2998, 2947, 2832, 1738, 1713, 1659, 1605, 1505, 1480, 1432, 1231, 1030, 754; ^1H NMR (400 MHz, DMSO-d_6) δ ppm 2.26 (t, J = 7. 2 H, 2H), 2.63 (m, 2H), 2.85 (m, 2H), 3.06–3.13 (m, 2H), 3.55 (s, 3H), 3.66 (s, 3H), 3.67 (s, 3H), 4.42 (dd, J = 14.05, 7.15 H, 1H), 4.59 (t, J = 7.52, H, 1H), 6.66 (dd, J = 8.74, 2.04 H, 1H), 6.78 (m, 4H), 7.18 (d, J = 8.64 H, 2H), 7.22 (d, J = 8.65 H, 2H), 7.51 (s, 1H) , 8.22 (d, J = 7.13 H, 1H), 10.65 (s, 1H), 11.2 (s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6)δ ppm 29.62, 36.05, 37.45, 38.08, 49.53, 52.27, 53.11, 54.16, 55.19, 55.83, 101.57, 111.76, 112.17, 113.86, 118.87, 121.94, 126.98, 127.10, 127.9, 128.56, 128.64, 129.08, 129.28, 129.58, 131.77, 135.82, 136.01, 153.81, 158.04, 171.46, 171.97, 208.34; MS (EI^+): m/z: 533.3 $[M^- H]^+$.

4.23. (2*S*) Methyl 3-(1-*H*-imidazol-4-yl)-2- [6-(5-methoxy-1it H-indol-3yl))-4-oxo-6-(4-(trifluoromethyl)phenyl)hexanamido] propanoate (9k)

Yield 75%, mp 111–112 °C; R ν (neat, cm^{-1}): 3263 (br), 3005, 2950, 2843, 1742, 1714, 1610, 1510, 1459, 1367, 1248, 1218, 1177, 771; ^1H NMR (400 MHz, DMSO-d_6)δ ppm 2.26 (t, J = 6.57 H, 2H), 2.73 (m, 2H), 2.91 (m, 2H), 3.12 (m, 2H), 3.56 (s, 3H), 3.68 (s, 3H), 4.45 (dd, J = 13.74, 7.54 H, 1H), 4.60 (t, J = 7.33, H, 1H), 6.69 (d, J = 8.43, H, 2H), 7.1 (s, 1H), 7.13 (d, J = 8.11 H, 1H), 7.25 (d,J = 8.57 H, 2H),7.27(d,J = 8.61 Hz, 1H), 7.31 (s, 1H), 7.7.48 (s, 1H) 7.8 (s, 1H), 8.24 (d, J = 7.13 H, 1H), 10.65 (s, 1H), 11.07 (s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6)δ ppm 28.69, 28.89, 36.85, 37.36, 47.73, 51.68, 52.36, 55.30, 100.16, 110.19, 112.00, 116.67, 122.66, 123.27, 124.90, 124.93, 125.44, 126.32, 126.41, 126.56, 128.38, 131.47, 134.77, 150.02, 152.88, 171.24, 172.03, 207.44; MS (EI^+): m/z: 571.2 $[M^- H]^+$.

4.24. General procedure for the synthesis of 2-[6-(5-substituted-1*H*-indol-3-yl)-6-(4-substituted phenyl)-4-oxohexanamido]-propanoic acid (10a-k)

To a solution of alkyl esters 9a–k (0.742 mmol) in THF (20 mL) and ethanol (10 mL) at room temperature was added an aqueous solution of lithium hydroxide (2.92 mmol). The reaction was stirred at room temperature for 3 h. The progress of the reaction was monitored by TLC. After the solution was concentrated in vacuo to one-third of its original volume, ethylacetate (60 mL), 1N HCl (10 mL) and water (20 mL) were added and the organic layer was separated. The aqueous solution was extracted with ethylacetate (2×50 mL). Organic solutions were combined, dried with sodium sulfate anhydrous, filtered, and concentrated to afford the desired final target compounds 10a–k.

4.25. (2*S*)-3-(4-Hydroxyphenyl)-2-[6-(5-methoxy-1*H*-indol-3yl)-6-(4- methoxyphenyl)-4-oxo hexanamido]propanoic acid (10a)

Yield 82%, mp 156–157 °C; $[\alpha]_D^{22}$ −11.03 (c 0.82%, methanol). IR ν (neat, cm^{-1}): 3525–3150 (br), 3005, 2932, 2832, 1709, 1658, 1612, 1511, 1441, 1246, 1215, 1175; ^1H NMR (400 MHz, *DMSO*-d_6) δ ppm 2.22 (t, J = 7.1 H, 2H), 2.58 (m, 2H), 2.88 (m, 2H), 3.11–3.23 (m, 2H), 3.66 (s, 3H), 3.67 (s, 3H), 4.28 (dd, J = 13.6, 8.28 H, 1H), 4.57 (t, J = 7.49, H, 1H), 6.64 (d, J = 7.58 H, 2H), 6.67 (d, J = 1.93 H, 1H), 6.6.78 (d, J = 8.68 H, 3H), 6.98 (d, J = 7.68 H, 2H), 7.16 (s, 1H), 7.18 (s, 1H), 7.22 (d, J = 8.61 H, 2H), 8.08 (d, J = 7.79 H, 1H), 9.20 (s, 1H), 10.65 (s, 1H), 12.57 (br, 1H); ^{13}C NMR (400 MHz, aceton-d_6) δ ppm 37.33, 38.03, 38.55, 49.98, 54.58, 55.34, 55.77, 101.9, 112.1, 112.6, 114.3, 115.9, 123.12, 128.0, 128.6, 129.5, 131.2, 133.0, 137.9, 154.4, 157.0, 158.8, 172.3, 173.1, 208.1; MS (EI^+): m/z: 544.2 $[M^-]^+$.

4.26. (2*S*)-3-(1*H*-Indol-3-yl)l)-2-[6-(5-methoxy-1*H*-indol-3yl)-6-(4- methoxyphenyl)-4-oxo hexanamido]propanoic acid (10b)

Yield 85%, mp 139–140 °C; $[\alpha]_D^{26}$ −23.42 (c 0.37%, methanol); IR ν (neat, cm^{-1}): 3572–3200 (br), 3055, 2998, 2836, 1707, 1659, 1622, 1583, 1510, 1484,1457, 1360, 1246; ^1H NMR (400 MHz, DMSO-d_6)δ ppm 2.24 (t, J = 7. 2 H, 2H), 2.65–2.95 (m, 2H), 3.0–3.1 (m, 2H), 3.22 (m, 2H), 3.661 (s, 3H), 3.67 (s, 3H), 4.4 (dd, J = 13.6, 8.3 H, 1H), 4.56 (t, J = 7.53, Hz, 1H), 6.66 (dd, J = 8.73 H, 1H), 6.78 (d, J = 8.64 H, 2H), 6.8 (d, J = 2.07 H, 1H), 6.9 (t, J = 6.28 H, 1H), 7.05 (t, J = 7.49 H, 1H), 7.11 (s, 1H), 7.16 (s, 1H), 7.18 (s, 1H), 7.22 (d, J = 8.59 H, 2H), 7.32 (d, J = 8.02 H, 1H), 7.51 (d, J = 7.87 H, 1H), 8.1(d, J = 7.14 H, 1H), 10.65 (s, 1H), 10.82 (s, 1H), 12.59 (br, 1H); ^{13}C NMR (400 MHz, *CDCl*$_3$)δ ppm 18.3, 26.8, 29.3, 37.1, 49.2, 55..1, 55.7, 59.3, 101.6, 111.4, 111.7, 111.9, 113.7, 123.4, 126.9, 128.5, 131.7, 135.9, 157.8, 173, 174.1, 209.2; MS (EI+): m/z: 567.2 $[M^-]^+$.

4.27. (2*S*)-2-[6-(5-Methoxy-1*H*-indol-3yl)-6-(4-methoxyphenyl)-4- oxohexanamido]-3-phenyl propanoic acid (10c)

Yield 85%, mp 132–1133 °C; $[\alpha]_D^{26}$ −28.78 (c 0.31%, methanol); IR ν (neat, cm^{-1}): 3523–3100 (br), 3059, 2991, 2833, 1710, 1661, 1609, 1583, 1510, 1484, 1455, 1247, 1214, 1175; ^1H NMR (400 MHz, DMSO-d_6)δ ppm 2.22 (t, J = 7.1 H, 2H), 2.55-2.59 (m, 2H), 2.84 (m, 2H), 3.22–3.25 (m, 2H), 3.66 (s, 3H), 3.67 (s, 3H), 4.37 (dd, J = 13.6, 8.28 H, 1H), 4.57 (t, J = 7.49, H, 1H), 6.67 (dd, J = 8.72, 2.38 H, 1H), 6.79 (d, J = 8.7 H, 3H), 7.15–7.3 (m, 9H), 8.16 (d, J = 8.04 H, 2H), 10.66 (s, 1H), 12.54 (br, 1H): ^{13}C NMR (400 MHz, aceton-d_6)δ ppm 37.9, 38.09, 38.4, 41.3 49.9, 54.2, 55.3, 55.7, 101.9, 112.1, 112.6, 114.2, 115.9, 123.08, 127.3, 128.0, 129.04, 129.5, 130.1, 133.0, 137.3, 154.3, 158.8, 158.8, 172.2, 172.99, 208.07; MS (EI+): m/z: 528.2 $[M^- H]_+$

4.28. (2*S*)-3-(4-Hydroxyphenyl)-2-[6-(5-methoxy-1*H*-indol-3yl))-4- oxo-6-(4-trifluoromethyl) phenyl) hexanamido]propanoic acid (10d)

Yield 89%, mp 130–131 °C; $[\alpha]_D^{28}$ −14.88 (c 0.91%, methanol); IR ν (neat, cm^{-1}): 3515–3200 (br), 3066, 2940, 2832, 1709, 1652, 1616, 1515, 1484, 1441, 1324, 1215, 1118; ^1H NMR (400 MHz, DMSO-

d_6)δ ppm 2.23 (t, J = 7.1 H, 2H), 2.59–2.6 (m, 2H), 2.8 (m, 2H), 3.3 (m, 2H), 3.66 (s, 3H), 4.28 (dd, J = 13.48, 8.07 H, 1H), 4.73 (t, J = *1M,* Hz, 1H), 6.64 (d, J = 7.38 H, 2H), 6.67 (dd, J = 8.92, 2.28 H, 1H), 6.83 (d, J = 1.68 H, 1H), 6.98 (d, J = 7.12 H, 2H), 7.19 (d, J = 8.74 H, 1H), 7.24 (s, 1H), 7.57 (s, 4H), 8.1 (d, J = 7.89 H, 1H), 9.19 (s, 1H), 10.75 (s, 1H), 12.57 (br, 1H),^{13}C NMR (400 MHz, aceton-d_6)δ ppm 28.8, 30,23, 37.32, 38.33, 49.2, 54.56, 55.75, 101.5, 112.3, 112.8, 115.9, 118.2, 123.4, 125.7, 125.8, 127.8, 128.6, 129.4, 131.2, 150.9, 154.5, 157.0, 172.2, 173.1, 207.6; MS (EI$^+$): m/z: 583.2 [M$^\cdot$ H]$^+$.

4.29. (2*S*)-3-(1*H*-Indol-3-yl)-2-[6-(5-methoxy-1*H*-indol-3yl)-4-oxo-6- (4-(trifluoromethyl)phenyl) hexanamido]propanoic acid (10e)

Yield 79%, mp 143–144 °C; $[\alpha]_D^{26}$ −20.71 (c 0.43%, methanol); IR ν (neat, cm^{-1}): 3403 (br), 3005, 2936, 2836, 1711, 1660, 1619, 1583, 1530, 1484,1457, 1324, 1214, 1121; ^{1}H NMR (400 MHz, DMSO-d_6)δ ppm 2.27 (t, J = 7. 2 H, 2H), 2.60–2.63 (m, 2H), 2.73–2.97 (m, 2H), 3.10–3.15 (m, 2H), 3.66 (s, 3H), 4.4 (dd, J = 13.6, 8.3 H, 1H), 4.73 (t, J = 7.33, H, 1H), 6.67 (dd, J = 8.29 H, 1H), 6.82 (d, J = 2.07 H, 1H), 6.96 (t, J = 6.28 H, 1H), 7.04 (t, J = 7.49 H, 1H), 7.11 (s, 1H), 7.19(d, J = 8.73 H, 1H), 7.24 (s, 1H), 7.32 (d, J = 8.07 Hz, 1H), 7.51 (d, J = 7.66 H, 1H), 7.56 (s, 4H), 8.13 (d, J = 7.48 H, 1H), 10.7 (s, 1H), 10.82 (s, 1H), 12.59 (br, 1H); ^{13}C NMR (400MHz, *aceton*-d_6)δ ppm 28.1, 30.28 38.3, 38.4, 49.2, 53.9, 55.7, 101.6, 110.9, 112.1, 112.4, 112.8, 118.2, 119.3, 119.5, 122.1, 123.5, 124.4, 125.8, 127.9, 128.7, 129.5, 133.0, 137.5, 150.9, 154.6, 172.3, 173.4, 207.6; MS (EI$^+$): m/z: 605.2 [M$^\cdot$]$^+$.

4.30. (2*S*)-2-[6-(5-Methoxy-1*H*-indol-3yl)-4-oxo-6-(4-trifluoromethyl) phenyl)hexanamido]-3-phenyl propanoic acid (10f)

Yield 91%, mp 156-157 °C; $[\alpha]_D^{28}$ −22.64 (c 0.65%, methanol); IR ν (neat, cm^{-1}): 3314 (br), 3026, 2938, 1724, 1660, 1612, 1557, 1484, 1455, 1366, 1324,1215, 1123; ^{1}H NMR (400 MHz, DMSO-d_6)δ ppm 2.32 (t, J = 7.2 H, 2H), 2.63 (m, 2H), 2.82 (m, 2H), 3.04 (m, 2H), 3.66 (s, 3H), 4.38 (dd, J = 13.48, 8.07 H, 1H), 4.74 (t, J = *1M,* Hz, 1H), 6.68 (dd, J = 8.61, 2.58 H, 1H), 6.83 (d, J = 2.39 H, 1H), 7.1–7.24 (m, 7H), 7.57 (s, 4H), 8.3 (s, 1H), 10.75 (s, 1H), 12.57 (br, 1H); ^{13}C NMR (500 MHz, DMSO-d_6)δ ppm 28.89, 30.23, 36.86, 37.5, 47.73, 53.83, 55.30, 100.60, 110.92, 112.02, 116.68, 122.68, 123.29, 124.91, 125.44, 126.16, 126.32, 126.42, 126.58, 127.99, 128.39, 129.11, 129.24, 12983, 131.49, 137.96, 150.03, 152.89, 170.90, 173.44, 207.83; MS (EI+): m/z: 566.2 [M$^\cdot$]$^+$.

4.31. (2*S*)-2-[6-(5-Bromo-1*H*-indol-3yl)-6-(4-methoxyphenyl)-4- oxohexanamido]-3-(4-hydroxy phenyl)propanoic acid (10g)

Yield 85%, mp 137–138 °C; $[\alpha]_D^{28}$ −15.16 (c 0.72%, methanol); IRv (neat, cm^{-1}):3307, 3070, 3016, 2932, 2832, 1719, 1708, 1650, 1612, 1511, 1455, 1365, 1246, 1176; ^{1}H NMR (400 MHz, *DMSO*-d_6) δ ppm 2.23 (t, J = 7.1 H, 2H), 2.6 (m, 2H), 2.7–2.84 (m, 2H) 3.05–3.11 (m, 2H), 3.67 (s,3H), 4.28 (dd, J = 13.42, 8.56 H, 1H), 4.60 (t, J = 7.54, H, 1H), 6.64 (d, J = 8.15 Hz, 2H), 6.79 (d, J = 8.51 H, 2H),, 6.98 (d, J = 8.15 H, 2H), 7.21 (d, J = 8.32 H, 2H), 7.27 (d, J = 8.57 H, 2H), 7.3 (d, J = 7.63 H, 1H), 7.33 (s, 1H), 8.09 (d, J = 8.01 H, 1H), 9.19 (s, 1H),11.06 (s, 1H), 12.53 (s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6)δ ppm 28.85, 36.07 36.19, 37.55, 48.55, 54.14, 54.89, 110.80, 113.33, 113.55, 114.83, 117.92, 120.86, 123.36, 127.90, 128.12, 128.43, 130.02, 135.02, 136.73, 155.74, 157.37, 170.87, 173.41, 207.71; MS (EI$^+$): m/z: 592.1

4.32. (2*S*)-2-[6-(5-Bromo-1*H*-indol-3yl)-6-(4-methoxyphenyl)-4- oxohexanamido]-3-(1*H*-indol-3yl) propanoic acid (10h)

Yield 86%, mp 204–205 °C; $[\alpha]_D^{27}$ −18.82 (c 0.69%, methanol); IR ν (neat, cm^{-1}):3404, 3063, 3001, 2931, 2836, 1705, 1643, 1583, 1510, 1417, 1361, 1247, 1178, 1101, 745; ^{1}H NMR (400 MHz, DMSO-$d_£$)ppm 2.21 (t, J = 6.51 Hz, 2H), 2.67 (m, 2H), 2.92–2.98 (m, 2H) 3.04–3.11 (m, 2H), 3.67 (s, 3H), 4.26 (dd, J = 18.52, 9.11 H, 1H), 4.59 (t, J = 7.56, H, 1H), 6.78 (d, J = 8.41 H, 2H), 6.92 (t, J = 6.00 H, 1H), 7.02 (t, J = 7.23 H, 1H), 7.06 (s, 1H), 7.12 (d, J = 8.19 H, 1H), 7.20 (d, J = 8.42 H, 2H), 7.25 (s, 1H), 7.29 (d, J = 11.1 H, 2H), 7.46–7.49 (m, 2H), 10.72 (s, 1H),11.07 (s, 1H), 12.53 (s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm 28.68, 35.74, 36.85, 37.35, 47.72, 51.68, 53.16, 55.29, 100.61109.40, 110.92, 111.38, 112.00, 116.67, 117.93, 118.35, 120.90, 122.66, 123.27, 124.90,125.44, 126.32, 126.56, 127.05128.95, 131.47, 136.03, 150.01, 152.88, 171.27, 172.37, 207.45; MS (EI$^+$): m/z: 615.1 [M$^\cdot$]$^+$, 617.1[M$^\cdot$+2]$^+$.

4.33. (2*S*)-2-[6-(5-Bromo-1*H*-indol-3yl)-4-oxo-6-(4- trifluoromethyl)phenyl)hexanamido]-3-(4-hydroxyphenyl) propanoic acid (10i)

Yield 83%, mp 233–234 °C; $[\alpha]_D^{26}$ −12.39 (c 0.85%, methanol); IR ν (neat, cm^{-1}): 3328, 3016, 1720, 1645, 1615, 1514, 1445, 1324, 1231, 1165, 1121, 1067; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.23–2.27 (m, 2H), 2.59–2.67 (m, 2H), 2.96–271 (m, 2H), 2.88–2.92 (m, 2H), 4.37 (dd, J = 13.6, 8.28 H, 1H), 4.77 (t, J = 7.19, H, 1H), 6.61 (d, J = 7.44 H, 2H), 6.95 (d, J = 7.58 H, 2H), 7.13 (dd, J = 8.41, 1.17 H, 1H), 7.28 (d, J = 8.58 H, 1H), 7.40 (s, 1H), 7.56–7.60 (m, 5H), 9.19 (br, 1H), 11.19 (s, 1H), 12.54 (br, 1H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm 28.96, 36.36, 36.50, 37.52, 47.77, 54.51, 55.98, 111.05, 113.44, 114.75, 116.77, 120.65, 122.52, 123.24, 123.57, 123.81, 125.03, 125.06, 125.40, 126.47, 126.72, 127.95, 128.36, 130.03, 134.97, 149.78, 155.64, 170.63, 207.36; MS (EI$^+$): m/z: 630.1 [M$^\cdot$]$^+$; 632.1[M$^\cdot$+2]$^+$.

4.34. (2*S*)-3-(1*H*-imidazol-4-yl)-2-[6-(5-methoxy-1*H*-indol-3yl))-6- (4-metoxyphenyl)-4-oxo hexanamido]propanoic acid (10j)

Yield 79%, mp 136–137 °C; $[\alpha]_D^{27}$ +12.97 (c 0.78%, methanol); IR ν (neat, cm^{-1}): 3393 (br), 2956, 2927, 2851, 1715, 1705, 1668, 1623, 1509, 1485, 1248, 1215, 1175, 1031, 832; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.24 (t, J = 7. 2 H, 2H), 2.63 (m, 2H), 2.80(m, 2H), 3.21 (m, 2H), 3.66 (s, 3H), 3.67 (s, 3H), 4.42 (dd, J = 14.05, 7.15 H, 1H), 4.59 (t, J = 7.52, H, 1H), 6.65 (dd, J = 8.69, 1.99 H, 1H), 6.77 (m, 4H), 7.17 (d, J = 8.62 H, 2H), 7.23 (d, J = 8.63 H, 2H), 7.51 (s, 1H), 8.22 (d, J = 7.13 H, 1H), 10.66 (s, 1H), 12.2 (s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6)δ ppm 20.99, 26.16, 28.69, 30.20, 36.86, 37.30, 47.72, 51.10, 55.31, 100.16, 110.19, 112.00, 116.67, 122.66, 123.27, 124.90, 124.93, 125.44, 126.32, 126.41, 126.56, 128.38, 131.47, 134.77, 150.02, 152.88, 171.24, 172.03, 207.44 ; MS (EI$^+$): m/z: 519.2 [M$^\cdot$ H]$^+$.

4.35. (2S)-3-(1*H*-Imidazol-4-yl)-2-[6-(5-methoxy-1*H*-indol-3yl) -4-oxo-6-(4- (trifluoromethyl) phenyl)hexanamido]propanoic acid (10k)

Yield 82%, mp 147–147 °C; $[\alpha]_D^{27}$ +15.10 (c 0.89%, methanol); IR ν (neat, cm^{-1}): 3258 (br), 3143, 3011, 2943, 2901, 2832, 1714 (br), 1670, 1648, 1619, 1541, 1485, 1440, 1416, 1325, 1215, 1165, 1117, 1067; ^{1}H NMR (400 MHz, DMSO-d_6)δ ppm 2.28 (t, J = 6.75 H, 2H), 2.66 (m, 2H), 2.97 (m, 2H), 3.27 (m, 2H), 3.67 (s, 3H), 4.50 (dd, J = 13.62, 6.75 H, 1H), 4.73 (t, J = 7.38, H, 1H), 6.68 (dd, J = 8.73, 2.15, H, 1H), 6.83 (s, 1H), 7.2 (d, J = 8.75 H, 1H), 7.26 (d, J = 2.01 H, 1H), 7.35 (s, 1H), 7.57 (m, 4H) 8.31 (d, J = 8.08 H, 1H), 8.98 (s, 1H), 10.78 (s, 1H), 14.2 (s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6)δ ppm 26.16, 28.69, 36.85, 37.29, 47.72, 51.10, 55.31, 100.66, 110.85, 112.00, 116.59, 116.76, 122.68, 123.25, 124.91, 125.41, 126.31, 126.38, 126.56, 128.07, 128.37, 129.30, 130.04, 131.48, 133.55, 149.98, 152.85, 171.31, 171.95, 207.43; MS (EI$^+$): m/z: 557.1 [M$^\cdot$ H]$^+$.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.07.084.

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