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Development of a potent 2-oxoamide inhibitor of secreted phospholipase A_2 guided by molecular docking calculations and molecular dynamics simulations

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ABSTRACT

Inhibition of group IIA secreted phospholipase A_2 (GIIA sPLA$_2$) has been an important objective for medicinal chemists. We have previously shown that inhibitors incorporating the 2-oxoamide functionality may inhibit human and mouse GIIA sPLA$_2$s. Herein, the development of new potent inhibitors by molecular docking calculations using the structure of the known inhibitor **7** as scaffold, are described. Synthesis and biological evaluation of the new compounds revealed that the long chain 2-oxoamide based on (*S*)-valine **GK241** led to improved activity (IC_{50} = 143 nM and 68 nM against human and mouse GIIA sPLA$_2$, respectively). In addition, molecular dynamics simulations were employed to shed light on **GK241** potent and selective inhibitory activity.

1. Introduction

Phospholipases A_2 (PLA$_2$) are a superfamily of enzymes whose main role is the hydrolysis of the ester bond of the membrane glycerophospholipids at the *sn*-2 position.[1] The products of this enzymatic activity are mainly unsaturated fatty acids which can act as bioactive mediators, correlating with numerous pathological conditions. PLA$_2$ enzymes are categorized into six types according to their dependency on calcium, their amino acid sequence domain, their molecular weight and their evolutionary relationship.[1]

Approximately, one third of PLA$_2$ enzymes belong to the secreted phospholipase (sPLA$_2$) type, whose members are characterized by a low-molecular weight.[2-4] They can act either by catalyzing reactions as enzymes or by binding to receptors and hence, they are involved in the activation of several biological pathways. To date, 11 gene products of sPLA$_2$s (IB, IIA, IIC, IID, IIE, IIF, III, V, X, XIIA and XIIB) have been identified in mammals. Their domains are highly stabilized by six common disulfide bonds and additionally one or two that are typical of each enzyme. Their active site contains the His/Asp dyad that is utilized in the catalytic mechanism. Their activation may require concentration of Ca^{2+} in the mM range and thus these proteins are localized in the extracellular space, targeting both cellular glycerophospholipids and soluble lipids.

The up-regulation or down-regulation of the expression of these enzymes is related to pathological conditions, such as atherosclerosis, cardiovascular disease and cancer, as it has been summarized in a number of recent reviews.[5,6] As a consequence, a variety of synthetic inhibitors of sPLA$_2$ have been developed by pharmaceutical companies and research institutes.[1,7-9] In particular, GIIA sPLA$_2$ has been related to inflammation since it was identified in 1989.[10] GIIA sPLA$_2$ is highly expressed in synovial cells, while its concentration increases in the plasma of patients who suffer from coronary artery disease, making it a useful tool for prognostic purposes.

Many attempts for selective inhibition of GIIA sPLA$_2$ have been made with many positive outcomes. Varespladib[11] (**1a**, Fig. 1), which is a potent but not selective GIIA sPLA$_2$ inhibitor, was advanced into clinical trials as an intravenously-administered therapy for sepsis-induced systemic inflammatory response syndrome. Although it was found to have an acceptable safety profile in patients with severe sepsis, its development was terminated because Phase II studies resulted in little efficacy. Also, Varespladib methyl (**1b**, Fig. 1) which functions as a prodrug and is rapidly converted *in vivo* to Varespladib, was synthesized as an attempt to improve Varespladib efficacy. Both inhibitors were claimed by Anthera Pharmaceuticals as new agents for the treatment of cardiovascular diseases and underwent clinical trials from

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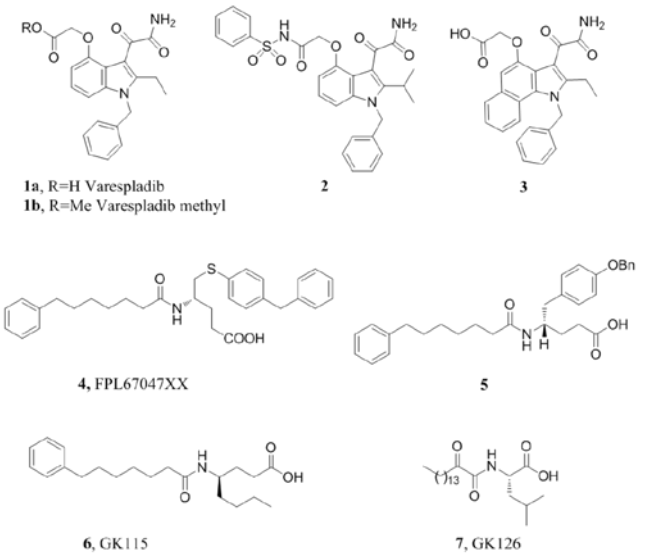


Figure 1. Common inhibitors of secreted PLA_2.

2006 to 2012. However, on 2012 phase III clinical studies were terminated due to lack of efficacy.

The expression of the full set of human and mouse groups I, II, V, X, and XII $sPLA_2$s in *Escherichia coli* and insect cells provided pure recombinant enzymes for detailed comparative interfacial kinetic and binding studies.[12] Among the class of inhibitors of $sPLA_2$s based on substituted indoles, 6,7-benzoindoles and indolizines,[13,14] it was found that compound **2** (Fig. 1) was a selectively potent inhibitor against hGX over all other human and mouse $sPLA_2$ enzymes, while compound **3** (Fig. 1) inhibited nearly all human and mouse $sPLA_2$s in the low nanomolar range. Another important class of $sPLA_2$ inhibitors is amides based on non-natural amino acids. Compound FPL67047XX[15] (**4**, Fig. 1) was found to be a potent inhibitor of human platelet $sPLA_2$, and its precise binding interactions with the human nonpancreatic $sPLA_2$ were determined by high-resolution X-ray crystallography. The structurally related compound **5** (Fig. 1) was co-crystallized with GIIA $hnpsPLA_2$ and the crystal structure revealed a chelation to a Ca^{2+} ion through its carboxylate and amide oxygen atoms, H-bonding through an amide NH group to His48, multiple hydrophobic contacts and a T-shaped aromatic group - His6 interaction.[16] In addition, compound GK115[17] (**6**, Fig. 1) was found to inhibit GV $sPLA_2$ ($X_I(50)$ = 0.003 ± 0.0004) without affecting the activities of intracellular GIVA $cPLA_2$ and GVIA $iPLA_2$. In a continuation of our studies on a novel class of $cPLA_2$ inhibitors,[18,19] the 2-oxoamide based on the natural •-amino acid (*S*)-leucine GK126,[20] (**7**, Fig. 1), demonstrated a promising IC_{50} value of 0.30 μM against GIIA $sPLA_2$.[20]

Computer-aided drug design has recently offered powerful tools for the rational design of PLA_2 inhibitors, raising the potential for the development of new compounds with improved inhibitor properties. A recent review article demonstrates various applications of rational design on PLA_2 inhibitors.[21] For example, docking calculations have led to the design of new indole inhibitors of $sPLA_2$.[22] The aim of this work was to develop improved inhibitors of GIIA $sPLA_2$ by keeping the 2-oxoamide functionality of the lead 2-oxoamide inhibitor **7** and altering its other structural features. Using molecular docking simulations, we designed new 2-oxoamides based on •-amino acids. Herein, the synthesis of new 2-oxoamides derivatives and their *in vitro* activities against various $sPLA_2$s are presented. Moreover, we report our results from docking, molecular dynamics simulations and free energy calculations of the complexes, in an attempt to understand their structure-activity relationships.

2. Results and discussion

2.1. Design and docking of new 2-oxoamides

New 2-oxoamides were designed based on the structure of the lead inhibitor **7** (Fig. 2). Proteinogenic •-amino acids of both *S* and *R* configuration were used to replace the (*S*)-leucine part of **7** and the resulted derivatives were docked in the active site of GIIA $sPLA_2$ using GOLD v5.2[23] program by CCDC. The evaluation of the generated poses was mainly based on the number of interactions they formed with the residues of the active site upon binding. The • E_{Clash}, • $E_{Internal}$, *Chemscore.Internal.Correction.Weighted* and *Chemscore.Rot.Weighted* penalty terms of the *ChemScore* scoring function were also considered. The highly scored penalty terms usually indicate poor geometry of the bound inhibitors.

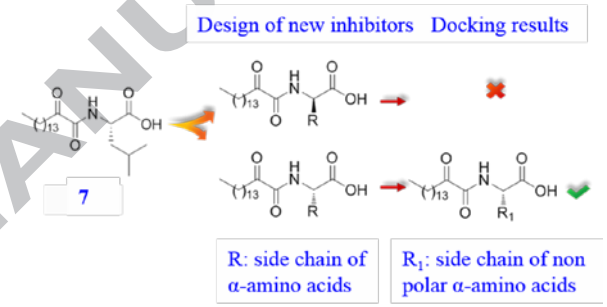


Figure 2. The workflow of the development of new potential inhibitors based on the inhibitor **7** guided by molecular docking calculations.

According to the docking results, the derivatives containing •-amino acids with *R* configuration did not generate favorable conformations. This, in combination with the low inhibitory activity of similar derivatives previously reported[20] led us to discard these structures.

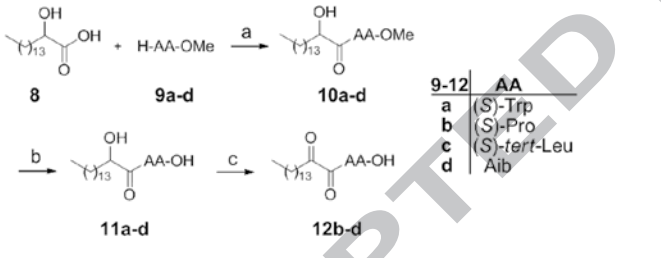
The derivatives containing non-polar •-amino acids with *S* configuration frequently generated favorable binding modes with comparatively good scoring values. Therefore, in order to better understand this behavior, simulated annealing was employed to generate 30 possible conformations for each of these molecules, which subsequently were docked in GOLD. By docking multiple conformations of each ligand, improved statistical analysis of the docking results was achieved.

Indeed, the recurrence statistics of the desirable binding motif of (*S*)-valine, (*S*)-alanine and (*S*)-proline derivatives was high, according to their docking solutions. More specifically, the carboxyl group of the derivatives chelates the calcium ion and also forms a hydrogen bond with either Gly31 or Lys62 residues. In addition, the 2-carbonyl group or the oxygen atom of the amide group point to Ca^{2+} ion. A hydrogen bond is formed between Gly29 and the 2-carbonyl group in most cases (Supporting info). Similar results were generated when derivatives of two non-natural amino acids ((*S*)-*tert*-leucine and 2-aminoisobutyric acid) were docked. Moreover, some 2-hydroxy amides were also docked and no significant differences between the generated poses and the corresponding 2-oxoamides ones were observed. The two (*S*)-Glu derivatives were docked for comparison.

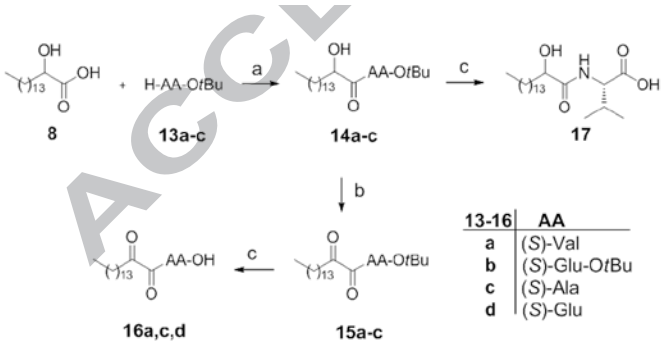
Synthesis and biological evaluation of the docked ligands were accomplished. According to the experimental data, which are discussed later, the (*S*)-valine derivative **16a** (**GK241**) demonstrated inhibitory activity against GIIA $sPLA_2$. Therefore, an effort to modify its structure, in order to succeed a higher potency, was made. It is well established that when a ligand has multiple conformations while unbound, then narrowing down this number to the only favorable conformations in the binding pocket, is entropically expensive. Thus, the replacement of **16a** long aliphatic chain by a shorter, carrying an aromatic system (phenyl, biphenyl or naphthyl), was a reasonable concept. New analogues were designed and some of the most promising structures, according to the docking simulations, were synthesized and tested.

2.2. Synthesis of inhibitors

The synthesis of 2-oxoamides and 2-hydroxyamides is presented in Schemes 1-3. The protected (*S*)-• -amino acids **9a-d** and **13a-c** were coupled with 2-hydroxyhexadecanoic acid using 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (WSCI·HCl) as a condensing agent in the presence of 1-hydroxybenzotriazole (HOBt). The • -hydroxyamide methyl esters **10a-d** were saponified to produce the acids **11a-d**. Compounds **11b-d** were then oxidized to the corresponding 2-oxoamide acids **12b-d** using Dess-Martin periodinane. In addition, • -hydroxyamide *tert*-butyl esters **14a-c** were first oxidized using Dess-Martin reagent to afford **15a-c** and subsequently, the protecting group was removed by trifluoroacetic acid to afford the desired 2-oxoamides **16a,c,d**. The synthesis of compound **21** is described in Scheme 3.

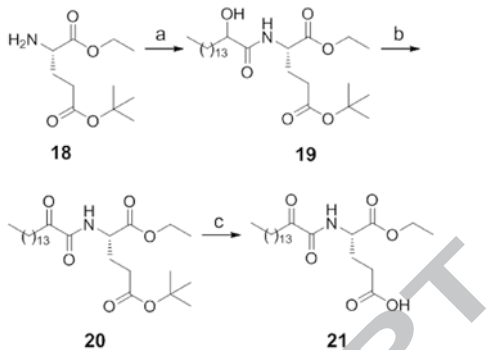


Scheme 1. Reagents and conditions: (a) WSCI·HCl, HOBt, Et_3N, CH_2Cl_2; (b) 1N aq. NaOH, MeOH; (c) Dess-Martin periodinane, CH_2Cl_2.



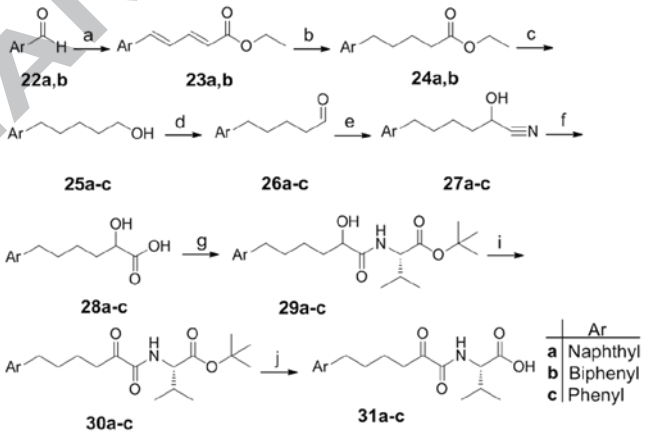
Scheme 2. Reagents and conditions: (a) WSCI·HCl, HOBt, Et_3N, CH_2Cl_2; (b) Dess-Martin periodinane, CH_2Cl_2; (c) CF_3COOH, CH_2Cl_2.

The synthesis of 2-oxoamide (*S*)-valine analogues, where the long chain has been replaced by a medium one carrying an aromatic system, is depicted in Scheme 4. Horner-Wadsworth-Emmons olefination of 1,1'-biphenyl-4-carbaldehyde and 2-naphthaldehyde **22a**,**b** led to esters **23a**,**b** and subsequently catalytic hydrogenation to saturated products **24a**,**b**. The ethyl

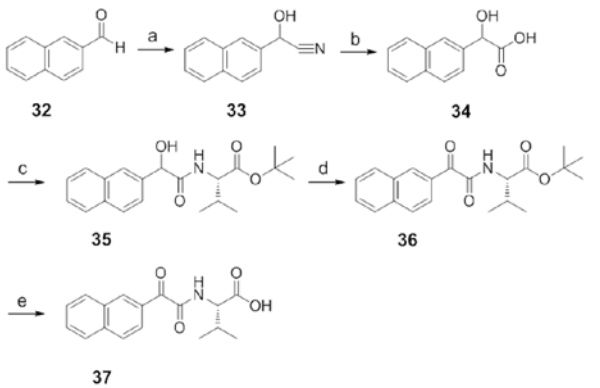


Scheme 3. Reagents and conditions: (a) $CH_3(CH_2)_{13}CHOHCOOH$ (**8**), WSCI·HCl, HOBt, Et_3N, CH_2Cl_2; (b) Dess-Martin periodinane, CH_2Cl_2; (c) CF_3COOH, CH_2Cl_2.

esters were then converted to alcohols **25a**,**b** using the di-isobutyl aluminum hydride (DIBAL-H) reducing agent, followed by the NaOCl/AcNH-TEMPO oxidation method, to form the corresponding aldehydes **26a**,**b**. The synthesis of **31c** started from 5-phenyl-1-pentanol **25c**, which was converted to the corresponding aldehyde **26c**, as it described above. Cyanohydrin reaction followed by acid and then alkaline conditions afforded the desirable • -hydroxy acids **28a-c**. These acids were coupled with (*S*)-valine, followed by oxidation and



Scheme 4. Reagents and conditions: (a) $C_2H_5OOCH{=}CHCH_2P(=O)(OC_2H_5)_2$, $LiOH{\cdot}H_2O$, dry THF; (b) H_2/Pd, THF; (c) DIBAL-H, Et_2O; (d) $NaBr/H_2O$, toluene/EtOAc, AcNH-TEMPO, 0.5• NaOCl/$NaHCO_3$/• $_2$• ; (e) (i) $NaHSO_3$, $CHCl_3$ (ii) H_2O, 6M KCN; (f) (i) HCl conc. (ii) EtOH/H_2O, KOH; (g) (*S*)-Val-O*t*Bu, WSCI·HCl, HOBt, Et_3N, CH_2Cl_2; (i) Dess-Martin periodinane, CH_2Cl_2; (j) CF_3COOH, CH_2Cl_2.



Scheme 5. Reagents and conditions: (a) EtOAc/THF/80% CH_3COOH, 6M KCN; (b) (i) HCl conc. (ii) EtOH/H_2O, KOH; (c) (*S*)-Val-O*t*Bu, WSCI·HCl, HOBt, Et_3N, CH_2Cl_2; (d) Dess-Martin periodinane, CH_2Cl_2; (e) CF_3COOH, CH_2Cl_2.

removal of the protecting group to afford the final products **31a-c**. The synthesis of **37** was accomplished by similar methods (Scheme 5).

2.3. *In vitro* inhibition of GIIA sPLA$_2$

The new 2-oxoamide derivatives were tested for inhibitory activity against a panel of human and mouse sPLA$_2$ enzymes, using a previously described continuous fluorometric assay.[24] None of the compounds **12b-d**, **16c**,**d** and **21** presented inhibition of GIIA sPLA$_2$ higher than 25% at 1 μM concentration and thus we did not further study them. However, the (*S*)-valine derivative **16a**, exhibited high and selective potency for inhibition of GIIA sPLA$_2$ and the IC$_{50}$ values are summarized in Table 1. Compound **16a** demonstrates a two times higher potency for GIIA sPLA$_2$ than the previously reported inhibitor **7** and it is ten times more selective for GIIA than GV sPLA$_2$. The IC$_{50}$ values for varespladib (**1a**, Fig. 1) using the same assay conditions, are included in Table 1 for comparison.

Table 1. IC$_{50}$ values of **1a** and **16a** on hGIIA, hGV, and mGIIA.

	IC$_{50}$ value (nM)		
Compound	**hGIIA**	**hGV**	**mGIIA**
1a	125 ± 20[13,14]	500 ± 50[13,14]	70 ± 20[13,14]
16a	143 ± 50	1200 ± 50	68 ± 16

In addition, **16a** does not exhibit any appreciable inhibition against various other human and mouse sPLA$_2$ (Table 2).

Table 2. Inhibitory activity of **16a** on other sPLA$_2$ enzymes.

Isoform	% remaining activity of the enzymes at 1000 nM	**Isoform**	% remaining activity of the enzymes at 1000 nM
hGX	95	**mGX**	98
hGIB	98	**mIID**	99
hGIIE	93	**mGV**	90
hGIIF	94		

Moreover, the lack of inhibitory activity of 2-hydroxyamide derivatives (data not shown) indicates that indeed the 2-oxoamide functionality has a crucial contribution to the binding affinity of these compounds.

Table 3. Inhibitory activity of analogues of **16a** on sPLA$_2$ enzymes.

No	**% Inhibition***			
	hGIIA	**hGIB**	**hGV**	**hGX**
31a	27	20	26	51
31b	21	30	50	54
31c	11	56	40	40
37	50	50	53	56

*10 μM final concentration of the inhibitor.

The analogues of **16a** were also tested against several sPLA$_2$s and the results are summarized in Table 3. The *in vitro* activities of **31a-c** and **37** failed to meet our docking predictions. Molecular dynamics simulations were applied on **16a** and on one of its analogues in an attempt to understand the *in vitro* data.

2.4. Molecular dynamics simulations

The lowest energy docking poses of **16a** and **31c** were subjected to MD simulations in AMBER v12 molecular dynamics package.[25] Poses were selected so that the key interactions between the ligands and the binding site to be present. The total simulation time was 200 ns in explicit solvent and RMSD calculations were performed with respect to the starting coordinates of each complex.

In the initial poses, the inhibitor interacts with the calcium ion via its carboxyl group and the 2-carbonyl group of the amide (Fig. 3 & 4). During the first 20 ns of the simulation, both molecules were reoriented within the active site in such a way that the carboxylate oxygen atoms chelated the metal in bidentate fashion (Fig. 3 & 4). This interaction lasted until the end of the simulation of both complexes.

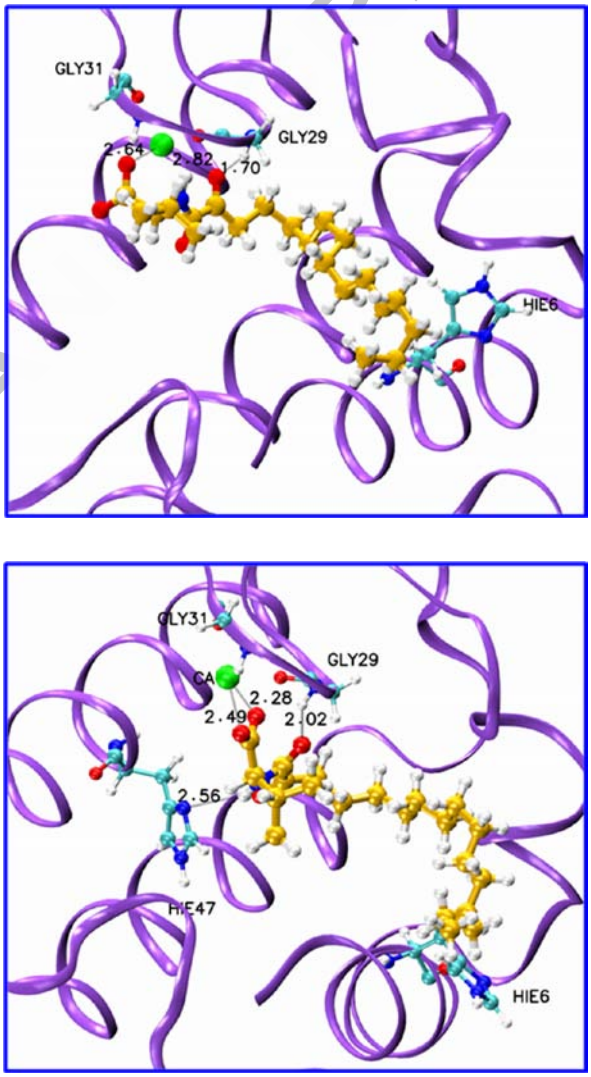


Figure 3. Conformational changes of the complex **16a**-GIIA sPLA$_2$ at the beginning (up) and at the end (down) of the MD simulation.

During the MD simulation of **16a** complex, two hydrogen bonds are formed between the amide group and the residues His47 and Gly29 (Table 4), resulting in the stable binding of **16a** to the enzyme. The RMSD distribution further supports the low mobility of the **16a** functional groups, which allows the formation of this H-bonds network (Fig. 5). However, the 2-oxoamide group of **31c** does not form any H-bond with the residues of the catalytic centre, during the simulation time (Table 4). Due to conformational changes of **31c**, the 2-oxoamide group moves away from the catalytic centre and periodically interacts only with the solvent molecules.

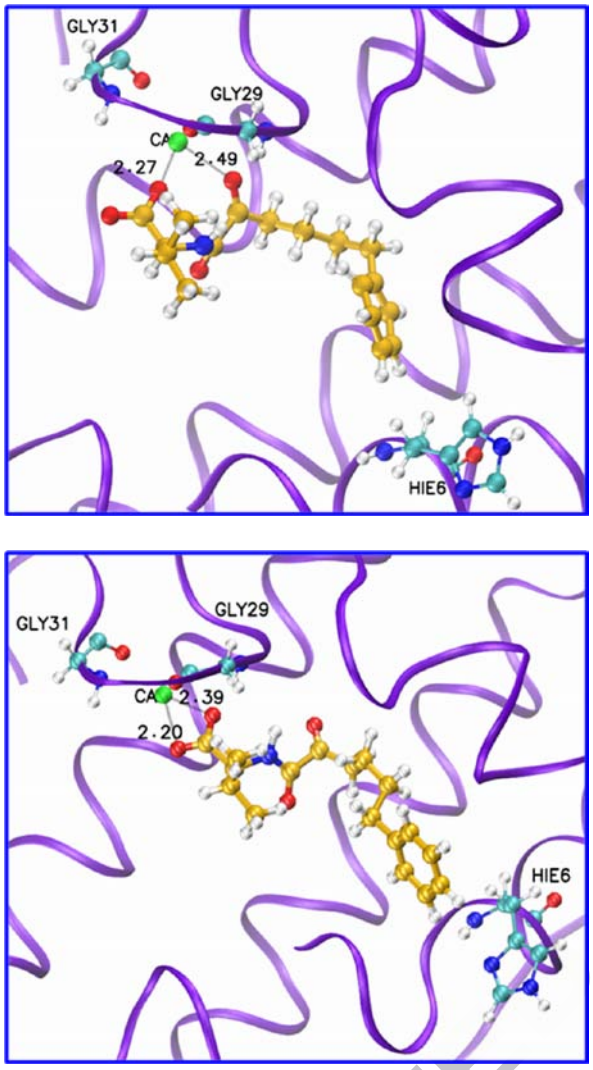


Figure 4. Conformational changes of the complex **31c**-GIIA $sPLA_2$ at the beginning (up) and at the end (down) of the MD simulation.

Table 4. Main H-bond interactions between the ligands and the residues of the GIIA $sPLA_2$ active site. The numbers describe the H-bond distance and the occurrence as the percentage of simulation time that the interaction exists.

	16a	**31c**
GLY29	COO^- group 3.05 Å 52% NHCO group 2.9 Å 51%	COO^- group 2.94 Å 75.8%
HIS47	NHCOCO group 3.18 Å 30.47%	

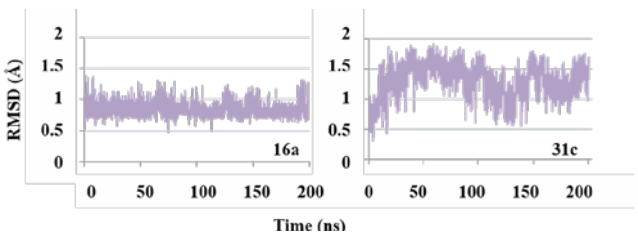


Figure 5. RMSD for the **16a** and **31c**.

The fluctuations of the RMSD values manifest the high mobility of the **31c** in the active site (Fig. 5). The RMSD values were calculated for the part of the molecules which form the key interactions and consists of the C• atom of (*S*)-valine, the two carbon atoms of the 2-oxoamide moiety and the nitrogen atom of the amide.

Using the MD trajectories, free energy calculations were performed with the Molecular Mechanics / Poisson-Boltzmann Surface Area method (MM-PBSA) (Table 5). Frames from the second half of the simulation were used for the calculations. Analysis of the energetic terms for each complex, revealed a high contribution from van der Waals forces.

Table 5. Energetic analysis for **16a**-GIIA $sPLA_2$ and **31c**-GIIA $sPLA_2$ complexes.

Energy* **(kcal·mol^{-1})**	**16a**	**31c**
ΔE_{vdW}	-36.86 ± 4.34	-30.12 ± 3.85
ΔE_{elec}	-515.69 ± 12.53	-501.60 ± 10.45
ΔG_{PB}	519.78 ± 11.93	506.27 ± 9.42
ΔG_{NP}	-4.90 ± 0.19	-3.76 ± 0.10
ΔG_{solv}	514.88 ± 11.88	502.51 ± 9.407
$\Delta E_{MM,\ gas}$	-552.55 ± 12.51	-531.73 ± 8.69
$\Delta H_{(MM+solv)}$	-37.67 ± 5.16	-29.21 ± 4.62
$-T\cdot\Delta S_{tot}$	20.09 ± 9.03	22.59 ± 11.10
$\Delta G_{MM\text{-}PBSA}$	-17.58 ± 3.37	-6.62 ± 1.81

*Errors represent the standard deviation error out of the total number of trajectory snapshots used in MM-PBSA calculations.

16a creates two additional H-bonds within the cavity of the enzyme compared to **31c**, which is reflected on the difference between the • *E* electrostatic term of the two complexes (Table 5). Moreover, the entropic term does not greatly differ between the two complexes. Partly, this could be due to the fact that both molecules are similarly flexible. Interestingly, in the case of **16a** the overall flexibility of the compound does not increase the mobility of the 2-oxoamide group, which creates the main interactions with the residues. The difference in binding energies between **16a** and **31c** is 10.96 kcal·mol^{-1}. This indicates that **16a** complex formation is more favored over **31c** complex formation, in agreement with the *in vitro* results.

3. Conclusion

Structural modifications were applied on the GIIA $sPLA_2$ inhibitor **7** and the new compounds were docked using GOLD. The results suggested that the non-polar •-amino acids derivatives with *S* configuration could generate favorable conformations. Their *in vitro* evaluation revealed that the 2-oxoamide inhibitor based on (*S*)-valine **16a** is a potent and selective inhibitor of GIIA $sPLA_2$. Further attempts to optimize the **16a** activity by altering its structure, did not lead to any desirable outcomes.

Thus, molecular dynamics simulations were used in order to understand how the replacement of the long aliphatic chain of **16a** resulted in the decrease of its potency. It was suggested that the aliphatic chain, which creates van der Waals interactions, adopts a conformation that keeps the 2-oxoamide moiety close to the key residues of the catalytic centre. Subsequently, the 2-oxoamide moiety forms a permanent H-bond network which further stabilize the **16a** binding. The replacement of the long aliphatic chain by a four C atoms chain carrying a phenyl ring led to an increase of 2-oxoamide group's mobility and the loss of the H-bonding interactions it crated with the active side. Although its carboxylic group chelates the Ca^{2+} ion and creates an H-bond with Gly29, these

interactions seems to be not enough to keep the derivative bound to the enzyme.

Further work will be needed to test the *in vivo* potency of **16a**. Molecular dynamics simulations are a useful tool for explaining and evaluating the experimental data and should be used towards this direction.

4. Experimental section

4.1. Computational methods

4.1.1. Preparation of ligands' structures

The preparation of the structures was accomplished using SYBYL v8.0.[26] The charges were defined at physiological pH and the *S* or *R* configuration was assigned. Several minimization steps were performed to accomplish optimization of the structures, using initially the steepest descent algorithm for 300 iteration steps followed by the conjugate gradients method for another 300 iteration steps and finally the Powell algorithm for 700 iteration steps.[27] The Tripos force field[28] was applied during optimization and each method was terminated when a convergence of 0.05 kcal·mol^{-1}·Å^{-1} was reached. For the Powell algorithm, the simplex method[29] was used for its initial optimization.

The simulated annealing method[30] was used in order to sample 30 relevant conformations for each structure. Molecules were heated at 600 K for 1500 fs and annealed at 250 K for 2000 fs. The Tripos force field was used and the non-bonded cutoff was set to 8.0 Å. The structures produced were optimized using 200 iteration steps with the Powell algorithm with a gradient of 0.05 kcal·mol^{-1}·Å^{-1}. The simplex method was used as well.

4.1.2. Docking

The atomic coordinates of the human secreted phospholipase group IIA protein were downloaded from Protein Data Bank[31] (PDB code: 1KQU).[16] Missing loops were added with the Prime module of Schrödinger Suite.[32] Hydrogen atoms were added in GOLD and atoms types were automatically assigned by the program.

Docking simulations were performed in GOLD. The cavity of the catalytic centre of GIIA sPLA$_2$ was oriented within 5 Å around the crystallographic ligand including Leu2, Phe5, His6, Gly29, Gly31, His47, Asp48, Lys52, Lys62 and Asp91 amino acids. Eight crystal water molecules in the spatial vicinity to the catalytic center were kept and assigned as toggle and spin, while the rest were deleted. His6 was assigned to have flexible side chain (1 rotamer). H-bond donors / acceptors were treated as solvent accessible. The options 'flip amide bonds and ring corners' were set on for ligand flexibility. The *ChemScore* scoring function (1) along with chemscore.p450_csd.params parameters file were used to rank the generated binding modes.

$$ChemScore = -(\bullet G + \bullet E(\text{clash}) + \bullet E(\text{int})) \quad (1)$$

where $\bullet G$ is the free energy change upon ligand binding, $\bullet E$ (clash) is the protein-ligand clash penalty and $\bullet E$ (int) is the ligand internal torsion strain penalty. It was established via additional computational experiments that this combination of scoring function and parameters were suitable for the docking. Internal ligand energy offset was enabled and the preset genetic algorithm parameter settings were used and set to 100,000 operations.

4.1.3. Molecular dynamics simulations

MD simulations in explicit solvent were performed using AMBER.[25] The Antechamber tool was used to generate the partial atomic charges of each ligand with the AM1-BCC[33] method. Modification of the force field parameters of each structure was made by Parmchk program. The AMBER force field *ff12SB*[34] and the general AMBER force field (GAFF)[35] were loaded in tLEaP module to create the parameters and topologies of the protein and the organic molecule, respectively. The complex was loaded in tLEaP along with the parameters file of calcium ions, which were created by a combination of crystallographic data,[16] GAFF force field parameters[35] and the Aqvist *et al.* method.[36] Disulfide bonds between Cys83-Cys59, Cys124-Cys49, Cys117-Cys26, Cys50-Cys90, Cys28-Cys44, Cys43-Cys97 and Cys88-Cys77 were assigned, as well as the coordination between the metal ion of the catalytic centre and the residues His27, Gly29 and Gly31. The complex was then immersed in a truncated octahedral water box using the TIP3P water model with 12.0 Å periodic boundary conditions. Finally, the system was neutralized by adding 18 Cl^- counter ions using the Joung and Cheatham ion parameters[37] and its coordinate and parameter topology files were saved.

Prior to the MD simulation, an initial minimization was performed using 1500 iteration steps of steepest descent and 1500 iteration steps of conjugate gradient in SANDER,[38] keeping the complex almost fixed with a restraint set to 400 kcal·mol^{-1}·Å^{-1}. In addition, four sets of steepest descent and conjugate gradient steps were applied to the system, with the strength of the restraint being gradually reduced to 8 kcal·mol^{-1}·Å^{-1}. The system was then smoothly heated from 0 to 300 K in three steps under constant volume. During these steps of total time 100 ps, the restraint was retained to 8 kcal·mol^{-1}·Å^{-1}, hydrogen atoms were constrained using the SHAKE algorithm and the Langevin thermostat was used for temperature control. Two steps of equilibration were applied keeping the restraint to 8 kcal·mol^{-1}·Å^{-1} for the first 50 ps and then removed it for the next 50 ps, under constant pressure. MD simulations were performed with the pmemd.cuda[39] implementation for 200 ns and a 8 Å nonbonded cutoff was applied. SHAKE was kept during the production runs to allow the use of 2 fs time step, and the long-range electrostatic interactions were accounted for using the particle mesh Ewald (PME) method.[40] Analysis on the produced trajectories (RMSD, distances, H-bond interactions) was performed with the ptraj analysis tool available in the AmberTools13.[41]

The relative binding energies of each system (Table 5) were estimated using the MM-PBSA.[42] The $\bullet E_{\text{elect}}$ term is the electrostatic interaction energy and the $\bullet E_{\text{vdW}}$ term is the van der Waals interaction energy.

The Poisson-Boltzmann (PB) model[43] was used for the estimation of the electrostatic contribution to solvation energies ($\bullet G_{\text{PB}}$) and the solvent-accessible surface area (SASA) was used for the estimation of the non electrostatic contributions ($\bullet G_{\text{NP}}$). The SASA is calculated by the following equation:

$$\bullet G_{\text{NP}} = \gamma \text{SASA} + \beta \quad (2)$$

where the SASA term is the surface area of the solute, the γ term is 0.00542 kcal·mol^{-1}·Å^{-2} while β is -1.008000 kcal·mol^{-1}.[44] The γ and β are constants related with the surface tension coefficient and the offset, respectively.

The entropic term $T \cdot S$ was calculated using the *nmode* program available in AMBER. The metal ions were removed during the calculation (strip_mask) to avoid misleading results.

4.2. Chemistry

Merck Silica Gel 60 (70-230 or 230-400 mesh) z dv#kvhg#ru# vkh#fkurp dwjudsklf#sxulilfdwrq#ri#surgxfw#dqg#Mdfd#ho#93# I587#doxp lqxp#sodwv#ir u#vkh#vklq-od|hu#fkurp dwjudsk|#+WOF,1#XY#dj kw#dqg2ru#skrvskrp rq|eglf#df lg#lq#HwRK#z dv# hp s or|hg#ir u#ylvxdd}lqj#vsr wl#D#E˙fkl#530 apparatus was used to estimate melting points and they were uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury at 200 MHz or 300 MHz and 50 MHz respectively. Samples were diluted in $CDCl_3$, CD_3OD or DMSO. Chemical shifts are given in ppm, and coupling constants (*J*) in Hz. Peak multiplicities are typified as: s, singlet, d, doublet, t, triplet and m, multiplet. Electron spray ionization (ESI) mass spectra were recorded on a Finnigan Surveyor MSQ Plus spectrometer. Specific rotations of the compounds were measured at 25 ºC on a Perkin-Elmer 343 polarimeter using a 10 cm cell. Dichloromethane, diethylether and toluene were dried by standard procedures and stored over molecular sieves. No further purification of other solvents and chemicals needed as they were reagent grade. HRMS spectra were recorded on a Bruker Maxis Impact QTOF Spectrometer.

Compounds **23b**,[45] **24b**,[45] **25a**,[46] **26a**,[46] **25b**,[47] **26b**[48] have been described elsewhere and their analytical data are in accordance with literature.

4.2.1. Coupling method. To a stirred solution of hydrochloride amino component (1.0 mmol) in CH_2Cl_2 (10 mL), Et_3N (0.3 mL, 2.2 mmol) and subsequently 1-(3-dimethyl-aminopropyl)-3-ethyl carbodiimide hydrochloride (WSCI·HCl) (0.21 g, 1.1 mmol) and 1-hydroxybenzotriazole (HOBt) (0.14 g, 1.0 mmol) were added at 0˚C. The acid reactant (1.0 mmol) was added and the reaction mixture was stirred for 1 h at 0˚C and then overnight at room temperature. After the completion of the reaction, the solvent was evaporated under reduced pressure and EtOAc (20 mL) was added. The organic layer was washed consecutively with brine, 1N HCl, brine, 5% $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated under reduced pressure. Purification by flash column chromatography eluting with the appropriate mixture of EtOAc/petroleum ether (bp 40-60 °C) afforded the product.

4.2.1.1. (2*S*)-Methyl 2-(2-hydroxyhexadecanamido)-3-(1*H*-indol-3-yl)propanoate (mixture of diastereomers) (10a). Yield 52%; White solid; mp 78-85 °C; $[\bullet]^{20}_D$ 41.1 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 8.22 (s, 1H, NH), 7.61-7.50 (m, 1H, arom), 7.43-6.88 (m, 5H, arom & NH), 5.08-4.88 (m, 1H, NHC*H*), 4.14-3.97 (m, 1H, C*H*OH), 3.81-3.67 (m, 3H, $COOCH_3$), 3.34 (d, *J* = 5.6 Hz, 2H, NHCHCH_2), 1.87-1.64 (m, 2H, CH_2CHOH), 1.62-1.15 (m, 24H, CH_2), 0.89 (t, *J* = 6.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 173.6, 172.3, 169.0, 168.4, 135.9, 122.7, 122.2, 119.6, 119.5, 118.5, 118.3, 111.3, 109.8, 80.6, 72.0, 52.4, 34.6, 31.9, 29.6, 29.5, 29.4, 29.3, 27.6, 24.9, 22.6, 14.1; MS (ESI) m/z (%): 473.3 (100) $[M + H]^+$; Anal. Calcd for $C_{28}H_{44}N_2O_4$: C, 71.15; H, 9.38; N, 5.93. Found: C, 71.03; H, 9.45; N, 5.87.

4.2.1.2. (2*S*)-Methyl 1-(2-hydroxyhexadecanoyl) pyrrolidine -2-carboxylate (mixture of diastereomers) (10b). Yield 64%; White solid; mp 42-55 °C; $[\bullet]^{20}_D$ -50.0 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 4.75-4.43 (m, 1H, NCH), 4.29-4.14 (m, 1H, C*H*OH), 3.71 (s, 3H, CH_3), 3.62-3.44 (m, 2H, NCH_2), 3.06 (br, 1H, OH), 2.57-1.74 (m, 6H, $NCH_2CH_2CH_2$ & CH_2CHOH), 1.73-1.42 (m, 4H, CH_2), 1.23 (s, 20H, CH_2), 0.85 (t, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 173.6, 172.4, 81.9, 69.5, 59.4, 59.1, 56.7, 52.5, 46.7, 45.6, 34.4, 32.7, 32.1, 29.9, 29.7, 29.6, 29.2, 28.9, 25.3, 24.9, 22.9, 14.3; MS (ESI) m/z (%): 384.4 (100) $[M + H]^+$; Anal. Calcd for $C_{22}H_{41}NO_4$: C, 68.89; H, 10.77; N, 3.65. Found: C, 68.63; H, 10.84; N, 3.56.

4.2.1.3. (2*S*)-Methyl 2-(2-hydroxyhexadecanamido)-3,3-dimethylbutanoate (mixture of diastereomers) (10c). Yield 84%; White solid; mp 73-75 °C; $[\bullet]^{20}_D$ 2.30 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.29-7.07 (m, 1H, NH), 4.47-4.37 (m, 1H, NHC*H*), 4.21-4.04 (m, 1H, C*H*OH), 3.70 (s, 3H, CH_3), 1.93-1.70 (m, 2H, CH_2CHOH), 1.52-1.16 (m, 24H, CH_2), 0.96 (s, 9H, CH_3), 0.85 (t, *J* = 6.8 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 174.0, 172.1, 171.7, 72.2, 71.9, 59.5, 59.4, 51.7, 34.7, 31.8, 29.6, 29.3, 29.2, 26.4, 25.0, 24.9, 22.6, 14.0; MS (ESI) m/z (%): 400.2 (100) $[M + \bullet]^+$; Anal. Calcd for $C_{23}H_{45}NO_4$: C, 69.13; H, 11.35; N, 3.51. Found: C, 69.05; H, 11.51; N, 3.32.

4.2.1.4. Methyl 2-(2-hydroxyhexadecanamido)-2-methylpropanoate (racemic mixture) (10d). Yield 54%; White solid; mp 53-56 °C; $[\bullet]^{20}_D$ 2.35 (*c* 1.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.13 (s, 1H, NH), 4.09-3.93 (m, 1H, C*H*• •), 3.72 (s, 3H, $COOCH_3$), 3.53 (br, 1H, OH), 1.84-1.58 (m, 2H, CH_2CHOH), 1.53 (s, 6H, CH_3), 1.34-1.10 (m, 24H, CH_2), 0.86 (t, *J* = 6.8 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 174.9, 173.7, 71.8, 55.9, 52.5, 34.5, 31.8, 29.6, 29.4, 29.3, 24.8, 24.6, 22.6, 14.0; MS (ESI) m/z (%): 372.2 (100) $[M + \bullet]^+$; Anal. Calcd for $C_{21}H_{41}NO_4$: C, 67.88; H, 11.12; N, 3.77. Found: C, 67.66; H, 11.29; N, 3.72.

4.2.1.5. (2*S*)-*tert*-Butyl 2-(2-hydroxyhexadecanamido)-3-methylbutanoate (mixture of diastereomers) (14a). Yield 62%; White solid; mp 49-53 °C; $[\bullet]^{20}_D$ 9.80 (*c* 1.01, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.17 (d, *J* = 9.0 Hz, ½H, NH), 7.05 (d, *J* = 9.0 Hz, ½H, NH), 4.46-4.31 (m, 1H, CH), 4.18-4.03 (m, 1H, C*H*OH), 4.00 (d, *J* = 4.8 Hz, ½H, OH), 3.83 (d, *J* = 4.8 Hz, ½H, OH), 2.24-2.03 (m, 1H, NHCHC*H*), 1.90-1.52 (m, 2H, CH_2CHOH), 1.44 (s, 9H, CH_3), 1.34-1.13 (m, 24H, CH_2), 0.97-0.75 (m, 9H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 174.1, 174.0, 171.3, 170.9, 82.0, 81.9, 72.2, 71.9, 57.0, 56.8, 34.8, 31.8, 31.3, 31.2, 29.6, 29.5, 29.3, 27.9, 25.0, 24.8, 22.6, 18.9, 18.8, 17.5, 17.4, 14.0; MS (ESI) m/z (%): 428.3 (95) $[M + \bullet]^+$; Anal. Calcd for $C_{25}H_{49}NO_4$: C, 70.21; H, 11.55; N, 3.28. Found: C, 70.03; H, 11.70; N, 3.19.

4.2.1.6. (2*S*)-di-*tert*-Butyl 2-(2-hydroxyhexadecanamido) pentanedioate (mixture of diastereomers) (14b). Yield 76%; Yellowish oil; $[\bullet]^{20}_D$ 8.1 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.24-7.06 (m, 1H, NH), 4.61-4.38 (m, 1H, NHC*H*), 4.22-4.02 (m, 1H, C*H*OH), 3.61 (br, 1H, OH), 2.44-1.71 (m, 6H, CH_2), 1.53-1.38 (m, 18H, CH_3), 1.36-1.17 (m, 24H, CH_2), 0.97-0.78 (m, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 174.2, 174.0, 172.0, 170.8, 82.3, 82.2, 80.7, 71.9, 51.7, 34.7, 31.8, 31.5, 29.6, 29.3, 29.2, 27.9, 27.6, 24.9, 22.6, 14.0; MS (ESI) m/z (%): 514.6 (90) $[M + H]^+$; Anal. Calcd for $C_{29}H_{55}NO_6$: C, 67.80; H, 10.79; N, 2.73. Found: C, 67.62; H, 10.88; N, 2.67.

4.2.1.7. (2*S*)-*tert*-Butyl 2-(2-hydroxyhexadecanamido) propanoate (mixture of diastereomers) (14c). Yield 40%; Colorless oil; $[\bullet]^{20}_D$ 7.7 (*c* 0.99, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.22-7.04 (m, 1H, NH), 4.56-4.35 (m, 1H, NHC*H*), 4.17-4.02 (m, 1H, C*H*OH), 3.45 (br, 1H, OH), 1.93-1.55 (m, 2H, CH_2CHOH), 1.45 (s, 9H, CH_3), 1.36 (d, *J* = 7.0 Hz, 3H, NHCHCH_3), 1.32-1.18 (s, 24H, CH_2), 0.86 (t, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 173.9, 173.7, 172.3, 172.0, 81.9, 71.9, 48.1, 34.7, 34.6, 31.8, 29.6, 29.5, 29.4, 29.3, 29.2, 27.8, 24.9, 22.6, 18.4, 14.0; MS (ESI) m/z (%): 400.4

(100) [M + H]$^+$; Anal. Calcd for $C_{23}H_{45}NO_4$: C, 69.13; H, 11.35; N, 3.51. Found: C, 68.91; H, 11.46; N, 3.43.

4.2.1.8. (2*S*)-5-*tert*-Butyl 1-ethyl-2-(2-hydroxyhexadecanamido)pentanedioate (mixture of diastereomers) (19). Yield 53%; Yellow oil; [•]$^{20}_D$ 5.80 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.33-7.19 (m, 1H, NH), 4.55-4.38 (m, 1H, NHC*H*), 4.18-3.95 (m, 3H, C*H*OH & COOCH_2), 2.33-1.74 (m, 6H, CH_2 & CH_2CHOH), 1.34 (s, 9H, CH_3), 1.29-1.07 (m, 27H, CH_2 & CH_3), 0.76 (t, *J* = 6.8 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 174.6, 174.4, 171.8, 171.5, 80.6, 71.8, 61.4, 51.1, 34.5, 34.5, 34.4, 31.7, 31.2, 29.4, 29.2, 29.1, 27.8, 27.1, 24.8, 24.7, 22.4, 13.9; MS (ESI) m/z (%): 486.4 (100) [M + H]$^+$; Anal. Calcd for $C_{27}H_{51}NO_6$: C, 66.77; H, 10.58; N, 2.88. Found: C, 66.48; H, 10.72; N, 2.78.

4.2.1.9. (2*S*)-*tert*-Butyl 2-(2-hydroxy-6-(naphthalen-2-yl) hexanamido)-3-methylbutanoate (mixture of diastereomers) (29a). Yield 73%; Colorless oil; [•]$^{20}_D$ 10.1 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.86-7.68 (m, 3H, arom), 7.63-7.54 (m, 1H, arom), 7.51-7.24 (m, 3H, arom), 7.08 (d, *J* = 8.8 Hz, ½H, NH), 6.91 (d, *J* = 8.8 Hz, ½H, NH), 4.51-4.39 (m, 1H, NHC*H*), 4.21-4.06 (m, 1H, C*H*OH), 3.02 (br, 1H, OH), 2.78 (t, *J* = 7.4 Hz, 2H, CH_2), 2.27-2.06 (m, 1H, NHCHC*H*), 2.01-1.64 (m, 4H, CH_2), 1.62-1.40 (m, 11H, CH_2 & CH_3), 0.98-0.80 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 173.9, 173.8, 171.3, 170.9, 139.8, 133.5, 131.8, 127.7, 127.5, 127.3, 127.2, 126.2, 125.7, 124.9, 82.1, 82.0, 72.0, 71.8, 57.0, 56.8, 35.8, 35.8, 34.7, 34.6, 31.3, 31.2, 31.0, 27.9, 24.6, 18.9, 18.8, 17.5, 17.3; MS (ESI) m/z (%): 412.2 (100) [M - H]$^-$; Anal. Calcd for $C_{25}H_{35}NO_4$: C, 72.61; H, 8.53; N, 3.39. Found: C, 72.38; H, 8.69; N, 3.20.

4.2.1.10. (2*S*)-*tert*-Butyl 2-(6-([1,1'-biphenyl]-4-yl)-2-hydroxyhexanamido)-3-methylbutanoate (mixture of diastereomers) (29b). Yield 68%; Colorless oil; [•]$^{20}_D$ 10.8 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.64-6.90 (m, 10H, arom & NH), 4.47 (dd, J_1 = 9.0 Hz, J_2 = 4.6 Hz, 1H, NHC*H*), 4.25-4.05 (m, 1H, C*H*OH), 3.72-3.33 (m, 1H, OH), 2.67 (t, *J* = 7.4 Hz, 2H, CH_2), 2.31-2.08 (m, 1H, NHCHC*H*), 1.98-1.34 (m, 15H, CH_2 & CH_3), 1.00-0.81 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 174.0, 171.3, 170.9, 141.4, 141.0, 138.5, 128.8, 128.6, 126.8, 82.1, 82.0, 72.0, 71.8, 57.0, 56.8, 35.3, 34.6, 31.3, 31.2, 31.1, 27.9, 24.6, 18.9, 18.8, 17.5, 17.4; MS (ESI) m/z (%): 438.4 (100) [M - H]$^-$; Anal. Calcd for $C_{27}H_{37}NO_4$: C, 73.77; H, 8.48; N, 3.19. Found: C, 73.55; H, 8.65; N, 3.14.

4.2.1.11. (2*S*)-*tert*-Butyl 2-(2-hydroxy-6-phenylhexanamido) -3-methylbutanoate (mixture of diastereomers) (29c). Yield 92%; Colorless oil; [•]$^{20}_D$ 8.38 (*c* 0.99, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.33-6.96 (m, 6H, arom & NH), 4.42 (dd, J_1 = 9.0 Hz, J_2 = 4.6 Hz, 1H, NHC*H*), 4.21-4.03 (m, 1H, C*H*OH), 3.72 (br, 1H, OH), 2.61 (t, *J* = 7.4 Hz, 2H, CH_2), 2.30-2.06 (m, 1H, NHCHC*H*), 1.96-1.52 (m, 6H, CH_2), 1.47 (s, 9H, CH_3), 0.97-0.82 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 174.1, 174.0, 171.3, 170.8, 142.3, 128.2, 128.1, 125.5, 82.0, 81.9, 71.9, 71.8, 56.9, 56.7, 35.7, 34.5, 31.1, 27.9, 24.6, 18.8, 17.5, 17.4; MS (ESI) m/z (%): 362.4 (100) [M - H]$^-$; Anal. Calcd for $C_{21}H_{33}NO_4$: C, 69.39; H, 9.15; N, 3.85. Found: C, 69.27; H, 9.27; N, 3.75.

4.2.1.12. (2*S*)-*tert*-Butyl 2-(2-hydroxy-2-(naphthalen-2-yl) acetamido)-3-methylbutanoate (mixture of diastereomers) (35). Yield 66%; Yellow oil; [•]$^{20}_D$ 7.9 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.88-7.68 (m, 4H, arom), 7.55-7.38 (m, 3H, arom), 7.29-6.77 (m, 1H, NH), 5.20 (d, *J* = 3.0 Hz, 1H, C*H*OH), 4.47-4.33 (m, 1H, NHC*H*), 2.20-1.97 (m, 1H, NHCHC*H*), 1.46-1.33 (m, 9H, CH_3), 0.93-0.66 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 172.1, 172.0, 170.7, 170.6, 136.7, 133.1, 133.0, 128.5, 128.3, 128.0, 127.5, 126.2, 126.1, 126.0, 125.9, 124.0, 123.9, 82.1, 74.3, 74.1, 57.3, 57.1, 31.3, 27.8, 18.7, 17.4, 17.2; MS (ESI) m/z (%): 356.2 (100) [M - H]$^-$; Anal. Calcd for $C_{21}H_{27}NO_4$: C, 70.56; H, 7.61; N, 3.92. Found: C, 70.38; H, 7.70; N, 3.86.

4.2.2. Saponification of methyl esters. To a stirred solution of a methyl ester (1.0 mmol) in water, 1N NaOH (1 mL, 1.0 mmol) was added and the mixture was left overnight at room temperature. After the completion of the reaction, the mixture was washed with EtOAc, acidified with 1N HCl to pH 1 and extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure.

4.2.2.1. (2*S*)-2-(2-Hydroxyhexadecanamido)-3-(1*H*-indol-3-yl) propanoic acid (mixture of diastereomers) (11a). Yield 80%; White solid; mp 144-146 °C; [•]$^{20}_D$ 18.7 (*c* 1.00, MeOH); ^{1}H NMR (CD_3OD, 200 MHz): • 7.63-7.51 (m, 1H, NH), 7.36-7.26 (m, 1H, arom), 7.15-6.92 (m, 3H, arom), 4.82-4.67 (m, 1H, NHC*H*), 4.01-3.88 (m, 1H, C*H*OH), 3.44-3.19 (m, 2H, NHCHCH_2), 1.77-1.50 (m, 2H, CH_2CHOH), 1.48-1.12 (m, 24H, CH_2), 0.89 (t, *J* = 6.6 Hz, 3H, CH_3); ^{13}C NMR (CD_3OD, 50 MHz): • 177.0, 175.0, 142.0, 128.9, 124.5, 122.4, 119.9, 119.4, 112.2, 110.4, 72.5, 53.3, 35.5, 33.1, 30.8, 30.5, 28.5, 25.9, 23.8, 14.5; HRMS (ESI) calcd for $C_{27}H_{42}N_2NaO_4$ [M + Na]$^+$: 481.3037. Found: 481.3057; Anal. Calcd for $C_{27}H_{42}N_2O_4$: C, 70.71; H, 9.23; N, 6.11. Found: C, 70.59; H, 9.35; N, 6.01.

4.2.2.2. (2*S*)-1-(2-Hydroxyhexadecanoyl)pyrrolidine-2-carboxylic acid (mixture of diastereomers) (11b). Yield 80%; White solid; mp 71-74 °C; [•]$^{20}_D$ -5.2 (*c* 1.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 4.82-4.38 (m, 1H, NCH), 4.37-4.08 (m, 1H, C*H*OH), 3.82-3.30 (m, 2H, NCH_2), 2.56-1.76 (m, 4H, NCHCH_2CH_2), 1.73-1.00 (m, 26H, CH_2), 0.86 (t, *J* = 6.8 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 174.6, 167.8, 81.5, 69.5, 59.5, 56.4, 46.7, 45.4, 33.9, 32.3, 31.8, 29.5, 29.4, 29.3, 28.9, 28.4, 25.0, 24.5, 22.6, 22.1, 14.0; HRMS (ESI) calcd for $C_{21}H_{39}NNaO_4$ [M + Na]$^+$: 392.2771. Found: 392.2785; Anal. Calcd for $C_{21}H_{39}NO_4$: C, 68.25; H, 10.64; N, 3.79. Found: C, 68.14; H, 10.73; N, 3.65.

4.2.2.3. (2*S*)-2-(2-Hydroxyhexadecanamido)-3,3-dimethylbutanoic acid (mixture of diastereomers) (11c). Yield 80%; White solid; mp 99-101 °C; [•]$^{20}_D$ 1.8 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.52-7.01 (m, 1H, NH), 4.51-4.25 (m, 1H, NHC*H*), 4.24-4.03 (m, 1H, C*H*OH), 1.92-1.11 (m, 26H, CH_2), 0.96 (s, 9H, CH_3), 0.89-0.72 (m, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 175.5, 174.6, 174.4, 74.5, 59.8, 34.6, 34.5, 31.9, 29.7, 29.6, 29.5, 29.3, 26.5, 25.1, 24.5, 22.6, 14.1; MS (ESI) m/z (%): 386.2 (100) [M + H]$^+$; Anal. Calcd for $C_{22}H_{43}NO_4$: C, 68.53; H, 11.24; N, 3.63. Found: C, 68.44; H, 11.35; N, 3.52.

4.2.2.4. 2-(2-Hydroxyhexadecanoylamino)-2-methylpropanoic acid (racemic mixture) (11d). Yield 72%; White solid; mp 81-83 °C; [•]$^{20}_D$ 1.7 (*c* 1.02, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.20 (br s, 1H, NH), 4.21-3.92 (m, 1H, C*H*OH), 1.94-1.03 (m, 32H, CH_2 & CH_3), 0.88 (t, *J* = 6.8 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 173.1, 96.9, 96.4, 72.0, 34.3, 31.9, 29.8, 29.6, 29.5, 29.3, 25.1, 24.7, 24.5, 22.7, 14.1; MS (ESI) m/z (%): 358.3 (100) [M + H]$^+$; Anal. Calcd for $C_{20}H_{39}NO_4$: C, 67.19; H, 10.99; N, 3.92. Found: C, 67.02; H, 11.08; N, 3.86.

4.2.3. Oxidation of 2-hydroxyamides. To a solution of 2-hydroxyamide (1.0 mmol) in dry CH_2Cl_2 (10 mL) Dess-Martin periodinane was added (0.64 g, 1.5 mmol) and the mixture was stirred for 1-3 h at room temperature. The organic solvent was

evaporated under reduce pressure and Et_2O (30 mL) was added. The organic phase was washed with saturated aqueous $NaHCO_3$ (20 mL) containing $Na_2S_2O_3$ (1.5 g, 9.5 mmol), H_2O (20 mL), dried over Na_2SO_4 and the organic solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography [EtOAc/petroleum ether (bp 40-60 °C), 2:8 or $CHCl_3$/MeOH, 9:1].

4.2.3.1. (*S*)-1-(2-Oxohexadecanoyl)pyrrolidine-2-carboxylic acid (rotamers) (12b). Yield 80%; Colorless semi solid; $[\bullet]^{20}_D$ -3.2 (*c* 1.01, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.40 (br, 1H, COOH), 4.86-4.31 (m, 1H, NCH), 3.95-3.41 (m, 2H, NCH_2), 3.06-2.60 (m, 2H, CH_2CO), 2.46-1.12 (m, 28H, NCHCH_2CH_2 & CH_2), 0.88 (t, *J* = 6.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 200.0, 177.4, 163.0, 61.3, 48.3, 47.4, 39.1, 38.5, 31.9, 29.7, 29.5, 29.3, 29.1, 25.1, 23.1, 22.7, 22.6, 14.0; HRMS (ESI) calcd for $C_{21}H_{38}NO_4$ $[M + H]^+$: 368.2795. Found: 368.2794; Anal. Calcd for $C_{21}H_{37}NO_4$: C, 68.63; H, 10.15; N, 3.81. Found: C, 68.51; H, 10.22; N, 3.74.

4.2.3.2. (*S*)-3,3-Dimethyl 2-(2-oxohexadecanamido)butanoic acid (12c). Yield 62%; Colorless oil; $[\bullet]^{20}_D$ 1.8 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.50 (d, *J* = 9.2 Hz, 1H, NH), 4.31 (d, *J* = 9.2 Hz, 1H, NHC*H*), 2.91 (t, *J* = 6.8 Hz, 2H, CH_2CO), 1.74-1.49 (m, 2H, CH_2CH_2CO), 1.43-1.18 (m, 22H, CH_2), 1.01 (s, 9H, CH_3), 0.88 (t, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 198.5, 175.7, 159.8, 60.8, 36.8, 34.7, 31.9, 29.6, 29.4, 29.3, 29.0, 26.5, 23.0, 22.6, 14.1; HRMS (ESI) calcd for $C_{22}H_{41}NNaO_4$ $[M + Na]^+$: 406.2928. Found: 406.2945; Anal. Calcd for $C_{22}H_{41}NO_4$: C, 68.89; H, 10.77; N, 3.65. Found: C, 69.11; H, 10.88; N, 3.59.

4.2.3.3. 2-Methyl 2-(2-oxo-hexadecanoylamino)propanoic acid (12d). Yield 95%; Colorless oil; ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.77 (br s, 1H, COOH), 2.88 (t, *J* = 6.8 Hz, 2H, CH_2CO), 1.66-1.40 (m, 8H, CH_3 & CH_2CH_2CO), 1.39-1.15 (m, 22H, CH_2), 0.88 (t, *J* = 6.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 199.0, 198.8, 180.6, 160.0, 57.2, 36.4, 31.9, 29.6, 29.5, 29.4, 29.3, 29.1, 24.2, 23.0, 22.6, 14.1; HRMS (ESI) calcd for $C_{20}H_{37}NNaO_4$ $[M + Na]^+$: 378.2615. Found: 378.2627; Anal. Calcd for $C_{20}H_{37}NO_4$: C, 67.57; H, 10.49; N, 3.94. Found: C, 67.36; H, 10.61; N, 3.87.

4.2.3.4. (*S*)-*tert*-Butyl 3-methyl-2-(2-oxohexadecanamido) butanoate (15a). Yield 92%; Yellow oil; $[\bullet]^{20}_D$ 12.7 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.40 (d, *J* = 9.0 Hz, 1H, NH), 4.40 (dd, J_1 = 9.2 Hz, J_2 = 4.4 Hz, 1H, NHC*H*), 2.91 (t, *J* = 7.2 Hz, 2H, CH_2COCO), 2.33-2.10 (m, 1H, NHCHC*H*), 1.71-1.53 (m, 2H, CH_2CH_2CO), 1.48 (s, 9H, CH_3) 1.40-1.16 (m, 22H, CH_2), 1.01-0.81 (m, 9H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 198.2, 169.6, 159.5, 81.9, 57.1, 36.4, 31.5, 31.1, 29.2, 29.0, 28.7, 27.6, 22.8, 22.3, 18.6, 17.1, 13.7; MS (ESI) m/z (%): 387.2 (100) [M - *t*Bu + NH_4]$^+$; Anal. Calcd for $C_{25}H_{47}NO_4$: C, 70.54; H, 11.13; N, 3.29. Found: C, 70.43; H, 11.24; N, 3.21.

4.2.3.5. (*S*)-di-*tert*-Butyl 2-(2-oxohexadecanamido) pentanedioate (15b). Yield 84%; Yellow oil; $[\bullet]^{20}_D$ 9.18 (*c* 1.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): • 7.47 (d, *J* = 8.4 Hz, 1H, NH), 4.49-4.37 (m, 1H, NHC*H*), 2.88 (t, *J* = 7.2 Hz, 2H, CH_2COCO), 2.38-2.08 (m, 3H, CH_2C*H*H), 2.05-1.87 (m, 1H, C*H*H), 1.66-1.51 (m, 2H, CH_2CH_2CO), 1.46 (s, 9H, CH_3), 1.42 (s, 9H, CH_3), 1.35-1.16 (m, 22H, CH_2), 0.86 (t, *J* = 6.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 198.3, 171.7, 170.0, 159.8, 82.7, 80.8, 52.1, 36.7, 31.8, 31.3, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 28.0, 27.9, 27.4, 23.0, 22.6, 14.0; MS (ESI) m/z (%): 529.5 (100) [M + NH_4]$^+$; Anal. Calcd for $C_{29}H_{53}NO_6$: C, 68.06; H, 10.44; N, 2.74. Found: C, 67.90; H, 10.55; N, 2.68.

4.2.3.6. (*S*)-*tert*-Butyl 2-(2-oxohexadecanamido)propanoate (15c). Yield 95%; White solid; mp 35-36 °C; $[\bullet]^{20}_D$ 5.0 (*c* 1.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.44 (d, *J* = 7.0 Hz, 1H, NH), 4.52-4.33 (m, 1H, NHC*H*), 2.90 (t, *J* = 7.4 Hz, 2H, CH_2CO), 1.76-1.38 (m, 14H, CH_3 & CH_2CH_2CO), 1.38-1.17 (m, 22H, CH_2), 0.86 (t, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 198.5, 171.1, 159.4, 82.4, 48.3, 36.6, 31.9, 29.6, 29.0, 27.9, 23.1, 22.6, 18.2, 14.1; MS (ESI) m/z (%): 415.3 (100) [M + NH_4]$^+$; Anal. Calcd for $C_{23}H_{43}NO_4$: C, 69.48; H, 10.90; N, 3.52. Found: C, 69.34; H, 10.96; N, 3.43.

4.2.3.7. (*S*)-5-*tert*-Butyl 1-ethyl 2-(2-oxohexadecanamido) pentanedioate (20). Yield 85%; Colorless oil; $[\bullet]^{20}_D$ 11.6 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): • 7.52 (d, *J* = 8.2 Hz, 1H, NH), 4.60-4.49 (m, 1H, NHC*H*), 4.21 (q, *J* = 7.2 Hz, 2H, $COOCH_2$), 2.89 (t, *J* = 7.2 Hz, 2H, CH_2COCO), 2.41-2.17 (m, 3H, CH_2C*H*H), 2.08-1.92 (m, 1H, CH*H*), 1.66-1.52 (m, 2H, CH_2CH_2CO), 1.43 (s, 9H, CH_3), 1.37-1.17 (m, 25H, CH_2 & CH_3), 0.85 (t, *J* = 6.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 198.2, 171.6, 170.8, 159.9, 80.9, 61.7, 51.7, 36.7, 31.8, 31.2, 29.6, 29.4, 29.3, 29.0, 28.0, 27.2, 23.0, 22.6, 14.0; MS (ESI) m/z (%): 428.2 (100) [M - *t*Bu + H]$^+$; Anal. Calcd for $C_{27}H_{49}NO_6$: C, 67.05; H, 10.21; N, 2.90. Found: C, 66.96; H, 10.29; N, 2.81.

4.2.3.8. (*S*)-*tert*-Butyl 3-methyl-2-(6-(naphthalen-2-yl)-2-oxohexanamido)butanoate (30a). Yield 91%; Colorless oil; $[\bullet]^{20}_D$ 11.1 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.86-7.68 (m, 3H, arom), 7.65-7.53 (m, 1H, arom), 7.52-7.18 (m, 4H, arom & NH), 4.39 (dd, J_1 = 9.2 Hz, J_2 = 4.6 Hz, 1H, NHC*H*), 2.99 (t, *J* = 7.2 Hz, 2H, CH_2), 2.80 (t, *J* = 7.0 Hz, 2H, CH_2CO), 2.32-2.08 (m, 1H, NHCHC*H*), 1.86-1.58 (m, 4H, CH_2), 1.48 (s, 9H, CH_3), 1.00-0.82 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 198.3, 169.9, 159.8, 139.4, 133.5, 131.8, 127.8, 127.5, 127.3, 127.2, 126.3, 125.8, 125.0, 82.3, 57.4, 36.5, 35.6, 31.4, 30.6, 27.9, 22.7, 18.9, 17.4; MS (ESI) m/z (%): 356.2 (100) [M - *t*Bu + H]$^+$; Anal. Calcd for $C_{25}H_{33}NO_4$: C, 72.96; H, 8.08; N, 3.40. Found: C, 72.85; H, 8.17; N, 3.26.

4.2.3.9. (*S*)-*tert*-Butyl 2-(6-([1,1'-biphenyl]-4-yl)-2-oxohexanamido)-3-methylbutanoate (30b). Yield 82%; Yellow oil; $[\bullet]^{20}_D$ 12.2 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.75-7.16 (m, 10H, arom & NH), 4.48-4.29 (m, 1H, NHC*H*), 3.11-2.87 (m, 2H, CH_2), 2.80-2.55 (m, 2H, CH_2CO), 2.34-2.09 (m, 1H, NHCHC*H*), 1.85-1.60 (m, 4H, CH_2), 1.48 (s, 9H, CH_3) 1.04-0.82 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 198.3, 169.9, 159.8, 141.1, 141.0, 138.6, 128.7, 128.6, 127.0, 126.9, 82.3, 57.4, 36.5, 35.1, 31.4, 30.7, 27.9, 22.7, 18.9, 17.4; MS (ESI) m/z (%): 436.4 (100) [M - H]$^-$; Anal. Calcd for $C_{27}H_{35}NO_4$: C, 74.11; H, 8.06; N, 3.20. Found: C, 74.02; H, 8.17; N, 3.09.

4.2.3.10. (*S*)-*tert*-Butyl 3-methyl-2-(2-oxo-6-phenylhexanamido)butanoate (30c). Yield 74%; Colorless oil; $[\bullet]^{20}_D$ 11.3 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.50-6.94 (m, 6H, arom & NH), 4.36 (dd, J_1 = 9.2 Hz, J_2 = 4.6 Hz, 1H, NHC*H*), 2.93 (t, *J* = 6.8 Hz, 2H, CH_2), 2.62 (t, *J* = 6.8 Hz, 2H, CH_2CO), 2.31-2.06 (m, 1H, NHCHC*H*), 1.73-1.55 (m, 4H, CH_2), 1.46 (s, 9H, CH_3), 1.00-0.74 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 197.9, 169.7, 159.8, 142.0, 128.3, 125.6, 82.3, 57.1, 36.5, 35.5, 31.4, 30.7, 27.9, 22.6, 18.9, 17.4; MS (ESI) m/z (%): 360.4 (100) [M - H]$^-$; Anal. Calcd for $C_{21}H_{31}NO_4$: C, 69.78; H, 8.64; N, 3.87. Found: C, 69.61; H, 8.73; N, 3.81.

4.2.3.11. (*S*)-*tert*-Butyl 3-methyl-2-(2-(naphthalen-2-yl)-2-oxoacetamido)butanoate (36). Yield 95%; Yellow oil; $[\bullet]^{20}_D$ 7.03 (*c* 1.01, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 9.21-8.96 (m, 1H, arom), 8.23-7.05 (m, 7H, arom & NH), 4.55 (dd, J_1 = 9.2 Hz, J_2 = 4.6 Hz, 1H, NHC*H*), 2.43-2.08 (m, 1H,

NHCHC*H*), 1.50 (s, 9H, CH_3), 1.09-0.79 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 186.6, 170.0, 161.6, 136.0, 134.8, 134.7, 132.2, 130.4, 130.2, 129.2, 128.2, 127.6, 126.7, 125.1, 82.3, 57.6, 31.5, 27.9, 19.0, 18.9, 17.6; MS (ESI) m/z (%): 354.1 (100) $[M - H]^-$; Anal. Calcd for $C_{21}H_{25}NO_4$: C, 70.96; H, 7.09; N, 3.94. Found: C, 70.74; H, 7.20; N, 3.86.

4.2.4. Cleavage of *tert*-butyl protecting group. A solution of the *tert*-butyl ester derivative (1.0 mmol) in 50% TFA/CH_2Cl_2 (0.5M) was stirred for 1-3 h at room temperature. After the completion of the reaction, the organic solvent was evaporated under reduced pressure and the residue was purified by recrystallization.

4.2.4.1. (*S*)-3-Methyl 2-(2-oxohexadecanamido)butanoic acid (16a, GK241). Yield 72%; White solid; mp 55-58 °C; $[\bullet]^{20}_D$ 5.04 (*c* 1.03, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.43 (d, *J* = 8.0 Hz, 1H, NH), 4.59-4.35 (m, 1H, NHC*H*), 2.91 (t, *J* = 6.8 Hz, 2H, C*H*$_2$CO), 2.42-2.16 (m, 1H, NHCHC*H*), 1.74-1.50 (m, 2H, C*H*$_2$CH$_2$CO), 1.47-1.15 (m, 22H, CH_2), 1.08-0.79 (m, 9H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 198.4, 176.0, 160.2, 57.4, 36.8, 31.9, 31.0, 29.7, 29.4, 29.3, 29.1, 23.1, 22.7, 19.1, 17.5, 14.1; HRMS (ESI) calcd for $C_{21}H_{40}NO_4$ $[M + H]^+$: 370.2952. Found: 370.2938; Anal. Calcd for $C_{21}H_{39}NO_4$: C, 68.25; H, 10.64; N, 3.79. Found: C, 68.09; H, 10.76; N, 3.70.

4.2.4.2. (*S*)-2-(2-Oxohexadecanamido)propanoic acid (16c). Yield 90%; White solid; mp 98-101 °C; $[\bullet]^{20}_D$ 5.8 (*c* 1.03, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 8.92 (br, 1H, COOH), 7.43 (d, *J* = 7.6 Hz, 1H, NH), 4.69-4.50 (m, 1H, NHC*H*), 2.91 (t, *J* = 7.2 Hz, 2H, CH_2CO), 1.70-1.48 (m, 5H, C*H*$_2$CH$_2$CO & CH_3), 1.44-1.19 (s, 22H, CH_2), 0.88 (t, *J* = 6.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 198.3, 176.8, 159.6, 47.8, 36.7, 31.9, 29.6, 29.5, 29.4, 29.3, 29.0, 23.0, 22.6, 17.7, 14.1; HRMS (ESI) calcd for $C_{19}H_{36}NO_4$ $[M + H]^+$: 342.2639. Found: 342.2650; Anal. Calcd for $C_{19}H_{35}NO_4$: C, 66.83; H, 10.33; N, 4.10. Found: C, 66.61; H, 10.45; N, 4.01.

4.2.4.3. (*S*)-2-(2-Oxohexadecanamido)pentanedioic acid (16d). Yield 90%; White solid; mp 100-103 °C; $[\bullet]^{20}_D$ 30.5 (*c* 1.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 9.67 (br, 1H, COOH), 7.63 (d, *J* = 8.0 Hz, 1H, NH), 4.72-4.56 (m, 1H, NHC*H*), 2.91 (t, *J* = 7.0 Hz, 2H, CH_2CO), 2.64-2.43 (m, 2H, NHCHCH$_2$C*H*$_2$), 2.38-2.20 (m, 2H, NHCHC*H*$_2$), 1.72-1.51 (m, 2H, C*H*$_2$CH$_2$CO), 1.44-1.18 (m, 22H, CH_2), 0.88 (t, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 198.1, 178.5, 176.2, 159.8, 51.2, 36.7, 31.9, 29.6, 29.5, 29.4, 29.3, 29.0, 23.0, 22.6, 14.1; HRMS (ESI) calcd for $C_{21}H_{37}NNaO_6$ $[M + Na]^+$: 422.2513. Found: 422.2513; Anal. Calcd for $C_{21}H_{37}NO_6$: C, 63.13; H, 9.33; N, 3.51. Found: C, 62.99; H, 9.44; N, 3.43.

4.2.4.4. (2*S*)-2-(2-Hydroxyhexadecanamido)-3-methylbutanoic acid (mixture of diastereomers) (17). Yield 93%; White solid; mp 95-99 °C; $[\bullet]^{20}_D$ 17.1 (*c* 1.00, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.01 (d, *J* = 9.2 Hz, 1H, NH), 4.57-4.42 (m, 1H, NHC*H*), 4.24-4.10 (m, 1H, C*H*OH), 3.68 (br, 1H, OH), 2.39-2.15 (m, 1H, NHCHC*H*), 1.95-1.53 (m, 2H, C*H*$_2$CHOH), 1.51-1.12 (s, 24H, CH_2), 1.10-0.76 (m, 9H, 3xCH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 176.2, 175.3, 174.4, 72.2, 57.0, 56.7, 34.5, 34.4, 31.8, 30.6, 30.5, 29.6, 29.3, 25.0, 22.6, 19.0, 17.4, 14.1; HRMS (ESI) calcd for $C_{21}H_{41}NNaO_4$ $[M + Na]^+$: 394.2928. Found: 394.2947; Anal. Calcd for $C_{21}H_{41}NO_4$: C, 67.88; H, 11.12; N, 3.77. Found: C, 67.73; H, 11.23; N, 3.69.

4.2.4.5. (*S*)-5-Ethoxy-5-oxo-4-(2-oxohexadecanamido) pentanoic acid (21). Yield 93%; White solid; mp 64-66 °C; $[\bullet]^{20}_D$ 13.4 (*c* 1.03, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 9.75 (br, 1H, COOH), 7.57 (d, *J* = 8.2 Hz, 1H, NH), 4.67-4.49 (m, 1H, NHC*H*), 4.22 (q, *J* = 7.0 Hz, 2H, $COOCH_2$), 2.89 (t, *J* = 7.2 Hz, 2H, CH_2CO), 2.36-1.94 (m, 2H, NHCHCH$_2$C*H*$_2$), 1.70-1.48 (m, 2H, NHCHC*H*$_2$), 1.46-1.08 (m, 24H, CH_2), 0.87 (t, *J* = 6.8 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 198.1, 177.8, 170.7, 160.0, 61.9, 51.5, 36.7, 31.8, 29.9, 29.6, 29.3, 29.2, 29.0, 26.9, 23.0, 22.6, 14.0; HRMS (ESI) calcd for $C_{23}H_{41}NNaO_6$ $[M + Na]^+$: 450.2826. Found: 450.2826; Anal. Calcd for $C_{23}H_{41}NO_6$: C, 64.61; H, 9.67; N, 3.28. Found: C, 64.49; H, 9.74; N, 3.22.

4.2.4.6. (*S*)-3-Methyl 2-(6-(naphthalen-2-yl)-2-oxohexanamido)butanoic acid (31a). Yield 83%; Colorless solid; $[\bullet]^{20}_D$ -2.8 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.85-7.69 (m, 3H, arom), 7.64-7.58 (m, 1H, arom), 7.51-7.28 (m, 4H, arom & NH), 5.94 (br, 1H, COOH), 4.52 (dd, J_1 = 9.2 Hz, J_2 = 4.6 Hz, 1H, NHC*H*), 2.98 (t, *J* = 6.8 Hz, 2H, CH_2), 2.81 (t, *J* = 7.0 Hz, 2H, CH_2CO), 2.41-2.21 (m, 1H, NHCHC*H*), 1.88-1.59 (m, 4H, CH_2), 1.08-0.89 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 198.1, 175.4, 159.9, 139.4, 133.5, 131.9, 127.8, 127.5, 127.3, 127.2, 126.3, 125.8, 125.0, 56.9, 36.5, 35.6, 31.0, 30.5, 22.7, 19.0, 17.4; HRMS (ESI) calcd for $C_{21}H_{26}NO_4$ $[M + H]^+$: 356.1856. Found: 356.1841; Anal. Calcd for $C_{21}H_{25}NO_4$: C, 70.96; H, 7.09; N, 3.94. Found: C, 70.84; H, 7.16; N, 3.87.

4.2.4.7. (*S*)-2-(6-([1,1'-Biphenyl]-4-yl)-2-oxohexanamido)-3-methylbutanoic acid (31b). Yield 92%; White solid; mp 123-124 °C; $[\bullet]^{20}_D$ -6.1 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.70-7.08 (m, 10H, arom & NH), 4.56-4.26 (m, 1H, • • C*H*), 3.12-2.80 (m, 2H, CH_2), 2.76-2.46 (m, 2H, CH_2CO), 2.39-2.08 (m, 1H, NHCHC*H*), 1.83-1.44 (m, 4H, CH_2), 1.10-0.68 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 198.1, 176.2, 160.1, 141.0, 140.9, 138.6, 128.7, 128.6, 127.0, 126.9, 57.5, 36.6, 35.2, 30.7, 27.3, 22.6, 19.1, 17.5; HRMS (ESI) calcd for $C_{23}H_{28}NO_4$ $[M + H]^+$: 382.2013. Found: 382.1995; Anal. Calcd for $C_{23}H_{27}NO_4$: C, 72.42; H, 7.13; N, 3.67. Found: C, 72.64; H, 7.24; N, 3.25.

4.2.4.8. (*S*)-3-Methyl 2-(2-oxo-6-phenylhexanamido)butanoic acid (31c). Yield 50%; Colorless oil; $[\bullet]^{20}_D$ 5.8 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 9.43 (br, 1H, COOH), 7.37 (d, *J* = 9.0 Hz, 1H, NH), 7.32-7.03 (m, 5H, arom), 4.50 (dd, J_1 = 9.0 Hz, J_2 = 4.6 Hz, 1H, NHC*H*), 3.04-2.86 (m, 2H, CH_2), 2.72-2.50 (m, 2H, CH_2CO), 2.41-2.16 (m, 1H, NHCHC*H*), 1.79-1.50 (m, 4H, CH_2), 1.11-0.81 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 198.0, 175.8, 159.9, 141.9, 128.2, 125.7, 57.1, 36.5, 35.5, 30.9, 30.6, 22.5, 19.0, 17.4; HRMS (ESI) calcd for $C_{17}H_{24}NO_4$ $[M + H]^+$: 306.1700. Found: 306.1695; Anal. Calcd for $C_{17}H_{23}NO_4$: C, 66.86; H, 7.59; N, 4.59. Found: C, 66.65; H, 7.71; N, 4.52.

4.2.4.9. (*S*)-3-Methyl 2-(2-(naphthalen-2-yl)-2-oxoacetamido) butanoic acid (37). Yield 80%; Yellow solid; mp 122-124 °C; $[\bullet]^{20}_D$ -25.3 (*c* 1.03, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): • 9.58 (br, 1H, COOH), 9.15 (s, 1H, arom), 8.17 (d, *J* = 8.6 Hz, 1H, NH), 8.00 (d, *J* = 7.8 Hz, 1H, arom), 7.94-7.76 (m, 2H, arom), 7.75-7.44 (m, 3H, arom), 4.71 (dd, J_1 = 9.0 Hz, J_2 = 4.6 Hz, 1H, NHC*H*), 2.54-2.26 (m, 1H, NHCHC*H*), 1.18-0.95 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 186.4, 176.4, 161.9, 136.0, 135.0, 132.2, 130.3, 130.2, 129.4, 128.4, 127.6, 126.8, 125.0, 57.1, 31.1, 19.1, 17.5; HRMS (ESI) calcd for $C_{17}H_{18}NO_4$ $[M + H]^+$: 300.1230. Found: 300.1216; Anal. Calcd for $C_{17}H_{17}NO_4$: C, 68.21; H, 5.72; N, 4.68. Found: C, 68.12; H, 5.80; N, 4.59.

4.2.5. (2*E*,4*E*)-Ethyl 5-(naphthalen-2-yl)penta-2,4-dienoate (23a). To a stirred solution of aldehyde (1.0 mmol) in dry THF (10 mL) and molecular sieves (1.5 g/mmol aldehyde) under Ar, $C_2H_5OOCH{=}CHCH_2P(=O)(OC_2H_5)_2$ (1.5 mmol) was added at 0 °C. Then, $LiOH{\cdot}H_2O$ (1.5 mmol) was added

dropwise and the reaction mixture heated at reflux for 24 h. The reaction was filtered over celite and the organic solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography [EtOAc/petroleum ether (bp 40-60 ˚C), 5:95]. Yield 50%; White solid; mp 100-102 ºC; ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.90-7.60 (m, 5H, arom), 7.55-7.45 (m, 3H, arom & CH), 7.10-6.95 (m, 2H, CH), 6.04 (s, 1H, CH), 4.26 (m, 2H, CH_2), 1.28 (t, J = 7.0 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 167.1, 144.6, 140.4, 133.5, 133.4, 128.5, 128.2, 128.1, 127.7, 126.6, 126.5, 126.4, 123.3, 121.3, 60.4, 14.3; MS (ESI) m/z (%): 253 (100) $[M + H]^+$; Anal. Calcd for $C_{17}H_{16}O_2$: C, 80.93; H, 6.39. Found: C, 80.85; H, 6.55.

4.2.6. Ethyl 5-(naphthalen-2-yl)pentanoate (24a). To a stirred solution of the alkene (1.0 mmol) in absolute EtOH (10 mL), Pd/C 10% was added. The reaction left overnight under H_2 at room temperature. The reaction was filtered over celite and the organic solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography [EtOAc/petroleum ether (bp 40-60 ˚C), 5:95]. Yield 80%; Colorless oil; ^{1}H NMR ($CDCl_3$, 200 MHz): • 8.03-7.18 (m, 7H, arom), 4.34-4.06 (m, 2H, COOCH_2CH$_3$), 3.03-2.69 (m, 2H, CH_2), 2.51-2.31 (m, 2H, CH_2), 1.91-1.69 (m, 4H, CH_2), 1.41-1.22 (m, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): • 173.4, 139.4, 133.4, 131.8, 127.6, 127.4, 127.2, 127.1, 126.2, 125.7, 124.9, 60.0, 35.5, 34.0, 30.5, 24.4, 14.1; MS (ESI) m/z (%): 274.1 (100) $[M + NH_4]^+$; Anal. Calcd for $C_{17}H_{20}O_2$: C, 79.65; H, 7.86. Found: C, 79.39; H, 8.00.

4.2.7. General method of oxidation of primary alcohols to aldehydes and then to cyanohydrins. To a solution of primary alcohol (1.0 mmol) in a mixture of toluene (3 mL) and EtOAc (3 mL), a solution of NaBr (0.11 g, 1.1 mmol) in water (0.5 mL) was added and the mixture was cooled down to -5 ºC. To the resulting biphasic system, AcNH-TEMPO (2.2 mg, 0.01 mmol) was added, followed by the drop-wise addition of an aqueous solution of 0.5 M NaOCl (2.2 mL, 1.1 mmol) and $NaHCO_3$ (0.24 g, 3.0 mmol) over a period of 1 h under vigorous stirring. After the mixture was stirred for a further 15 min at 0 ºC, EtOAc (10 mL) and H_2O (10 mL) were added. The aqueous layer was separated and washed with EtOAc (2 x 10 mL). The combined organic layers were washed consecutively with 5 % aqueous citric acid (10 mL) containing KI (0.04 g), 10 % aqueous $Na_2S_2O_3$ (10 mL), and brine and dried over Na_2SO_4. The solvents were evaporated under reduced pressure and the aldehyde was used straight to the next reaction without further purification.

To a solution of the aldehyde (1.0 mmol) in CH_2Cl_2 (1.3 mL), an aqueous solution of $NaHSO_3$ (0.25 mL, 1.5 mmol) was added and the mixture was stirred for 30 min at room temperature. The organic solvent was evaporated under reduced pressure and H_2O (5 mL) was added. The mixture was cooled down to 0 ºC and KCN 6 M (0.25 mL, 1.5 mmol) was added drop-wise under vigorous stirring. The reaction was left stirring for 18 h at room temperature. After the completion of the reaction, the organic layer was extracted with CH_2Cl_2 (2 x 20 mL), washed with brine and dried over Na_2SO_4. The organic solvent was evaporated under reduced pressure and the compound was purified with flash column chromatography [EtOAc/petroleum ether (bp 40-60 °C), 2:8].

4.2.8. 2-Hydroxy-6-(naphthalen-2-yl)hexanenitrile (27a). Yield 56%; Yellow oil; ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.95-7.19 (m, 7H, arom), 4.41 (t, J = 6.6 Hz, 1H, CHOH), 2.99 (br, 1H, OH), 2.88-2.66 (m, 2H, CH_2), 1.98-1.42 (m, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 50 MHz): • 139.3, 133.4, 131.8, 127.8, 127.5, 127.3, 127.1, 126.3, 125.9, 125.1, 119.9, 61.0, 35.6, 34.9, 30.5, 24.1; MS (ESI) m/z (%): 257.1 (100) $[M + NH_4]^+$; Anal. Calcd for $C_{16}H_{17}NO$: C, 80.30; H, 7.16; N, 5.85. Found: C, 80.07; H, 7.32; N, 5.66.

4.2.9. 6-([1,1'-Biphenyl]-4-yl)-2-hydroxyhexanenitrile (27b). Yield 80%; Pink solid; mp 78-82 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.69-7.25 (m, 9H, arom), 4.50-4.46 (m, 1H, CHOH), 3.64 (br, 1H, OH), 2.83-2.70 (m, 2H, CH_2), 1.97-1.90 (m, 2H, CH_2), 1.80-1.61 (m, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 50 MHz): • 140.9, 140.7, 138.5, 128.6, 126.9, 126.7, 120.0, 60.8, 35.0, 34.7, 30.5, 24.1; MS (ESI) m/z (%): 282.9 (100) $[M + NH_4]^+$; Anal. Calcd for $C_{18}H_{19}NO$: C, 81.47; H, 7.22; N, 5.28. Found: C, 81.25; H, 7.39; N, 5.23.

4.2.9.1. 2-Hydroxy-6-phenylhexanenitrile (27c). Yield 78%; Colorless oil; ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.44-7.15 (m, 5H, arom), 4.56-4.32 (m, 1H, CHOH), 4.03 (br, 1H, OH), 2.81-2.59 (m, 2H, CH_2), 2.01-1.48 (m, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 50 MHz): • 141.7, 128.1, 125.5, 119.9, 60.7, 35.3, 34.6, 30.4, 23.9; MS (ESI) m/z (%): 207.3 (90) $[M + NH_4]^+$; Anal. Calcd for $C_{12}H_{15}NO$: C, 76.16; H, 7.99; N, 7.40. Found: C, 76.04; H, 8.11; N, 7.30.

4.2.10. 2-Hydroxy-2-(naphthalen-2-yl)acetonitrile (33). To a stirring solution of naphthalene-2-carbaldehyde (1.0 mmol) in EtOAc/THF/AcOH 4:4:2, aqueous solution of NaCN 6M (0.25 mL, 1.5 mmol) was added. After the completion of the reaction, the organic layer was extracted with CH_2Cl_2 (2 x 20 mL), washed with brine and dried over Na_2SO_4. The organic solvent was evaporated under reduced pressure and the compound was purified with flash column chromatography [EtOAc/petroleum ether (bp 40-60 °C), 2:8]. Yield 95%; White solid; mp 110-113 ˚C; ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.99-7.24 (m, 7H, arom), 5.78-5.59 (m, 1H, CHOH), 2.08 (d, J = 2.4 Hz, 1H, OH); ^{13}C NMR ($CDCl_3$, 50 MHz): • 133.6, 132.8, 132.3, 129.4, 128.3, 127.7, 127.2, 123.6, 63.8; MS (ESI) m/z (%): 157.1 (73) $[M - CN]^+$; Anal. Calcd for $C_{12}H_9NO$: C, 78.67; H, 4.95; N, 7.65. Found: C, 78.49; H, 5.10; N, 7.56.

4.2.11. Converting the nitriles into corresponding carboxylic acids. The nitrile (1.0 mmol) was dissolved in HCl conc. (2.5 mL) and stirred for 18 h at room temperature. H_2O (5 mL) was then added and the organic layer was extracted with CH_2Cl_2, washed with brine and dried over Na_2SO_4. The organic solvent was evaporated under reduced pressure and the amide derivative was crystallized over cold petroleum ether.

To a solution of the amide derivative (0.8 mmol) in MeOH/H_2O (2:1, 6 mL), KOH (8.0 mmol) was added and the reaction mixture was heated under reflux for 2 h. Then, methanol was evaporated under reduced pressure, H_2O was added and the aqueous layer was acidified with 1N HCl to pH 1. The organic layer was extracted with EtOAc (3 x 15 mL), dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The compound was purified by recrystallization [CH_2Cl_2/petroleum ether (bp 40-60 °C)].

4.2.11.1. 2-Hydroxy-6-(naphthalen-2-yl)hexanoic acid (28a). Yield 98%; White solid; mp 115-118 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.81-7.25 (m, 7H, arom), 4.35-4.10 (m, 1H, CHOH), 2.78 (t, J = 7.4 Hz, 2H, CH_2), 2.07-1.31 (m, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 50 MHz): • 177.1, 139.8, 133.4, 131.7, 127.6, 127.4, 127.2, 127.2, 126.1, 125.7, 124.9, 69.9, 49.7, 49.2, 48.8, 35.7, 33.9, 30.9, 24.5; MS (ESI) m/z (%): 257.2 (100) $[M - H]^-$; Anal. Calcd for $C_{16}H_{18}O_3$: C, 74.39; H, 7.02. Found: C, 74.31; H, 7.18.

4.2.11.2. 6-([1,1'-Biphenyl]-4-yl)-2-hydroxyhexanoic acid (28b). Yield 93%; White solid; ^{1}H NMR (DMSO, 200 MHz): • 7.48-7.11 (m, 9H, arom), 4.06-3.94 (m, 1H, CHOH), 2.58-2.50

(m, 2H, CH_2), 1.74-1.37 (m, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 50 MHz): • 177.1, 141.4, 140.9, 138.4, 128.6, 128.5, 126.8, 126.8, 69.9, 35.2, 33.9, 31.0, 24.5; MS (ESI) m/z (%): 282.9 (100) [M - H]$^-$; Anal. Calcd for $C_{18}H_{20}O_3$: C, 76.03; H, 7.09. Found: C, 75.77; H, 7.23.

4.2.11.3. 2-Hydroxy-6-phenylhexanoic acid (28c). Yield 63%; White solid; mp 100-102 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.41-7.10 (m, 5H, arom), 4.36-4.20 (m, 1H, C*H*OH), 2.64 (t, *J* = 7.2 Hz, 2H, CH_2), 2.02-1.37 (m, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 50 MHz): • 179.5, 142.3, 128.4, 128.3, 125.7, 70.1, 35.7, 34.0, 31.1, 24.4; MS (ESI) m/z (%): 208.1 (100) [M]$^-$; Anal. Calcd for $C_{12}H_{16}O_3$: C, 69.21; H, 7.74. Found: C, 69.04; H, 7.89.

4.2.11.4. 2-Hydroxy-2-(naphthalen-2-yl)acetic acid (34). Yield 90%; White solid; mp 57-60 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): • 7.89-7.24 (m, 7H, arom), 5.28 (s, 1H, C*H*OH), 3.32-3.08 (m, 1H, OH); ^{13}C NMR ($CDCl_3$, 50 MHz): • 175.1, 135.8, 133.1, 128.2, 127.9, 127.5, 126.2, 126.1, 126.1, 125.8, 124.1, 72.6; MS (ESI) m/z (%): 202.21 (100) [M + H]$^+$; Anal. Calcd for $C_{12}H_{10}O_3$: C, 71.28; H, 4.98. Found: C, 71.11; H, 5.13.

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Supplementary Material

Code numbers of tested compounds, ^{1}H, ^{13}C NMR and HRMS spectra of inhibitor **16a** are given. Docking results of the synthesized compounds and MD simulations of **16a** and **31c** are provided. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/

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Graphical Abstract

Development of a Potent 2-Oxoamide Inhibitor of Secreted Phospholipase A_2 Guided by Molecular Docking Calculations and Molecular Dynamics Simulations

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