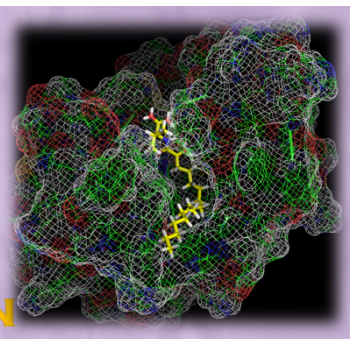

Hydrophobic α-Amino Acids Favour the Inhibition of Human GIIA Phospholipase A_2 by 2-Oxoamides

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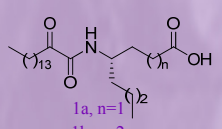
Introduction

Phospholipases A_2 (PLA_2) are a superfamily of enzymes involved in various biochemical processes -such as arachidonic acid (AA) metabolism- and therefore are pharmaceutical targets. They catalyze the hydrolysis of the *sn-2* ester bond of glycerophospholipids, producing free fatty acids including arachidonic acid and lysophospholipids.[1]

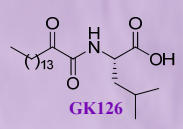
The secreted PLA_2 ($sPLA_2$) family (groups IB, IIA, IIC, IID, IIE, IIF, III, V, X and XII) are mainly small secreted proteins of 14-18 kDa. GIIA was among the first groups of $sPLA_2$ identified and was found in the synovial fluid of patients with rheumatoid arthritis. It has also been detected in human atherosclerotic lesions and is believed to enhance lipid accumulation in arterial intima.[2]

The domain of GIIA $sPLA_2$ contains three long α-helices, two-stranded β-sheets referred as β-wings, and a conserved Ca^{2+} binding loop. Most of the residues of the GIIA $sPLA_2$ surface are basic, which may cause the selectivity of the enzyme of binding to anionic vesicles. The active site consists of the catalytic dyad His47/Asp91, the Ca^{2+} ion and a long lipophilic tunnel which tends to enfold the aliphatic parts of substrates when they occur.

The discovery of potent and selective PLA_2 inhibitors is of great pharmaceutical importance.[3] Kokotos and cowokers have developed a novel class of inhibitors (**1a**, **1b**) for cytosolic phospholipase A_2 (GIVA $cPLA_2$).[4] Most recently, it was found that the long chain 2-oxoamide **GK126** based on the amino acid (*S*)-leucine displayed inhibition of human and mouse GIIA $sPLA_2$ (IC_{50} 300 nM and 180 nM, respectively).[5] Here, we present a series of docking experiments of long chain 2-oxoamide derivatives based on the proteinogenic α-amino acids and the synthesis of the most promising molecules according to the docking results.



1a, n=1
1b, n=2



GK126

Simulated Docking

Simulated docking was used to calculate possible binding modes of new structures at the active site of GIIA $sPLA_2$. Based on previous work,[5] Sybyl 8.0 by TRIPOS was used for the design, the energy minimisation and the simulated annealing of the structures. The ligands were sketched taking into account the correct ionization and tautomeric states of the molecules, and the Powell algorithm was used for the energy minimisation. The programme Gold 5.1 by Gold was used for the docking.

For the docking process the crystal structure of GIIA $sPLA_2$ was retrieved from the Brookhaven Protein Databank (PDB code: 1KQU). From this structure, water molecules within a distance of 5Å from the active site were set to 'Toggle' and 'Spin' state and all water molecules in a greater distance were deleted. Setting up the active site, all the protein residues within 6.0Å distance of the bound ligand were marked with their charge in physiological pH in order to form sensible interactions with the substrate. The calcium ion was treated in order to have the correct geometry and formal charge. **Table 1** summarizes the docking results of the 2-oxoamide derivatives of all the proteinogenic α-amino acids plus some non-natural amino acids as well.

According to the docking results, derivatives based on polar and amide groups of (*S*)-amino acids showed poor calculations and no desirable conformations for a successful binding and therefore were not part of our synthetic goal. Derivative based on (*S*)-pheylalanine had given poor *in vitro* results in an earlier study[6] and so aromatic group was out of our synthetic interest, as well. Derivatives based on non polar group of (*S*)-amino acids had some promising computational results. In addition, given the previous good *in vitro* results of (*S*)-leucine derivative **GK126**,[5] we decided to synthesise derivatives based on amino acids with similar to (*S*)-leucine side chain and polarity (**Table 2**).

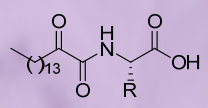
Synthesis

The synthesis of the new 2-oxoamides is depicted in **Schemes 1** and **2**. The methyl or *tert*-butyl ester of the (*S*)-amino acid was coupled with 2-hydroxyhexadecanoic acid using 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (WSCI) as a condensing agent in the presence of 1-hydroxybenzotriazole (HOBt). In case of the methyl ester, saponification took place after coupling, followed by oxidation using Dess-Martin periodinane. In the case of *tert*-butyl ester, oxidation took place (either using Dess-Martin periodinane or NaOCl/AcNH-TEMPO method) after coupling, followed by treatment with trifluoroacetic acid to afford the corresponding acid.

Results and Discussion

The 2-oxoamide derivatives **GK241**, **GK259**, **GK260**, **GK261**, **GK262** were tested *in vitro* for their inhibition against GIIA $sPLA_2$. The so far *in vitro* results reveal (*S*)-valine derivative **GK241** to have a better IC_{50} record than that of (*S*)-leucine **GK126**. Based on this result, we could claim a possible successful binding mode for **GK241** shown in **Figure 1**. The key interactions are described in **Table 3**.

Table 1. Docking results of 2-oxoamides derivatives based on (*S*)-amino acids.

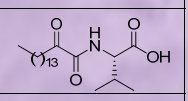
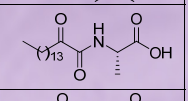
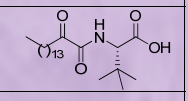
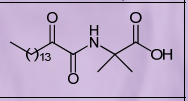
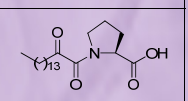


R=side chain of the corresponding amino acid

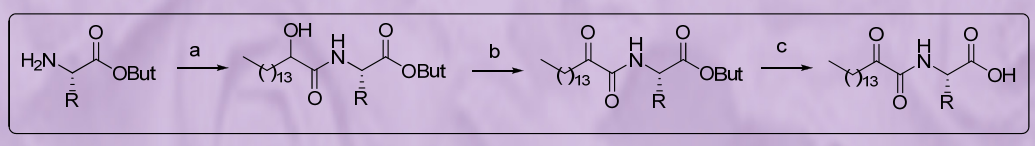
R	Gsc. Fit.	Binding Energy	R	Gsc. Fit.	Binding Energy
Nucleophilic			**Aromatic**		
Serine	33.13	- 36.75	Phenylalanine	36.35	- 37.36
Threonine	34.81	- 38.29	Tryptophan	37.45	- 39.86
Cysteine	35.15	- 38.94	Tyrosine	34.65	- 35.07
Small			**Polar**		
Alanine	33.14	- 38.16	Aspartic acid	32.96	- 35.38
Glycine	35.41	- 36.67	Glutamic acid	29.82	- 32.33
NonPolar			Histidine	32.69	- 35.06
Valine	34.36	- 38.40	Lysine	32.75	- 34.42
Leucine	35.18	- 36.42	Arginine	34.12	- 37.15
Isoleucine	36.51	- 38.10	**Amide**		
Methionine	39.96	- 44.88	Asparagine	29.77	- 30.75
Proline	38.97	- 41.03	Glutamine	30.80	- 32.07

Not natural	Gsc. Fit.	Binding Energy
tert-Leucine	34.56	- 37.99
2-aminoisobutiric acid	34.60	- 37.92

Table 2. 2-Oxoamides synthesized in this study.

Compound	Structure
GK241	
GK259	
GK260	
GK261	
GK262	

Scheme 1. Synthesis of 2-oxoamide derivatives based on (*S*)-Valine and (*S*)-Alanine.
Reagents and conditions: (a) $CH_3(CH_2)_{13}CHOHCOOH$, Et_3N, WSCI, HOBt, CH_2Cl_2; (b) NaOCl, AcNH-TEMPO, NaBr, $NaHCO_3$, EtOAc, H_2O, toluene, or Dess-Martin, CH_2Cl_2; (c) CF_3COOH, CH_2Cl_2.



Scheme 2. Synthesis of 2-oxoamide derivatives based on (*S*)-Proline, tert-Leucine, 2-aminoisobutyric acid.
Reagents and conditions: (a) $CH_3(CH_2)_{13}CHOHCOOH$, Et_3N, WSCI, HOBt, CH_2Cl_2; (b) 1N aq. NaOH, MeOH; (c) CH_2Cl_2, Dess-Martin.

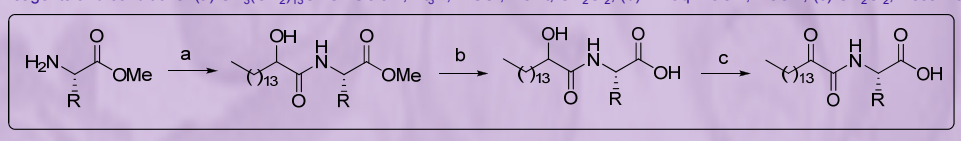


Table 3. The key interactions between **GK241** and the active site of $sPLA_2$ GIIA.

H bond/aliphatic- aliphatic/ aliphatic-aromatic interactions between		
inhibitor		$sPLA_2$ residues
carboxylic acid group	COO^- ··· HOH ··· O=C COO^- ··· HOCO	Asp48 Lys52
1-carbonyl group	NHCO ··· HN	Asp48
2-carbonyl group	NHCOCO ··· HN	Gly29
2-carbonyl group	NHCOCO ··· Ca^{++}	Ca^{++}
valine side chain		located close to Lys52
long aliphatic chain		Leu2, Phe5, His6

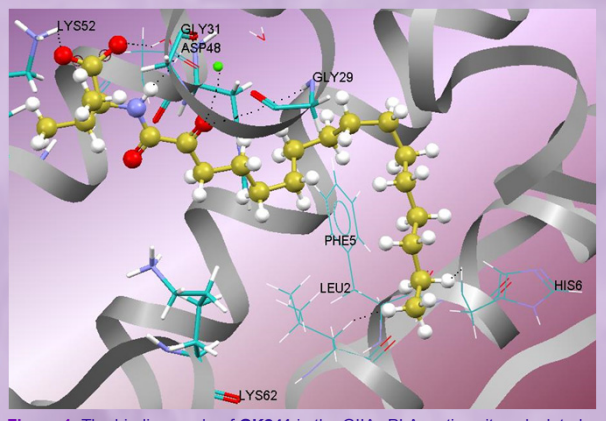


Figure 1. The binding mode of **GK241** in the GIIA $sPLA_2$ active site calculated using GOLD 5.1.

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