Inhibition of GIIA Phospholipase A_2 by 2-Oxoamides Based on α-Amino Acids: Exploring the Role of the α-Amino Acid

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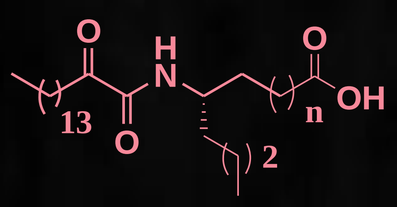
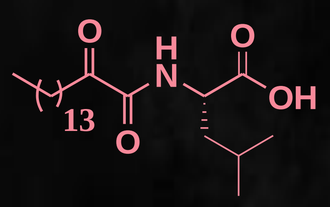
Introduction

Group IIA secreted phospholipase A_2 (GIIA $sPLA_2$) is a member of the mammalian $sPLA_2$ enzyme superfamily which are characterised by their ability to catalyse the hydrolysis of the sn-2 ester bond of glycerophospholipids releasing free fatty acids.[1] The crystal structure of the enzyme reveals a highly conserved Ca^{2+} binding loop and a catalytic dyad consisting of His47/Asp91. The mechanism of the substrate hydrolysis includes the activation of a water molecule by His47 and consequently an attack to the sn-2 ester carbonyl carbon of the substrate. GIIA $sPLA_2$ is associated with various inflammatory conditions and therefore is considered to be an anti- inflammatory drug target.[2]

*Kokotos et al have developed a novel class of inhibitors (**1a**, **1b**) for cytosolic phospholipase A_2 (GIVA $cPLA_2$).[3] Most recently, they found that the long chain 2-oxoamide **2** based on the amino acid (S)-leucine displayed inhibition of human and mouse GIIA $sPLA_2$ (IC_{50} 300 nM and 180 nM, respectively).[4] Here, we present a series of docking experiments of long chain 2-oxoamide derivatives based on the proteinogenic a-amino acids. The structures were designed using the same motif: a long chain 2-oxoamide (same for all structures) and an amino acid unit.*



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



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Performing Docking Experiments

Simulated docking was used for the study of new structures with potential inhibitory activity towards GIIA $sPLA_2$. Based on previous work,[4] Sybyl 8.0 by TRIPOS was used for the design, the energy minimisation and the simulated annealing of the structures while Gold 5.0.1 by Gold company was chosen for the docking. The ligands were sketched taking in to account the correct ionization and tautomeric states of the molecules and the Powell algorithm was used for the energy minimisation.

For the docking process the crystal structure of GIIA $sPLA_2$ was retrieved from the Brookhaven Protein Databank (PDB code: 1DB4). 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.

*Substrate **2** was docked first in order to reassure that all the parameters were set up correctly and to ensure the production of reliable docking results. **Table 1** summarizes all the docking results of the 2-oxoamide derivatives of all the proteinogenic a-amino acids.*

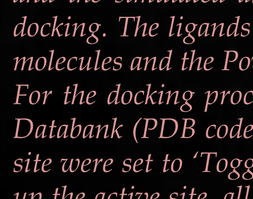
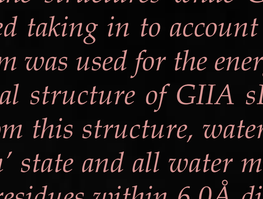
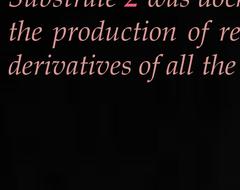
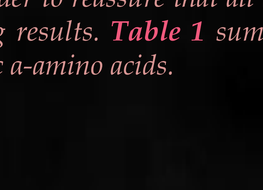
Compound	GoldScoreFitness (kJ/mol)	Compound	GoldScoreFitness (kJ/mol)
 **3** Glutamic acid ethylester	100.74	 **5** Histidine	97.15
 **4** Glutamic acid	97.43	 **2** **Leucine**	83.85

Table 1: Docking Score Fitness of all the 2-oxoamide derivatives of α-amino acids.

a- amino acid 	GoldScoreFitness (kJ/mol)
Aspartic acid	97.56
Methionine	97.40
Glutamine	90.63
Proline	89.76
Arginine	89.37
Cysteine	88.86
Threonine	86.39
Tryptophan	85.05
Asparagine	85.79
Serine	87.14
Lysine	84.76
Phenilalanine	80.84
Valine	78.22
Glycine	77.12
Alanine	79.80
Tyrosine	79.18
Isoleucine	79.10

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Results and Discussion

*Evaluating the docking results, among the structures with Score Fitness higher than 97kJ/mol, the stuctures based on glutamic acid ethylester **3**, glutamic acid **4**, histidine **5** were of the outmost interest. Only a slight change in the position of these ligands in the active site was observed, comparing to the reference ligand **2** placement. More specifically, the conformation they take up is similar to the ligand **2** (**Figure 1**) and the key interactions with the active site are preserved (**Figure 2**). Moreover, in the case of ligand **4**, it is the carbonyl oxygen of the side chain of the a-amino acid that interacts with the Ca^{2+} and not the oxygen of the carbonyl group as it happens in ligand **2**. This new interaction led us to design ligand **3** which gave improved results. Synthesis of the molecules **3-5**, which show the higher docking scoring, is in progress in an attempt to test the parallelism between docking scoring and bioactivity.*

Figure 1: Conformation of the ligands **2- 5** in the active site of GIIA $sPLA_2$.

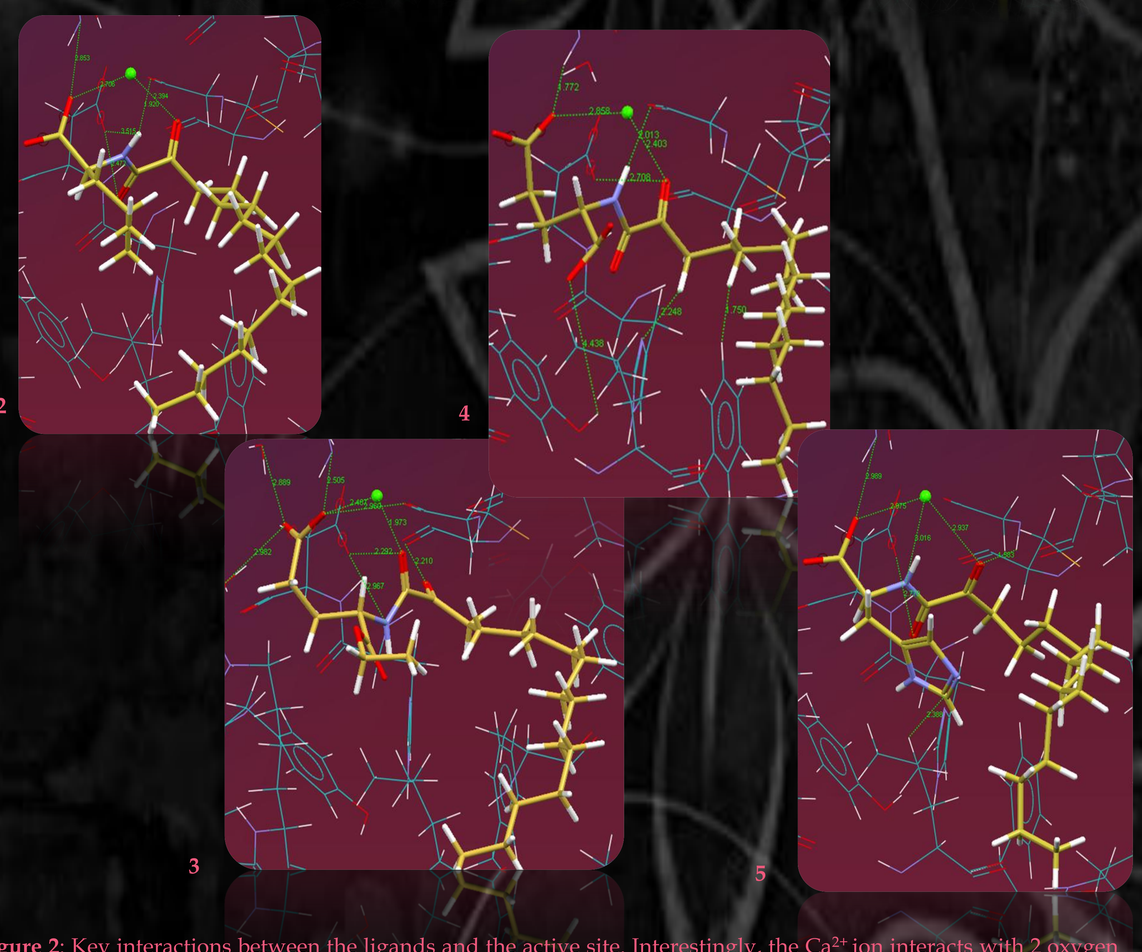

Figure 2: Key interactions between the ligands and the active site. Interestingly, the Ca^{2+} ion interacts with 2 oxygen atoms of the ligand (important for the inhibition) in all structures. The H-bonds interactions between the amino and carbonyl groups of the structures and the enzyme's residues are also important for the binding.

References

1. Dennis, E. A.; Cao, J.; Hsu, Y. H.; Magrioti, V.; Kokotos, G. *Chem. Rev.* **2011**, accepted.
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4. Mouchlis, V. D.; Magrioti, V.; Barbayianni, E.; Cermak, N.; Oslund, R. C.; Mavromoustakos, T. M.; Gelb, M. H.; Kokotos, G. *Bioorg. Med. Chem.* **2011**, 19, 735.