Rational Modification of Human Synovial Fluid Phospholipase A_2 Inhibitors†

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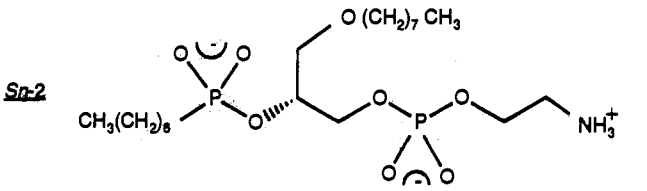
Mammalian nonpancreatic secretory phospholipase A_2 (PLA_2) splits the 2-acyl bond in 1,2-diacylphosphatides.[1] This enzyme has been found in high concentrations in the synovial fluid of patients with rheumatoid arthritis,[2] and it has been suggested that inhibitors of this enzyme may have therapeutic value. The three-dimensional structure of human synovial fluid PLA_2 (HSF-PLA_2) is known both in its native form[3] and in a complex with the transition-state analogue (TSA) L-1-*O*-octyl-2-heptylphosphonyl-*sn*-glycero-3-phosphoethanolamine,[4] **1**. The present work is a part of our program to develop PLA_2 inhibitors and describes the successful rational modifications introduced into **1** aimed at enhancing its affinity toward HSF-PLA_2, based on the combined use of biochemical information, molecular graphics analysis,[5] molecular orbital[6] and molecular mechanics calculations,[7] and the GRID[8] and LUDI[9] programs.

Hydrocarbon chain length is a critical factor for the activity of potential PLA_2 inhibitors. Studies with phospholipid analogues demonstrated that 10 carbons are required in the *sn*-2 acyl chain for optimum binding to cobra venom PLA_2,[10] whereas the optimal length for the *sn*-1 alkyl chain is four carbons in the case of porcine pancreatic PLA_2.[11] These findings can be rationalized in terms of the observed number of contacts between the phospholipid analogs and the enzyme in known PLA_2–inhibitor complexes.[12] Analysis of the HSF-PLA_2 structure with the GRID program suggests similar structure–activity relationships[13] (Figure 1).

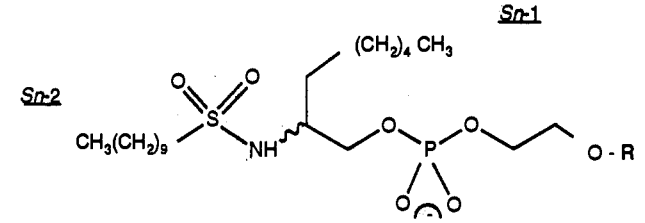
The capacity of TSAs to bind with high affinity has been shown by Gelb *et al.* who introduced a phosphonate group into compound **1**.[14] In contrast, the substitution of acyl by sulfonyl, which is extensively used as a TSA of an ester group undergoing hydrolysis, has been reported by de Haas *et al.*[15] not to improve inhibitory properties. Despite this discouraging data, we decided to introduce the sulfonamide group on the basis of the following rationale: Yu and Dennis[16a] showed that the pK_a of the catalytically active His-48 is 6.1. Therefore, this residue is predominantly unprotonated under physiological conditions. Thus, in order for a TSA to function effectively at physiological pH, the bioisostere of the ester should be chosen so that, in addition to possessing tetrahedral features to resemble the transition state, it has a proton available to form a hydrogen bond to the Nδ atom of His-48, as this hydrogen bond has been shown to provide 1.5 kcal/mol of binding energy.[16a] Monosubstituted sulfonamides have a range of pK_a values that fulfills this requirement at physiological pH.[16b] Moreover, the sulfonamide group may release some strain energy in the molecule. The C–O–P–C dihedral angle of the methylphosphonate moiety of **1** in the complex is 121.2°, giving rise to a strain energy of 0.6 to 0.9 kcal/mol[17] (Figure 2). In contrast, the C–N–S–C dihedral angle of the *N*-methylmethanesulfonamide group has a global energy minimum at 120.0° [18] (Figure 2). These data indicate that sulfonamide-based inhibitors could be at least as effective as the phosphonate-based ones. Accordingly, 2-((decylsulfonyl)amino)-1-octylphosphoglycol **2**,[19] which fulfills the chain length features described above and has a sulfonamide group, is an effective inhibitor of HSF-PLA_2 activity with an $X_i(50) = 0.026$[20] in a mixed vesicle model. In this model, compound **1** inhibited the enzyme with an $X_i(50)$ value of 0.025. Therefore, sulfonamide-based TSAs are effective PLA_2 inhibitors. A molecular model accounting for the interaction of compound **2** with HSF-PLA_2 was built.[21] In this model, carbons 8–10 of the *sn*-2 acyl chain fit in a hydrophobic pocket within the hydrophobic channel[22] surrounded by residues Ala-18, Ala-19, Leu-2, Val-3, Phe-5, and His-6. There are no large conformational differences between this complex and the X-ray structure of HSF-PLA_2 + **1** (rms (Cα) = 0.53 Å; rms (all non-hydrogen atoms) = 3.1 Å).

The result obtained with **2** encouraged us to design new modifications. Thus, the modeled complex of HSF-PLA_2 with **2** was used to search with the GRID program[23] for additional ligand binding sites in the enzyme that could be exploited by further modification of **2**. Favorable aromatic interactions were found within what we have termed the "hydrophobic cage", a hydrophobic pocket delimited by residues Val-46, Thr-130, Pro-131, Gly-33 and the disulfide bridge linking Cys-50 and Cys-133 (Figure

Scheme 1. Structural Formulas of Compounds 1–3.



L-1-*O*-octyl-2-heptylphosphonyl-*sn*-glycero-3-phosphoethanolamine (**1**)



R= -H (**2**)
R= -C_6H_5 (**3**, LM-1228)

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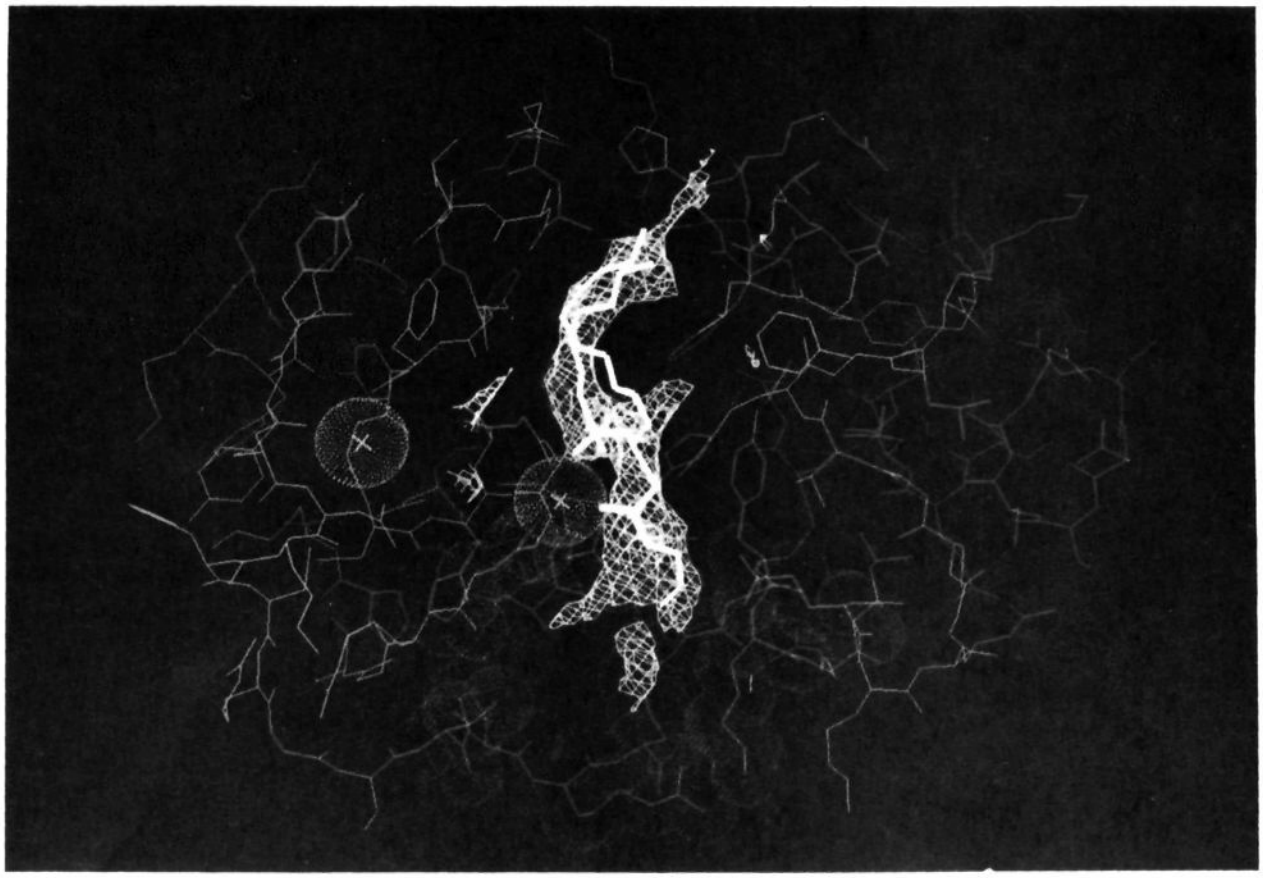


Figure 1. Crystal structure of HSF-PLA_2 complexed with the TSA (1). The GRID energy contours for an aromatic carbon probe plotted at −2.25 kcal mol^{-1} delineate sites of favorable interaction with the protein and highlight possible sites of substitution on **2** to improve its binding affinity. These surround the inhibitor but also show a region where the inhibitor could be extended. The van der Waals surface shows the shape of the "additional cage" surrounding these GRID contours. The two calcium ions are also shown with van der Waals surfaces.

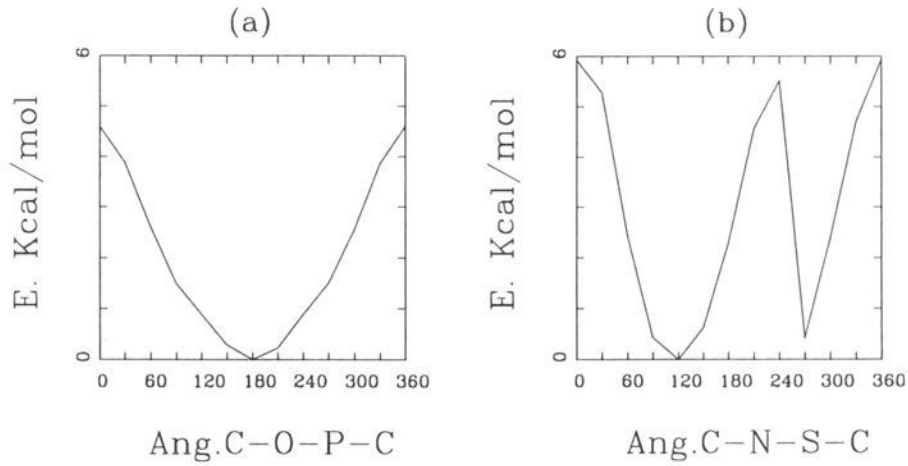


Figure 2. Energy dependence on rotation around dihedrals. (a) C–O–P–C in methyl methanephosphonate. (b) C–N–S–C in *N*-methylmethanesulfonamide.

1). This pocket, created by a seven-residue C-terminal extension of the enzyme, is one of the features distinguishing group I and group II PLA_2s[24] and is, therefore, a target for incorporating pharmacological selectivity into the inhibitors. In addition, the OH group of **2** is located close to this hydrophobic cage (Figure 1), providing a convenient anchor for further substitution. The LUDI program was then employed[25] in order to identify possible substituents. It suggested a number of possible fragments, some of which formed hydrogen bonds with the enzyme. A representative selection of these is given in Table 1. Compound **3**[26] (LM-1228), the *O*-benzyl ether derivative of **2**, was the most readily available candidate compound fulfilling both the GRID and the LUDI suggestions. The complex was modeled[27] (Figure 3), and the ability of **3** to inhibit HSF-PLA_2 activity was tested,[20] yielding $X_i(50)$ values of 0.0036. The porcine pancreas enzyme is a type I PLA_2[24] which lacks the heptapeptide C-terminal extension forming the hydrophobic cage (GRID contour map not shown). Therefore, in the absence of this additional

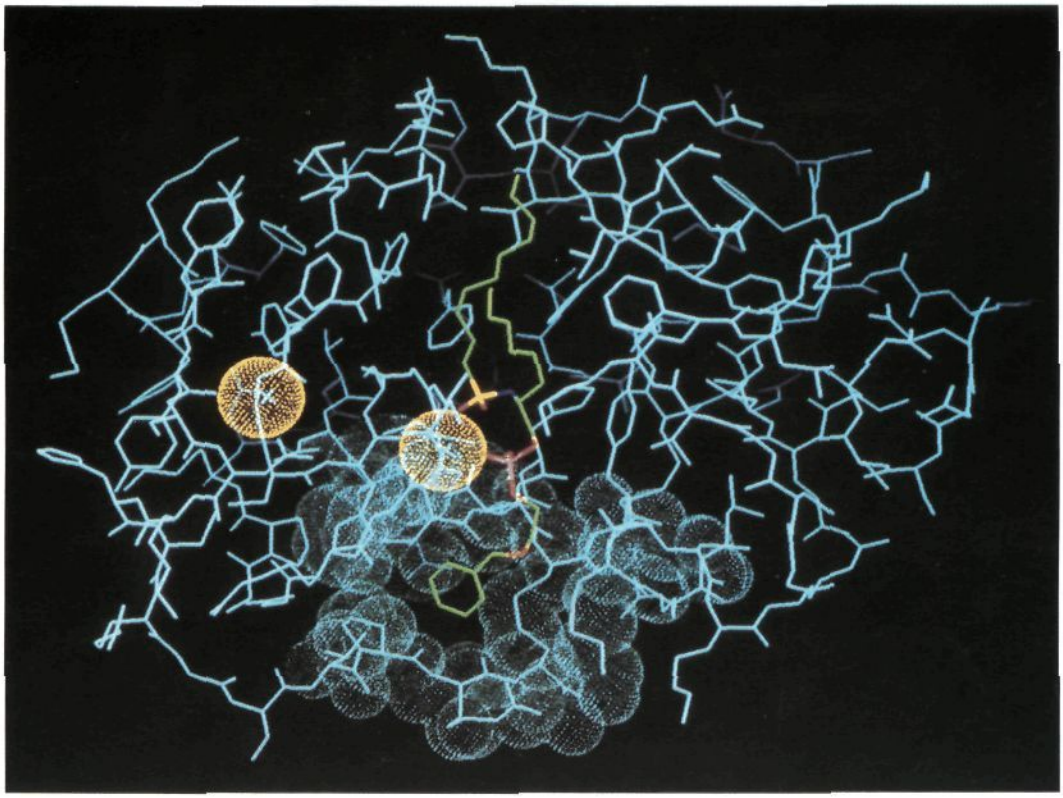
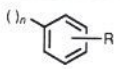
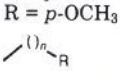


Figure 3. The energy-minimized structure of the HSF-PLA_2 + **3** complex. HSF-PLA_2 is shown in blue, and inhibitor **3** is shown colored by atom type. Calcium ions are shown in orange with van der Waals surfaces.

Table 1. Substituent Fragments Suggested by the LUDI Program To Fit in the "Hydrophobic Cage"

formula	n	H-bonds with HSF-PLA_2
		
R = *m*-CH_2OH	0	Gly-33 (–CO–)
R = *p*-$C(NH)NH_2$	0	His-28 (–CO–)
		Gly-33 (–CO–)
		Thr-130 (–CO–)
		Pro-131 (–CO–)
R = H	1	
R = *p*-OH	1	Gly-33 (–CO–)
R = H	2	
R = *p*-OH	2	Pro-131 (–CO–)
R = *p*-OCH_3	2	Lys-53 ($-NH_3$)
		
R = CH_2OH	2	Gly-33 (–CO–)
R = $CH(CH_3)_2$	2	
R = CH_3	4	

interaction, the difference in the inhibitory behavior of compounds **2** and **3** observed for HSF-PLA_2 was not expected for porcine pancreatic PLA_2. Experiments showed that both compounds have comparable inhibitory effects against porcine pancreatic PLA_2. PLA_2 activities were measured as described earlier[20] at a constant mole fraction of inhibitor of 0.05. Compounds **2** and **3** give rise respectively to 66% and 96% inhibition with the HSF enzyme and 43% and 38% inhibition with the porcine pancreatic enzyme. Although not conclusive, these results suggest that the increase in potency is due to better inhibitor–enzyme complementarity. Compound **3** (LM-1228) is one of the most potent HSF-PLA_2 inhibitors described so far, and represents a new and encouraging lead compound.

The present work demonstrates that the design of tight-binding inhibitors of HSF-PLA_2 is possible and provides an example of the usefulness of computer-assisted methods in improving our understanding of the interactions of inhibitors with their receptors. Our results also indicate that the combined use of programs GRID and LUDI can be a powerful general strategy in drug design. GRID is a useful method for discovering additional binding sites that can be exploited in order to modify a known inhibitor, but it is not always simple to translate the GRID binding energies into chemical structures. On the other hand, LUDI may not be easy to use if the potential ligand binding sites are unknown. Together, these programs enable molecular fragments to be positioned in additional binding sites, providing valuable ideas on how to modify an existing ligand. Forthcoming publications will provide details of a series of compounds related to those described here.

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(17) (a) Torsional energy barriers for CH_3–O–PO_2–CH_3 and for CH_3–NH–SO_2–CH_3 were calculated by semiempirical molecular orbital methods, using both the AM1[6b] and the MNDO[6c] Hamiltonians, as implemented in the MOPAC 6.0 package.[6a] A grid scan around the torsional angles τ(C–O–P–C) and τ(C–N–S–C) was carried out with a grid resolution of 30°. At each angle, the molecular geometry was optimized using the BFGS geometry optimization procedure. Quantitatively similar values were obtained with the AM1 and MNDO methods. For CH_3–NH–SO_2–CH_3, the results using MNDO are qualitatively similar to those obtained with *ab initio* methods and a 6-31G* basis set,[18] although the energy barriers are considerably smaller. (b) Thatcher, G. R. J.; Campbell, A. S. Phosphonates as Mimics of Phosphate Biomolecules: *Ab Initio* Calculations on Tetrahedral Ground States and Pentacoordinate Intermediates for Phosphoryl Transfer. *J. Org. Chem.* **1993**, *58*, 2272–2281.

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(21) The 3D structure of HSF-PLA_2 cocrystallized as a complex with 1 at 2.1-Å resolution[4] was employed. Both subunits in the asymmetric unit cell were superimposed. Significant differences between the two active sites were not found, and therefore, subunit A was chosen for study. Molecular mechanics parameters within the DISCOVER force field were developed for CH_3–NH–SO_2–CH_3.[18b] Compound 1 was substituted by the *R* enantiomer of compound 2. All hydrogen atoms were added, and the complex was surrounded by a 5-Å shell of TIP3P water molecules. The complex was optimized using the cff91 DISCOVER force field[7] with a 10-Å cutoff for the nonbonded interactions and a distance dependent dielectric constant. Hydrogen atoms were reoriented and optimized by using steepest descent energy minimization (200 steps) while keeping the non-hydrogen atoms frozen. Then, steepest descent minimization (200 steps) was applied to the hydrogen atoms and water molecules while keeping the rest of the system frozen. Next, the hydrogen atoms, the water molecules, and the inhibitor were minimized by using steepest descent (500 steps) while keeping the enzyme frozen. Then, the energy of the whole system was relaxed by using 500 steps of steepest descent and 1500 steps of conjugate gradient energy minimization. There were no large conformational differences between the complexes after minimization.

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(23) The inhibitor and all the hydrogen atoms and water molecules were removed from the modeled complex HSF-PLA_2 + **2**. Interactions with the HSF-PLA_2 were calculated for water (OH_2) and aromatic carbon (C1=) probes by following the same protocol as used for the crystallographic HSF-PLA_2. Regions of the energy maps close to the inhibitor were analyzed with the aid of the molecular graphics program INSIGHT.

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(25) The modeled and energy-minimized complex of HSF-PLA_2 + **2** without water molecules was used. Calculations were performed with the LUDI program in order to append fragments to the already existing inhibitor in the "additional cage" region that the GRID

calculations showed was favorable. Interaction sites were calculated within a radius of 5.0 Å of several points in this region with the link and standard modules of LUDI. (In the link module, LUDI attempts to append fragments to a specific position of the inhibitor.) The fit was achieved with a maximum rms deviation of 0.5 Å from the interaction sites for the fragments. The default fragment data base was used.

(26) The synthetic route to compounds **2** and **3**[19] have been presented at the VIII Congreso de la Sociedad Española de Química Terapéutica, 28 September 1993, Salamanca, Spain.

(27) By using the HSF-PLA_2 + **2** complex with no water molecules and the position initially assigned by LUDI to the benzyl fragment, an initial model with the *R* enantiomer of compound **3** was built. The position of the benzyl derivative was optimized by running a trajectory of 10 ps of molecular dynamics at 300 K, with coordinates saved every 0.1 ps, during which only the benzyl moiety was allowed to move. All the saved conformations were energy minimized by using the steepest descent algorithm until the rms deviation of the energy gradient was less than 5 kcal mol^{-1} $Å^{-1}$. Then, the VA09A Quasi-Newton-Raphson algorithm was used until the rms deviation of the energy gradient was less than 0.1 kal mol^{-1} $Å^{-1}$. The lowest energy conformation was selected as representing the complex. A 5-Å shell of TIP3P water molecules was then added around the complex, and the complex system was optimized by following the same energy minimization protocol as used for the HSF-PLA_2 + **2** complex. The final rms deviations with respect to the X-ray structure with **1** are: 0.52 Å (Cα) and 3.1 Å (all non-hydrogen atoms), respectively.