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Communications to the Editor

Discovery of New Non-Phospholipid Inhibitors of the Secretory Phospholipases A_2

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Phospholipases A_2 (PLA_2s)[1] catalyze the hydrolysis of fatty acids from the *sn*-2 position of aggregated glycerophospholipids. The first PLA_2 enzymes to be characterized were the closely related, low molecular weight secretory proteins ($sPLA_2$s), isolated from such sources as mammalian pancreas (designated as group I $sPLA_2$), snake venom (groups I and II $sPLA_2$), bee venom (group III $sPLA_2$), and human platelets (group II $sPLA_2$). More recently, however, a quite different, high molecular weight cytosolic PLA_2 was described.[2] This enzyme ($cPLA_2$) selectively cleaves arachidonic acid from membrane phospholipids and is considered to catalyze the rate-limiting step in the cellular generation of biologically active eicosanoid products. Due to this pivotal role in the formation of a variety of proinflammatory lipid mediators, both the mammalian group II $sPLA_2$[3] and $cPLA_2$[4] have been widely implicated in the initiation and exacerbation of the inflammatory response in animals. In attempt to discover novel antiinflammatory agents, considerable effort has been expended in the design of potential inhibitors of both of these enzyme classes, but to date, no *bona fide* selective inhibitor has been reported to be undergoing clinical development.[5] The properties of these enzymes and the associated reasons for the slow progress in inhibitor design have been discussed in a number of recent reviews.[1]

In a previous publication,[6] we described the design and synthesis of some phospholipid substrate analogue inhibitors of pancreatic $sPLA_2$ and defined the binding interactions in the enzyme–inhibitor complex using NMR and molecular modeling techniques. The usual ambiguities associated with *in vitro* screening of these enzymes were resolved using parallel assays with both monomerically dispersed and aggregated substrate systems. Phosphocholine **1** was one of the more potent compounds we described, but due to its physical properties and metabolic instability represented a poor drug candidate. In this communication we describe the discovery of a novel series of highly potent, non-phospholipid inhibitors of both the porcine pancreatic and human group II $sPLA_2$ enzymes.

Inhibitor Design and Structure–Activity Relationships. Pancreatic (Group I) PLA_2. During our earlier enzyme–inhibitor interaction studies,[6] we concluded that one of the phosphate oxygen atoms of compounds such as **1** forms an ionic interaction with the Ca^{2+} ion at the active site of pancreatic PLA_2 while another accepts a hydrogen bond from the OH of mobile residue, Tyr-69. Furthermore, we observed that replacement of the quaternary N atom of **1** with an OH group produced a phosphoglycol derivative with enhanced activity over the phosphocholine. These findings led us to speculate that it may be possible to achieve similar strength of binding with a simple anionic group in place of the phosphocholine moiety present in the parent inhibitor.

Biochemical screening (Table 1) of a newly synthesized series of carboxylate analogues of **1** showed the foregoing hypothesis to be correct. Inspection of the activities of compounds **2a–d** indicates that direct replacement of the phosphocholine moiety by carboxylate (compound **2b**) produces an inhibitor with comparable potency to **1**. Homologue **2c** was similarly active, whereas **2a** and **2d** with shorter and longer methylene chains, respectively, were less active.

In an attempt to reduce the overall inhibitor lipophilicity, the hexadecanoyl chain of **2b** was replaced by a decanoyl moiety to give **3**, a molecule with somewhat reduced activity. However, as was previously observed[6] in the phosphocholine series, we found that the even less lipophilic *trans*-dec-3-enoyl analogue (**4**) displayed a similar level of potency to the longer chain compound (**2b**). A combination of 2-D NMR and molecular modeling techniques indicated that the binding interactions between

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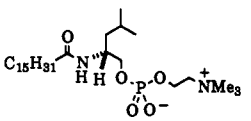
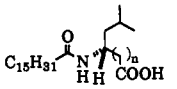
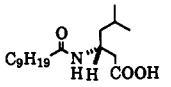
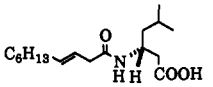
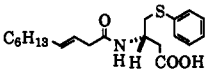
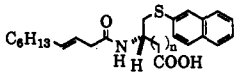
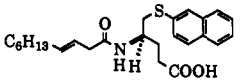
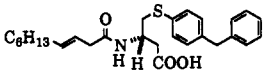
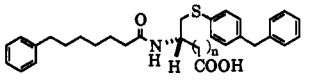
Table 1. *In Vitro* Activity of Substrate Analogue Inhibitors of PLA_2

compd	structure		IC_{50} (μM)[a] panc PLA_2 mono[b]	panc PLA_2 DOC[c]	human platelet PLA_2[d]
1			0.30 ± 0.05 (15)	30 (4)	NT
2		**2a**: $n = 0$	15 (2)	>30 (2)	NT
		2b: $n = 1$	0.19 (5)	7.3 (3)	NT
		2c: $n = 2$	0.20 (2)	4.5 (2)	NT
		2d: $n = 3$	1.3 (2)	19 (2)	NT
3			1.4 (2)	15 (2)	NT
4			0.30 (3)	8.5 (2)	NT
5			0.095 (2)	2.2 (2)	NT
6		**6a**: $n = 1$	0.023 (2)	0.79 ± 0.06 (10)	NT
		6b: $n = 2$	0.016 (2)	0.38 (2)	1.85 (2)
7			>250 (2)	>100 (2)	12 (3)
8			0.030 (2)	0.12 (2)	3.8 (2)
9		**9a**: $n = 1$	NT	NT	0.065 (2)
		9b: $n = 2$	0.015 (2)	0.030 (4)	0.021 ± 0.004 (33)

[a] Mean of at least two separate results (number of individual results shown in parentheses); data for small sets (individual results <10) falls within a range of 2-fold either side of the mean; statistical analysis performed on larger sets, 95% confidence limits of mean indicated. [b] Porcine pancreatic enzyme and monomerically dispersed substrate.[6] [c] Porcine pancreatic enzyme and micellar substrate with deoxycholate (DOC).[6] [d] $sPLA_2$ isolated from human platelets and assayed using an enzyme system based on the release of ^{14}C-labeled oleate from *E. coli* membranes.[11]

carboxylate 4 and pancreatic PLA_2 were very similar to those seen between a phosphocholine derivative and the same enzyme in our earlier studies. In particular, the investigation[7] indicated that one of the oxygen atoms of the carboxylate anion does indeed form an ionic interaction with the active-site Ca^{2+} ion in the enzyme–inhibitor complex while the other oxygen atom forms a hydrogen bond to the OH of Tyr-69. Thus, despite the difference in geometry (sp^2 rather than sp^3), the carboxylate group can effectively mimic the role of the phosphate moiety in spanning the distance between Ca^{2+} and Tyr-69 in the enzyme active site.

The isobutyl group attached to the asymmetric carbon atom in 1 was shown to bind into the hydrophobic pocket of the enzyme which normally accommodates the *sn*-1 chain of the natural substrate.[6] Molecular modeling indicated that the isobutyl group was not an optimal fit for this pocket and that larger groups might be accommodated in this cavity. Indeed, increasing the steric bulk of this substituent in the carboxylate series resulted in an increase in inhibitor activity. In particular, (phenylthio)methyl derivative 5 was 3 times more active, and (naphthylthio)methyl analogue **6a** demonstrated more than an order of magnitude increase in potency over 4. The homologue of **6a** (**6b**) was similarly active. The stereospecificity of the enzyme–inhibitor interaction is indicated by the 4 orders of magnitude difference between the activity of **6b** and that of its enantiomer (7) in the monomeric substrate assay. Further optimization[8] of the activity against the pancreatic enzyme in this chemical series resulted in the discovery of the highly potent benzyl-substituted analogue 8 and the even more active phenylheptanoyl derivative **9b**.

The roughly parallel structure–activity relationships observed between the monomeric and aggregated substrate screens, coupled with the very high levels of potency and stereospecificity achieved in this series, give added support to the belief that these compounds inhibit by direct interaction with the enzyme rather than by substrate perturbation.

Human Platelet (Group II) PLA_2. In contrast to the results obtained with the pancreatic enzyme, carboxylic acid derivatives **6b** and 8 showed only moderate potency against the $sPLA_2$ isolated from human platelets (Table 1). This weaker interaction is further reflected in the smaller potency difference between the enantiomers **6b** and 7 when tested against the latter enzyme. However, a dramatic increase in activity was seen when the dec-3-enoyl substituent of 8 was replaced by the phenylheptanoyl residue as exemplified by the activity of **9a**. The homologue of **9a** (**9b**) demonstrated a further 3-fold increase in activity.

The difference in structure–activity relationships for this series of compounds between porcine pancreatic PLA_2 and the human platelet enzyme is not unexpected. While there is a large degree of homology between the enzymes from these two sources, there are some key differences in those residues situated around the active-site regions which

bind the hydrophobic chains of the substrate.[9] The consequences of these differences on the activity of the inhibitors will be discussed in more detail in a subsequent publication.

Summary. A novel series of highly potent, non-phospholipid inhibitors of the secreted PLA_2s has been discovered. The design of these molecules was based on the hypothesis that the carboxylate function may provide interactions with the active sites of the enzymes which are of similar magnitude to those of the phosphocholine moiety present in molecules which we have previously described. It is likely that such simplified compounds will have improved metabolic stability compared with the phospholipid analogues. There are clearly some significant differences in the inhibitor structure–activity relationships between the pancreatic (group I) and human platelet (group II) enzymes, and these are presently being rationalized in molecular modeling studies using the published X-ray crystal structures of the different proteins. This study provides one of the first examples of the successful use of the carboxylic acid function as a bioisosteric replacement for a phosphodiester group in the rational design of biologically active molecules.[10]

The most potent inhibitor (**9b**) to be described in this communication has been designated as FPL 67047XX. The compound has an IC_{50} against the human platelet $sPLA_2$ of 21 ± 4 nM. The properties of this molecule are presently being investigated in animal models with the objective of identifying the role which the group II $sPLA_2$ may play in inflammatory disease.

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Supplementary Material Available: Detailed synthetic procedures for the synthesis of **9b** as well as a description of the biochemical assay for screening inhibitors of the human platelet $sPLA_2$ (7 pages). Ordering information is given on any current masthead page.

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(8) Since the IC_{50}s of compounds **6a** and **6b** were now approximately half of the enzyme concentration (30 nM) used in the monomeric substrate assay, it is clear that stoichiometric binding was occurring and that this screen could not be used for the identification of more potent analogues. However, the higher activities associated with the benzylphenylthio and 7-phenylheptanoyl substituents (compounds **8** and **9b**, respectively) can be clearly observed in the DOC mixed micelle assay (enzyme concentration 5 nM) (Table 1).

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