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# A β-LACTAM INHIBITOR OF CYTOSOLIC PHOSPHOLIPASE $A_2$ WHICH ACTS IN A COMPETITIVE, REVERSIBLE MANNER AT THE LIPID/WATER INTERFACE

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Cytosolic phospholipase $A_2$ ($cPLA_2$) catalyzes the selective release of arachidonic acid from the *sn*-2 position of phospholipids and is believed to play a key cellular role in the generation of arachidonic acid. When assaying the human recombinant $cPLA_2$ using membranes isolated from [$^3$H]arachidonate-labeled U937 cells as substrate, 3,3-Dimethyl-6-(3-lauroylureido)-7-oxo-4-thia-1-azabicyclo[3,2,0]heptane-2-carboxylic acid (**1**) was found to inhibit the enzyme in a dose-dependent manner ($IC_{50} = 72\,\mu M$). This β-lactam did not inhibit other phospholipases, including the human nonpancreatic secreted phospholipase $A_2$. The inhibition of $cPLA_2$ was found not to be time-dependent. This, along with the observation that the degradation of the inhibitor was not catalyzed by the enzyme, demonstrates that the inhibition does not result from the formation of an acyl-enzyme intermediate with the active site serine residue. Moreover, the ring-opened form of **1** is also able to inhibit $cPLA_2$ with near-equal potency. To further characterize the mechanism of inhibition, an assay in which the enzyme is bound to vesicles of 1,2-dimyristoyl-*sn*-glycero-3-phosphomethanol containing 6–10 mole percent of 1-palmitoyl-2-[1-$^{14}$C]-arachidonoyl-*sn*-glycero-3-phosphocholine was employed. With this substrate system, the dose-dependent inhibition was defined by kinetic equations describing competitive inhibition at the lipid/water interface. The apparent dissociation constant for the

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Abbreviations used: $cPLA_2$, cytosolic phospholipase $A_2$; [$^{14}$C]PAPC, 1-palmitoyl-2-arachidonoyl-[*arachidonoyl*-1-$^{14}$C]-*sn*-glycero-3-phosphocholine; DMPM, 1,2-dimyristoyl-*sn*-glycero-3-phosphomethanol; hnp-$sPLA_2$, human non-pancreatic secreted phospholipase $A_2$.



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inhibitor bound to the enzyme at the interface ($K_I^{*app}$) was determined to be $0.5 \pm 0.1$ mole% versus an apparent dissociation constant for the arachidonate-containing phospholipid of $0.4 \pm 0.1$ mole%. Thus, **1** represents a novel structural class of inhibitors of $cPLA_2$ which partitions into the phospholipid bilayer and competes with the phospholipid substrate for the active site.

*Keywords:* Cytosolic phospholipase $A_2$; Inhibition; Interfacial catalysis

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## INTRODUCTION

Cytosolic phospholipase $A_2$ ($cPLA_2$) catalyzes the hydrolysis of the *sn*-2 arachidonoyl ester of phospholipids.[1,2] Because of its apparent role in the generation of leukotrienes and prostaglandins, potent inflammatory mediators metabolized from arachidonate, $cPLA_2$ has received considerable interest as a target for novel anti-inflammatory agents.

The $cPLA_2$ is a calcium-dependent enzyme which is present in a number of different tissues and cells including monocytes, neutrophils, and platelets.[3-5] The enzyme is normally located in the cytosol, but translocates to the membrane in response to submicromolar concentrations of calcium.[6,7] Moreover, agonist-induced phosphorylation at Ser-505 of $cPLA_2$ has been proposed to regulate the enzyme *in vivo*,[8] although phosphorylation of the enzyme does not appreciably affect its catalytic activity.[9] It has also been shown that enzyme phosphorylation and an influx of calcium are not sufficient to activate $cPLA_2$ in U937 cells, and that activation of a pertussis toxin-sensitive G-protein is required.[10] Additionally, the enzyme shows a cooperative behavior, with respect to the mole fraction of arachidonate-containing phospholipids, which may account for another mechanism of regulation.[11]

The catalytic mechanism of $cPLA_2$ is thought to be much like that of serine esterases and proteases: the enzyme forms an acyl enzyme intermediate between arachidonate of the phospholipid substrate and an active site serine residue. Site-directed mutagenesis has been used to provide evidence that serine-228 is this putative active site nucleophile.[12,13] Other evidence for this mechanism comes from the fact that the enzyme also catalyzes, to a small extent, a transacylase reaction which is most simply explained by invoking an acyl enzyme intermediate.[14,15] Additionally, the arachidonoyltrifluoromethyl ketone, which is a potent inhibitor of the enzyme, shows kinetic and spectroscopic characteristics consistent with the formation of a hemiketal with a serine residue in the active site.[16,17] The presence of glycerol, which enhances the activity of the enzyme,[11,18] results in the formation of monoarachidonoyl glycerol which further demonstrates that the enzyme possesses a transacylase activity.[15]

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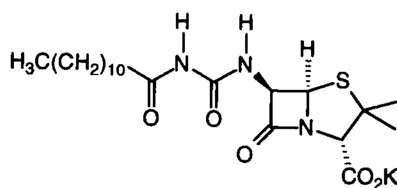
Since the phospholipid substrate on which the enzyme acts is in the form of an aggregate rather than water soluble monomers, the enzyme must first bind to the surface of the lipid/water interface before a phospholipid molecule can bind to the active site.[19] The enzyme contains a calcium-dependent interfacial binding site, separate from the active site, which is responsible for the translocation of the enzyme to the interface. The location of this interfacial binding site is at the N-terminal end of the enzyme.[7,20] This portion of the enzyme has significant sequence homology to the CaLB lipid binding domains of both protein kinase C and phospholipase $C_{\gamma 1}$.[7]

The interfacial binding step has presented a great challenge when performing kinetic analysis of the enzyme. However, the analysis of phospholipase catalysis at the interface has received considerable investigation and has led to kinetic schemes describing the action of phospholipases.[21,22] The kinetics can be described using classical Michaelis-Menten kinetic theory applied to interfacial catalysis,[23] which facilitates determination of numerous kinetic parameters and binding constants.[11,24]

In this paper, we provide a detailed characterization of the inhibition of $cPLA_2$ by a β-lactam-containing inhibitor, **1**, which was identified by high-throughput screening of a compound library. Surprisingly, the inhibitor is shown to act not by acylating the enzyme, but by inhibiting the enzyme in a reversible, competitive manner when the enzyme is at the lipid-water interface.




**1**

## MATERIALS AND METHODS

### Enzymes and Substrates

All non-radiolabeled phospholipids were obtained from Avanti Polar Lipids except for DMPM, which was from Calbiochem. The radiolabeled [$^{14}$C]PAPC was from DuPont/NEN (55 mCi/mmol). The human,

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premonocytic U937 cell line was obtained from the American Type Culture Collection. [$^3$H]arachidonate-labeled U937 membranes were prepared from U937 cells which had been prelabeled with [$^3$H]arachidonate (100 Ci/mmol) as previously described.[9]

The human, recombinant $cPLA_2$ was expressed in Sf9 insect cells and highly purified as previously described.[11] The hnps-$PLA_2$, which was purified from human platelets according to the procedure of Raghupathi and Franson,[25] was obtained from Dr. R.C. Franson (Virginia Commonwealth University) along with the [1-$^{14}$C]-oleic acid-labeled *E. coli* membrane substrate (3000 dpm/nmol phospholipid).

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### Enzyme Assays

Sonicated phospholipid covesicles comprised of DMPM and containing [$^{14}$C]PAPC or other phospholipids, were prepared using the general methods described previously.[1,26,27] With this synthetic substrate, enzymatic assays were performed using the general procedure of Burke *et al.*[11] in which $cPLA_2$ (210–390 ng/mL) was added to solutions of the radiolabeled covesicles (150–270 µM bulk phospholipid) in 25 mM BisTris Propane containing 7 mM $CaCl_2$, 0.4 mg/mL albumin (BSA, essentially fatty acid free), and 4 M glycerol at pH 8. All components except enzyme were incubated at 37°C for 5 min before the addition of enzyme. At various times, 100 µL aliquots were removed and quenched by addition into 1.9 mL tetrahydrofuran. The hydrolyzed, radiolabeled fatty acid was then isolated using aminopropyl solid-phase extraction columns as described previously.[28] Under these assay conditions, the high calcium concentration catalyzes vesicle fusion with continuous exchange of phospholipids.[11,29]

Assays of $cPLA_2$ activity using the [$^3$H]arachidonate-labeled U937 membranes as substrate used 0.6 µg/mL enzyme and membrane substrate (22 µM) in 20 mM HEPES buffer, pH 8, containing 6 mM $CaCl_2$, 0.9 mg/mL HSA and 0–4 M glycerol. Enzyme assays were allowed to proceed for 20 min at 37°C before quenching with THF. The radiolabeled fatty acid product was isolated as described above.

Assays of hnps-$PLA_2$ utilizing radiolabeled *E. coli* membrane as substrate were performed as described previously.[28]

### Degradation of Compound 1 in the Assay

Enzyme (440 ng/mL) was added to a solution of **1** (50 µM) in an assay mixture containing the non-radiolabeled covesicles of PAPC/DMPM

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(270 μM bulk phospholipid, 8 mole% PAPC) in 25 mM BisTris Propane containing 7 mM $CaCl_2$, and 0.4 mg/mL albumin (BSA, essentially fatty acid free) at pH 8. This mixture was then incubated at 37°C. At various times, aliquots were removed and analyzed by HPLC for the amount of **1** remaining. A control without enzyme was also analyzed.

HPLC analysis employed a Waters μ-Bondapak c18 reverse phase column (3.9 × 150 mm) and an isocratic elution with 75:25 $CH_3CN$/50 mM $K_2HPO_4$ at 1.5 mL/min. Under these conditions, **1** had a retention time of 5.6 min.

## Synthesis

*3,3-Dimethyl-6-(3-lauroylureido)-7-oxo-4-thia-1-azabicyclo[3,2,0]heptane-2-carboxylic acid, potassium salt* (**1**) was prepared according to the procedure of Naito *et al.*[34]

*Lauroylureidopenicilloic acid, mono potassium salt* (**2**) A solution of 3,3-dimethyl-6-(3-lauroylureido)-7-oxo-4-thia-1-azabicyclo[3,2,0]heptane-2-carboxylic acid, potassium salt (1.00 g, 2.08 mmol) in water (40 mL) was treated dropwise at 22°C with 1 M KOH (2.0 mL, 2.0 mmol) and the resulting mixture was stirred for 3 h. After adjusting to pH 5.8 with 0.2N sulfuric acid, the solution was then chromatographed on reversed phase silica gel (μ-Bondapak c-18). Elution with a gradient of acetonitrile (0–50%) in water gave 0.426 g (41%) of lauroylureidopenicilloic acid mono potassium salt as a white powder after lyophilization. $[\alpha]^{22}D + 66.6°$ (c 1.0, $H_2O$). IR (KBr) $\nu_{max}$ ($cm^{-1}$): 1690 (C=O of amide) and 1600 (C=O of carboxylate). $^1H$ NMR 400 MHz ($D_2O$) δ (ppm): 0.90 (3H, t, J = 6.5 Hz, $CH_3$), 1.28 and 1.59 (2 × 3H, 2s, 2 × $CH_3$), 1.3 and 1.7 (16H and 2H, 2m, $(CH_2)_9$), 2.45 (2H, t, J = 7.3 Hz, $\underline{CH_2}CO$), 3.63 (1H, s), 4.24 and 5.21 (2 × 1H, 2d, J = 4.9 Hz).

# RESULTS AND DISCUSSION

## β-Lactam 1 is a Selective Inhibitor of $cPLA_2$

U937 cells treated with [$^3$H]arachidonate incorporate this radiolabeled fatty acid into phospholipid pools. An assay using membranes isolated from these radiolabeled U937 cells as substrate was used to measure the activity of human, recombinant $cPLA_2$. Synthetic compounds from the Bristol-Myers Squibb compound collection were tested, resulting in the identification of

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the $\beta$-lactam, **1**, as an inhibitor of the enzyme. As shown in Figure 1, the dose-dependent inhibition gave an $IC_{50}$ value of 72 µM.

The human, nonpancreatic secreted phospholipase $A_2$ (hnp-$sPLA_2$) is an enzyme which shows no structural, genetic, or mechanistic homology to the $cPLA_2$ even though the two enzymes catalyze the same reaction (for a review, see Reference 30). As depicted in Figure 1, **1** showed much less activity against the hnp-$sPLA_2$ with less than 25% inhibition at concentrations as high as 250 µM. Thus, **1** showed a marked selectivity for the $cPLA_2$ as compared to the hnp-$sPLA_2$.

Moreover, **1** inhibited neither other types of phospholipases such as phospholipase $A_1$, phospholipase C or phospholipase D, nor arachidonate-metabolizing enzymes such as cyclooxygenase and 5-lipoxygenase (results not shown).

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## Noncovalent Mechanism of Inhibition by 1

Since the $cPLA_2$ is a serine esterase, it may not seem surprising that a $\beta$-lactam such as **1** inhibits the enzyme. Indeed, the inhibition of D-alanine carboxypeptidase, another serine protease, by $\beta$-lactam-containing penicillins results from an acylation of the active site serine.[31]

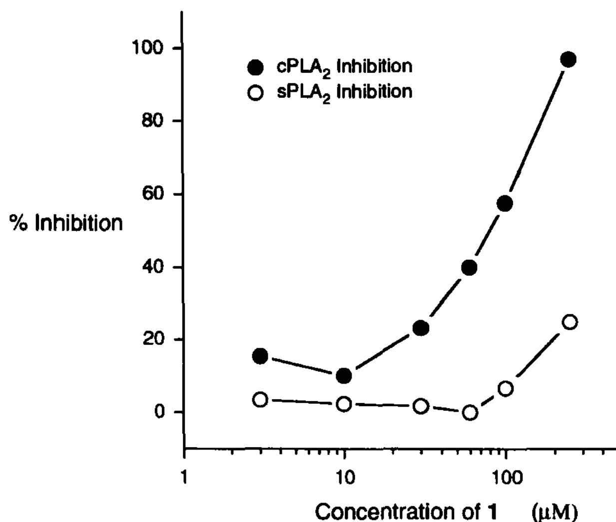


FIGURE 1 Dose-Dependent Inhibition of $cPLA_2$ and hnp-$sPLA_2$ by **1**. *Closed circles*, Inhibition of the activity of $cPLA_2$ assayed using membranes from [$^3$H]arachidonate-labeled U937 cells as substrate; *open circles*, inhibition of hnp-$sPLA_2$. See Materials and Methods for details. The data represent the average of duplicate measurements.

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To detemine whether **1** inhibits $cPLA_2$ by a similar mechanism, the time-dependency of the inhibition was measured. As shown in Figure 2, the rate of hydrolysis of covesicle substrate comprised of [$^{14}$C]PAPC dispersed within DMPM is, as expected, linear. The further observation that the inhibition observed with **1** is immediate rather than time-dependent, however, is inconsistent with an inhibition mechanism in which the active site serine is acylated. It would have been predicted that covalent modification of the serine would have resulted in a time-dependent inhibition (inactivation) of the enzyme.[32]

This lack of time-dependent inhibition can be reconciled with an acylation mechanism if a putative acyl-enzyme intermediate formed between **1** and the active site serine is quickly hydrolyzed. That is, **1** may function as a substrate for the enzyme. This possibility was investigated by determining the rate of degradation of **1** by $cPLA_2$ under the conditions of the assay. As shown in Figure 3, this rate of degradation was not significantly different than the surprisingly fast nonenzymatic rate of degradation ($t_{1/2} = 3.2 \pm 0.2$ h and $3.8 \pm 0.5$ h, respectively). This data indicates that **1** is not acting by acylating the active site serine of $cPLA_2$, even in a transient manner.

Definitive evidence against an acylation mechanism of inhibition comes from the use of **2**, the ring-opened analog of **1**. When assayed using the [$^3$H]arachidonate-labeled membranes from U937 cells as substrate, a

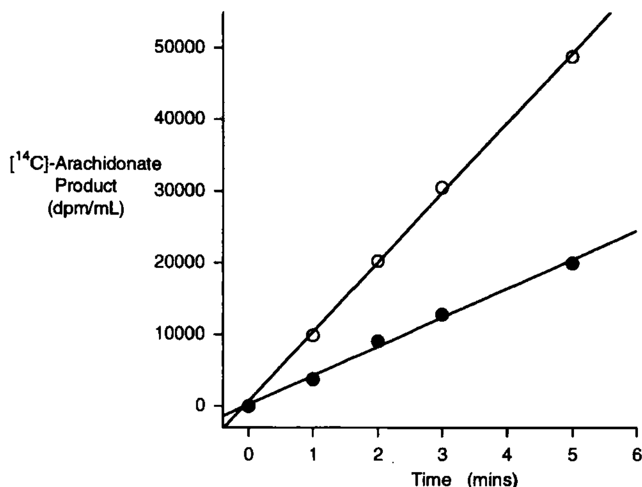


FIGURE 2 Effect of **1** on the $cPLA_2$-catalyzed hydrolysis of [$^{14}$C]PAPC dispersed in covesicles with DMPM. [$^{14}$C]Arachidonate produced in the absence of inhibitor (*closed circles*), and in the presence of 25 μM **1** (*open circles*). [Enzyme] = 207 ng/mL, [phospholipid] = 150 μM. See Materials and Methods for details. The data represent the average of duplicate measurements.

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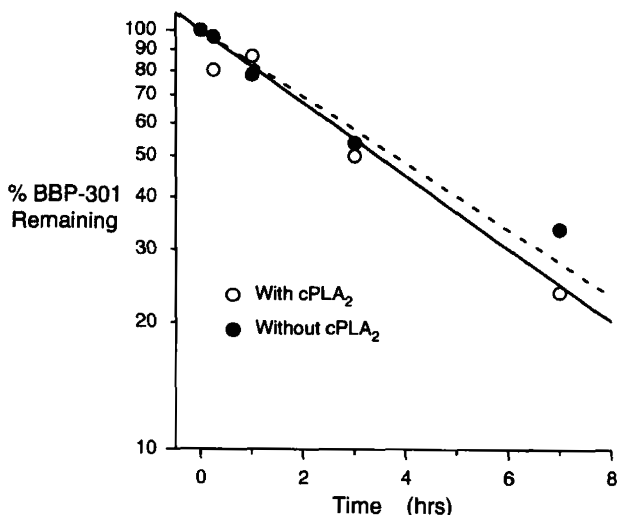
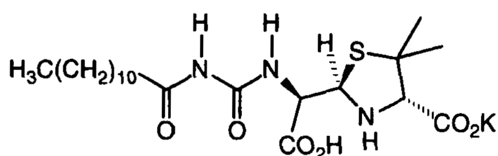



FIGURE 3 Rate of degradation of **1** in the $cPLA_2$ assay. The inhibitor, **1**, was incubated in the presence (*open circles, solid line*) or absence (*closed circles, dotted line*) of $cPLA_2$ and the amount of compound remaining over time (%BBP-301) as compared to time = 0 was determined by HPLC. See Materials and Methods for details.

dose-dependent inhibition by **2** was observed with an $IC_{50}$ value of 81 μM (data not shown). The observation that **2**, which is unable to acylate the serine residue, inhibits $cPLA_2$ with a potency nearly equal to that observed with **1** demonstrates that the mechanism of inhibition does not proceed through an acyl-serine intermediate.




**2**

## Inhibition of $cPLA_2$ by 1 at the Lipid/Water Interface

The equilibrium binding of $cPLA_2$ to the substrate involves a dependency on two processes as defined in Scheme 1.[11] These include the intrinsic equilibrium binding to the interface (defined by a dissociation constant, $K_S$) and the equilibrium binding of phospholipid substrate to the active site at the interface (defined by a dissociation constant, $K_M{}^*$).

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SCHEME 1 Kinetic Scheme for Binding of $cPLA_2$ to Phospholipid. E is defined as the free enzyme; A is the phospholipid vesicle, EA* is the enzyme bound to the lipid/water interface; S is the phospholipid substrate within the vesicle; and EAS* is the interface-bound enzyme containing an active-site-bound phospholipid substrate.

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A competitive, reversible inhibitor of phospholipase $A_2$ which acts at the lipid/water interface is a compound which partitions into the phospholipid bilayer and binds to the active site of the enzyme when the enzyme is bound to the interface.[33] This type of inhibitor is competitive: It competes with the individual phospholipids for the active site.

Definitive proof that **1** is acting as a competitive, reversible inhibitor at the lipid/water interface comes from the use of covesicles of [$^{14}$C]PAPC dispersed within DMPM. This covesicle substrate allows for the determination of the equilibrium dissociation constants of phospholipids and inhibitors from the active site at the interface.[11] Using these covesicles, the concentration of **1** was varied while measuring the $cPLA_2$-catalyzed rate of hydrolysis of the covesicles containing different mole percentages of [$^{14}$C]PAPC. As long as the bulk phospholipid concentration is large enough to ensure that essentially all of the enzyme is at the interface (e.g., at 270 μM phospholipid over 98% of the enzyme is interface-bound),[9] the following equation describing competitive inhibition of $cPLA_2$ at the interface is valid:[11,35,36]

$$\frac{(v_0)^o}{(v_0)^I} = 1 + \left(\frac{1/K_I^{*app}}{1 + \frac{X_S{}^o}{K_M^{*app}}}\right) X_I/(1 - X_I), \qquad (1)$$

where $(v_0)^o/(v_0)^I$ is the ratio of initial rates in the absence to that in the presence of a competitive inhibitor; $K_M^{*app}$ and $K_I^{*app}$ are defined as the apparent dissociation constants for the substrate and inhibitor, respectively;[37] $X_I$ is the concentration of inhibitor (in units of mole fraction); and $(X_S{}^o)$ is the mole fraction of radiolabeled substrate ([$^{14}$C]PAPC) in the absence of inhibitor. The unit of concentration in the interface is mole fraction which is related to the surface concentration of substrate, rather than bulk concentration which has units of molarity.

Using these covesicles as substrate for $cPLA_2$, the dose-dependent inhibition by **1** was plotted in Figure 4 as $(v_0)^o/(v_0)^I$ versus $X_I/(1 - X_I)$ at each $(X_S{}^o)$ value.[38] As predicted by equation (1), linear correlations were obtained. Nonlinear regression analysis of the data as fit to equation (1)

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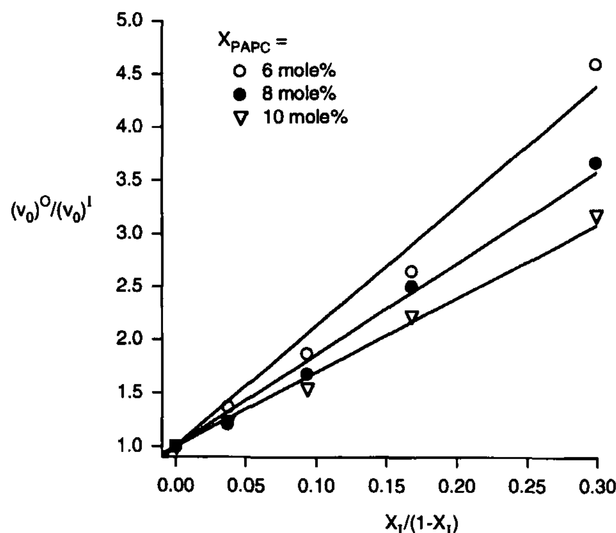


FIGURE 4 Correlation of the inhibition of hydrolysis of [$^{14}$C]PAPC/DMPM covesicles with the concentration of **1** ($X_I$). Enzyme was assayed in a solution containing radiolabeled covesicles under fusing conditions. These [$^{14}$C]PAPC/DMPM covesicles contained 0–23 mole% **1** and had $X_S{}^o$ values (in terms of [$^{14}$C]PAPC concentrations) of: *open circles*, 6 mole%; *closed circles*, 8 mole%; *open triangles*, 10 mole%. The data was fitted to equation (1) which describes competitive inhibition at the interface. [Enzyme] = 390 ng/mL, [phospholipid] = 270 µM. See Materials and Methods for details. The data represent the average of duplicate measurements. To determine the $X_I$ values, the inhibitor was assumed to be completely partitioned into the bilayer.

yielded $K_M^{*app}$ and $K_I^{*app}$ values of $0.4 \pm 0.1$ and $0.5 \pm 0.1$ mole%, respectively. The value of $K_M^{*app}$ determined here is in good agreement with the value of $0.3 \pm 0.1$ mole% determined previously.[11]

That the inhibition of $cPLA_2$ can be defined by equation (1) demonstrates, unequivocally, that **1** is a competitive, reversible inhibitor at the interface. That a compound such as **1** becomes incorporated into the bilayer is not surprising since an energetic hydrophobic interaction between the *n*-alkyl chain of the inhibitor and the fatty acid tails of the phospholipids comprising the bilayer would be expected.

This mechanism is of particular relevance to the design of pharmacologically active compounds since inhibitors which act at the interface will always show inhibition due to the requirement that the enzyme must be at the interface in order to hydrolyze the phospholipid. Inhibitors of this type may be pharmacologically advantageous *in vivo* as compared to inhibitors which act on the enzyme in the aqueous phase since the degree of inhibition will not be dependent on the fraction of enzyme bound to the interface.

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In conclusion, $\beta$-lactams and peptide analogs such as **1** and **2** represent an important step in the development of potent inhibitors of $cPLA_2$ for use as novel antiinflammatory agents. Work is ongoing in our laboratory to determine which chemical moieties of **1** and **2** provide for interactions with the active site.

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## *References*


[1] Diez, E., Louis-Flamberg, P., Hall, R.H. and Mayer, R.J. (1992). *J. Biol. Chem.*, **267**, 18342.

[2] Diez, E., Chilton, F.H., Stroup, G., Mayer, R.J., Winkler, J.D. and Fonteh, A.N. (1994). *Biochem. J.*, **301**, 721.

[3] Nakamura, T., Lin, L.-L., Kharbanda, S., Knopf, J.L. and Kufe, D. (1992). *EMBO J.*, **11**, 4917.

[4] Kramer, R.M., Roberts, E.F., Manetta, J.V., Hyslop, P.A. and Jakubowski, J.A. (1993). *J. Biol. Chem.*, **268**, 26796.

[5] Ramesha, C.S. and Ives, D.L. (1993). *Biochim. Biophys. Acta*, **1168**, 37.

[6] Channon, J.Y. and Leslie, C.C. (1990). *J. Biol. Chem.*, **265**, 5409.

[7] Clark, J.D., Lin, L.-L., Kriz, R.W., Ramesha, C.S., Sultzman, L.A., Lin, A.Y., Milona, N. and Knopf, J.L. (1991). *Cell*, **65**, 1043.

[8] Schievella, A.R., Regier, M.K., Smith, W.L. and Lin, L.-L. (1995). *J. Biol. Chem.*, **270**, 30749.

[9] Burke, J.R., Guenther, M.G., Witmer, M.R., Tredup, J.A., Micanovic, R. and Villafranca, J.J. (1997). *Arch. Biochem. Biophys.*, **341**, 177.

[10] Burke, J.R., Davern, L.B., Gregor, K.R., Todderud, G., Alford, J.G. and Tramposch, K.M. (1997). *Biochim. Biophys. Acta*, **1341**, 223.

[11] Burke, J.R., Witmer, M.R., Tredup, J., Micanovic, R., Gregor, K., Lahiri, J., Tramposch, K.M. and Villafranca, J.J. (1995). *Biochemistry*, **34**, 15165.

[12] Sharp, J.D., Pickard, R.T., Chiou, X.G., Manetta, J.V., Kovacevic, S., Miller, J.R., Varshavsky, A.D., Roberts, E.F., Strifler, B.A., Brems, D.N. and Kramer, R.M. (1994). *J. Biol. Chem.*, **269**, 23250.

[13] Huang, Z., Laliberte, F., Tremblay, N.M., Weech, P.K. and Street, I.P. (1994). *Anal. Biochem.*, **222**, 110.

[14] Reynolds, L.J., Hughes, L.L., Louis, A.I., Kramer, R.M. and Dennis, E.A. (1993). *Biochim. Biophys. Acta*, **1167**, 272.

[15] Hanel, A.M. and Gelb, M.H. (1995). *Biochemistry*, **34**, 7807.

[16] Street, I.P., Lin, H.-K., Laliberte, F., Ghomaschchi, F., Wang, Z., Perrier, H., Tremblay, N.M., Huang, Z., Weech, P.K. and Gelb, M.H. (1993). *Biochemistry*, **32**, 5935.

[17] Trimble, L.A., Street, I.P., Perrier, H., Tremblay, N.M., Weech, P.K. and Bernstein, M.A. (1993). *Biochemistry*, **32**, 12560.

[18] Ulevitch, R.J., Watanabe, Y., Sano, M., Lister, M.D., Deems, R.A. and Dennis, E.A. (1988). *J. Biol. Chem.*, **263**, 3079.

[19] Verger, R. (1980). *Meth. Enzymol.*, **64**, 340–392.

[20] Nalefski, E.A., Sultzman, L.A., Martin, D.M., Kriz, R.W., Towler, P.S., Knopf, J.L. and Clark, J.D. (1994). *J. Biol. Chem.*, **269**, 18239.

[21] Ramirez, F. and Jain, M.K. (1991). *Proteins*, **9**, 229.

[22] Yu, L. and Dennis, E.A. (1992). *Bioorg. Med. Chem. Lett.*, **2**, 1343.

[23] Berg, O.G., Yu, B.Z., Rogers, J. and Jain, M.K. (1991). *Biochemistry*, **30**, 7283.

[24] Gelb, M.K., Jain, M.K. and Berg, O. (1992). *Bioorg. Med. Chem. Lett.*, **2**, 1335.

[25] Raghupathi, R. and Franson, R.C. (1987). *Fed. Proc.*, **46**, 1945.

[26] Jain, M.K., Yuan, W. and Gelb, M.H. (1989). *Biochemistry*, **28**, 4135.

[27] Jain, M.K. and Gelb, M.H. (1991). *Meth. Enzymol.*, **197**, 112.

[28] Tramposch, K.M., Steiner, S.A., Stanley, P.L., Nettleton, D.O., Franson, R.D., Lewin, A.H. and Carroll, F.I. (1992). *Biochem. Biophys. Res. Commun.*, **189**, 272.


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[29] Jain, M.K., Rogers, J., Jahagirdar, D.V., Marecek, J.F. and Ramirez, F. (1986). *Biochem. Biophys. Acta*, **860**, 435.
[30] Glaser, K.B., Mobilio, D., Chang, J.Y. and Senko, N. (1993). *Trends Pharm. Sci.*, **14**, 92.
[31] Silverman, R.B. (1992). *The Organic Chemistry of Drug Design and Drug Action*, pp. 181–185. San Diego: Academic Press.
[32] Jung, M.J. and Metcalf, B.W. (1975). *Biochem. Biophys. Res. Commun.*, **67**, 301.
[33] Burke, J.R., Gregor, K.R. and Tramposch, K.M. (1995). *J. Biol. Chem.*, **270**, 274.
[34] Naito, T., Nakagawa, S., Okumura, J., Konishi, M. and Kawaguchi, H. (1965). *J. Antibiotics, Ser. A.*, **18**, 145.
[35] Burke, J.R., Witmer, M.R., Tredup, J., Micanovic, R., Gregor, K., Lahiri, J., Tramposch, K.M. and Villafranca, J.J. (1997). *Biochemistry*, **36**, 6854.
[36] This equation is valid since the active site dissociation constant for DMPM has been shown to be more than 330-times greater than the value for PAPC (see Reference 35).
[37] $K_M^{*app}$ and $K_I^{*app}$ are related to the intrinsic dissociation constants ($K_M^*$ and $K_I^*$) by the equations: $K_M^{*app} = K_M^*(1 + 1/K_L^*)$ and $K_I^{*app} = K_I^*(1 + 1/K_L^*)$ where $K_L^*$ is the active site dissociation constant for DMPM at the interface (see Reference 35).
[38] When determining the equilibrium dissociation constants from the active site, the mole amount of substrate ([$^{14}$C]PAPC in this case) and the mole amount of DMPM were held constant while varying the mole amount of inhibitor (see References 11 and 35). This has the effect of actually decreasing the mole fraction of both substrate and DMPM as the inhibitor concentration is increased. Thus, $X_S{}^o$ in equation (1) equals the mole fraction of substrate phospholipid without inhibitor. The mole fraction of substrate in the presence of the inhibitor is correspondingly less.

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