Biochemical Pharmacology, Vol. 51, pp. 737–742, 1996.

ISSN 0006-2952/96/$15.00 + 0.00
SSDI 0006-2952(95)02172-8

4-Alkoxybenzamidines as New Potent Phospholipase A_2 Inhibitors

Mina Aitdafoun, Carine Mounier,† Françoise Heymans,* Carine Binisti,* Cassian Bon† and Jean-Jacques Godfroid*‡*

*Unité de Recherche "Chimie et Pharmacologie," Laboratoire de Pharmacochimie Moléculaire, Université Paris 7, 2 place Jussieu, 75251 Paris, Cedex 05, France; and †Unité des Venins, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France

ABSTRACT. A series of 4-alkoxybenzamidines was synthesized, varying the number of carbons of the alkyl chain, and their potency as phospholipase A_2 (PLA_2) inhibitors was evaluated. The relationship between their capacity to inhibit PLA_2 activity and their lipophilicity was examined. The optimum of the inhibitory effect against two extracellular PLA_2s from rabbit platelets and bovine pancreas was observed with compounds bearing an alkyl chain of 12 and 14 carbons. These 4-dodecyl and tetradecyloxybenzamidines inhibited bovine pancreatic and rabbit platelet lysate PLA_2s with IC_{50} values of 3 μM and 5–5.8 μM, respectively. The mechanism of inhibition was of the competitive type. In addition, 4-tetradecyloxybenzamidine was shown to exert an anti-inflammatory effect *in vivo* on the carrageenan-induced rat paw oedema. These results show that 4-tetradecyloxybenzamidine will serve as an interesting tool to investigate the physiological role of mammalian-secreted PLA_2, both *in vitro* and *in vivo*. BIOCHEM PHARMACOL 51;6:737–742, 1996.

KEY WORDS. extracellular PLA_2; benzamidine; PLA_2 inhibitor; carrageenan-induced oedema

PLA_2s§ are ubiquitous enzymes that catalyze the hydrolysis of the ester bound at the *sn*-2 position of glycerophospholipids, thereby releasing free fatty acids and lysophospholipids [1]. In the inflammatory process, these two products may be converted into potent pro-inflammatory mediators: Eicosanoids (prostaglandins, leukotrienes, thromboxane) are derived from arachidonic acid, and PAF from lyso-PAF [2]. PLA_2s are found in both cell-associated and extracellular forms [3]. According to the similarity of their polypeptide sequence, extracellular PLA_2s have been classified into three groups [4, 5]: group I (PLA_2-I) including PLA_2s from mammalian pancreatic secretions and *Elapidae* venoms; group II (PLA_2-II) comprising PLA_2s from *Crotalidae* and *Viperidae* venoms as well as the mammalian nonpancreatic secretory PLA_2 [6, 7]; and group III PLA_2s (PLA_2-III) from bee and lizard venoms.

Mammalian PLA_2-II has been postulated to play a central role in inflammatory diseases [8, 9]. Indeed, high levels of PLA_2-II activity have been found in the synovial fluid and serum of patients suffering from rheumatoid arthritis, peritonitis, and psoriatic lesions, as well as in the case of endotoxin shock. In addition, intradermal injection of PLA_2-II purified from human platelets and synovial fluids produces an inflammatory reaction in the mouse paw [10], and injection of purified rat platelet PLA_2-II exacerbates the paw oedema in rats with adjuvant arthritis [11]. Further, the recombinant form of human PLA_2-II also elicits inflammatory responses [12, 13]. Taken together, these results strongly suggest an involvement of PLA_2-II in inflammation. However, depending on the inflammatory model, the pro-inflammatory effect of this enzyme may be due either to its ability to produce lipid mediators or its ability to induce mast cell degranulation via the liberation of serotonin and histamine [9]. Thus, a potent inhibitor of PLA_2-II would allow a better understanding of the precise involvement of this enzyme in inflammatory processes, and might also have therapeutic potential if it exerts an anti-inflammatory effect *in vivo*.

Several inhibitors of PLA_2-II have been described and discussed [14, 15]. Most of them are substrate analogues (or "transition state mimics"), but the ability of these inhibitors to exert their inhibitory effect in cellular and physiological systems is unknown [16, 17]. Other molecules, either from natural origin such as manoalide [18], or synthetic compounds such as parabromophenacyl bromide, have contributed to develop new PLA_2 inhibitors; but the irreversibility of their action and their sometimes toxic effect on cells has reduced interest in them for *in vivo* studies. The last class of inhibitors contains molecules that act on the substrate, such as mepacrin [14] and lipocortins [19], and may present nonspecific effects. Finally, except for some recently described compounds unre-

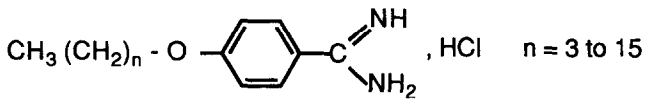
‡ Corresponding author: J. J. Godfroid, Unité de Recherche "Chimie et Pharmacologie," Laboratoire de Pharmacochimie Moléculaire, Université Paris 7, case postale 7066, 2 place Jussieu, 75251 Paris Cedex 05 France. Tel. (33-1) 44.27.56.26. FAX (33-1) 44.27.56.41.

Received 10 March 1995; accepted 30 September 1995.

§ *Abbreviations:* 10-pyrene PA, 1-palmitoyl-2-(10-pyrenyldecanoyl)-sn-glycero-3-monomethyl phosphatidic acid; BSA, bovine serum albumin; PAF, platelet activating factor; PLA_2, phospholipase A_2; and TLC, thin layer chromatography.

lated to phospholipid analogues [20–22], there is no common inhibitor of mammalian PLA_2-II that can be used in different models, and the development of new candidates still remains an important field of research.

This paper describes the synthesis of 4-alkoxybenzamidines of the general formula:



and examines their PLA_2 inhibitory effect as a function of the length of the alkyl chain, using an *in vitro* assay. Further, the effect of one of these inhibitors has been examined both on the carrageenan-induced rat paw oedema, as a model of acute inflammation, and on the phorbol ester-induced inflammation of the ear in mice, as a model of chronic inflammation.

MATERIALS AND METHODS

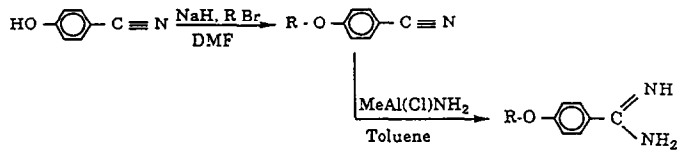
Materials

All chemicals were of reagent quality and were used without further purification. Silica gel 60 for column chromatography and TLC plastic sheets (silica gel 60 F 254, layer thickness 0.2 mm) were from Merck (Darmstadt, Germany). Bovine pancreatic PLA_2 and fatty acid-free BSA were purchased from Sigma (St. Louis, MO, U.S.A.). Rabbit platelets were prepared as described by Mounier *et al.* [23]. The fluorescent substrate for PLA_2 assay, 1-palmitoyl-2-(10-pyrenyldecanoyl)-*sn*-glycero-3-monomethyl phosphatidic acid (10-pyrene PA), was obtained from Interchim (Montluçon, France).

Methods

SYNTHETIC PROCEDURES. All compounds were prepared following Scheme 1. As an example, only the synthesis of 4-tetradecyloxybenzonitrile will be described in detail. The purity of each compound was checked by TLC. Spots were revealed under ultraviolet lamp as well as with iodine. All elemental analyses, C, H, N, and Cl, were within ±0.4% of theoretical values. The melting points were determined in a digital melting point apparatus (Electrothermal). All inhibitors were characterized by 200 MHz ^{1}H-NMR using a Brüker spectrometer with hexamethyl disiloxane (HMDS) as internal standard.

Scheme I



SYNTHESIS OF 4-TETRADECYLOXYBENZONITRILE (COMPOUND *1e*). A solution of 11.9 g (100 mmol) of 4-hydroxybenzonitrile in 25 mL of dry dimethyl formamide (DMF) was added dropwise, at room temperature, to a stirred suspension of 4 g (100 mmol) of NaH (60% dispersion in mineral oil) in 50 mL dry DMF. After the end of hydrogen release, 27.7 g (100 mmol) of tetradecyl bromide were added dropwise, and the reaction mixture stirred overnight at 80°C. The DMF was removed by evaporation and the residue dissolved in ether. After washing the ether solution with water until pH 7 and drying over $MgSO_4$, evaporation of the solvent yielded the crude compound *1e*, which was then purified on a silica gel column, using 20% ether in petroleum ether as eluent. Yield: 78%. m.p.: 54.8°C (petroleum ether). R_F = 0.41 in ether/petroleum ether (20:80, v/v). ^{1}H-NMR ($CDCl_3$): δ, 0.82 (t, 3H, CH_3), 1.23 (large s, 22H, alkyl), 1.50 (quintet, 2H, bCH_2), 3.92 (t, 2H, aCH_2), 6.87 and 7.50 (2d, 4H, aromatic H).

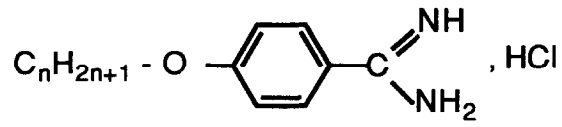
SYNTHESIS OF 4-TETRADECYLOXYBENZAMIDINE (COMPOUND *2e*, TABLE 1). The nitrile function was converted into amidine in one step by a modification of the procedure of Garigipati [24]. Then, 30 mL of a 0.67 M solution of methyl chloroaluminium amide, freshly prepared according to Levin *et al.* [25], was added dropwise at room temperature to a stirred solution of 3.15 g (10 mmol) of *1e* in 10 mL dry toluene. The mixture was then refluxed for 48 hr under argon atmosphere. After cooling, the aluminium complex was decomposed by carefully pouring the solution into a stirred suspension of 30 g silicagel in 100 mL of chloroform. After 15 min additional stirring, the silica gel was filtered and the filtercake washed twice with chloroform (100 mL). The filtercake was then taken up in methanol (100 mL), stirred for 15 min, then filtered again and washed twice with methanol (100 mL). The methanolic solution was evaporated *in vacuo* to give a residue of crude compound *2e*, which was purified on a silica gel column using 10% methanol in chloroform as eluent. Yield: 91%. m.p.: 116.5°C (methanol/ether). R_F = 0.44 in chloroform/methanol/acetic acid (80:20:4, v/v/v). ^{1}H-NMR (DMSO D_6): δ, 0.80 (t, 3H, CH_3), 1.20 (large s, 22H, alkyl), 1.67 (quintet, 2H, bCH_2), 4.01 (t, 2H, aCH_2), 7.08 and 7.76 (2d, 4H, aromatic H), 9.05 (large s, disappears with D_2O, 4H, NH, and NH^+).

SYNTHESIS OF 4-HEXADECYLOXYBENZAMIDINE (COMPOUND *2f*, TABLE 1). This compound was obtained by the same process as described for compound *2e* starting from 4-hexadecyloxybenzonitrile *1e* (m.p.: 61.9°C (petroleum ether), R_F = 0.42 in ether/petroleum ether 20:80, v/v). Yield: 89%. m.p.: 120.1°C (methanol/ether). R_F = 0.48 in chloroform/methanol/acetic acid (80:20:4, v/v/v).

SYNTHESIS OF OTHER COMPOUNDS. The synthesis of compounds *2a*, *2b*, *2c*, and *2d* has already been described, following another process [26]. Their melting points were determined and compared to the data in the literature. Compound *2a*: 184°C (lit.: 185–186). Compound *2b*: 126.7°C (lit.: 129). Compound *2c*: 120.7°C (lit: 118–119). Compound *2d*: 116.5°C (lit.: 114–115).

PLA_2 ASSAY. PLA_2 activity was evaluated by the method of Radvanyi *et al.* [27] using the fluorescent phospholipid analogue 10-pyrene PA as substrate, and bovine pancreatic PLA_2

TABLE 1. Effect of benzamidine derivatives on extracellular PLA_2s



Compound	n	Bovine pancreatic PLA_2 IC_{50} (μM)*	Rabbit platelet lysate IC_{50} (μM)*
2a	4	Inactive	Inactive
2b	8	18	36
2c	10	7	20
2d	12	3	5.8
2e	14	3	5
2f	16	150	155

* IC_{50} values are the means of three independent experiments. The standard errors were less than 10%.

or rabbit platelet lysate as the enzyme or source of enzyme, respectively. This assay has been extensively controlled and its specificity for detecting secretory PLA_2 already shown, since the cytosolic PLA_2 was not active on substrate with a pyrene group at the *sn*-2 position [28].

In a total volume of 1 mL, the standard reaction mixtures contained: 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM EGTA, 2 μM substrate, fatty acid-free BSA solution in water (15 μM or 0.1%) except when specified, and 5 nM of pancreatic PLA_2 or 10 μL of platelet lysate (≈0.4 μg of proteins). The fluorescence of the enzymatic reaction medium (blank) was recorded for 1 min with a spectrofluorimeter SFM 25 (Kontron Instruments) equipped with a Xenon lamp. The reaction was then initiated by the addition of $CaCl_2$ (10 mM, final concentration). When used, the inhibitor was added to the reaction medium after introduction of BSA. The activity was expressed in micromoles of fluorescent 10-pyrene PA hydrolyzed per min and per mg of protein, or per cells. Usually, the specific activity was 100 μmol/min · mg for the pancreatic enzyme and 200 nmol/min · 10^9 cells for the PLA_2 contained in the platelet lysate. The standard error of the means of three independent experiments was less than 10%. The reversibility of inhibitors was checked by dilution. Bovine pancreatic PLA_2 was preincubated with the inhibitor (20 μM), for 1 min. Then, aliquots (10 μL) were assayed for their PLA_2 activity following a 100-fold dilution, the final concentration of the inhibitor in the assay being 0.2 μM.

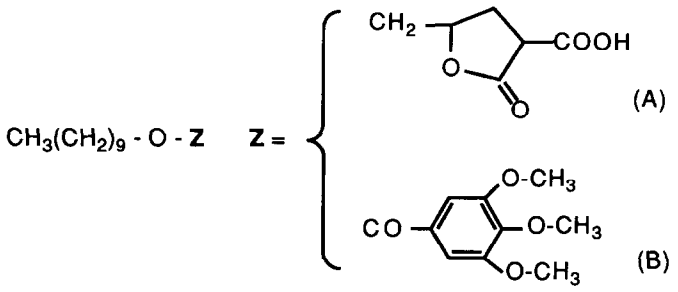
THIN-LAYER CHROMATOGRAPHY. TLC was performed after extraction of the reaction compounds from the assay medium, as described by Mounier *et al.* [23]. The fluorescent reaction product, 10-pyrenyldecanoic acid, as well as the fluorescent substrate, were identified by TLC under UV illumination. Hydrolysis with bovine pancreatic PLA_2 was performed in the presence or absence of BSA and/or inhibitor.

CARRAGEENAN-INDUCED RAT PAW OEDEMA. Four-week-old male Crj:CD(SD) rats were purchased from Charles River. Paw oedema was induced by a subcutaneous injection of 0.05 mL of saline containing 1% carrageenan into the right hind paw. The volume of the right hind paw was measured before and 1, 2, 3, 4, and 5 hours after carrageenan administration, using a plethysmometer (UNICOM, TK-101). Indomethacin was used as a positive control for the inhibition of the oedema, and was given by oral administration at 3 mg/kg one hour before the injection of carrageenan. Saline was used as a negative control with the same experimental procedure. Compound 2*e*, which shows the greatest ability to inhibit PLA_2 activity, was chosen to examine the anti-inflammatory effect of the 4-alkoxybenzamidines. This drug was given at 3 mg/kg by oral administration one hour before the injection of carrageenan. The oedema rate was expressed as percent of the increase in paw volume (mL) calculated by subtracting the basal volume (volume before the injection of carrageenan).

PHORBOL 12-MYRISTATE 13-ACETATE (PMA)-INDUCED INFLAMMATION. Five μg of PMA in 20 μL ethanol:water (8:2) was applied topically to the anterior and posterior surfaces of the right ear of mice 30 minutes following similar application of either the vehicle (ethanol used as negative control) or 10 mg of compound 2*e*. As positive anti-inflammatory drugs, dexamethasone and indomethacine were used at 3 mg. Ear swelling (representing the difference between the swelling in the presence of the drug and the swelling in the presence of the vehicle only) was then measured by a Dyer model micrometer gauge after 6 hours.

RESULTS AND DISCUSSION

In a preliminary investigation [29], several compounds with a similar alkyl chain of 10 carbons and various chemical groups were synthesized and examined for their ability to inhibit PLA_2 activity, such as compound (A) and compound (B) with the following formula:



With the exception of the 4-alkoxybenzamidines, the synthesized compounds did not show any inhibitory effect on either pancreatic PLA_2-I or rabbit platelet PLA_2-II using a fluorescent assay described in Materials and Methods to detect secretory PLA_2 (data not shown). For this reason, we have developed a series of 4-alkoxybenzamidines whose alkyl chain vary in length to examine the structure-activity relationships of these compounds.

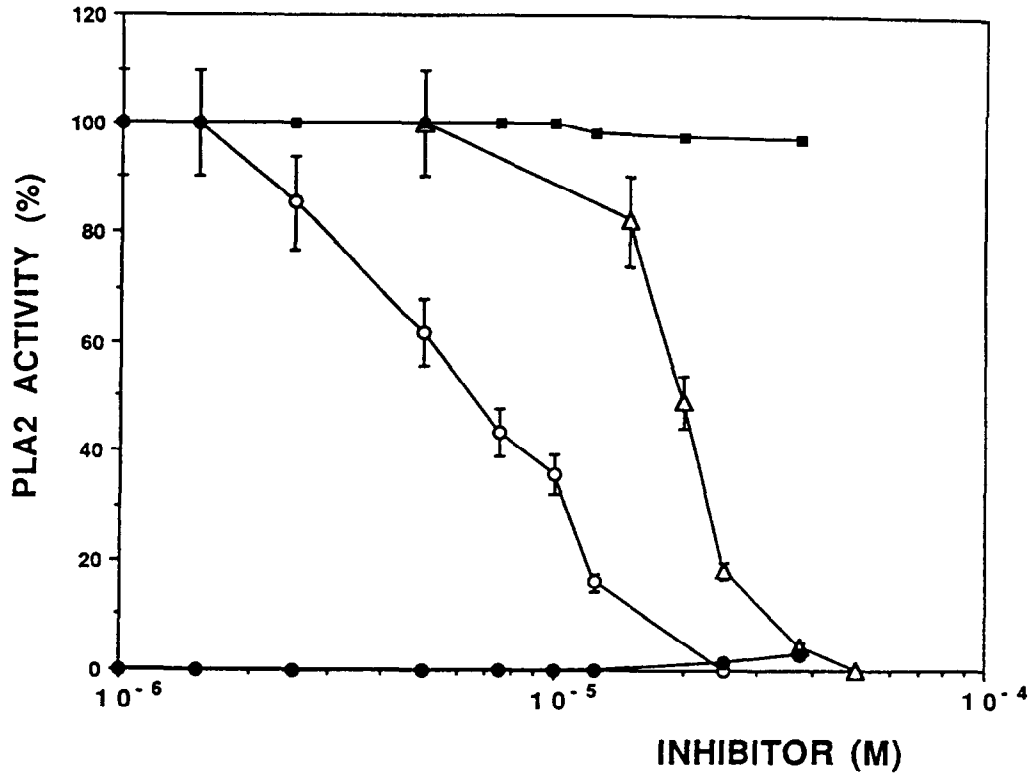


FIG. 1. Inhibition of extracellular PLA_2s by compound 2c. The conditions of the assay are described in Materials and Methods. PLA_2 activity is expressed as percentage of the control in the absence of inhibitor. Inhibition of the activity of bovine pancreatic PLA_2 (○) and the activity of rabbit platelet lysate (△) by compound 2c. The effect of inhibitor on basal fluorescence in the absence of enzyme and of $CaCl_2$ was measured at the indicated concentrations (●). Maximal fluorescence (■), obtained at the end of enzymatic reaction after addition of the inhibitor at the indicated concentration, is expressed as percent of the control in the absence of inhibitors. The experimental points represent the means of three experiments; the bars represent standard deviation.

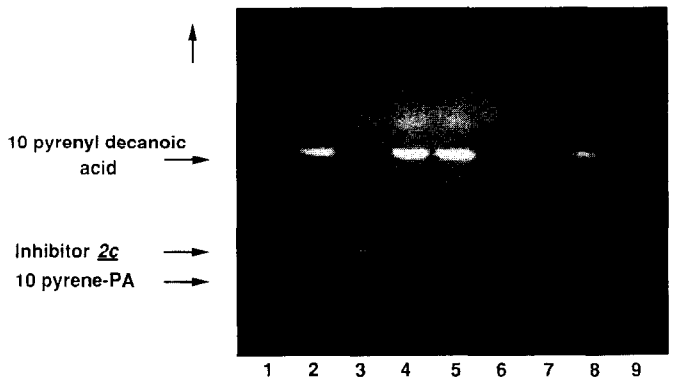


FIG. 2. Evaluation by TLC of the hydrolysis of the substrate by bovine pancreatic PLA_2. The experiment was performed as described in Materials and Methods. The picture of TLC plate was taken under ultraviolet light. 10-pyrene PA used as standard 2 μM in ethanol (column 1), or extracted from the reaction medium (column 2), PLA_2 assay in the presence of 0.1% BSA and compound 2c at 10 μM (column 3), 1 μM (column 4), 0.1 μM (column 5) or in the absence of BSA but in the presence of compound 2c at 10 μM (column 6), 1 μM (column 7), 0.1 μM (column 8), inhibitor at 10 μM (column 9).

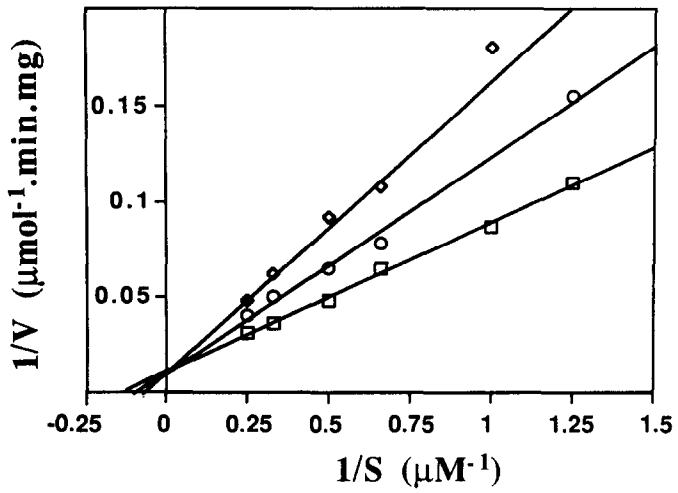


FIG. 3. Double reciprocal plot for the inhibition of bovine pancreatic PLA_2 activity by compound 2c. The reaction rate, expressed in mmoles/min/mg protein, was measured with concentrations of substrate varying from 0.4 to 4 μM in the absence (□) or in the presence of 3 μM (○) and 5 μM (◇) inhibitor. Values are the means of at least three determinations. SEM are ±10%.

TABLE 2. Effect of 4-alkoxybenzamidines on carrageenan-induced hind paw oedema in rats

Drug	Dose mg/kg	No. of animals	Swelling (%) 1 hr*	2 hr	3 hr	4 hr	5 hr
Control	0	10	17.6 ± 2.3†	30.6 ± 4.4	46.5 ± 2.9	56.6 ± 2.9	59.4 ± 2.6
Compound *2e*	3	10	8.4 ± 1.7§ (52)‡	13.5 ± 2.3§ (56)	20 ± 2.8§ (57)	27.4 ± 3.8§ (52)	34 ± 3.4§ (42)
Indomethacin	3	10	8.9 ± 1.2§ (49.5)	11.4 ± 1§ (63)	16.9 ± 1.7§ (64)	20.7 ± 2.5§ (63)	25.7 ± 2.6§ (57)

* Time after administration of carrageenan.
† Mean ± SEM.
‡ In parentheses, inhibition compared to control (%).
§ Significantly different from control group at $P < 0.01$.

Table 1 shows the IC_{50} values of the various 4-alkoxybenzamidines, characterizing their inhibitory effects on the enzymatic activity of pancreatic PLA_2 and rabbit platelet PLA_2. Compound *2a* was inactive in both cases, while all other compounds were active, IC_{50} values being similar for both enzymes. It is interesting that in this series, compounds *2d* and *2e* are more efficient inhibitors than compounds with shorter or longer alkyl chains. Figure 1 shows the profile of inhibition versus concentration for compound *2c*. Since the PLA_2 assay monitors the enhancement of fluorescence intensity upon binding of the fluorescent product of enzymatic reaction, 10-pyrenyl decanoic acid, to BSA [27], TLC experiments were performed to ensure that compound *2c* did not perturb the formation of the complex. Figure 2 shows that 20 μM of compound *2c* completely prevented hydrolysis of 10-pyrene PA by bovine pancreatic PLA_2 in the presence (column 3) as well as in the absence (column 6) of BSA. Moreover, TLC revealed that compound *2c* was more efficient in the absence of BSA (columns 3 to 5) than in its presence (columns 6 to 8). This was confirmed by PLA_2 assays with different concentrations of BSA: The IC_{50} of compound *2c* on bovine pancreatic PLA_2 increased from 7 μM with 10 μM BSA (standard conditions) to 20 μM in the presence of 50 μM BSA (data not shown). These results indicate that the IC_{50} value of the inhibitory effect of each compound would be lower in the absence of BSA than the value determined under standard PLA_2 assay conditions.

It was verified in the case of the most active compounds, *2c* to *2e*, that the inhibition was fully reversible. When bovine pancreatic PLA_2 was preincubated with the inhibitor and then diluted as described in Methods, only a slight inhibition was observed, indicating reversibility in less than 1 min. Michaelis-Menten kinetics have been used to describe the inhibitory effect of various molecules on PLA_2 activity and to characterize competitive and noncompetitive inhibitors [21–23, 30]. In our case, a double reciprocal plot (Fig. 3) shows that compound *2c*, taken as an example, behaves kinetically as a competitive inhibitor with a Ki of 5 μM.

The inhibitory effect of 4-alkoxybenzamidines clearly varies with the length of the alkyl chain. As shown in Table 1, maximal inhibition was obtained for 12 or 14 carbons. Inhibition efficiency decreases more rapidly for longer than for shorter chains, indicating that the lipophilicity of the compounds plays a critical role in their interaction with the active site. This result suggests that the active site of secreted mammalian PLA_2-I and PLA_2-II is a hydrophobic pocket able to bind an alkyl chain of 12 to 14 carbons in length. This observation is in good agreement with the report of Yu and Dennis [16], who established, for the venom PLA_2-I from *Naja naja naja*, that the effective size of the catalytic site is approximately 10 carbons in length with respect to the sn-2 acyl group. The slight difference observed may be due to the fact that the inhibitor used in our study was smaller than the thioether amide phospholipid analogues used by Yu and Dennis [16]. The closed inhibitory action of these compounds on both pancreatic PLA_2-I and platelet PLA_2-II has to be correlated with the very similar active site of these enzymes, involving in particular the amino acids His48 and Asp99.

The influence of compound *2e* (the more potent inhibitor of this series) has been investigated on acute responses where mammalian PLA_2-II is involved, such as in carrageenan-induced oedema in the rat paw [31, 32] and carrageenan-induced pleurisy in rats [3, 33]. Table 2 shows that the oral administration of compound *2e* strongly inhibited carrageenan-induced rat paw oedema during the early and late phases of the response, with the same efficiency as indomethacin, a well-known anti-inflammatory agent. Unlike indomethacin and dexamethasone, which both strongly reduced swelling with values of 5.8 ± 1.3 mm and 3 ± 0.6 mm, respectively, versus 11.2 ± 0.7 mm for the control, the compound *2e* was not able to reduce phorbol ester-induced inflammation of the ear in mice (swelling of 12.6 ± 1.3 mm), a model representative of a chronic inflammation in which the involvement of PLA_2-II has not been demonstrated. Taken together, these observations demonstrate an inhibitory action of compound *2e* on the acute inflammation model in which the involvement of the PLA_2-II has been established. It is therefore tempting to hypothesize that the inhibitory effect of compound *2e* on acute inflammation induced by carrageenan is due to its ability to inhibit *in vivo* the PLA_2-II, as observed *in vitro*. Compound 2e might therefore offer an additional drug to control acute inflammation in addition to indomethacin and glucocorticoids, which are characterized by a larger spectrum of activities.

In conclusion, 4-alkoxybenzamidines, and in particular compound *2e*, are good candidates for examining the physiological role of the mammalian PLA_2-II in inflammatory pro-

cesses. They act as competitive inhibitors against different PLA_2 *in vitro*, and exert a powerful inhibitory effect on acute inflammation in a model where PLA_2-II is involved. These compounds therefore appear to constitute interesting tools to examine the involvement of PLA_2-II on cellular processes such as arachidonic acid production by inflammatory cells and blood platelets and mast cell degranulation.

The financial assistance of ADIR & Compagnie (Courbevoie, France) is gratefully acknowledged.

References

1. Waite M, Approaches to the study of mammalian cellular phospholipases. *Journal of Lipid Research* **26:** 1379–1388, 1985.
2. Burgoyne RD and Morgan A, The control of free arachidonic levels. *TIBS* **15:** 365–366, 1990.
3. Tesson F, Ruffié C, Hidi R, Silva PMR, Vazeux G, Vargaftig BB and Bon C, Cell-associated and soluble phospholipases A_2 increase during carrageenan and zymosan-induced pleuresy in rat. *Eur J Pharmacol* **248:** 27–32, 1993.
4. Heinrickson RL, Krueger ET and Keim PS, Amino acid sequence of phospholipase A_2 from the venom of *Crotalus adamanteus*. A new classification of phospholipases A_2 based upon structural determinants. *J Biol Chem* **252:** 4913–4921, 1977.
5. Davidson FF and Dennis EA, Evolutionary relationships and implications for the regulation of phospholipase A_2 from snake venom to human secreted forms. *J Mol Evol* **31:** 228–238, 1990.
6. Seilhamer JJ, Pruzanski W, Vadas P, Plant S, Miller JA, Kloss J and Johnson LK, Cloning and recombinant expression of phospholipase A_2 present in rheumatoid arthritic synovial fluid. *J Biol Chem* **264:** 5335–5338, 1989.
7. Kramer RM, Hession C, Johansen B, Hayes G, McGray P, Chow EP, Tizard R and Pepinsky RB, Structure and properties of human and non-pancreatic phospholipase A_2. *J Biol Chem* **264:** 5768–5775, 1989.
8. Pruzanski W and Vadas P, Phospholipase-A_2—A mediator between proximal and distal effectors of inflammation. *Immunol Today* **12:** 143–146, 1991.
9. Pruzanski W, Vadas P and Browning J, Secretory non-pancreatic group II phospholipase A(2)—Role in physiologic and inflammatory processes. *J Lipid Mediators* **8:** 161–167, 1993.
10. Chang J, Marshall LM and Carlson RP, Proinflammatory effects of phospholipase A2 (PLA2) in several *in vitro* and *in vivo* systems. In: *Advances in Prostaglandin, Thromboxane, and Leukotriene Research* (Eds. Samuelsson B, Wong PYK and Sun FF), vol. 19, pp. 549–597. Raven Press, Ltd., New York, 1989.
11. Murakami M, Kudo I, Nakamura H, Yokoyama Y, Mori H and Inoue K, Exacerbation of rat adjuvant arthritis by intradermal injection of purified mammalian 14-kDa group-II phospholipase A2, *FEBS Lett* **268:** 113–116, 1990.
12. Bomalaski JS, Lawton P and Browning JL, Human extracellular recombinant phospholipase-A2 induces an inflammatory response in rabbit joints. *J Immunol* **146:** 3904–3910, 1991.
13. Cirino G, Cicala C and Sorrentino L, Human recombinant platelet phospholipase A2 exacerbates poly-L-arginine induced rat paw edema. *Inflammation* **18:** 59–66, 1994.
14. Chang J, Musser JH and McGregor H, Phospholipase A_2: Function and pharmacological regulation. *Biochem Pharmacol* **36:** 2429–2436, 1987.
15. Gelb MH, Jain MK and Berg OG, Inhibition of phospholipase A2. *FASEB* **8:** 916–924, 1994.
16. Yu L and Dennis EA, Defining the dimensions of the catalytic site of phospholipase A2 using amide substrate analogues. *J Am Chem Soc* **114:** 8757–8763, 1992.
17. Vandenberg L, Franken PA, Verheij HM, Dijkman R and De Haas GH, Competitive inhibition of lipolytic enzymes. VI. Inhibition of 2 human phospholipases-A2 by acylamino phospholipid analogues. *Biochim Biophys Acta* **1124:** 66–70, 1992.
18. Jacobson PB, Marschall LA, Sung A and Jacobs RS, Inactivation of human synovial fluid phospholipase A2 by the natural product, manoalide. *Biochem Pharmacol* **39:** 1557–1564, 1990.
19. Davidson FF, Lister MD and Dennis EA, Binding and inhibition studies on lipocortins using phosphatidylcholine vesicles and phospholipase A_2 from snake venom, pancreas, and a macrophage-like cell line. *J Biol Chem* **265:** 5602–5609, 1990.
20. Oinuma H, Takamura T, Hasegawa T, Nomoto K-I, Naito T, Daiku Y, Hamano S, Kakisawa H and Minami N, Synthesis and biological evaluation of substituted benzenesulfonamides as novel potent membrane-bound phospholipase A2 inhibitors. *J Med Chem* **34:** 2260–2267, 1991.
21. Köhler T, Heinisch M, Kirchner M, Peinhardt G, Hirschelmann R and Nuhn P, Phospholipase A2 inhibition by alkylbenzoylacrylic acids. *Biochem Pharmacol* **44:** 805–813, 1992.
22. Miyake A, Yamamoto H, Takebayashi Y, Imai H and Honda K, The novel natural product YM-26567-1 [(+)-trans-4-(3-dodecanoyl-2,4,6-trihydroxyphenyl)-7-hydroxy-2-(4-hydroxyphenyl)chroman]: A competitive inhibitor of group II phospholipase A2. *J Pharmacol Exp Ther* **263:** 1302–1307, 1992.
23. Mounier C, Hatmi M, Faili A, Bon C and Vargaftig BB, Competitive inhibition of phospholipase A2 activity by the platelet-activating factor antagonist Ro 19-3704 and evidence for a novel suppressive effect on platelet activation. *J Pharmacol Exp Ther* **264:** 1460–1467, 1993.
24. Garigipati RS, An efficient conversion of nitriles to amidines. *Tetrahedron Lett* **31:** 1969–1972, 1990.
25. Levin JI, Tures E and Weinreb SM, An alternative procedure for the aluminium-mediated conversion of esters to amides. *Synth Comm* **12:** 989–993, 1982.
26. Schubert H and Zascke H, *J Prakt Chem* **312:** 494–506, 1970.
27. Radvanyi F, Jordan L, Russo-Marie F and Bon C, A sensitive and continuous fluorometric assay for phospholipase A_2 using pyrene-labeled phospholipids in the presence of serum albumin. *Analytic Biochem* **177:** 103–109, 1989.
28. Mounier C, Faili A, Vargaftig BB, Bon C and Hatmi M, Secretory phospholipase A2 is not required for arachidonic acid liberation during platelet activation. *Eur J Biochem* **216:** 169–175, 1993.
29. Aitdafoun M, Phospholipase A2 inhibitors: synthesis, *in vitro* pharmacological study and structure-activity correlation. Unpublished Ph.D. thesis, University Paris 7 Denis Diderot, 1993.
30. Tanaka K, Matsutani S, Matsumota K and Yoshida T, A novel type of phospholipase A2 inhibitor, thielocin A1β, and mechanism of action. *J Antibiotics* **45:** 1071–1078, 1992.
31. Di Rosa M, Giroud JP and Willoughby DA, Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenin and turpentine. *J Pathol* **104:** 15–29, 1971.
32. Cirino G, Peers SH, Flower RJ and Pepinsky RB, Human recombinant lipocortin 1 has acute local anti-inflammatory properties in the rat paw oedema test. *Proc Natl Acad Sci USA* **86:** 3428–3432, 1989.
33. Kakutani M, Murakami M, Okamoto H, Kudo I and Inoue K, Role of type II phospholipase A2 in the inflammatory process of carrageenan-induced pleuresy in rats. *FEBS Lett* **339:** 76–78, 1994.