Novel 2-Oxoamide Inhibitors of Human Group IVA Phospholipase A_2

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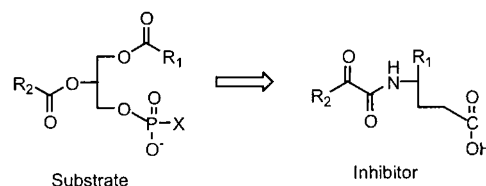
Abstract: A novel class of potent human cytosolic phospholipase A_2 (GIVA PLA_2) inhibitors was developed. These inhibitors were designed to contain the 2-oxoamide functionality and a free carboxyl group. Among the compounds tested, a long-chain 2-oxoamide containing L-γ-norleucine was the most potent inhibitor, causing a 50% decrease in GIVA PLA_2 activity at 0.009 mole fraction.

Introduction. The phospholipases A_2 (PLA_2) superfamily consists of a broad range of enzymes defined by their ability to catalyze the hydrolysis of the ester bond at the sn-2 position of phospholipids.[1] Among the various PLA_2's, human cytosolic PLA_2, which has been referred to as $cPLA_2$ and now is known as Group IVA PLA_2 (GIVA PLA_2),[1] plays a central role in lipid mediator biosynthesis. Mice made deficient in GIVA PLA_2 showed a marked decrease in eicosanoid biosynthesis, allergic symptoms, and postischemic brain injury.[2] GIVA PLA_2 is essential for proinflammatory prostaglandin, leukotriene, and PAF production, and therefore, its inhibitors are very attractive targets as agents for treating inflammatory and other diseases.

Arachidonyl trifluoromethyl ketone (AATFK), an arachidonic acid analogue in which the −COOH group is replaced by $-COCF_3$, has been shown to be a slow, tight-binding, reversible inhibitor of GIVA PLA_2.[3] Another fatty acid analogue, methyl arachidonyl fluorophosphonate, has been reported to irreversibly inactivate Group IVA PLA_2.[4] The inhibition of GIVA PLA_2, in comparison with GVI PLA_2, by fatty acid tricarbonyls[5] and trifluoromethyl ketones[6] has been studied. Recently, pyrrolidine-based inhibitors of GIVA PLA_2 were reported,[7] which block arachidonic acid release in a variety of mammalian cells.[8]

The catalytic mechanism of GIVA PLA_2 proceeds through a serine−acyl intermediate using Ser-228 as the nucleophilic residue.[4b,9] The X-ray crystal structure of GIVA PLA_2 has recently been solved,[10] indicating that the enzyme consists of an N-terminal calcium-dependent lipid-binding/C2 domain and a catalytic unit whose topology is distinct from that of other lipases. The enzyme possesses an unusual catalytic Ser−Asp dyad located in a deep cleft at the center of a hydrophobic funnel.

Scheme 1



Many synthetic inhibitors of serine proteases contain an activated carbonyl group, for example, fluorinated ketones[11] and α-ketoamides,[12] replacing the scissile amide bond. The mechanism of action of these electrophilic inhibitors most likely involves a nucleophilic addition of the active site enzymatic serine hydroxy group to the carbonyl group of the inhibitor, with formation of a metastable hemiacetal adduct that mimics the tetrahedral species involved in the enzymatic cleavage of peptide bonds. A number of lipophilic 2-oxoamides[13] and 2-oxoamide and bis-2-oxoamide triacylglycerol analogues[14] have been synthesized and shown to inhibit pancreatic and gastric lipases, enzymes containing a classical catalytic triad (Ser−His−Asp).

The aim of this work was to develop a novel class of GIVA PLA_2 inhibitors. These novel inhibitors were designed taking into consideration the chemical structure of phospholipids, which are the natural substrates of phospholipases (Scheme 1). The 2-oxoamide functionality was chosen as the group to replace the scissile ester bond of the natural substrate. To mimic the negative charge of the substrate phosphate moiety, a carboxyl group was analogously incorporated into the 2-oxoamide derivative.

Chemistry. A number of simple primary and secondary 2-oxoamides (Table 1, entries 1−4) as well as four derivatives containing a free carboxyl group (Table 1, entries 5−8) were synthesized.[15] The synthesis of compound **6** (entry 7), as a representative example, is depicted in Scheme 2.

Boc-protected amino alcohol **1**,[16] prepared from Boc-L-norleucine as previously described,[17] was oxidized to the corresponding aldehyde by NaOCl in the presence of a catalytic amount of 4-acetamido-2,2,6,6-tetramethylpiperidine-1-yloxy free radical (AcNH-TEMPO),[18] and the aldehyde was directly used in the next step without any purification. Treatment of the aldehyde with benzyl (triphenylphosphoranylidene)acetate in THF under reflux led to α,β-unsaturated ester **2**. The Boc group was removed, and the amino component **3** was coupled with 2-hydroxydodecanoic acid using 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide[19] as a condensing agent in the presence of hydroxybenzotriazole. Catalytic hydrogenation of the double bond of **4** with simultaneous removal of the benzyl group led to compound **5**, which was oxidized by PDC to the target compound **6**. Since amino aldehydes are sensitive to racemization, the enantiomeric purity of the α,β-unsaturated γ-amino

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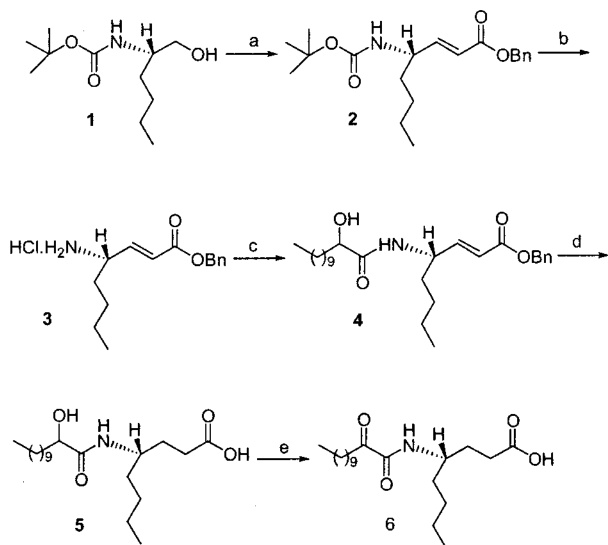
Table 1. Inhibitory Activity of 2-Oxoamides against GIVA PLA_2

Entry	Structure	$X_I(50)$
1		inactive[a]
2		22 ± 7 % inhibition[b]
3		inactive[a]
4		inactive[a]
5		inactive[c]
6		0.017 ± 0.009
7		0.009 ± 0.004
8		0.068 ± 0.005

[a] 0.01 mole fraction. [b] 0.02 mole fraction. [c] 0.05 mole fraction.

Scheme 2[a]



[a] Conditions: (a) [i] AcNH-TEMPO, NaBr, NaOCl, $NaHCO_3$, AcOEt/$PhCH_3$/H_2O, −10 °C; [ii] $Ph_3P{=}CHCOOBn$, THF, reflux; (b) 4 N HCl/Et_2O; (c) $CH_3(CH_2)_9CH(OH)COOH$, WSCI, HOBt, Et_3N, CH_2Cl_2; (d) H_2, 10% Pd/C, EtOH; (e) PDC, CH_3COOH.

ester **3** was checked by NMR analysis of the corresponding (*S*)-(−)- and (*R*)-(+) Mosher amides.[20] The α-H was well-resolved, and the absence of any diastereomeric proton signal in the spectrum of each MTP amide indicated that the chiral center configuration was retained.

Results and Discussion. All compounds were tested for their ability to inhibit GIVA PLA_2 in a GIVA PLA_2 specific assay that utilizes mixed micelles of substrate, 1-palmitoyl-2-arachidonoyl phosphatidylcholine (PAPC), phosphatidylinositol 4,5-bisphosphate (PIP_2), and detergent Triton X-100 (97:3:400 μM).[21,22] Inhibitors of lipolytic enzymes are best reported in terms of mole fraction of inhibitor in the interface (number of moles of inhibitor to the total number of moles of lipid, detergent, and inhibitor in the micelle).[6,23] This is because inhibitors of these enzymes partition into the membrane or micelle surface. Thus, the surface concentration in mole fraction, not the bulk concentration in molarity, best reflects how much inhibitor the enzymes see. An initial screen was carried out at 0.05 mole fraction inhibitor (25 μM) except where low solubility necessitated using 0.01 or 0.02 mole fraction, as noted in Table 1. Entries 6−8 showed inhibition in the initial screen, so their relative inhibitory strengths were determined by varying the mole fraction of the inhibitor in the assay. The mole fractions of each inhibitor required to produce 50% inhibition, designated as $X_I(50)$, are summarized in Table 1. As an example, an $X_I(50)$ of 0.01 mole fraction for an inhibitor is the same at both 0.1 mM total lipid surface and 1 mM total lipid surface. At the same two concentrations, the IC_{50} values change from 1 to 10 μM, a misleading 10-fold difference. Thus, $X_I(50)$ is correctly used over IC_{50} for enzymes acting at the lipid/water interface of membranes and micelles.

AATFK gave an $X_I(50)$ of 0.036 mole fraction for GIVA PLA_2 in an assay similar to the one described here.[6] The $X_I(50)$ was enhanced 8-fold with a 4 h preincubation.[6] Given this possible mode of slow-binding inhibition, we tested the effect of preincubating GIVA PLA_2 with the compounds for up to 30 min. The inhibition levels were the same with or without preincubation (not shown). To ensure that the inhibitors were directly interacting with GIVA PLA_2 and not simply interfering with the high-affinity GIVA PLA_2 ligand, PIP_2, select assays were also performed without PIP_2 as described previously.[22] The same trend of inhibition was seen without PIP_2 (not shown).

The primary 2-oxoamide (entry 1) was inactive. Among aliphatic secondary 2-oxoamides (entries 2−4), only one (entry 2) presented a very weak inhibitory effect. The comparison of the inhibition data obtained for 2-oxoamides containing a γ-amino acid residue (entries 5−8) led to interesting structure−activity results. The γ-aminobutyric acid derivative (entry 6) inhibited GIVA PLA_2 with an $X_I(50)$ of 0.017 mole fraction. A long chain at the 2-oxo acid moiety is essential for the inhibition (entries 5, 6), and the presence of a carboxyl group is also necessary (entries 6, 2). Compound **6** (entry 7) was the most potent inhibitor in this study, causing a 50% decrease in GIVA PLA_2 activity at 0.009 mole fraction. The chirality of the γ-amino acid moiety influences the inhibition. The (*S*) enantiomer (entry 7) is 2-fold more potent than the nonchiral derivative (entry 6) and 8-fold more potent than the (*R*) enantiomer (entry 8). Compound **6** (entry 7) presents an inhibitory effect of the same order of magnitude with a recently reported pyrrolidine-based inhibitor that exhibits an $X_I(50)$ value of 0.002 mole fraction.[8]

In conclusion, 2-oxoamides containing a γ-amino acid residue constitute a novel class of potent GIVA PLA_2 inhibitors. The optimization of the structural elements

of such inhibitors (work in progress) could potentiate the inhibition by increasing the protein/inhibitor interactions.

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Supporting Information Available: Details of experimental procedures for the synthesis of compounds, activity assays, and analytical characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

Note Added after ASAP Posting. This manuscript was released ASAP on 6/7/2002 with minor errors in the capitalization of Group IVA PLA_2 and in the titles of refs 5 and 6. The correct version was posted on 6/27/02.

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