# Broad Platform for Development of Selective, Phospholipase $A_2$ Inhibitors for Neurological Diseases

**Background:** The past two decades has resulted in a marked increase in our knowledge about phospholipase $A_2$ ($PLA_2$) enzymes. This enzyme superfamily hydrolyzes fatty acids from membrane phospholipids, and when released the by-products arachidonic acid and lysophospholipids can be metabolized to form various bioactive lipid mediators. Phospholipase $A_2$ enzymes have been classified into four main types: secreted $sPLA_2$s, cytosolic $cPLA_2$s, calcium-independent $iPLA_2$s, and lipoprotein-associated $LpPLA_2$s, also known as platelet activating factor acetylhydrolase.

Each of the four types of $PLA_2$ enzymes has links to diverse kinds of lipid metabolism and disease progression, justifying interest from the pharmaceutical and biotechnology industry in developing selective inhibitors to specific types. Various diseases of the central nervous system are characterized by induction of inflammatory events, which involve formation of prostaglandins. Production of prostaglandins is regulated by the activity of phospholipases $A_2$. In addition to inflammation, the downstream metabolic products of $PLA_2$ activity have also been linked to demyelination, a hallmark of diseases such as multiple sclerosis and spinal cord injuries.

**Technology Description:** Researchers at UC San Diego and their collaborators have developed a portfolio of issued and pending patents disclosing the compositions and methods for selectively inhibiting members of the s-, i-, and c-$PLA_2$ enzymes associated with specific diseases, with a focus on targeting drugs against many different neurological illnesses. The group of collaborators has developed novel amide, oxoamide, and fluoroketone compounds for treating hyperalgesia, multiple sclerosis, and spinal cord injuries.

A decade of experimentation has permitted the team of inventors to develop a suite of potent inhibitors of $PLA_2$ that exhibit a high degree of differential specificity for the secreted ($sPLA_2$), cytosolic ($cPLA_2$), and calcium-independent ($iPLA_2$) types of $PLA_2$ (*Refs. 1, 10*). Their experimental data indicate that sPLA2 inhibitors markedly improves functional recovery, enhance tissue protection and axon regeneration in the mouse spinal cord contusion injury model, while cPLA2 and iPLA2 inhibitors reduce the severity of experimental autoimmune encephalomyelitis (EAE) in mice, a widely used animal model of MS.

> **Cytosolic $PLA_2$ (cPLA2)**: Group IVA $PLA_2$ enzymes are characterized by a requirement for $Ca^{2+}$ for activation, this is the only $PLA_2$ with a preference for arachidonic acid in the *sn*-2 position of phospholipids. Arachidonic acid is the precursor for the generation of eicosanoids, and this enzyme is now generally considered to be a central enzyme in mediating many inflammatory processes. Recognition of the importance of the $cPLA_2$ in inflammatory diseases, as well as important structural discoveries has made it a very attractive drug target, and

many different laboratories have attempted to develop inhibitors. Focusing on cytostolic phospholipase (cPLA2) the inventors have developed a composition of oxoamides leading to selective inhibition of cPLA2 *in vitro* and *in vivo*. Novel cPLA2 inhibitors represent a potential therapy for the treatment of multiple sclerosis (MS). *Details of these technologies are described in references 1,3,7-9,11,12,14*

**$Ca^{2+}$ Independent $PLA_2$ (iPLA2)**: Group VI-A $PLA_2$ enzymes are characterized by no requirement for $Ca^{2+}$ for catalytic activity, calcium-independent phospholipase A2 (iPLA2) plays a variety of roles across cell and tissue types. They are known to influence the regulation of many cellular activities, including cell proliferation, apoptosis, bone formation, sperm development, and glucose-induced insulin secretion. The inventors have developed the use of a dipeptide or pseudodipeptide coupled to an oxoamide to specifically target iPLA2 (*Refs: 2, 6,12,14*). These inhibitors represent a potential therapy for the treatment of MS.

**Stage of Development.** *In vitro and in vivo* experiments validate a suite of potent inhibitors of $PLA_2$, with differential specificity for the $sPLA_2$, $cPLA_2$, and $iPLA_2$. Specifically, $sPLA_2$ inhibitors markedly improve functional recovery and enhance tissue protection and axon regeneration in the mouse spinal-cord contusion injury model, while $cPLA_2$ and $iPLA_2$ inhibitors reduce the severity of disease in a widely used animal model of MS (experimental autoimmune encephalomyelitis, or EAE) and iPLA2 inhibitors protect in rat models of hyperalgesia.

**Intellectual Property**

**United States issued Patent 5,464,754:** ASSAY AND SUBSTRATE FOR ARACHIDONOYL-SPECIFIC PHOSPHOLIPASE A2 (issued 07-Nov-1995)

A non-radioactive, spectrophotometric, microtiter plate assay for human cytosolic phospholipase $A_2$ ($cPLA_2$) is described. The assay utilizes a novel synthetic thiol-phospholipid analog as a substrate. In one embodiment, the substrate is a phosphatidylcholine derivative with an arachidonoylthioester in the sn-2 position and an alkenyl-ether or alkenyl-ether in the sn-1 position. The alkyl-ether and the alkenyl-ether in the sn-1 position of the substrate ensures that the assay will only measure $cPLA_2$ activity and will not be complicated by metabolism of the lysophospholipid product by the enzyme's lysophospholipase activity.

**U.S. Patent Application 20050148549:** COMPOSITIONS AND METHODS FOR INHIBITION OF PHOSPHOLIPASE A2 MEDIATED INFLAMMATION (published 07-July-2005)

Specific, highly potent 2-oxo-amide based inhibitors of phospholipase A.sub.2 (PLA.sub.2) activity are provided. A role for PLA.sub.2 activity in spinally mediated

inflammatory processes is established, and a method for treating hyperalgesia and other inflammatory conditions associated with PLA.sub.2 activity is provided.

**International Patent Application WO/2007/022443:** SYSTEMIC AND INTRATHECAL EFFECTS OF A NOVEL SERIES OF PHOSPHOLIPASE A2 INHIBITORS ON HYPERALGESIA AND SPINAL PGE2 RELEASE (published 22-Feb-2007)

Phospholipase $A_2$ ($PLA_2$) forins are expressed in spinal cord whose inhibition induces a potent antihyperalgesia. $PLA_2$ inhibitor compounds are provided that include a common motif consisting of a 2-oxoamide with a hydrocarbon tail and a four carbon tether. The compounds block Group IVA calcium dependent $PLA_2$ ($cPLA_2$) and/or Group VIA calcium independent $PLA_2$ ($iPLA_2$) and/or Group V secreted $PLA_2$ ($sPLA_2$).

**International Patent Application WO/2003/076389:** COMPOSITIONS AND METHODS FOR INHIBITION OF PHOSPHOLIPASE A2 MEDIATED INFLAMMATION (published 18-Sep-2003)

Specific, highly potent 2-oxo-amide based inhibitors of phospholipase $A_2$ ($PLA_2$) activity are provided. A role for $PLA_2$ activity in spinally mediated inflammatory processes is established, and a method for treating hyperalgesia and other inflammatory conditions associated with $PLA_2$ activity is provided.

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**Key Words**: phospholipase $A_2$, $PLA_2$, inhibitor, inflammation, demyelination, oxoamides, amides, fluroketones, cytosolic, calcium-independent, secretory, lipoprotein-associated, PAF acetyl hydrolase, inhibitor, inhibition, inflammation, demyelination, multiple sclerosis, spinal cord injury, hyperalgesia, neurogenic disease, amides, dipeptides, oxoamides, pseudodipeptides, fluroketones, prostaglandins, eicosanoids, pain

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