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Synthetic and Natural Inhibitors of Phospholipases A_2: Their Importance for Understanding and Treatment of Neurological Disorders

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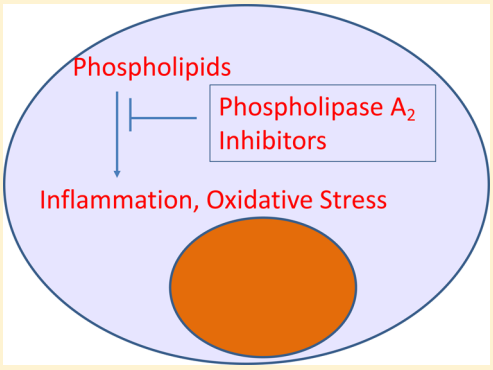
ABSTRACT: Phospholipases A_2 (PLA_2) are a diverse group of enzymes that hydrolyze membrane phospholipids into arachidonic acid and lysophospholipids. Arachidonic acid is metabolized to eicosanoids (prostaglandins, leukotrienes, thromboxanes), and lysophospholipids are converted to platelet-activating factors. These lipid mediators play critical roles in the initiation, maintenance, and modulation of neuroinflammation and oxidative stress. Neurological disorders including excitotoxicity; traumatic nerve and brain injury; cerebral ischemia; Alzheimer's disease; Parkinson's disease; multiple sclerosis; experimental allergic encephalitis; pain; depression; bipolar disorder; schizophrenia; and autism are characterized by oxidative stress, inflammatory reactions, alterations in phospholipid metabolism, accumulation of lipid peroxides, and increased activities of brain phospholipase A_2 isoforms. Several old and new synthetic inhibitors of PLA_2, including fatty acid trifluoromethyl ketones; methyl arachidonyl fluorophosphonate; bromoenol lactone; indole-based inhibitors; pyrrolidine-based inhibitors; amide inhibitors, 2-oxoamides; 1,3-disubstituted propan-2-ones and polyfluoroalkyl ketones as well as phytochemical based PLA_2 inhibitors including curcumin, *Ginkgo biloba* and *Centella asiatica* extracts have been discovered and used for the treatment of neurological disorders in cell culture and animal model systems. The purpose of this review is to summarize information on selective and potent synthetic inhibitors of PLA_2 as well as several PLA_2 inhibitors from plants, for treatment of oxidative stress and neuroinflammation associated with the pathogenesis of neurological disorders.



KEYWORDS: *Phospholipase A_2 inhibitors, eicosanoids, neuroinflammation, oxidative stress, neurological disorders, lipid mediators, lipids, neurodegeneration*

1. INTRODUCTION

1.1. Phospholipases A_2. Phospholipases A_2 (PLA_2) are a class of enzymes that hydrolyze the ester bond at the *sn*-2 position of glycerophospholipids to yield a free fatty acid and a lysophospholipid. The fatty acids that are produced by PLA_2 activity may undergo reacylation to glycerophospholipids, or may be further metabolized to bioactive products. Under physiological conditions, PLA_2 helps to maintain membrane structure and function, by removing oxidized and damaged phospholipids. Under pathological conditions, however, there is increased activity of PLA_2 which lead to increased generation of fatty acids and lysophospholipids, which in turn can be metabolized to second messengers and metabolites that contribute to neuroinflammation and propagation of neuronal injury. For instance, arachidonic acid (AA) is metabolized by cyclooxygenase (COX) and lipoxygenase (LO) into prostaglandins, leukotrienes, thromboxanes, and lipoxins.[1] These mediators are collectively known as eicosanoids and produce a wide range of biological actions. They include potent effects on inflammation, vasodilatation, vasoconstriction, apoptosis, and immune responses through interactions with eicosanoid receptors.[2] The nonenzymatic oxidation of AA results in the generation of 4 hydroxynonenal, isoprostanes, isoketals and isofurans.[2] These lipid mediators are important biomarkers for lipid peroxidation. The other product of the PLA_2 catalyzed reaction is a lysophospholipid. These are either reacylated into native phospholipids or acetylated to another bioactive mediator known as platelet activating factor (PAF), which also contributes to neuroinflammation.

Some PLA_2s release docosahexaenoic acid (DHA), which is further metabolized by 15-lipoxygenase into docosanoids (resolvins and neuroprotectins, and marsins) that have anti-inflammatory, antioxidant, antiapoptotic and neuroprotective properties[3] (Figure 1). In the brain, DHA modulates and facilitates neurotransmission through its effect on dopaminergic, noradrenergic, glutamatergic and serotonergic neurotransmitter receptors, and insulin, retinoid, and TGF-β receptors.[4] Detailed

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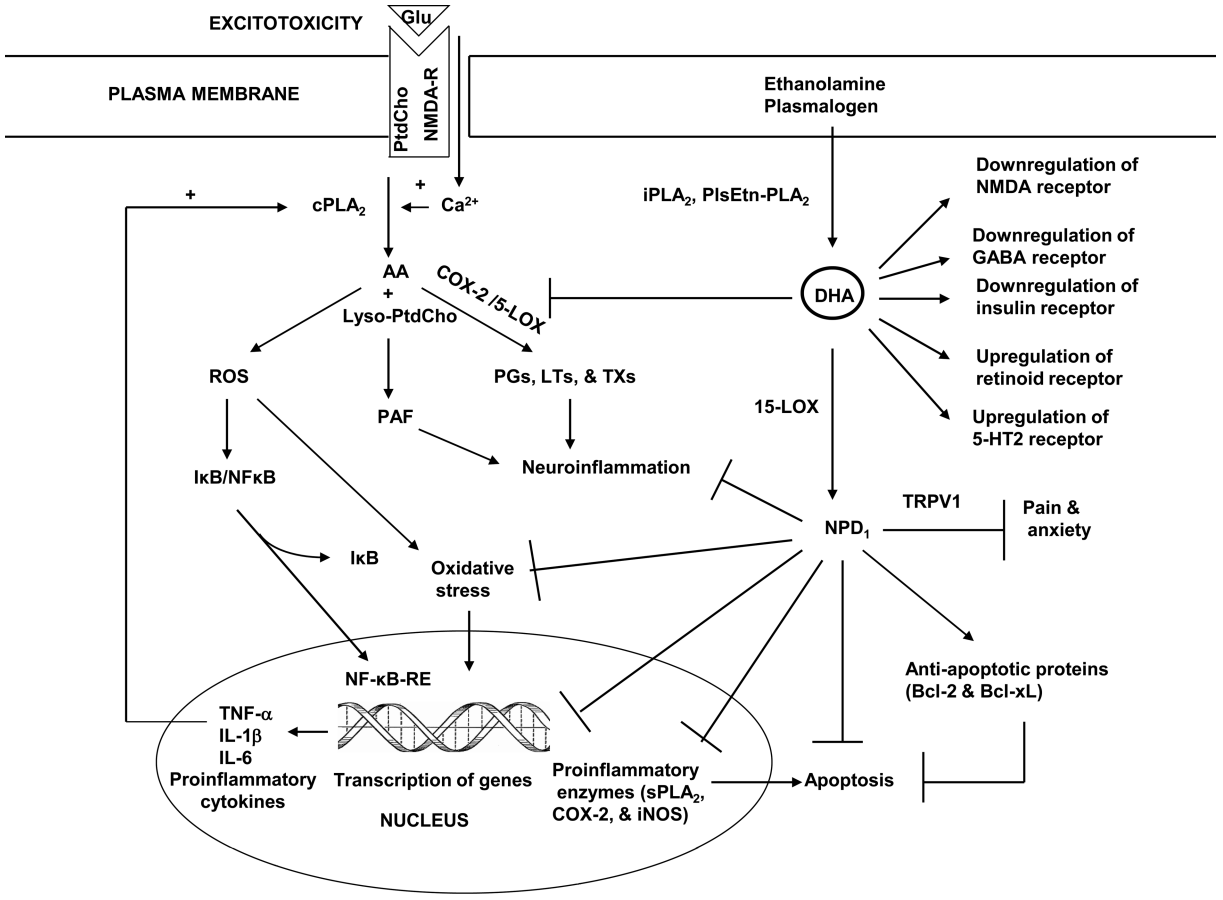

Figure 1. Interactions between AA- and DHA-derived metabolites and modulation of oxidative stress and neuroinflammation by docosahexaenoic acid (DHA) and neuroprotectin D1 (NPD1). *N*-Methyl-D-aspartate receptor (NMDA-R); glutamate (Glu); phosphatidylcholine (PtdCho); lyso-phosphatidylcholine (lyso-PtdCho); cytosolic phospholipase A_2 ($cPLA_2$); arachidonic acid (AA); prostaglandins (PGs); leukotrienes (LTs); thromboxanes (TXs); platelet activating factor (PAF); cyclooxygenase2 (COX-2); 5-lipoxygenase (5-LOX); 15-lipoxygenase (15-LOX); plasmalogen (PlsEtn); calcium independent phospholipase A2 ($iPLA_2$); plasmalogen-selective phospholipase A_2 (PlsEtn-PLA2); secretory phospholipase A_2 ($sPLA_2$); inducible nitric oxide synthase (iNOS); reactive oxygen species (ROS); tumor necrosis factor-alpha (TNF-α); interleukin 1beta (IL-1β); interleukin-6 (IL-6); and nuclear factor-κB (NF-κB).

investigations have been performed on neuroprotectin D1 (NPD1) in retinal pigment epithelial cells and brain.[5−9] NPD1 upregulates antiapoptotic proteins (Bcl-2 and Bcl-xL) but downregulates proapoptotic proteins (Bax and Bad) in response to cellular oxidative stress and cytokine activation, leading to an overall pro-survival transcriptome.[5,7−9]

1.2. Classification of Brain PLA_2s. Recent advances in molecular and cellular biology have led to the identification of 17 genes and more than 25 mammalian intracellular PLA_2 paralogs/splice variants/isozymes in the mammalian brain. PLA_2s can be classified into five main types on the basis of their specific features such as sequence, molecular weight, disulfide bonding patterns, and Ca^{2+} dependency. They include isoforms of cytosolic PLA_2 ($cPLA_2$), calcium independent PLA_2 ($iPLA_2$), secretory PLA_2 ($sPLA_2$), plasmalogen selective PLA_2 (PlsEtn-PLA_2), the platelet activating factor-acetylhydrolases (PAF-AH), and lipoprotein-PLA_2 (Lp-PLA_2, 45 kDa).[10,11] $cPLA_2$ requires 10 to 1000 nM calcium for binding to a phospholipid substrate. Various isozymes of $cPLA_2$ preferentially hydrolyze AA and are activated by phosphorylation on Ser-505, Ser-727, and Ser-515 mediated by MAPK, MEK1, and calcium- and calmodulin-dependent kinase II, respectively.[12] $cPLA_2$ is expressed at relatively high levels in the hypothalamus, brainstem, cerebellum, and spinal cord of normal rats.[13] Variant isozymes of $cPLA_2$ ($cPLA_2\alpha$, $cPLA_2\beta$, $cPLA_2\gamma$, $cPLA_2\delta$, $cPLA_2\varepsilon$, and $cPLA_2\zeta$.) have molecular mass of 85−110 kDa and are involved in signal transduction processes. $cPLA_2\alpha$ has been mapped to chromosome 1, $cPLA_2\beta$ to chromosome 15, and $cPLA_2\gamma$ to chromosome 19. Amino acid sequencing indicates that $cPLA_2\beta$ and $cPLA_2\delta$ have 120 and 135 amino acid inserts, respectively, between the C2 domain and catalytic domain A, whereas in $cPLA_2\alpha$ the two domains are adjacent to each other. In addition, $cPLA_2\beta$ has a unique N-terminal region composed of 242 amino acids, which is not required for enzymatic activity.[14] Most $cPLA_2$ isozymes prefer AA over other fatty acids and do not use Ca^{2+} for catalysis, although submicromolar Ca^{2+} concentrations are needed for membrane binding. $cPLA_2$ isoform (particularly $cPLA_2\alpha$) activity is regulated by a number of protein kinases, which phosphorylate $cPLA_2\alpha$ on different serine residues. For example, Ser 505 is phosphorylated by extracellular signal-regulated kinases (ERK1/2) and p38 mitogen-activated protein kinase (MAPK), Ser 515 by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), and Ser 727 by MAPK-interacting kinase (MNK1) or a closely related isoform.[15,16]

$iPLA_2$s are active without calcium and preferentially hydrolyze docosahexaenoic acid (DHA) from neural membrane phospholipids. They have a molecular mass of 80 kDa. These enzymes are involved in not only signal transduction processes but also

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ACS Chem. Neurosci. XXXX, XXX, XXX−XXX

phospholipid remodeling. Besides PLA_2 activity, isoforms of $iPLA_2$ exhibit lysophospholipase and transacylase activities.[17] $iPLA_2\alpha$, $iPLA_2\beta$, and $iPLA_2\gamma$ release fatty acids, while other $iPLA_2$ isoforms δ, ε, ξ, and η display triglyceride lipase and transacylase activities.[18] In addition, $iPLA_2\beta$ expresses an acyl-CoA thioesterase activity.[19] Members of the $iPLA_2$ family share homology with patatin, a lipid hydrolase with an unusual folding topology that differs from classical lipases. $iPLA_2$ is stabilized by ATP,[20] and the $iPLA_2\beta$ sequence contains a consensus nucleotide binding motif (GXGXXG) which is homologous to the nucleotide binding motif of protein kinases.[21] $iPLA_2\gamma$ plays an important role in regulation of glutamate receptor functions, long-term potentiation and depression, neuronal plasticity, and neurodegenerative processes.[22−24]

$sPLA_2$ isoforms have molecular mass (14−18 kDa) and are mainly associated with synaptosomes and the synaptic vesicle fraction.[25] They require millimolar concentrations of calcium and have no substrate specificity for fatty acids. Multiple forms of $sPLA_2$ exist, including $sPLA_2$-IB, $sPLA_2$-IIA, $sPLA_2$-IIC, $sPLA_2$-IID, $sPLA_2$-IIE, $sPLA_2$-IIF, $sPLA_2$-III, $sPLA_2$-V, $sPLA_2$-X, $sPLA_2$-XIIA, and $sPLA_2$-XIIB. $sPLA_2$ isoforms have 5−8 disulfide bonds. Phospholipid hydrolysis proceeds with the interaction of a water molecule through hydrogen bonding to the active site histidine residue. Adjacent to the histidine is a conserved aspartate residue at the catalytic dyad, which together with the calcium-binding loop, acts as a ligand cage for calcium. Isoforms of $sPLA_2$ are present in all regions of the mammalian brain. The highest activities of $sPLA_2$ are found in medulla oblongata, pons, and hippocampus, moderate activities in the hypothalamus, thalamus, and cerebral cortex, and low activities in the cerebellum and olfactory bulb.[26]

PlsEtn-PLA_2 hydrolyzes DHA from plasmalogen, a unique class of neural membrane phospholipids that has a long chain vinyl ether linkage at the *sn*-1 position of the glycerol moiety. This enzyme has a molecular mass of 39 kDa and does not require calcium for its activity.[27−29] No isoforms of PlsEtn-PLA_2 have been reported in the brain, and the enzyme is inhibited by bromoenol lactone. C2 ceramide stimulates PlsEtn-PLA_2 activity in a dose-dependent manner. However, at higher concentration, PlsEtn-PLA_2 activity is inhibited. Like ceramide, ceramide 1-phosphate stimulates PlsEtn-PLA_2 activity in a dose-dependent manner, and dihydroceramide, the inactive analog of C2 ceramide, has no effect on PlsEtn-PLA_2 activity.[29]

PAF acetyl hydrolase (PAF-AH, 26−45 kDa) selectively hydrolyzes short acyl chains (C2 to C9) at the *sn*-2 position of (PtdCho) or PtdEtn. This enzyme shows no activity with acyl chains longer than C9, but an unusual *sn*-2 acyl group containing a carbonyl group at the ω-end of the acyl chain acts as the substrate for this enzyme. Unlike $cPLA_2$ and $sPLA_2$, PAF-acetyl hydrolase is not an interfacial enzyme and has broad substrate specificity as an esterase. PAF-AH hydrolyzes PAF, its analogs, and short chain oxidized phospholipids in a calcium-independent manner. These substrates are more water-soluble than two long fatty acyl chains of native phospholipids that are interfacially hydrolyzed by $cPLA_2$ and $sPLA_2$.[30]

Lipoprotein-PLA_2 (Lp-PLA_2, 45 kDa) is found in blood circulation and is associated with apo-B100 of LDL. Elevated levels of Lp-PLA_2 are associated with coronary heart disease, stroke, and dementia.[10]

1.3. Significance of Paralogs/Splice Variants/Isozymes of PLA_2. The significance of occurrence of paralogs/splice variants/isozymes of PLA_2 in the brain is not fully understood. Studies to unveil the mechanisms of action of PLA_2 and factors regulating their activities in normal physiological and pathophysiological functions have demonstrated cross-talk among different groups of PLA_2s.[10,11,25] Thus, in brain tissue, PLA_2 isozymes are part of a complex signal transduction network through generation of lipid mediators. The activity of PLA_2 isozymes in neural cells is the rate-limiting step for release of AA and production of inflammatory lipid mediators (eicosanoids, lysophosphatidic acid, and platelet activating factor)[31] or DHA and derived anti-inflammatory lipid mediators (docosanoids and lipoxins). These processes provide neural cells with great versatility in ensuring that AA, DHA, and their oxygenated metabolites are efficiently utilized. In brain tissue, PLA_2 isozymes do not function interchangeably, but act in parallel to transduce signals among neurons, astrocytes, and microglial cells. It is likely that multiple forms of PLA_2 act on different cellular pools of phospholipid molecular species located in various subcellular organelles. The activity of PLA_2 isozymes may depend not only on structural, physicochemical, and dynamic properties of neural membranes but also on the interaction of extracellular signal with PLA_2-linked neural cell receptors such as dopamine, glutamate, serotonin, P2-purinergic, cytokine, and growth factor receptors.[25,32,33]

Overstimulation of glutamate receptors results in activation of NADPHoxidase (Nox). This leads to activation of PLA2 and generation of AA, and further metabolism of AA results in formation ROS and neuroinflammation in neurological disorders.[25,34,35] Brain cells are susceptible to the injurious effects of ROS. The latter also act as a signaling molecule to trigger inflammatory responses in the brain through the activation of the redox-sensitive transcription factors, including nuclear factor-κB (NF-κB) and activator protein-1 (AP-1).[36,37] Together, results indicate that members of the PLA_2 superfamily are involved in the synthesis of lipid mediators that have been implicated in fundamental cellular responses including growth, neuronal excitation, differentiation, adhesion, migration, inflammation, neurotransmitter exocytosis, cognitive and behavioral function, and apoptosis.[11,25,33,38]

1.4. Roles of PLA_2 in Neurodegeneration in Neurotraumatic, Neurodegenerative, and Neuropsychiatric Disorders. Under normal conditions, PLA_2 isozymes are involved in generation of lipid mediators that are closely associated with phospholipid turnover, neurotransmitter release, long-term potentiation, memory processes, membrane repair, ion channel function, and gene transcription processes.[39] In pathological conditions, however, increased degradation of phospholipids due to activation of PLA_2 isozymes leads to changes in membrane permeability and stimulation of enzymes involved in lipolysis, resulting in disruption of membrane structure.[32,33] Together with alterations in activities of membrane bound-enzymes, receptors, and ion channels, this produces nervous system dysfunction and cognitive impairment.[25,40,41] The effects are closely associated with the activation of microglia and astrocytes, which release inflammatory cytokines (TNF-α, IL-1β, and IL-6). These propagate and intensify neuroinflammation by a number of mechanisms including further upregulation of PLA_2, generation of platelet-activating factor, and stimulation of nitric oxide synthase.[4] High levels of free radicals, lipid peroxides, and eicosanoids in brain produce oxidative stress and inflammation, and these processes along with compromised energy metabolism may play a role in neurotraumatic (excitotoxicity, traumatic nerve and brain injury, cerebral ischemia), neurodegenerative/neuroimmune (Alzheimer disease, Parkinson disease, multiple sclerosis or

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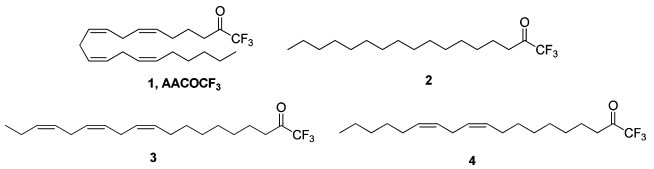
ACS Chem. Neurosci. XXXX, XXX, XXX−XXX

experimental allergic encephalitis; and prion diseases), chronic pain, and neuropsychiatric diseases (depression, schizophrenia, and autism).[25,42]

For treatment of the above neurotraumatic, neurodegenerative, and neuropsychiatric disorders, development of selective inhibitors of PLA_2 isozymes is very important. An ideal PLA_2 inhibitor not only should be able to inhibit neuroinflammation and block oxidative stress but also should cross the blood brain barrier (BBB). Inhibitors must reach the site where inflammatory processes are taking place for better efficiency,[25] and this may be achieved through better drug delivery systems that target the brain, or protect PLA_2 inhibitors from *in vivo* degradation or detoxification. The effects of PLA_2 inhibitors on genes and lipids can be monitored by microarray and lipidomic analyses. These studies can lead to better therapeutic agents for the treatment of neurological disorders involving glycerophospholipid alterations.

2. SYNTHETIC INHIBITORS OF PLA_2

2.1. Fluoroketones and Fluorophosphonates. Arachidonoyl trifluoromethyl ketone (**1**, $AACOCF_3$) was the first

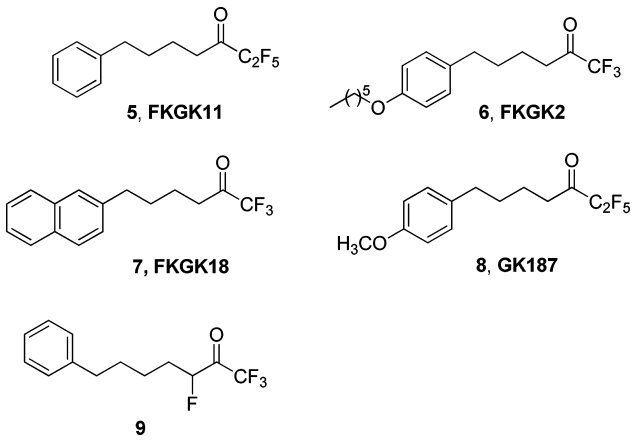


inhibitor of $cPLA_2$ reported in the literature.[43,44] It is a slow, tight binding inhibitor of $cPLA_2$, presenting 4 orders of magnitude more potent inhibition of this enzyme, compared to $sPLA_2$. $AACOCF_3$ inhibits $cPLA_2$ with an $X_I(50)$ value of 0.036 as determined using a mixed micelle assay.[45] The trifluoromethyl ketone analogues of palmitic (**2**), γ-linolenic (**3**), and linoleic acid (**4**) were also found to inhibit $cPLA_2$.[45,46] In contrast to $cPLA_2$, the saturated fatty acid derivative **2** is 4-fold more potent for $iPLA_2$ than $AACOCF_3$ (X_I (50) values 0.0075 and 0.028, respectively). It is clear, however, that $AACOCF_3$ is not a selective inhibitor, and $AACOCF_3$ (**1**) and palmitoyl trifluoromethyl ketone (**2**) also inhibit macrophage Ca^{2+}-independent PLA_2.[47] In addition, $AACOCF_3$ inhibits other enzymes such as COX.[48] Thus, any results obtained by using $AACOCF_3$ in cells and *in vivo* must be interpreted with caution.

$AACOCF_3$ has been reported to modulate pathogenesis of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis.[49] In addition, intracerebroventricular injection of $AACOCF_3$ significantly reduces responses to von Frey hair stimulation, 8 h and 1 day after facial carrageenan injection.[50] Intrathecal injection of $AACOCF_3$ dose-dependently modulates thermal hyperalgesia induced by carrageenan as well as formalin injections.[51] $cPLA_2$ is also proposed to be involved in prion disease, and daily treatment of prion-infected cell lines with $AACOCF_3$ for 7 days prevents accumulation of protease-resistant prion protein (PrPres).[52] Moreover, $cPLA_2$ reduction by $AACOCF_3$ ameliorates cognitive deficits in a mouse model of Alzheimer's disease.[53]

Kokotos, Dennis and co-workers developed a variety of polyfluoroketones.[54−56] Pentafluoroethyl ketone FKGK11 (**5**) was found to be a selective $iPLA_2$ inhibitor ($X_I(50)$ 0.0014),[55] while the trifluoromethyl ketone FKGK2 (**6**) is considered a pan-inhibitor of $iPLA_2$ ($X_I(50)$ 0.0169), $cPLA_2$ ($X_I(50)$ 0.0098), and even $sPLA_2$.[54] Structure−activity relationship studies led to the potent $iPLA_2$ inhibitor FKGK18 (**7**) ($X_I(50)$ 0.0002), which is 195 and >455 times more potent for $iPLA_2$ than for $cPLA_2$ and GV $sPLA_2$, respectively, making it a valuable tool to explore the role of $iPLA_2$ *in vitro* and *in vivo*.[55]

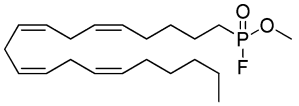
Recently, pentafluoroethyl ketone GK187 (**8**) was developed as the most potent inhibitor of $iPLA_2$ ever reported ($X_I(50)$ 0.0001).[56] Deuterium exchange mass spectrometry and molecular dynamics simulations show that the fluoroketone inhibitor **9** forms favorable interactions inside the active-site pocket $iPLA_2$ and blocks the entrance of phospholipid substrates.[57]



The polyfluoroketones, together with other selective PLA_2 inhibitors, for example 2-oxoamides, have been used to clarify the role of each PLA_2 class in neurological disorders.[58,59] For example, the role of various PLA_2 isoforms in the progression of EAE has been determined, using the inhibitors FKGK11, AX059, and FKGK2.[59] Inhibition of $iPLA_2$ by FKGK11 shows that the enzyme is a target for vasopressin signaling in the thick ascending limb of the kidney.[60]

Various reports, summarized in a recent review,[61] suggest that $iPLA_2$ plays an essential role in β-cell programmed cell death, and inhibitor FKGK18 is proposed as a candidate drug for prevention of β-cell apoptosis and diabetes.[62] Most recently, FKGK18 and a selective $cPLA_2$ inhibitor are used to discriminate between phospholipid pools of $cPLA_2$ and $iPLA_2$.[63]

Methyl arachidonyl fluorophosphonate (**10**, MAFP), another AA analog, is a potent irreversible $cPLA_2$ inhibitor without affecting human $sPLA_2$.[64] Intrathecal administration of MAFP dose dependently prevents thermal hyperalgesia induced by intraplantar carrageenan as well as formalin-induced flinching.[65]



2.2. Bromoenol Lactone. Bromoenol lactone (**11**, BEL) is an irreversible, covalent inhibitor of $iPLA_2$ (IC_{50} 60 nM).[47,66] It has shown a 1000-fold selectivity for $iPLA_2$ versus $cPLA_2$ and $sPLA_2$,[67] and thus it is considered a selective inhibitor of this enzyme and is commonly used to inhibit $iPLA_2$ in cellular systems. BEL is usually present as a racemate; however, the (*R*)- and (*S*)-enantiomers of BEL have different enzyme inhibitory properties.[68−70] (*S*)-BEL inhibited $iPLA_2\beta$ 10-fold more potently than (*R*)-BEL, while (*R*)-BEL inhibited $iPLA_2\gamma$ almost 10-fold more potently than (*S*)-BEL.[68,70] BEL inactivates $iPLA_2$ by generating a diffusible bromomethyl keto acid that alkylates cysteine thiols, rather than interacting with the active-site serine.[71] Recently, mass spectrometry studies demonstrate a highly reactive cysteine residue (C651) that interacts with the active site of the enzyme.[72]

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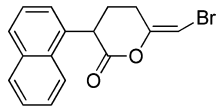
ACS Chem. Neurosci. XXXX, XXX, XXX−XXX

It is, however, noted that BEL also inhibits enzymes such as serine protease[73] magnesium-dependent phosphatidate phosphohydrolase-1,[73] and the possibility that BEL may be inhibiting these enzymes besides $iPLA_2$ must be considered when interpreting experiments using this inhibitor.



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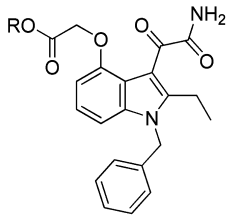
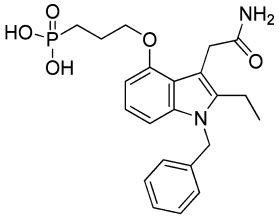
Studies using BEL report that prostaglandin E2 (PGE_2) generation may be partially mediated by $iPLA_2$, in addition to $sPLA_2$.[74] BEL reduces AA release and PGE_2 production upon stimulation in 3T6 fibroblast cultures.[75] In addition, BEL selectively increases AMPA receptor-mediated synaptic transmission.[76] Intracerebroventricular injection of BEL reduces responses to von Frey hair stimulation after facial carrageenan injection in both C57BL/6J (B6) and BALB/c mice.[50] BEL also decreases prostate cancer cell growth by p53-dependent and independent mechanisms[77] and activates p38 MAPK signaling pathways during cytostasis in prostate cancer cells.[78] These findings may provide some insights into signaling mechanisms involving $iPLA_2$ in the brain.

2.3. Indole-Based Inhibitors. A highly potent $sPLA_2$ inhibitor having a novel indole structure was discovered by



12, R=H Varespladib
13, R=Me Varespladib methyl

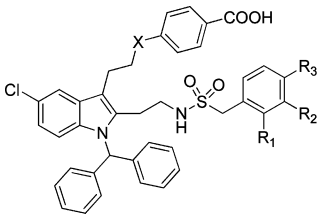


14, LY311727

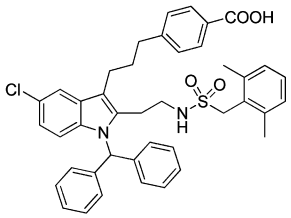
high-volume screening at Lilly, which cocrystallizes with human recombinant $sPLA_2$ and interacts with its active site.[79] Extensive work on indole-based inhibitors led to the development of varespladib (**12**) and varespladib methyl (**13**), which functions as a pro-drug of varespladib. Varespladib is a potent inhibitor of human $sPLA_2$ GIIA (IC_{50} 0.009 μM), which inhibits human GIB pancreatic $sPLA_2$ with an IC_{50} value of 0.228 and is inactive against $cPLA_2$ and COX.[80,81] Thus, varespladib (**12**) was selected for evaluation clinically as a $sPLA_2$ inhibitor. Another indole-based inhibitor is LY311727, which inhibits $sPLA_2$ with an IC_{50} value of 0.023 μM.[82]

Varespladib reached Phase II clinical trials for treatment of severe sepsis, but was terminated because efficacy was poorer than expected (http://clinicaltrials.gov/, Identifier: NCT00034476). Later, Anthera Pharmaceuticals disclosed varespladib and varespladib methyl as $sPLA_2$ inhibitors for the treatment of cardiovascular diseases,[83,84] but Phase III clinical trials were terminated in 2012 due to inability of the study to detect a statistically significant benefit of the drug.

Another series of very important and potent $cPLA_2$ inhibitors was developed by Wyeth, which include indole compounds such as **15**−**18**.[85,86] Ecopladib inhibits $cPLA_2$ with IC_{50} values of 0.15 μM using a GLU assay and 0.11 μM using an RWB assay, while efipladib presented IC_{50} 0.04 μM in a GLU assay and 0.07 μM in an RWB assay. WAY-196025 is even more potent (IC_{50} 0.01 μM in a GLU assay and 0.03 μM in an RWB assay).



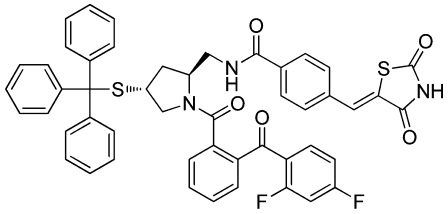
15, Ecopladib, X=O, R_1=H, R_2=R_3=Cl
16, Efipladib, X=CH_2, R_1=H, R_2=R_3=Cl
17, Giripladib, X=CH_2, R_1=CF_3, R_2=R_3=H



18, WAY-196025

Ecopladib (**15**) shows oral efficacy in inflammation models and advanced to phase I clinical trials, while efipladib (**16**) shows oral efficacy *in vivo*. Giripladib (**17**) was the most promising indole-based inhibitor and advanced to phase II clinical trial for osteoarthritis, but in 2007 the trial was terminated due to gastrointestinal and lipase events (http://clinicaltrials.gov/, Identifier: NCT00396955). Efipladib decreases nociceptive responses without affecting PGE_2 levels.[87]

2.4. Pyrrolidine-Based Inhibitors. Shionogi identified a series of pyrrolidine-based inhibitors of PLA_2.[88]



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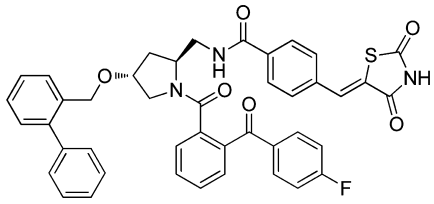

20

Studies on such derivatives[89−91] show that pyrrophenone (**19**) is a potent and reversible inhibitor of human $cPLA_2$ (IC_{50} 4.2 nM) that strongly inhibits AA release, PGE_2, and thromboxane B2 and leukotriene B4 formation in human whole blood.[90,91] A structurally related inhibitor, pyrroxyphene (**20**) presents an IC_{50} value of 0.078 μM and in cellular assays suppresses AA release and PGE2 synthesis from A23187 stimulated THP-1 cells with IC_{50} values of 0.32 and 0.26 μM, respectively.[91] It displays antiarthritic and antibone destructive action in a mouse arthritis model.[92] It is possible that these inhibitors may also be effective against brain $cPLA_2$.

2.5. Amide Inhibitors. A variety of amides based on non-natural amino acids have been reported as inhibitors of $sPLA_2$. FPL67047XX (**21**) is a potent inhibitor presenting an IC_{50} value against human platelet $sPLA_2$ of 21 nM,[93] and binding interactions with human nonpancreatic $sPLA_2$ were demonstrated by high-resolution X-ray crystallography.[94] Another amide inhibitor is based on D-tyrosine.[95] Inhibitor **22** (IC_{50} 0.029 μM) cocrystallizes with $sPLA_2$, and the crystal structure reveals chelation to a Ca^{2+} ion through carboxylate and amide oxygen atoms, H-bonding through an amide NH group to His48,

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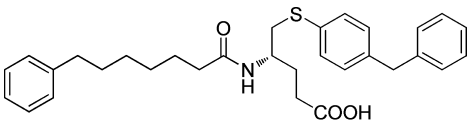
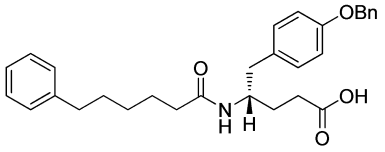
ACS Chem. Neurosci. XXXX, XXX, XXX−XXX



21, FPL67047XX



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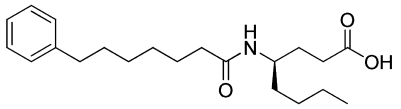
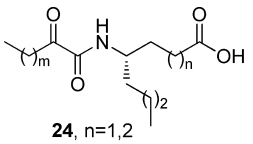


23, GK115

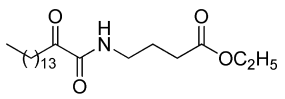
multiple hydrophobic contacts, and a T-shaped aromatic group−His6 interaction.[95] Oral or intravenous administration of this inhibitor protects the rat small intestine from ischemia reperfusion injury[96] and TNBS-induced colitis.[97] It also exhibits antifibrotic activity in young spontaneously hypertensive rats[98] and preserves bone architecture following ovariectomy in adult rats.[99] Another study reveals amide **23** (GK115), based on (*R*)-γ-norleucine, as a selective inhibitor of sPLA$_2$ (X_I(50) 0.003) that does not have significant inhibition against cPLA$_2$ or iPLA$_2$.[100] It is interesting to note that its enantiomer is inactive against all three PLA$_2$s (sPLA$_2$, cPLA$_2$, and iPLA$_2$).

GK115 improves locomotor function when administered 1 h after spinal cord injury, compared to vehicle controls.[101]

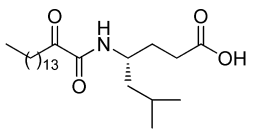
2.6. 2-Oxoamides. 2-Oxoamides were designed to target the active site serine of cPLA$_2$.[102−106] Long chain 2-oxoamides based



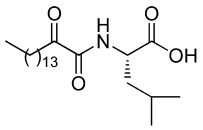
24, n=1,2
m=13



25, AX048



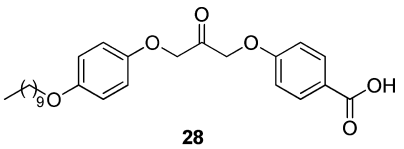
26, AX059



27, GK126

on γ- or δ-amino acids (**24**) containing a free carboxyl group are potent and selective inhibitors of cPLA$_2$, while the corresponding esters inhibit both cPLA$_2$ and iPLA$_2$. The location of the 2-oxoamide inhibitor AX007 (X_I(50) 0.009)[103] in the active site of cPLA$_2$ was determined by a combination of deuterium exchange mass spectrometry with molecular dynamics.[107] Long chain 2-oxoamides based on α-amino acids, for example compound **27**, exhibit inhibitory activity to sPLA$_2$ (IC$_{50}$ 300 nM for human sPLA$_2$ GIIA and 180 nM for mouse sPLA$_2$ GIIA).[108]

Inhibitor AX048 (**25**) (X_I(50) 0.022 for cPLA$_2$ and X_I(50) 0.027 for iPLA$_2$) is able to block spinal PGE$_2$ release and shows potent antihyperalgesic effect.[109] In addition, inhibitor AX059 demonstrates selective cPLA$_2$ inhibitory activity (X_I(50) 0.008) and modulates inflammation and degeneration by promoting regulatory T cells in rats with EAE.[110]



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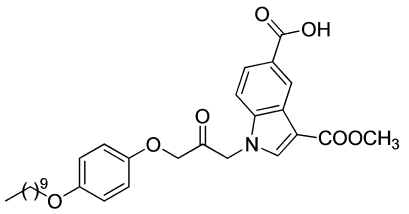
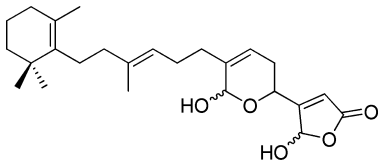


29

2.7. 1,3-Disubstituted Propan-2-ones. Astra Zeneca developed a series of potent inhibitors of cPLA$_2$ based on a 1,3-disubstituted propan-2-one skeleton.[111] Compound **28**, which contains a decyloxy lipophilic side chain and a benzoic acid group, inhibits cPLA$_2$ with an IC$_{50}$ value of 0.008 μM in a bilayer assay, 0.03 μM in a soluble assay, and 2.8 μM in a whole cell assay. Later, Lehr and co-workers presented a series of articles describing 1,3-disubstituted propan-2-one derivatives mainly incorporating an indole ring.[112−116]

Extended structure−activity relationship studies, on the influence of the position of the carboxylic acid group, the nature of the substituent of the indole ring, the introduction of a second substituent in position 3 of the indole ring, and the substitution of the octyl chain by a decyloxy chain, led to derivative **29**. This inhibitor presents an IC$_{50}$ of 0.0043 μM in a vesicle assay with isolated cPLA$_2$ enzyme.

2.8. Natural Products. The natural product manoalide (**30**), isolated in the early 1980s from the sponge Luffariella variabilis,



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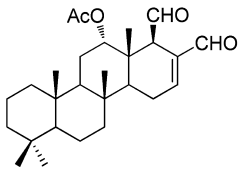

31

was reported to be the first inhibitor of cobra venom sPLA$_2$.[117] Manoalide reached phase II clinical trials as a topical antipsoriatic. However, its development was discontinued because of formulation problems.[118] Scalaradial (**31**), as well as 12-epi-scalaradial, are marine products that inhibit sPLA$_2$ and present in vivo anti-inflammatory activity.[119] Scalaradial has been demonstrated to inhibit human recombinant sPLA$_2$ GIIA (IC$_{50}$ 0.07 μM), but not cPLA$_2$.[120]

2.9. Other Inhibitors. Some other compounds have been claimed as PLA$_2$ inhibitors, but they are in general weak and not selective inhibitors and the mechanism of their action is not known. Thus, the interpretation of the in vivo effects of the inhibitors described in this section 2.9 has to be considered with caution.

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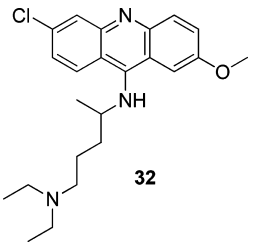
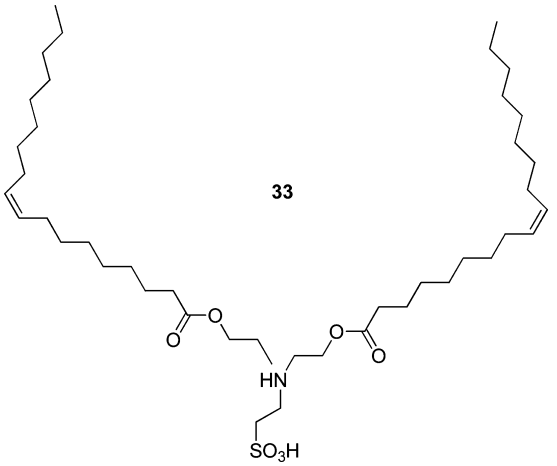
ACS Chem. Neurosci. XXXX, XXX, XXX−XXX

The antimalarial drug quinacrine (**32**) has been employed *in vivo* as an inhibitor of PLA_2, and attenuation of the release of AA in different cell lines has been demonstrated.[121] However, it is a nonspecific inhibitor that is able to inhibit both $cPLA_2$ and $sPLA_2$.[122] 7,7-Dimethyl-5,8-eicosadienoic acid (DEDA) is an AA analog that has been reported to inhibit macrophage PLA_2 activity with an IC_{50} of 16 μM.[123]



32

The nonapeptide CHEC-9 (CHEASAAQC) inhibits $sPLA_2$ and has been shown to inhibit neuron death and inflammation.[124] PX-18 (**33**) is a lipid compound and a nanocrystal formulation based on it presenting neuroprotective effects in cerebral ischemia/repefusion in gerlbils.[125] EPC-K1, a phosphate diester of α-tocopherol and ascorbic acid, has been reported to weakly inhibit $sPLA_2$ activity in human plasma in a concentration-dependent manner (IC_{50} 730 μM[126]).



33

3. ROLE OF PLA_2 INHIBITION IN NEUROLOGICAL CONDITIONS

3.1. Excitotoxicity. The binding of excitatory amino acids such as glutamate to NMDA, AMPA, and kainate classes of glutamate receptors leads to activation of PLA_2, release of AA from membrane lipids, free radical damage, inflammatory responses, and neuronal injury.[127,128] In return, PLA_2 activity can lead to increased exocytosis of neurotransmitters and may further propagate excitotoxicity.[129] All classes of PLA_2 inhibitors are effective in reducing glutamate and aspartate release into the extracellular milieu. The protein kinase C (PKC) inhibitor chelerythrine chloride reduced excitatory amino acid efflux, while

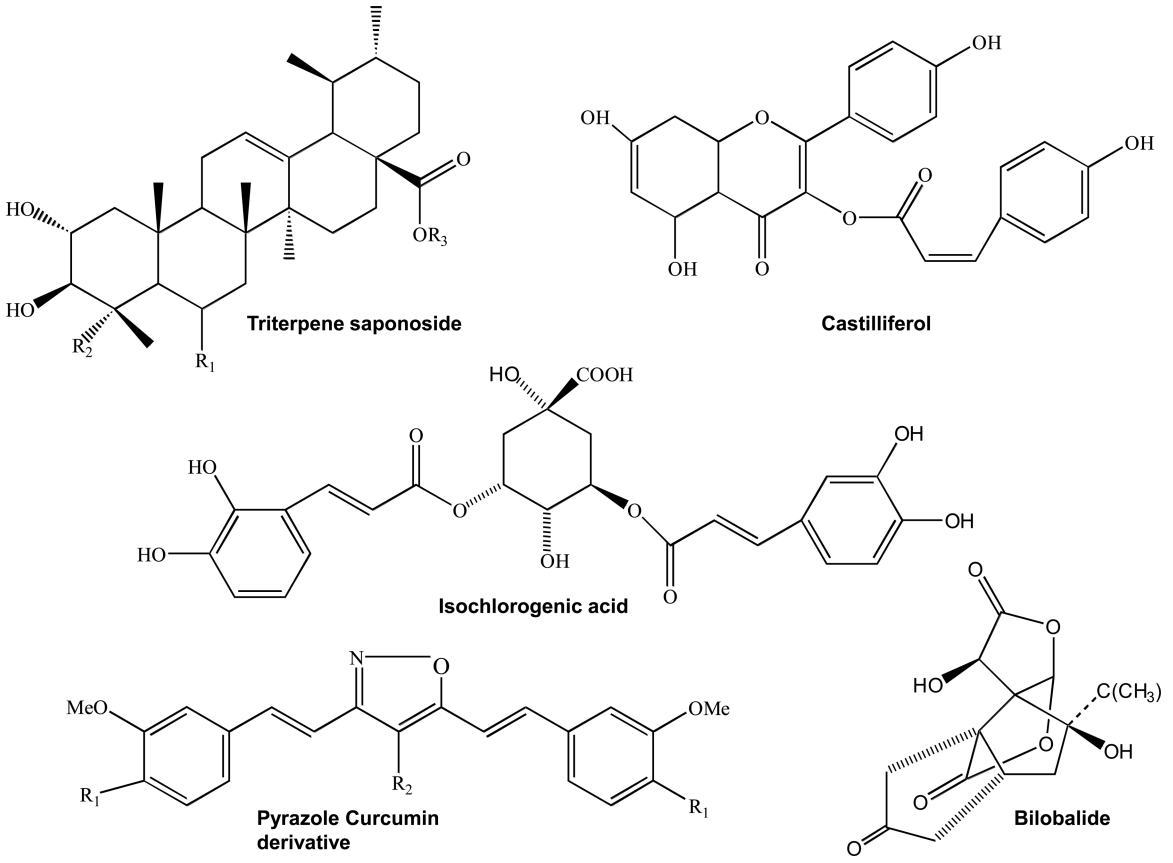


Figure 2. Chemical structures of active components of *Centella asiatica*, EGb 761, and curcumin. *Centella asiatica* contains triterpene saponoside (R_1 = H; R_2 = CH_2OH; R_3 = H), castilliferol, and isochlorogenic acid; EGb 761 contains glycosides of the flavonols quercetin, isorhamnetin, and kaempferol, the terpene-lactones bilobalide and ginkgolides A, B and C, M, J, and bilobalide, and ginkgolic acid. Curcumin powder contains many polyphenols.

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ACS Chem. Neurosci. XXXX, XXX, XXX−XXX

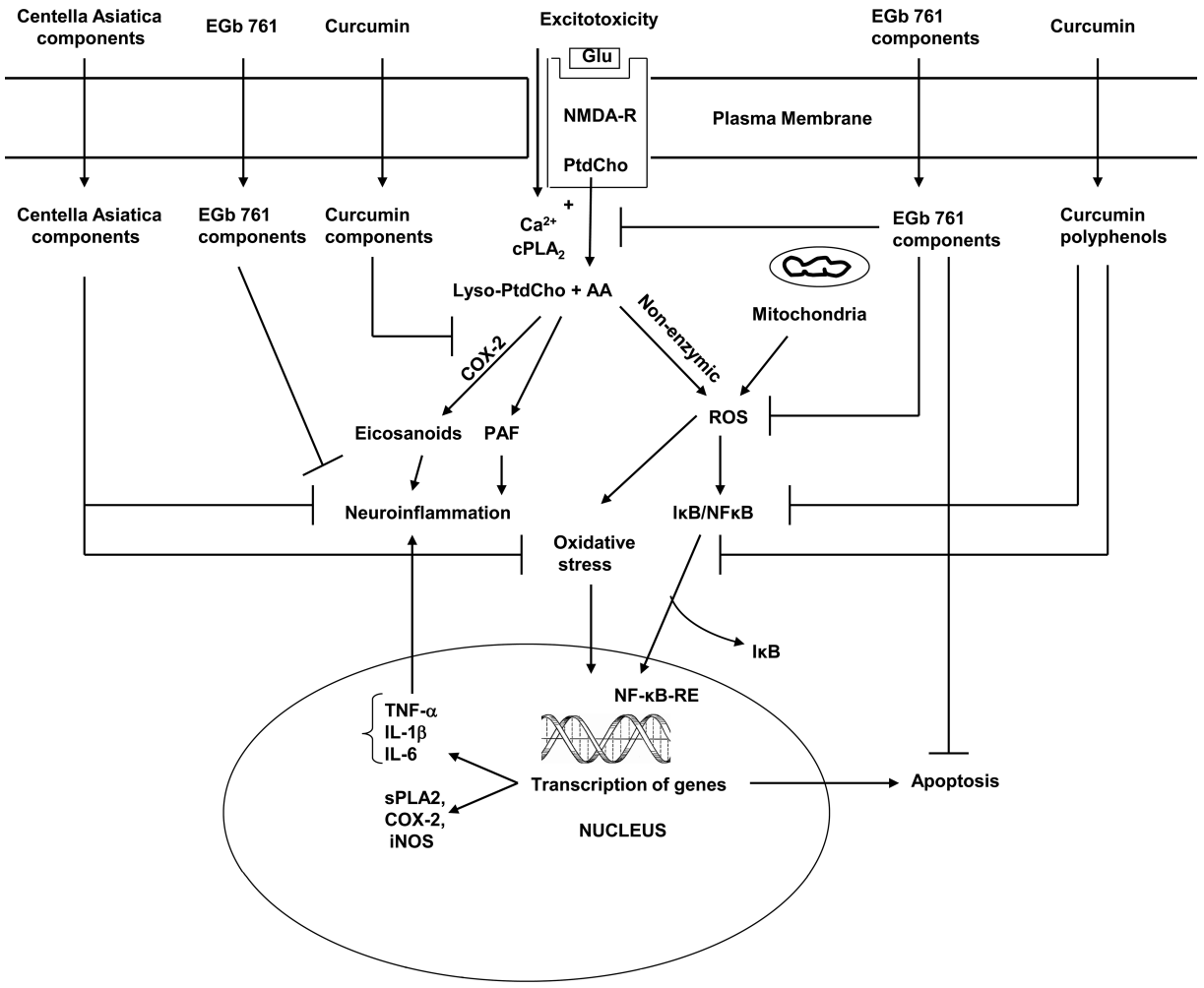

Figure 3. Modulation of neuroinflammation and oxidative stress by *Centella asiatica*, EGb 761, and curcumin. Arachidonic acid (AA). *N*-Methyl-D-aspartate receptor (NMDA-R); Glutamate (Glu); Phosphatidylcholine (PtdCho); cytosolic phospholipase A_2 ($cPLA_2$); lysophosphatidylcholine (lyso-PtdCho); cyclooxygenase (COX); lipoxygenase (LOX); arachidonic acid (AA); platelet activating factor (PAF); reactive oxygen species (ROS); nuclear factor-κB (NF-κB); nuclear factor-κB-response element (NF-κB-RE); inhibitory subunit of NF-κB (I-κB); tumor necrosis factor-α (TNF-α); interleukin-1β (IL-1β); interleukin-6 (IL-6); inducible nitric oxide synthase (iNOS); secretory phospholipase A_2 ($sPLA_2$);Mito (mitochondria); and Positive sign indicates stimulation.

the PKC activator phorbol 12-myristate 13-acetate (PMA) enhanced their release.[130] In addition, sPLA2-IIA is itself released from neuron-like SH-SY5Y neuroblastoma cells after kainate receptor activation, in a PKC dependent manner.[131] PLA_2 activity is itself upregulated after cells are exposed to glutamate. Brief exposure to a calcium ionophore or phorbol 12-myristate 13-acetate stably enhances PLA_2 activity, and downregulation of protein kinase C activity partially blocked glutamate's effects.[132,133]

Injection of the potent glutamate analog and excitotoxin, kainate, in rats results in seizures, upregulation of $cPLA_2$ mRNA expression,[129] and induction of $cPLA_2$ immunoreactivity in damaged neurons and reactive astrocytes of the hippocampus.[134] This is accompanied by increased $cPLA_2$ activity[26] and accumulation of the toxic lipid peroxidation product 4-hydroxynonenal, in lesioned areas.[135] Excitotoxicity is reduced by the $cPLA_2$ inhibitor, $AACOCF_3$, but not the $iPLA_2$ inhibitor BEL, in hippocampal slice cultures.[129] Damage is also reduced by treatment with some antimalarial drugs which are nonselective PLA_2 inhibitors.[136] As in kainate lesions, injection of iron chloride in the amygdala in rats induces seizures, upregulation of PLA_2, and elevated lipid peroxidation products.[137] $sPLA_2$-IIA is also activated in cortical neurons after stimulation of *N*-methyl-D-aspartate glutamate receptors (NMDA-R). Activation is dependent on Ca^{2+} and reactive oxygen/nitrogen species, and inhibition prevents neuronal apoptotic death.[138]

Studies have been carried out to explore the potential use of botanicals in excitotoxicity. *Ginkgo biloba* extract (EGb761), which contains many ginkgolides, flavonoids, and bilobalides (Figure 2), modulates glutamate and hydrogen peroxide induced increase in expression and activation of $cPLA_2$ and neuronal death after excitotoxicity. The extracellular signal-regulated kinase 1/2 9 (ERK1/2) signaling pathway is involved in EGb761's effect on modulation of $cPLA_2$ activation.[139] Another phytochemical which is reported to affect $cPLA_2$ activity is *Centella asiatica*. The latter is widely distributed in South America and Asia and is known as a therapeutic agent for improving memory and treatment of neurological disorders. Extracts contain triterpene saponoside, castilliferol, and isochlorogenic acid (Figure 2) that dose-dependently inhibits the activity of brain PLA_2, suggesting they may be useful in neurological conditions associated with increased activity of the enzyme[140] (Figure 3).

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ACS Chem. Neurosci. XXXX, XXX, XXX−XXX

3.2. Traumatic Nerve and Brain Injury. Induction of PLA_2 expression occurs after traumatic nerve or brain injury, which may lead to formation of lipid mediators and propagation of injury. Intracellular $cPLA_2$ and $iPLA_2$ also play important roles in Wallerian degeneration and axon regeneration after peripheral nerve injury.[101] Increased $cPLA_2$ immunoreactivity is observed in the septum after experimental axonal injury induced by fimbria-fornix transection. The increase is accompanied by accumulation of toxic AA peroxidation product, 4-hydroxynonenal (4-HNE) in target neurons of injured axons. Both $cPLA_2$ and 4-HNE immunoreactivity are blocked by intraperitoneal injections of the nonselective PLA_2 inhibitor, quinacrine. Results demonstrate the importance of PLA_2 in generation of AA metabolites, which may result in injury of postsynaptic neurons after axotomy.[141] Experimental transection of the fimbria-fornix in rats which leads to interruption of nerve projections from the septum to the hippocampus and vice versa, and fluid percussion injury of the brain, increases levels of 4-HNE, suggesting the harmful effects on neurons.[142] $sPLA_2$ also plays a role in axonal injury. An internal fragment of the human neuroprotective polypeptide DSEP (Diffusible Survival Evasion Peptide) CHEASAAQC or CHEC-9, that inhibits the enzymatic activity of secreted PLA_2 ($sPLA_2$), modulates inflammatory responses and preserves neurons after stab injury of the cortex.[143] In contrast to an increase in $cPLA_2$ and $sPLA_2$, a decrease in $iPLA_2$ appears to have a deleterious effect in traumatic brain injury. Dietary supplementation with a curcumin (diferuloylmethane) derivative (Figure 3) restores $iPLA_2$ expression and counteracts the effects of fluid percussion injury, possibly by upregulating mediators necessary for recovery from injury.[142]

PLA_2 also plays a role in spinal cord injury.[144] Total PLA_2 activity and $cPLA_2\alpha$ protein expression are increased, and upregulation of $sPLA_2$-IIA and IIE occurs 4 h after injury. This could result in formation of secondary mediators that induce loss of oligodendrocytes and propagation of injury.[145] Blocking $cPLA_2$ at 30 min after spinal cord injury, or $cPLA_2$ knockdown, reduces motor deficits and cell loss, indicating a neuroprotective effect.[146]

3.3. Cerebral Ischemia. PLA_2 enzymes appear to play an important role in damage to neural tissue after cerebral ischemia. An inhibitor of lipid peroxidation and of PLA_2 activity, EPC-K1 reduces deficits in spatial learning when given immediately or 30 min after the onset of reperfusion.[147] The nonselective PLA_2 inhibitor, quinacrine (5 mg/kg), also reduces infarct areas in the caudate putamen compared to saline treated rats.[148] Both p38 MAPK and $cPLA_2$ activation are markedly increased 1 day after reperfusion, and intracerebroventricular administration of a P38 MAPK inhibitor, SB203580, suppresses activation of $cPLA_2\alpha$ and attenuates BBB leakage and subsequent edema.[149] COX-2 induction and PGE_2 concentration are also greater in $cPLA_2\alpha$ +/+ compared to the $cPLA_2\alpha$ −/− ischemic cortex,[150] and neuronal swelling in ischemic regions is also greater in $cPLA_2\alpha$ +/+ than $cPLA_2\alpha$ −/− mouse brains.[151] In addition, the $cPLA_2$ inhibitor $AACOCF_3$ reduces brain PLA_2 activity and cortical and whole hemispheric infarct volume, compared to vehicle treatment.[151]

$cPLA_2$ expression is affected by statins. Treatment of rats that had undergone middle cerebral artery occlusion (MCAO) with a statin, atorvastatin significantly reduced brain water content and infarct sizes; in addition, expression of p38MAPK, phospho-p38MAPK, $cPLA_2$, and 12/15-LOX are decreased, suggesting the role of glycerophospholipid mediators in neuroprotective effects of statins.[152] In addition, the antidepressant nortriptyline induces a decrease in $cPLA_2$ mediated AA release, through attenuation of both PKC and Erk1/2 kinase expression in primary astrocyte cultures exposed to oxygen glucose deprivation.[153] Results indicate a role of $cPLA_2$, and potential clinical use of inhibitors of the enzyme in cerebral ischemia.

Upregulation of $sPLA_2$ is also reported after cerebral ischemia.[154] An $sPLA_2$ inhibitor, 7,7-dimethyleicosadienoic acid (DEDA), administered following ischemia reperfusion injury attenuates the activity of $sPLA_2$ and levels of lipid peroxidation products, reduces BBB leakage and infract volume, and improves neurological functions.[155] In addition, intraperitoneal injection of nanocrystals of the $sPLA_2$ inhibitor PX-18 reduces neuronal death, DNA damage, and glial activation after ischemia reperfusion induced neuronal injury.[125] PX-18 nanosuspension also preserves cerebrovascular reactivity to stimuli.[156] Results indicate that $sPLA_2$-derived oxidative products contribute to neurovascular damage and that treatment with DEDA or PX-18 reduces secondary injury.

Studies have been carried out to explore the potential use of botanicals for treatment of cerebral ischemia. Baicalein (5,6,7-trihydroxyflavone) originally isolated from the roots of *Scutellaria baicalensis* is an antioxidant and anti-inflammatory agent and was found to improve neurological deficit, reduce brain water content and infarct sizes, and downregulate the expression of 12/15-LOX, p38 MAPK, and $cPLA_2$ after MCAO.[152]

3.4. Alzheimer's Disease. Alzheimer's disease (AD) is a neurodegenerative disease, characterized by memory loss and other cognitive impairments. Two pathologic hallmarks of the disease are extracellular deposits of amyloid β ($A\beta$) and the presence of neurofibrillary tangles, consisting of hyperphosphorylated tau. There is evidence that $cPLA_2$ is a contributor to the neurodegenerative mechanisms of AD.[157] Analyses of cerebrospinal fluid glycerophospholipids showed a significant increase in the LPC-to-PC ratio, indicating increased PLA_2 activity in late onset Alzheimer's disease.[158] $A\beta$ activates NADPH oxidase[159] and enhances protein expression and phosphorylation of $cPLA_2$ and AA release by the NO signaling pathway.[160] Treatment of rat cortical neurons with low concentrations of soluble $A\beta$ (1−40) or $A\beta$ (1−42) results in early calcium-dependent release of AA. Both $cPLA_2$ antisense oligonucleotides and a selective inhibitor of $cPLA_2$ activity abolish the AA release from neurons and protect cells against apoptosis induced by $A\beta$. Inhibitors of PKC, p38, and MEK/ERK pathways that are involved in $cPLA_2$ phosphorylation and activation reduce $A\beta$-induced cell death.[161] $A\beta$ also induces activation of sphinogomyelinases and cell death, and these effects are inhibited by a $cPLA_2$ selective inhibitor or antisense oligonucleotide.[162] In addition, genetic ablation or reduction of $cPLA_2$ protects hAPP mice against $A\beta$-dependent deficits in learning and memory, behavioral alterations, and premature mortality.[53]

Tau hyperphosphorylation and neurofibrillary tangles are the other pathological hallmark of Alzheimer's disease. All six Tau isoforms that are expressed in the adult human brain are abnormally hyperphosphorylated and form neurofibrillary tangles in AD. *In vivo* PLA_2 inhibition decreases the levels of total (nonphosphorylated plus phosphorylated) Tau protein in rat brain.[163] An important mechanism through which both $A\beta$ and tau could be affected is via p25/Cdk5. The latter produces hyperphosphorylated tau and neurofibrillary tangles as well as aberrant amyloid precursor protein processing, and recent evidence indicates increased $cPLA_2$ is essential for triggering p25-mediated inflammatory and neurodegenerative pro-

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ACS Chem. Neurosci. XXXX, XXX, XXX−XXX

cesses.[164] Together, results suggest $cPLA_2$ as a therapeutic target for AD.

Studies have been carried out to explore the potential use of botanicals in the treatment of AD. The leaf extract of *Centella asiatica* is used as an alternative medicine for memory improvement in the Indian Ayurvedic system of medicine. Several studies have revealed its effect in ameliorating the cognitive impairment in rat models of AD, and *in vitro* studies reveal that $cPLA_2$ and $sPLA_2$ activities are inhibited *in vitro* by asiaticoside present in the water extract of *Centella asiatica*. This may be a candidate for the treatment of neurodegenerative diseases, because of its pharmacological activity in the brain and low toxicity as demonstrated by its long and popular use.[165]

3.5. Parkinson's Disease. Parkinson's disease (PD) is a neurodegenerative disorder characterized by not only hypokinesia but also mood and cognitive disorders. It involves loss of dopaminergic neurons in the substantia nigra pars compacta and deficits in nigrostriatal dopaminergic transmission, as well as secondary nondopaminergic abnormalities. Increased production of pro-inflammatory cytokines may contribute to the pathogenesis of PD. In unilaterally 6-OHDA and sham lesioned rats, the activity and protein levels of $cPLA_2$ and COX-2 are increased in the caudate putamen, frontal cortex, and remaining brain on the lesioned side, compared to the intact side, or sham controls.[166] Injection of a nonselective PLA_2 inhibitor, quinacrine, modulates MPTP or 6-OHDA induced reductions in striatal dopamine.[167] In contrast to $cPLA_2$, decreased $iPLA_2$ is encountered in some cases of PD. PLA2G6 or $iPLA_2$ is reported as the causative gene for early onset PARK14-linked dystonia-parkinsonism, and patients with the PLA2G6 mutation show heterogeneous phenotype such as dystonia-parkinsonism, dementia, and frontotemporal atrophy/hypoperfusion, with or without brain iron accumulation.[168]

Studies have been carried out to explore the use of nutraceuticals for treatment of PD. Treatment of mice with ethyl-eicosapentaenoate (E-EPA) attenuates the MPP(+) induced increase in $n-6$ fatty acids content and striatal dopaminergic turnover and prevents the increase of pro-apoptotic bax and caspase-3 mRNAs.[169] Likewise, feeding mice a 0.8% ethyl-eicosapentaenoate (E-EPA) diet prior to MPTP-P injections reduces hypokinesia induced by MPTP-P and ameliorates procedural memory deficits. Results suggest that omega 3 fatty acids, such as EPA, may be partially protective in the MPTP mouse model of PD.[170]

3.6. Multiple Sclerosis/Experimental Allergic Encephalitis. Multiple sclerosis (MS) is an inflammatory demyelinating disease of the CNS that results in motor and sensory deficits. Although MS and its animal model, experimental autoimmune encephalomyelitis (EAE), are associated with infiltrating T lymphocytes, the mechanisms underlying the lesions in the CNS are not fully understood. PLA_2 is highly expressed in EAE lesions, and inhibition leads to modulation of onset and progression of the disease.[49] Both $cPLA_2$ and $iPLA_2$ are central mediators in MS and EAE. $cPLA_2$ plays a role in onset, and $iPLA_2$, in onset and progression of the disease.[59] The CNS from myelin oligodendrocyte glycoprotein (MOG) immunized mice reveals extensive inflammatory lesions in the $cPLA_2\alpha \pm$ mice, whereas lesions are greatly reduced or absent in $cPLA_2\alpha -/-$ mice.[171] Inhibition of $cPLA_2$ from the onset of clinical EAE reduces duration of EAE relapses.[172] An inhibitor of $cPLA_2$, $AACOCF_3$, reduces clinical symptoms and attenuates the loss of mature, myelin producing, oligodendrocytes and axonal damage in the spinal cord white matter in EAE.[173] Blockade of $cPLA_2\alpha$ with a highly specific small-molecule inhibitor WAY-196025 during the tissue-damage phase abrogates the clinical features of disease, while therapeutic administration of $cPLA_2\alpha$ inhibitor starting from the peak of disease or during remission completely protects mice from subsequent relapses.[174] $sPLA_2$ may also play a role in MS. Patients show elevations in $sPLA_2$ enzyme activity, and mean levels of $sPLA_2$ increased 6-fold in the urine of patients with active disease and 4-fold for patients in remission, regardless of immunomodulating therapy.[175]

3.7. Pain. PLA_2 plays an important role in nociception, in both the PNS and CNS. Skin incision results in activation of keratinocytes and the increase in PLA_2 activating protein (PLAA). The latter may be regulated by miR-203, which is strongly downregulated in keratinocytes after incision, and could lead to increased PLAA expression and formation of proinflammatory mediators.[176] The importance of tissue/PNS $cPLA_2$ in nociception is supported by findings of the antinociceptive effect of orally administered $cPLA_2\alpha$ inhibitor, efipladib, which has limited ability to cross the blood−brain barrier.[87] Downstream from $cPLA_2$, treatment with a general LO inhibitor NDGA, a 5-LO inhibitor AA-861, and a 12-LO inhibitor baicalein results in the antinociceptive effect after carrageenan induced inflammation, suggesting the importance of LO in inflammatory pain.[177]

PLA_2 activity is increased in the CNS during inflammatory pain, where it may generate lipid mediators that have an effect on neurotransmission along the pain pathway. Lipidomic analyses of the caudal medulla and site of the spinal trigeminal nucleus after facial carrageenan injection shows decreases in phospholipids including phosphatidylethanolamine and phosphatidylinositol species, but increases in lysophospholipids, including lysophosphatidylethanolamine, lysophosphatidylinositol, and lysophosphatidylserine, indicating increased PLA_2 activity. Increased $sPLA_2$-III mRNA expression is found in the caudal medulla although there was no difference in $sPLA_2$-III protein expression. Together, results indicate an increase in brain PLA_2 enzyme activity after inflammatory orofacial pain, but no change in enzyme protein expression.[178] A study used inhibitors to $sPLA_2$ (12-epi-scalaradial), $cPLA_2$ ($AACOCF_3$), or $iPLA_2$ (BEL) to compare possible contributions of central nervous PLA_2 isoforms to the development of allodynia after carrageenan induced inflammation in mice. Intracerebroventricular injection of inhibitors to each of the three PLA_2 isoforms reduces responses to von Frey hair stimulation at 8 h and 1 day after facial carrageenan injection, but at 3 days after injection, only the $sPLA_2$ inhibitor has an effect.[50] The antinociceptive effects of PLA_2 inhibitors are not simply due to inhibition of AA formation, but also lysophospholipids.[50] Another study showed intrathecal administration of $cPLA_2$ inhibitors MAFP and $AACOCF_3$, but not the $iPLA_2$ inhibitor BEL, dose-dependently prevents thermal hyperalgesia induced by intraplantar carrageenan as well as formalin-induced flinching.[51] Knockdown of spinal $cPLA_2$ by antisense oligonucleotides does not change acute nociception (i.e., paw withdrawal thresholds to acute thermal stimuli and intradermal formalin-induced first phase flinching), but attenuates formalin-induced hyperalgesia (i.e., second phase flinching behavior).[179] As mentioned above, CNS $sPLA_2$ plays a role in spinal nociceptive processing, and intrathecal injection of an inhibitor LY311727 prevents intraplantar carrageenan-induced thermal hyperalgesia and formalin-induced flinching.[65] Together, results point to the important roles of $sPLA_2$ and $cPLA_2$ in nociceptive transmission.

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ACS Chem. Neurosci. XXXX, XXX, XXX−XXX

Besides inflammatory pain, PLA_2 also plays a role in neuropathic pain. Spinal nerve injury activates $cPLA_2$ in injured dorsal root ganglion neurons and likely contributes to tactile allodynia, where innocuous stimulation elicits pain behavior.[180] Such activation of $cPLA_2$ may involve CaM kinase II, since enzyme inhibition reduces $cPLA_2$ activation and prevents the development and expression of nerve injury-induced tactile allodynia.[181] Nerve injury also results in increased spinal cord $cPLA_2$ and $iPLA_2$ activities, with peaks at 1 h after injury. Both lysophosphatidic acid (LPA) production and neuropathic pain-like behaviors are abolished by intrathecal injection of $AACOCF_3$, or bromoenol lactone, at 1 h after injury.[182] Besides the ascending pain pathway, our preliminary data show that prefrontal cortical $iPLA_2\beta$ activity could be important in the supraspinal *antinociceptive* effect of certain antidepressants (Chew-WS and Ong-WY, unpublished observations).

Intrathecal and systemic injection of a 2-oxoamide $cPLA_2$ inhibitor, AX048, blocks carrageenan induced hyperalgesia. In addition, systemic delivery of AX048 modulates spinally mediated hyperalgesia induced by intrathecal substance P.[109] These findings are significant, as they demonstrate the effectiveness of a systemically administered PLA_2 inhibitor on the CNS. The ANXA1 protein also modulates nociceptive processing at the spinal level, by reducing synthesis of PGE_2 and modulating $cPLA_2$ and/or COX activity.[183] Treatment with minocycline inhibits LPA-induced microglial activation and neuropathic pain-like behavior,[184] while a recent study shows that (*E*)-4-(3,7-dimethylocta-2,6-dienylamino) phenol (LQFM-015) decreases PLA_2 and COX enzyme activity and behavioral responses to carrageenan-induced paw edema and pleurisy.[185] Together, results indicate a close link between anti-inflammatory and antinociceptive activities of compounds.

3.8. Depression. Changes in long-chain polyunsaturated fatty acids (PUFAs) are associated with depression.[186] On the other hand, treatment of mice with certain antidepressants increases $iPLA_2$ activity and endogenous release of PUFAs in brain tissue. PUFAs such as DHA may be metabolized by 15-LO to products such as resolvins that have effects on neuroplasticity. Lipidomic analyses of the prefrontal cortex of mice treated with the antidepressants maprotiline and paroxetine reveal significant decreases in phosphatidylcholine (PC) species (PC36:1, PC38:3, PC40:2p, PC40:6, PC40:5, PC42:7p, PC42:6p, and PC42:5p) but increases in lysophospholipid species (lysoPC16:0, lysoPC18:2, and lysoPC18:0), indicating elevated PLA_2 activity and endogenous release of long-chain fatty acids such as DHA.[177] Beneficial effects of maprotiline on depression-like behavior and the above-mentioned changes in lipids are abolished by intracortical injection of antisense oligonucleotide to $iPLA_2$, suggesting a role of the enzyme in antidepressant action.[187]

3.9. Bipolar Disorder. Bipolar disorder is a psychiatric condition characterized by recurrent manic and depressive episodes, without a characteristic neuropathology or clear etiology. Post-mortem samples of frontal cortex from bipolar disorder patients show significantly elevated levels of $cPLA_2$, $sPLA_2$IIA, COX-2, and membrane prostaglandin E synthase.[188] In contrast, mood stabilizers effective in treating mania (lithium, carbamazepine, valproate) or depression (lamotrigine) in bipolar disorder decrease transcription of $cPLA_2$ and COX-2 and decrease AA turnover in brain phospholipids, when administered chronically to rats.[189] A pilot study shows low-dose aspirin, which blocks COX-1 and COX-2, reduces the risk of clinical deterioration in subjects on lithium.[190] Results are consistent with the AA hypothesis of mood stabilizer action and reports of beneficial effects of *n*−3 PUFA rich- and/or *n*−6 PUFA poor diets in patients with bipolar disorder and migraine.[191]

3.10. Schizophrenia. Schizophrenia is a severe neuropsychiatric disorder associated with cognitive impairment. The condition is associated with changes in brain structure including reduction of gray matter, enlarged ventricles,[192] and loss of dendritic spines from pyramidal neurons in the cortex.[193] Accelerated phospholipid metabolism and reduced dopaminergic activity in the prefrontal cortex are postulated to play a role in schizophrenia.[194] Intracerebroventricular injection of PLA_2 in rats results in inhibition of apomorphine-induced locomotion compared to controls, indicating functional inhibition of dopaminergic postsynaptic receptors by PLA_2.[195] Moreover, mRNA and protein expression of $cPLA_2$, $sPLA_2$, and COX are elevated, together with increased cytokine and proinflammatory markers in schizophrenic brains (Rao et al., 2013). Brain membranes from the prefrontal cortex of schizophrenic patients demonstrate increased flexibility of fatty acid chains than controls, indicating an increase in PLA_2 activity.[196] First-episode schizophrenic patients show increased brain PLA_2 activity associated with structural alterations in the left prefrontal cortex and thalamus, while recurrent-episode patients demonstrate widespread associations between PLA_2 activity and structural changes in the left hemisphere and cerebellum.[197] The atypical antipsychotic clozapine increases mRNA and protein expression and activity of brain $iPLA_2$, as well as BDNF and of the postsynaptic marker drebrin, while decreasing COX activity and concentration of the AA metabolite, PGE_2. This suggests that some of the therapeutic effect of clozapine may involve increasing DHA anti-inflammatory metabolites such as resolvins and decreasing AA derived, proinflammatory mediators.[198]

3.11. Autism. Dysregulated phospholipid metabolism has been proposed as a component of neurodevelopmental disorders such as autistic disorder and attention-deficit/hyperactivity disorder (ADHD).[199] Lysophospholipids released by PLA_2 activity are metabolized to platelet activating factor, and recently, platelet-activating factor acetylhydrolase IB subunit alpha (also known as Lis1), an enzyme encoded by the PAFAH1B1 gene, is implicated as an important protein-network interaction node with high-risk autism spectrum disorder genes.[200] PAFAH1B1 is the noncatalytic subunit of an acetylhydrolase complex which inactivates platelet-activating factor by removing the acetyl group at the *sn*-2 position. The protein plays an important role in dendritic filopodia dynamics and spine turnover. Loss of Lis1 results in dysfunction of dynein protein motor and disruption of the actin cytoskeleton through dysregulated RhoGTPases.[200] Results suggest increased PAF activity, leading to alterations in dendritic spine formation and function in autistic disorders, and raise the possibility of PAF inhibitors in management of these conditions.

4. FUTURE DIRECTIONS AND CONCLUSION

Isozymes of PLA_2 along with cyclooxygenases have emerged as major players affecting inflammation and oxidative stress in brain tissue. Elucidation of the mechanism of action of the above-mentioned PLA_2 inhibitors *in vivo* is an important area of research due to the potential pharmacologic benefits of these compounds as therapeutic agents for the treatment of inflammation and oxidative stress in neurotrauma and neurodegenerative diseases.[201] PLA_2 inhibitors have been classified into selective and nonselective types. Selective inhibitors only inhibit PLA_2 activity at nanomolar concentrations. In contrast,

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ACS Chem. Neurosci. XXXX, XXX, XXX−XXX

nonselective inhibitors inhibit not only PLA_2 activity but also other enzymes and receptors.[202] For example, phytochemical based PLA_2 inhibitors (curcumin, *Ginkgo biloba*, and *Centella asiatica*) are complex mixtures of bioactive compounds. The levels of bioactive compounds may vary substantially depending upon many factors related to the growth of plants, harvesting, production, and storage conditions. These factors may cause alterations in chemical compositions of dietary phytochemicals resulting in batch variation. This is in contrast to pharmaceuticals that undergo extensive clinical trials in animals and humans prior to FDA approval.

Many chemical inhibitors of PLA_2 activity have been described in the literature.[201,203−207] Different mechanisms of action are possible; e.g., an inhibitor can produce alterations in enzymatic activity by perturbing the physicochemical properties of phospholipid bilayers. A PLA_2 inhibitor can interact directly with the active site of an isoform, or it can act on an allosteric site on the enzyme to bring about changes in enzymic activity. An inhibitor may also produce a detergent-like structure that can induce nonspecific changes in membrane properties *in vivo* through the interaction of their amphiphilic groups with other membrane components to produce changes in enzymatic activity. As mentioned above, even selective inhibitors of PLA_2 have been reported to produce nonspecific effects. For example, the dual $cPLA_2$ and $iPLA_2$ inhibitor methyl arachidonyl fluorophosphonate (MAFP) produces Tau phosphorylation at Ser214, supporting the view that inhibition of both $cPLA_2$ and $iPLA_2$ might influence several biochemical aspects of Tau proteins.[101,163,208] An $iPLA_2$ inhibitor (bromoenol lactone) and $cPLA_2$ inhibitor (palmitoyl trifluoromethyl ketone) retard insulin resistance.[209] Besides inhibiting PLA_2 activity, some inhibitors also modulate the expression of cytokines, growth factors, nuclear factor kappa B, and adhesion molecules and, thus, can block endogenous inflammatory reactions and oxidative stress indirectly.

Since the brain contains many isozymes of PLA_2, specific PLA_2 inhibitors must be designed for testing against individual PLA_2 isozymes to establish their physiological and pathological roles in the brain. The design of PLA_2 inhibitors should be focused on our rapidly emerging understanding of the role of signal transduction pathways in neurological disorders. At this time, it is quite difficult to predict the potential side effects of the chronic use of cell-permeable, specific or nonselective inhibitors of PLA_2. Hence, studies on the availability of specific, nontoxic potent inhibitors with greater blood−brain barrier permeability in animal models of neurodegenerative diseases are urgently needed.

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Notes

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