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Mini-Review

# Clarification of Arachidonic Acid Metabolic Pathway Intricacies

Hatem Tallima*

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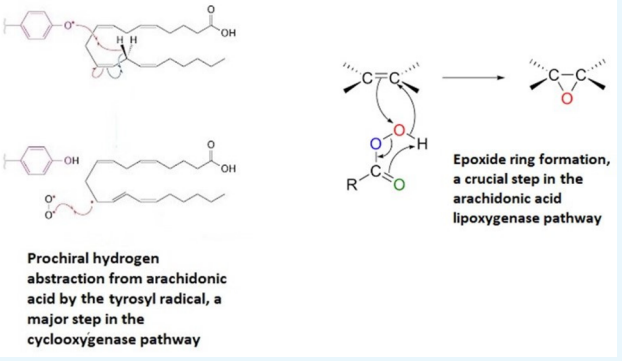
**ABSTRACT:** Surrounding inflammation activates phospholipase $A_2$, which cleaves and releases arachidonic acid (ARA) from cell membranes. The four cis double bonds are instrumental in ARA susceptibility to oxidation, resulting in the generation of numerous bioactive metabolites of critical importance for the immune system, namely inflammation in response to pathogens, resolution of inflammation, wound healing, and mood and energy balance. The ARA metabolism steps are replete with intricacies, deterring researchers from identifying targets, which could be useful in modulating the synthesis of ARA metabolites toward exclusive protection of the host from pathogens, endogenous excessive danger signals, pain, inflammation, stress, and anxiety disorders. While ARA metabolic pathways are reasonably defined, it was deemed mandatory to fully clarify the flow and direction of protons, electrons, and oxygen atoms and the intricacies behind formation and breakage of double bonds and cyclic structures. This in-depth novel information will perfect the development of strategies and drugs aimed at counteracting inflammation and promoting healing.




## INTRODUCTION

Arachidonic acid (ARA) is a 20-carbon chain, omega-6 (n-6) polyunsaturated fatty acid (PUFA), biochemically designated as *all-cis*-5,8,11,14-eicosatetraenoic.[1] In all eukaryotes, ARA is naturally found incorporated in phospholipids of the cell membrane conferring it with fluidity and flexibility, so necessary for the function of all cells. Free ARA modulates the function of ion channels and several receptors and enzymes, via stimulation as well as inhibition. These activities are key factors in the proper function of the brain and muscles and underlie its protective potential against cancer and schistosomiasis infection.[2,3] In humans and other mammals, different enzymes lead to cell membrane ARA oxidation, resulting in the generation of numerous pro-inflammatory and anti-inflammatory resolving mediators.[4,5] The double bonds are the ARA key for reacting with molecular oxygen. Peroxidation can happen nonenzymatically following ARA exposure to reactive oxygen (ROS) or reactive nitrogen (RNS) species, generating isoprostanes, mediators of oxidative stress and injury.[4−7] Arachidonic acid oxidation principally occurs through enzymatic reactions catalyzed by cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 leading to the generation of numerous pro-inflammatory and notable anti-inflammatory resolving mediators.[4−7] The intricacies behind formation and breakage of double bonds and cyclic structures remain unclear. This in-depth novel information will perfect the development of strategies and drugs aimed at counteracting inflammation and promoting healing.[8]

## CYCLOOXYGENASE PATHWAY

Arachidonic acid oxidation via the cyclooxygenase pathways has been detailed[4−7,9−12] but remains obscure for many researchers. Cyclooxygenase 1 and 2 (COX-1 and -2) are also termed prostaglandin H synthase (PGHS)-1 and -2, because they direct synthesis of the prostaglandins. They are bifunctional (see below), membrane bound hemoproteins (heme iron-containing) that catalyze the first committed steps in prostanoid biosynthesis, the conversion of ARA to prostaglandin $G_2$ ($PGG_2$) via cyclooxygenase catalysis, and the subsequent reduction of $PGG_2$ to $PGH_2$ through a classic heme peroxidase mechanism. Both isoforms contain a histidine-ligated heme group, which reacts with peroxides to form a two-electron oxidized intermediate (Compound I). Compound I can then undergo an intramolecular electron transfer, oxidizing a nearby tyrosine residue, Tyr385, by abstracting its hydroxyl hydrogen with its electron, leaving the hydroxyl oxygen with an unpaired electron (radical).[9,13] This is different from oxidation by extracting the hydrogen as a proton and leaving the oxygen with a negative charge (Figure 1A).

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$$Fe^{3+}\ (\text{of the enzyme's heme group}) + H_2O_2 \rightarrow [Fe^{4+}{=}O]R'\ (\text{Compound I}) + H_2O$$

$$[Fe^{4+}{=}O]R' + \text{substrate} \rightarrow [Fe^{4+}{=}O]R\ (\text{Compound II}) + \text{oxidized substrate}$$

$$[Fe^{4+}{=}O]R + \text{substrate (Tyr385)} \rightarrow Fe^{3+} + H_2O + \text{oxidized substrate (Tyr385 radical)}$$

The tyrosyl radical (oxidized by the abstraction of the hydroxyl hydrogen and left with an unpaired electron, generated from this redox reaction) is exceptionally reactive and promptly starts the COX's activity by abstracting the *Pro-S* hydrogen on carbon 13 (C-13) of a molecule of ARA.[14] Carbon-13 is a methylene carbon ($-CH_2-$) bonded to two different substituents and is dubbed a prochiral carbon. When a tetrahedral carbon can be converted to a chiral center (having four different substituents) by changing only one of the attached groups, it is referred to as a "prochiral" carbon. The two hydrogens on the prochiral carbon are described as 'prochiral hydrogens'. The tyrosyl radical abstracts the hydrogen on C-13 that would lead to an *S* rather than an *R* configuration on that carbon. By such an abstraction, the tyrosyl radical converts back to tyrosine while exporting the radical unpaired electron to ARA forming an arachidonyl radical[9,13−15] (Figure 1B).

The stabilization of the arachidonyl radical requires a rearrangement of its double bonds creating a pentadienyl radical at C-11. The engagement of the two carbons adjacent to the radical carbon in the double bonds has a short stabilization effect until the COX peroxidase activity exerts itself by adding a molecule of oxygen to C-11 and thus exporting the radical electron to the oxygen atom not bonded to the carbon, creating a peroxyl radical at C-11[14−16] (Figure 1C).

The 11-peroxyl radical cyclizes by bonding the radical oxygen to C-9 and exporting the radical electron to C-8 that makes use of the pentadienyl structure to cyclize bonding to C-12, creating the five-membered endoperoxide ring[16] (a ring having an O−O bridge). This cyclization will export the radical electron to C-15 to benefit from the allylic stabilization effect, and by this, it creates a second center for molecular oxygen addition on this carbon creating a peroxyl radical at C-15[17] (Figure 1C).

Finally, this peroxyl radical is reduced using tyrosine, generating a tyrosine radical that would repeat the cycle while forming prostaglandin G2 (PGG2). By this final step, the cyclooxygenase cycle catalyzed by COX-1 bifunctional activity: peroxidase (adding molecular oxygen twice) and synthase (ring formation by creating a new carbon−carbon bond) ends (Figure 1C). By a simple reduction of the peroxyl group on C-15 to a secondary alcohol, catalyzed by COX-2, prostaglandin H2 (PGH2) is formed[14−16] (Figure 1C).

Prostaglandin H2 is highly unstable; hence, it is converted by monooxygenase enzymes, belonging to the family of cytochrome P450 isomerases, to other prostaglandins and thromboxanes, which exert considerable physiological and immunological effects.[9−12] COX-1 and -2 are thus the essential enzymes in the biosynthesis of prostaglandins, the main precursors of several other important biological molecules including thromboxanes. Aspirin's ability to irreversibly inactivate the COX enzymes leads to suppression of prostaglandin and thromboxane production. The aspirin acetyl group covalently attaches to a serine residue in the active site of

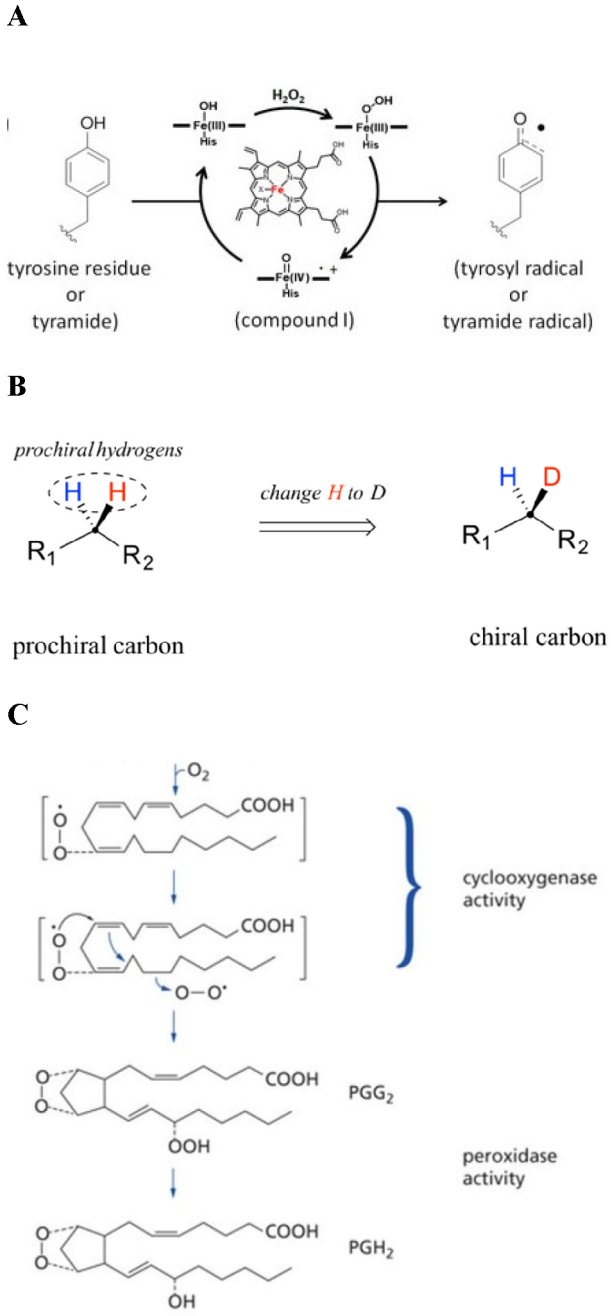


**Figure 1.** Tyrosine radical-catalyzed cyclooxygenase and peroxidase activities on arachidonic acid (ARA). (A) Generation of the tyrosyl radical by an intramolecular electron transfer undergone by heme-containing Compound I of cyclooxygenases (COXs). (B) Conversion of prochiral to chiral carbon by hydrogen abstraction. (C) COX-1 activities via first, adding two molecules of oxygen to ARA (peroxidase) and second, forming the endoperoxide ring (synthase).

the COX enzyme.[12,18,19] The acetylation of the COX's active site prevents the very first step of creating the tyrosyl radical that starts the cyclooxygenase cycle. Similarly to aspirin, the majority of the available nonsteroidal anti-inflammatory drugs (NSAIDs), especially those containing phenyl propionic or aryl acetic acid functional groups, is nonselective inhibitors of both COX's isoforms. Unlike aspirin, however, they form different chemical interactions with other protein residues of the active site of the enzyme. Ibuprofen uses its carboxylate to make an ion pair with an arginine and a tyrosine residue of the


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enzyme's constriction site, while flurbiprofen acts on the same amino acid residues but by forming hydrogen bonds. The aryl acetic acid-containing indomethacin forms a salt bridge with the same arginine and tyrosine residues, while its benzoyl group is stabilized by hydrophobic interactions with other residues.[20] Different from aspirin, the diaryl heterocyclic NSAIDs, like celecoxib, rofecoxib, and other coxibs, are mostly COX-2 selective inhibitors. They suppress the COX-2 activity by competitive reversible binding, followed by a time-dependent transition to a tightly bound enzyme−inhibitor complex where a valine residue of the enzyme's active site plays a key role.[19−21]

## LIPOXIGENASE PATHWAY

The arachidonate lipoxygenase family of enzymes comprises several members, prominent among which is arachidonate 5-lipoxygenase, ALOX5, a soluble, monomeric, nonheme iron-containing protein of 673 amino acids and molecular weight of ∼78 kDa. First, ALOX5 catalyzes the oxygenation of ARA ((5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate) to 5-hydroperoxyeicosatetraenoate (5-HpETE), followed by the epoxide formation of 5,6-epoxyeicosatetraenoate (leukotriene A4/LTA4), the first two steps in the biosynthesis of leukotrienes, which are potent mediators of inflammation.[4,5,11,12,22]

The enzyme possesses two catalytic activities. First, ALOX5 oxygenizes ARA via adding a hydroperoxyl ($HO_2$) residue to carbon 5 (C-5) of its 1,4-diene group (5*Z*,8*Z* double bonds), leading to formation of 5*S*-HpETE [5(*S*)-hydroperoxy-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid] (Figure 2A).

ARA oxygenation occurs in four consecutive steps: hydrogen abstraction, radical rearrangement, oxygen insertion, and peroxyradical reduction.[22] The iron of the ALOX5 in its ferric oxidation state (FeIII) will initiate the enzymatic activity of oxygenation by abstracting a hydrogen from C-7 of ARA with its electron leaving an unpaired electron on C-7 (radical) and achieving a ferrous oxidation state (FeII) by gaining this electron.[15,22] The reason behind the specific targeting of the hydrogen on C-7 of ARA for abstraction is the position of C-7 at the middle of a 1,4-pentadiene system formed from the double bonds at C-5 and C-8, which has a significant stabilizing effect on the radical formed by its ability to rearrange and export the radical to C-5[17] (Figure 2B).

The radical rearrangement paves the way for molecular oxygen insertion at C-5 creating a peroxyl radical, which will subsequently be reduced to form the hydroperoxide group in the fourth step of the oxygenation process by which 5*S*-HpETE is formed. The 5*S*-HpETE intermediate may then be released by the enzyme and rapidly reduced by cellular glutathione peroxidases to its corresponding alcohol, 5(*S*)-hydroxy-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid (5-HETE)[15,16,22] (Figure 2C).

Alternatively, 5*S*-HpETE is further metabolized by launching ALOX5's second activity. Epoxidase activity converts 5*S*-HpETE to its epoxide, leukotriene A4 (LTA4), via abstracting a hydroxy radical from the hydroperoxide group at C-5 and letting the other oxygen form the epoxide ring[15,22] (Figure 3).

LTA4 may then be acted upon by two separate, soluble enzymes. Leukotriene A4 hydrolase action leads to formation of the dihydroxyl product, leukotriene B4 (LTB4). LTC4 synthase or microsomal glutathione *S*-transferase 2 (MGST2) generates LTC4 via binding the sulfur of cysteine's thio (SH) residue of glutathione (glutamate-cysteine-glycine) to LTA4 carbon 6. γ-Glutamyltranspeptidase and a dipeptidase remove LTC4 glutamine and glycine residues to sequentially form LTD4 and LTE4[22] (Figure 4).

The transcellular ARA metabolism allows the action of platelet lipoxygenase 12 equal to lipoxin synthase upon LTA4 to yield the lipoxygenase interaction product known as lipoxin A4 (Figure 4), which, unlike the proinflammatory PG and LT, promotes resolution of acute inflammatory responses, especially in the airway, lung, and gastrointestinal tracts.[23−25]

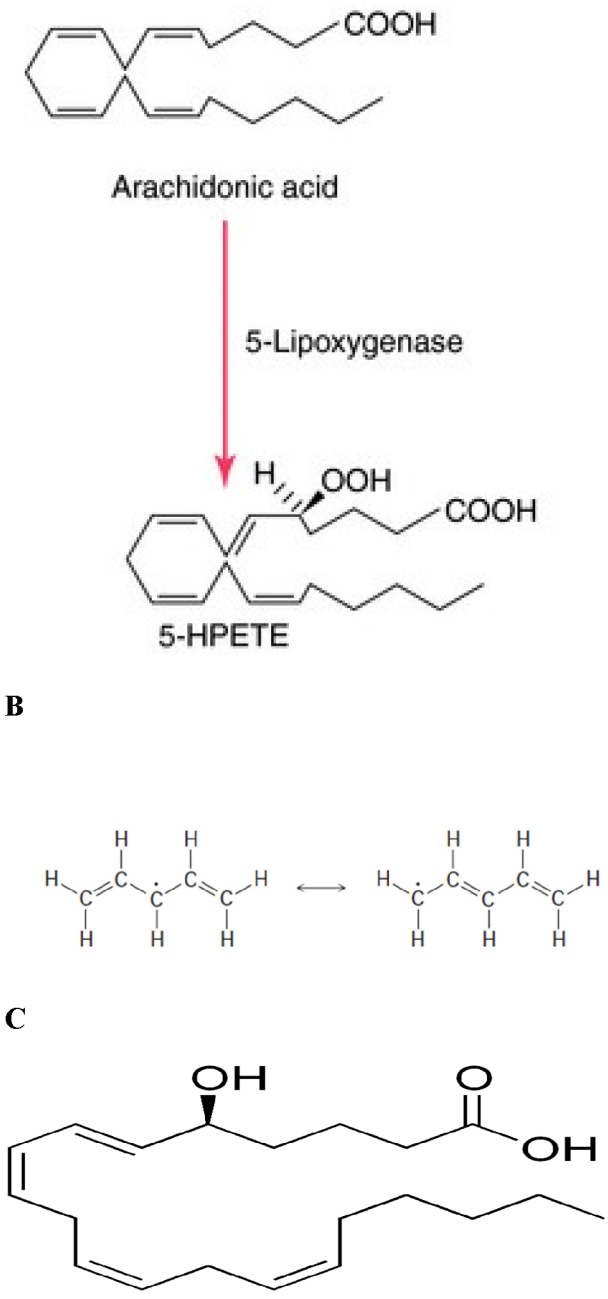


**Figure 2.** 5-Lipooxygenase activity on ARA for the biosynthesis of 5-HETE. (A) Dioxygenase activity of 5-lipooxygenase (ALOX5) via adding a hyroperoxyl residue to ARA at carbon 5 to form 5-HPETE. (B) Radical stabilization in the 1,4-pentadiene system after a central hydrogen abstraction. (C) Chemical structure of 5-HETE, a product of ALOX5 dioxygenase activity on ARA.



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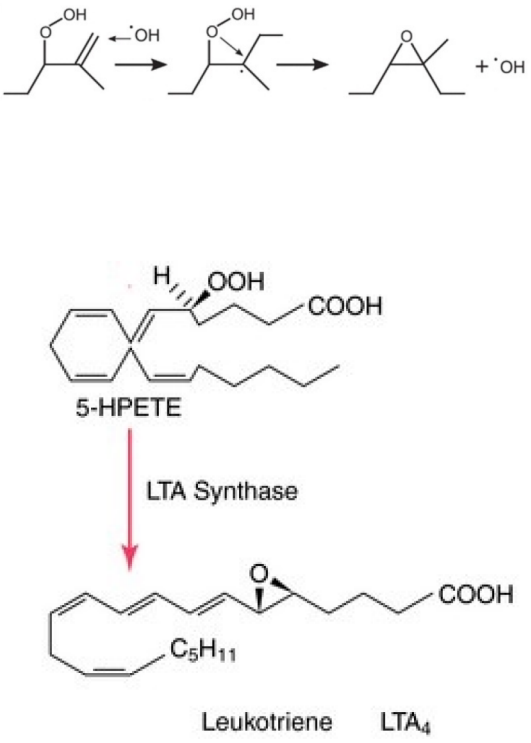


**Figure 3.** Leukotriene A4 biosynthesis by an epoxide ring formation.

## CONCLUSION

While many comprehensive research papers and review articles extensively covered the enzymatic oxidation of ARA through cyclooxygenase, lipoxygenase, and cytochrome P450 pathways, very few if any addressed the movement of electrons and protons throughout the various enzymatic reactions. This review aimed to clarify the intricacies of bond formation and breakage, ring closure and opening, and oxygenation and peroxidation that occur during the process of ARA oxidation. The types and mechanisms of organic reactions involved, bond rearrangements, and radical stabilization in the cyclooxygenase and lipoxygenase pathways have been investigated and explained. This work will be extended in a future review to also cover ARA oxidation through cytochrome P450 and to shed light on the intricacies of formation of the various ARA metabolites, an effort that could help develop strategies that counteract inflammation without the different side effects of the currently used NSAIDs.

## AUTHOR INFORMATION

### Corresponding Author

**Hatem Tallima** − *Department of Chemistry, School of Science and Engineering, American University in Cairo, New Cairo 11835, Cairo, Egypt;* orcid.org/0000-0003-3714-641X; Phone: 0020-100 6672 097; Email: htallima@aucegypt.edu

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.1c01952

### Notes

The author declares no competing financial interest.

### Biography

Hatem Tallima was a cum laude graduate of the Chemistry Department, School of Science and Engineering, The American University in Cairo in 2000. He obtained his M.Sc. and Ph.D. degrees in Biochemistry from the Faculty of Science, Cairo University in 2003 and 2006, respectively. He has authored 45 articles in international, peer-reviewed journals (https://pubmed.ncbi.nlm.nih.gov/?term=Tallima+H), *h* index 20. As Assistant Professor of Organic and Biochemistry at AUC and Senior Researcher at the Immunology

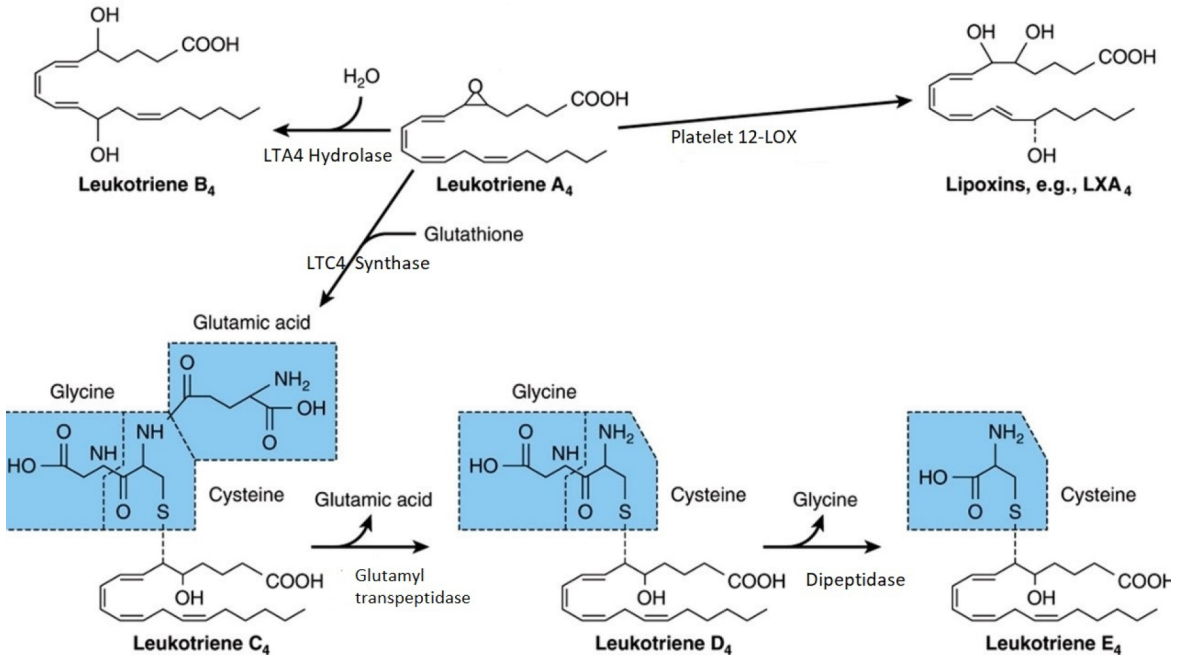


**Figure 4.** Biosynthetic pathways of leukotrienes and lipoxins. Leukotriene A4 (LTA4), the product of lipoxygenase activity on ARA, undergoes a series of conversions to form lipoxins via the action of platelet lipoxygenase 12 (platelet 12-LOX) or to form other leukotrienes, LTB4, LTC4, LTD4, and LTE4, via the action of LTA4 hydrolase, glutathione-*S*-transferase, glutamyl transpeptidase, and cysteinyl-glycine dipeptidase, respectively.



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Laboratories, Faculty of Science, Cairo University, he contributed to the development of a schistosomiasis drug: arachidonic acid and cysteine peptidase-based vaccines.

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