

Epoxyeicosatrienoic Acids, Hypertension, and Kidney Injury

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Kidney disease afflicts 33 million in the United States, and chronic kidney disease (CKD) accounts for >\$60 billion in Medicare costs.^{1,2} Hypertension afflicts 75 million in the United States, and significant portions of those patients develop CKD and progress to end-stage renal disease.¹⁻⁵ Interestingly, resistant hypertension which is defined as uncontrolled hypertension, despite 3 antihypertensive medication classes, increases the risk for cardiovascular diseases and end-stage renal disease.⁶ These recent findings in the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) highlights the fact that current treatments only slow the loss of kidney function, or have no benefit at all.^{5,6} New therapeutic approaches are urgently needed.

Development of drugs to increase a novel class of fatty acids, epoxyeicosatrienoic acids (EETs), represents a unique approach to treat hypertension and kidney disease. EETs are generated from the substrate arachidonic acid by cytochrome P450 (CYP) epoxygenase enzymes.^{7,8} There are 4 regioisomeric EETs formed: 5,6-EET; 8,9-EET; 11,12-EET; and 14,15-EET. These regioisomeric EETs are further metabolized to less active or inactive diols by the soluble epoxide hydrolase (sEH; *Ephx2*) enzyme. For clarity, EETs will be used as a general term and regioisomers mentioned when actions can be attributed to a specific regioisomeric EET. In the majority of circumstances, the primary EETs evaluated for cardiovascular and renal function have been 11,12-EET and 14,15-EET.⁷ Once formed, EETs act in an autocrine or paracrine manner to elicit biological responses. Vascular endothelial and renal epithelial cells are major sites for EET production.^{7,8} This localized EET generation aligns with the biological actions and contribution of EETs to cardiovascular and renal function. Prominent biological actions of EETs include their role as endothelial-derived hyperpolarizing factors and regulation of tubular sodium reabsorption by inhibiting epithelial sodium channel (ENaC) in the kidney.⁸⁻¹¹ These actions position EETs to increase blood flow to organs, decrease peripheral vascular resistance, and enhance sodium excretion. EETs also have anti-inflammatory actions that are beneficial in cardiovascular and renal diseases.^{7,12} The focus of this brief review is to discuss changes in EETs that contribute to hypertension and kidney injury and to discuss EET-based therapeutics being developed to combat cardiovascular and renal diseases.

Renal Disease

The link between decreased EETs and hypertension, especially salt-sensitive hypertension, has been strongly established.^{8,11,13-16} Decreased renal epoxygenase activity and decreased renal EET levels have been associated with angiotensin-dependent hypertension, salt-sensitive hypertension, and Lyon hypertensive rats.¹⁴⁻¹⁸ Transgenic rats overexpressing both human renin and angiotensinogen genes (dTGR) develop hypertension and renal failure that is associated with decreased kidney epoxygenase enzymatic activity and CYP2C11 and CYP2C23 protein levels.¹⁷ Likewise, we have found that an inability to increase renal cortical and vascular rat CYP2C11 and CYP2C23 or mouse *Cyp2c44* protein expression contributes to salt-sensitive hypertension.^{14,18} These CYP2C enzymes are primarily responsible for 11,12-EET and 14,15-EET formation in the rat and mouse kidneys.¹⁹ Rat CYP2C23 and mouse *Cyp2c44* are the predominant kidney epoxygenases which are upregulated by a high K⁺ (2.5%) or high Na⁺ (8%) salt diet.^{8,20} Another potential epoxygenase is the CYP2J5 protein that is abundantly expressed in the mouse kidney.²¹ However, the ability of CYP2J5 to generate EETs is questionable and *Cyp2j5*^{-/-} mice have demonstrated that CYP2J5 seems to contribute to blood pressure control by regulating estrogen rather than EET synthesis.²¹ However, genetic manipulation of CYP2C epoxygenase expression has provided additional support to the concept that CYP2C-derived EETs are essential in renal sodium handling and blood pressure regulation. *Cyp2c44*^{-/-} mice develop hypertension when fed a high K⁺ or high Na⁺ salt diet.^{8,11,22} Similarly, *Cyp4a10*^{-/-} mice have decreased renal *Cyp2c44* epoxygenase activity in response to high Na⁺ salt and develop salt-sensitive hypertension.²³ Differences in renal EET generation and blood pressure in response to dietary NaCl intake between the *Cyp2c44*^{-/-} mice and *Cyp4a10*^{-/-} mice provide additional evidence for a critical contribution for EETs in blood pressure regulation. Interestingly, *Cyp4a10*^{-/-} mice have decreased urinary EET levels and an elevated blood pressure on a normal salt (0.3% NaCl) diet.²³ Lowering dietary salt to 0.05% NaCl lowers blood pressure in *Cyp4a10*^{-/-} mice.²³ In contrast, *Cyp2c44*^{-/-} mice do not have decreased urinary EET levels or elevated blood pressures on a normal salt diet.¹¹ Both *Cyp2c44*^{-/-} and *Cyp4a10*^{-/-} mice demonstrate salt-sensitive hypertension in response to 8% NaCl feeding, which is

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associated with an inability to increase renal EET generation. The fact that amiloride lowers blood pressure in *Cyp2c44*^{-/-} and *Cyp4a10*^{-/-} mice fed a high-salt diet suggests a significant contribution for ENaC.^{11,22,23}

A major cellular mechanism responsible for salt-sensitive hypertension that results from decreased renal EET levels seems to be increased ENaC activity (Figure 1).^{8,11,22} Actions of 11,12-EET on basolateral inwardly rectifying K⁺ channels and apical ENaC channels on the cortical collecting duct epithelium can explain the salt-sensitive blood pressure regulation in response to high K⁺ or Na⁺ salt diets. Hypertensive *Cyp2c44*^{-/-} mice show a hyperactive ENaC and reduction in ERK1/2 and ENaC subunit phosphorylation.^{8,11} In regard to EET regioisomeric actions on ENaC, 11,12-EET inhibits ENaC to a greater extent than 14,15-EET and 8,9-EET had no effect on ENaC activity.¹¹ 11,12-EET can inhibit basolateral inwardly rectifying K⁺ channels that results in cell membrane depolarization to reduce the driving force for Na⁺ entry across the apical membrane.^{8,20} Another renal epithelial cell action attributed to 11,12-EET is stimulation of apical large-conductance Ca²⁺-activated K⁺ epithelial channels that could contribute to renal K⁺ secretion in response to high K⁺ intake.^{8,24,25} Interestingly, 11,12-EET is the major product of the mouse *Cyp2c44* and is generated in the cortical collecting duct and increases in response to a high K⁺ or Na⁺ salt diet.^{11,20} The inability of *Cyp2c44*^{-/-} mice to increase 11,12-EET in response to either a high Na⁺ or K⁺ diet and the lack of actions on K⁺ channels and ENaC in the cortical collecting duct results in salt-sensitive hypertension. Taken together, these findings clearly demonstrate a critical role for renal CYP2C enzymes in fluid and electrolyte homeostasis and blood pressure control.

Vascular Endothelial Dysfunction

EETs also contribute importantly to endothelial function in the pathology of hypertension and cardiovascular diseases (Figure 1).^{7,8} Numerous studies have shown that EETs are an endothelial-derived hyperpolarizing factor and are critical for proper regulation of resistance arteries and arterioles.^{7,9,10,26} EETs activate vascular smooth muscle cell large-conductance calcium-activated K⁺ channels (K_{Ca}) through a cAMP and

protein kinase A-dependent mechanism.^{27,28} Vascular expression of epoxygenase enzymes and generation of EETs is decreased in cardiovascular diseases.^{7,14,18,29} Decreased renal microvessel CYP2C11, CYP2C23, and CYP2J expression in the obese Zucker rat and in rats fed a high-fat diet is thought to contribute to increased blood pressure.²⁹ Vascular EET levels are further reduced by increased sEH expression in obese Zucker rats and this has been demonstrated to contribute to endothelial dysfunction.²⁹ Likewise, endothelial dysfunction and inflammation are associated with decreased plasma EET levels and increased sEH activity in humans with atherosclerotic disease.³⁰⁻³³ Reactive oxygen species that are elevated in hypertension can also reduce EET bioavailability and vasodilation in human coronary arterioles.^{34,35} Thus, decreased vascular EET levels significantly contribute to the progression of cardiovascular disease and organ damage in hypertension.

Inflammation

Inflammation is considered a major player in hypertension and the associated progression of kidney disease. Kidney-specific elevations in T cells have also been implicated in numerous animal models of hypertension.³⁶⁻³⁸ Recent studies have implicated kidney selective increases in tumor necrosis factor- α in the development of angiotensin II-dependent hypertension and associated kidney disease.³⁶ Likewise, a contribution for increased sEH activity and decreased EET levels has been demonstrated for the inflammation and renal injury associated with hypertension.^{7,18,22} On the flip side, increasing EET levels by genetic disruption of *Ephx2* decreased inflammation and attenuated the progression of renal damage associated with salt-sensitive hypertension.³⁹ Interestingly, expression of human *CYP2C8* or *CYP2J2* to increase mouse endothelial cell EET generation decreased blood pressure, enhanced vasodilatory responses, and decreased renal injury in angiotensin high-salt hypertension.²¹ These *CYP2C8* and *CYP2J2* transgenic mice or *Ephx2*^{-/-} mice also exhibited decreased vascular nuclear factor- κ B signaling and inflammation in response to endotoxin.⁴⁰ This is in agreement with the increasing amount of published data that EETs decrease vascular inflammation through inhibition of phospho-I κ B kinase-derived nuclear factor- κ B activation.^{7,12,39,40} Therefore, evidence indicates that decreased EETs or increased sEH activity contribute to the vascular inflammation and pathogenesis of renal injury in hypertension and that increasing EET bioavailability can counteract disease progression.

Human Polymorphisms

There is also evidence in humans that decreased EET levels contribute to hypertension. Human *CYP2C8* and *CYP2C9* are the major epoxygenases, whereas *CYP2J2* has both epoxygenase and ω -1 hydroxylase activity.⁴¹ Several *CYP2C8* and *CYP2C9* gene variants (*2C8*2*, *2C8*3*, *2C9*2*, and *2C9*3*) demonstrate reduced arachidonic acid epoxidation rates.^{30,41} Analysis of white and black cohorts failed to demonstrate an association between these variants and hypertension.⁴² However, the frequency of the *CYP2C9*3* allele was lower in a subset of Chinese women with hypertension.⁴³ A common polymorphism in the *CYP2J2* gene, *CYP2J2*7* allele reduces *CYP2J2* transcription, reduces plasma EET levels, and has

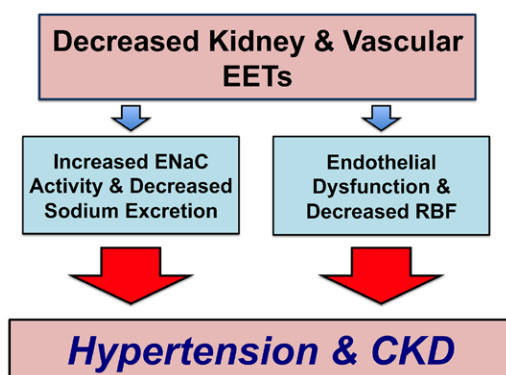


Figure 1. Cytochrome P450 epoxygenase metabolites, hypertension, and chronic kidney disease (CKD). Decreased epoxyeicosatrienoic acids (EETs) contribute to enhanced epithelial sodium channel (ENaC) activity, endothelial dysfunction, and decreased renal blood flow (RBF). These changes in kidney and vascular function contribute to hypertension and CKD.

been demonstrated to be associated with increased risk for essential hypertension in a Russian population.⁴⁴ However, other studies have found that *CYP2J2**7 allele associates with lower risk or no modification in the risk of developing hypertension.⁴⁵ Although polymorphisms of the sEH gene *EPHX2* have demonstrated associations to cardiovascular diseases, a majority of the studies have reported no association between *EPHX2* variants and essential hypertension.³⁰ Differences in the results of these genetic association studies could be attributed to factors, including ethnicity of the population studied, small cohorts, sex effects, and environmental factors.

Despite the discrepancies in the genetic population studies, there is more convincing evidence linking decreased EETs to hypertension when evaluating EET bioavailability and vascular responses. Genetic variations in *EPHX2* have been demonstrated to affect the magnitude of human forearm vasodilator responses.⁴⁶ There is a reduction in the forearm vasodilator response in white Americans who have the Arg55 variant allele, which increases sEH activity and would be expected to decrease EET availability⁴⁶; whereas, blacks who have the Gln287 variant allele that decreases sEH activity exhibit enhanced forearm bradykinin-mediated vasodilator responses.⁴⁶ Healthy human volunteers exhibit slightly reduced basal forearm blood flow in the presence of the CYP inhibitor fluconazole, whereas it did not alter radial artery blood flow in hypertensive patients in the presence or absence of nitric oxide inhibition.⁴⁷ In addition, fluconazole decreased local plasma EET levels in control but not in hypertensive patients.⁴⁷ Humans with hypertension also demonstrated decreased flow-mediated dilation, an indicator of endothelial dysfunction that was associated with a reduced EET levels.⁴⁸ These findings demonstrate that hypertensive patients where EET levels are genetically or pharmacological manipulated have vasodilator responses that differ from those of healthy volunteers. Thus in addition to nitric oxide, EET levels contribute importantly to endothelial function in hypertensive patients.

Overall, these experimental findings in rodents and humans have generated interest in developing pharmacological means to increase EETs that could potentially lower blood pressure and protect the kidney in hypertension.

Therapeutic Approaches: Hypertension and Kidney Diseases

During the past decade, EET and sEH enzyme-based drugs have been developed with antihypertensive and kidney protective properties that will be particularly beneficial for hypertensive patients that develop CKD (Figure 2).^{49,50} Carbamate urea sEH inhibitors were developed and demonstrated to lower blood pressure and decrease renal injury in animal models of hypertension.^{15,18,49} Further development of sEH inhibitors progressed rapidly and has resulted in clinical trials for hypertension, diabetes mellitus, and more recently, chronic obstructive pulmonary disease.^{49,51} This development of sEH inhibitors has been extensively chronicled in several excellent review articles.^{49,52,53}

More recent developments with sEH inhibitors are keeping enthusiasm for their potential use in hypertension and CKD at a high level. In a recent controlled clinical trial with peripheral arterial disease participants that were fed flaxseed containing

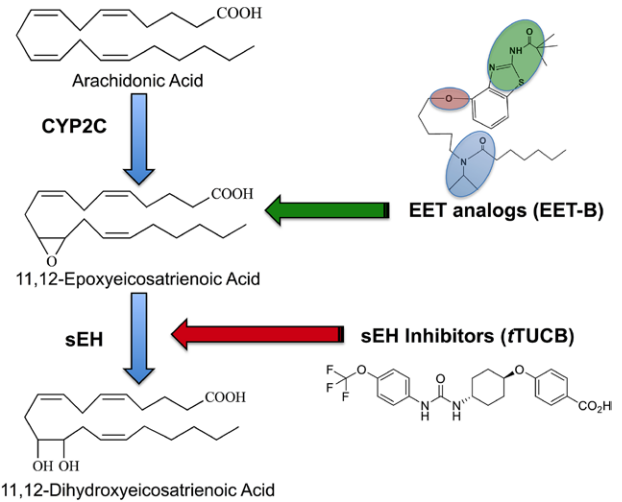


Figure 2. Therapeutic manipulation of epoxygenase metabolites. Arachidonic acid is converted to epoxyeicosatrienoic acids (EETs) by cytochrome P450 (CYP2C) epoxygenase enzymes. EETs primary metabolic fate is conversion to dihydroxyeicosatrienoic acids by the soluble epoxide hydrolase (sEH) enzyme. EET analogs and sEH inhibitors are 2 therapeutic approaches being tested to combat hypertension and kidney injury. EET-B has 3 structural attributes: (1) an acidic or hydrogen-bonding replacement (green) for the C(1)-carboxylate to avoid esterification and β -oxidation, (2) a *cis*- $\Delta^{8,9}$ -olefin or equivalent (red), and (3) an epoxide isostere (mimetic; blue) to obviate sEH metabolism.

α -linolenic acid for 6 months had decreased blood pressure.⁵⁴ α -Linolenic acid was demonstrated in an inhibitor screening assay to decrease sEH activity and the antihypertensive effects of flaxseed feeding were associated with a decrease in plasma sEH-derived oxylipins.⁵⁴ As for CKD, a recently published study demonstrated that *Ephx2* deficiency or sEH inhibition in mice decreased renal inflammation and fibrosis associated with unilateral ureteral obstruction.⁵⁵ The anti-inflammatory and fibroprotective effects in unilateral ureteral obstruction kidneys was via peroxisome proliferator-activated receptors activation and downregulation of nuclear factor- κ B, transforming growth factor- β 1/Smad3 inflammatory signaling.⁵⁵ Another of the more recent findings is that dietary fatty acid composition can enhance the effectiveness of sEH inhibitors in cardiovascular diseases.⁵⁶ Fish oil or ω -3 polyunsaturated fatty acid diet rich in eicosapentaenoic acid and docosahexaenoic acid coupled with sEH inhibitors lowers blood pressure and provides superior anti-inflammatory effects in angiotensin II-dependent hypertension.⁵⁶ Eicosapentaenoic acid-derived epoxyeicosatetraenoic acids and docosahexaenoic acid-derived epoxydocosapentaenoic acids are of particular interest because these epoxygenase metabolites of ω -3 polyunsaturated fatty acid have been demonstrated to protect from coronary heart disease and atrial fibrillation.^{33,57,58} These newer findings suggest that other fatty acid epoxides could be beneficial and that sEH inhibitors still have promise for hypertension and kidney disease.

Significant recent advancements in the development of robust EET analogs that mimic the actions of endogenous EETs position them as a potential therapeutic for renal and cardiovascular diseases. First generation EET analogs were methyl esters and sulfonimide substitutions of the carboxylic

acid, which obviated esterification and resisted β -oxidation.⁵⁹ The next generation of EET analogs removed the 1,4-diene responsible for autooxidation and replaced the labile epoxide with bioisosteres that resist metabolism (Figure 2).^{59,60} Studies of the second generation of EET analogs assessing vascular inflammation and dilation resulted in the following structural requirements: an acidic carboxyl group, Δ 8-olefin bond, 20-carbon chain length, and a *cis* epoxide.^{59,60}

EET analogs have substantial promise for the treatment of kidney and cardiovascular diseases. One such EET analog that has been successfully used *in vivo* in rodents is the aspartic amide of 11-nonyloxy-undec-8(Z)-enoic acid, NUDSA.^{61,62} NUDSA has been found to decrease blood pressure, improve metabolic status in metabolic syndrome, and provide cardioprotection in ischemic injury.^{61–63} Overall, the effects of NUDSA are linked to its ability to reduce inflammation and cell death, supporting the notion that EET analogs could be beneficial in renal pathologies. In support of this notion, orally active EET analogs, EET-A and EET-B, were found to protect the kidneys from cisplatin-induced nephrotoxicity.⁶⁴ Attenuated nephrotoxicity correlated with reduced inflammation, oxidative stress, and decreased apoptosis through a reduction in Bcl-2 protein-mediated proapoptotic signaling, reduced renal caspase-12 expression, and reduced renal caspase-3 activity.⁶⁴ EET-A and EET-B have been shown to decrease blood pressure and prevent hypertensive renal injury dramatically.^{22,65} EET-A lowers blood pressure in angiotensin-dependent hypertension and in *Cyp2c44*^{-/-} mice with salt-sensitive hypertension.²² Additional findings demonstrated that EET-A inhibits ENaC activity in cultured cortical collecting duct cells and reduced kidney expression of ENaC subunits in angiotensin II hypertension.²² Interestingly, kidney protection in Dahl SS rats independent of blood pressure lowering was demonstrated after 2 weeks of EET-B treatment. EET-B decreased renal injury by reducing oxidative stress, endoplasmic reticulum stress, and macrophage infiltration.⁶⁵ There are 2 potential explanations for the lack of blood pressure by EET-B in the Dahl SS rats. First, EET-B does not inhibit ENaC in the same manner as EET-A.⁶⁵ Although EET-B treated Dahl SS rats had decreased macrophage infiltration, EET-B failed to lower kidney T-cell levels, which is known to be a major contributor to the elevated blood pressure in this animal model of salt-sensitive hypertension.^{37,38,65} Taken together, these diverse biological actions and development of oral EET analogs demonstrate their therapeutic potential for hypertension and CKD.

Perspectives

It is now established that a reduction in EETs can contribute to hypertension and the associated renal injury and that approaches to increase EETs have therapeutic potential. As with every therapeutic approach, there is always a down side that is of concern. In the case of EETs, that concern has been their angiogenic and tumorigenic actions.^{49,66,67} Although initial studies demonstrated that EETs or sEH inhibition enhanced angiogenesis, tumorigenesis, and resulted in metastasis; recent studies have shown that sEH inhibition or *Ephx2* gene deficiency inhibits inflammatory bowel tumor development and supports the notion that EETs can inhibit cancer by blocking inflammation.^{68,69} Interestingly, dual inhibition of cyclooxygenase-2 and sEH synergistically

inhibits primary tumor growth and metastasis by suppressing tumor angiogenesis.⁷⁰ EET analogs also failed to increase cultured tumor cell proliferation and did not interfere with the ability of cisplatin to kill tumor cells.⁶⁴ Although these findings do not eliminate the concern for unwanted tumorigenesis with EET-based therapies, this concern seems to be considerably less than originally thought.

Other considerations for blood pressure regulation and hypertension are differences in sEH and EET levels between males and females and central nervous system effects. Cerebral vascular sEH expression is higher in male mice and females have increased EET-mediated protection from ischemic injury when compared with males.^{71,72} Furthermore, sEH inhibition abolishes sex-specific differences in endothelial cell survival and ischemic brain injury.^{71,72} Brain sEH inhibition via intracerebroventricular delivery of AUDA (12-[[tricyclo[3.3.1.1.3,7]dec-1-ylamino]carbonyl]amino]-dodecanoic acid) increases blood pressure and heart rate in spontaneously hypertensive rats.⁷³ In contrast, neuronal-specific expression of sEH to increase activity 3-fold failed to increase arterial blood pressure in mice.⁷⁴ Sex differences have also been found with regard to blood pressure regulation. Basal blood pressure in *Ephx2*^{-/-} mice was lower in males but not in females when compared with wild-type mice.⁷⁵ This decrease basal blood pressure in male *Ephx2*^{-/-} mice has not been observed when other colonies on various genetic backgrounds were generated.^{49,76} More recently, renal vascular EET levels were higher in female spontaneously hypertensive rats compared with that in males.⁷⁷ In this study, 10-day treatment with the sEH inhibitor AUDA increased EET levels but did not lower blood pressure in either male or female spontaneously hypertensive rats.⁷⁷ This finding is consistent with previous studies that have found variable effects of sEH inhibition on blood pressure in the spontaneously hypertensive rats.⁴⁹ These experimental findings highlight the need to consider brain actions of EETs and sex-specific actions of EETs when evaluating sEH inhibitors and EET analogs for hypertension and CKD.

The further development of EET analogs will be greatly enhanced if protein targets and receptors for EETs can be identified. Although the identity of EET-binding sites/receptors remain elusive, EETs activate renal and coronary vascular smooth muscle cell K_{Ca} channels through G protein (*Gas*)-dependent mechanism.^{9,10,26,27,78,79} Other investigations provide evidence that cAMP and protein kinase A are key signaling molecules required for K_{Ca} channel activation.^{26–28} Likewise, endothelial cell action of 11,12-EET are protein kinase A-dependent and require the Gs protein.⁸⁰ There are also differences in potency and activity when comparing 11,12-EET and 14,15-EET in various vascular tissues.^{7,9,10} 11,12-EET is more potent than 14,15-EET in renal arterioles, whereas rat mesenteric resistance arteries respond similarly to 11,12-EET and 14,15-EET.⁷ In addition, mesenteric resistance artery flow-induced dilation was inhibited by the 14,15-EET antagonist, 14,15-DHE5ZE, but unchanged by the 11,12-EET antagonist and 11,12,20-THE8ZE.⁸¹ These findings suggest unique biological activities and the potential for multiple vascular EET-binding sites/receptors.

Recent studies on the contribution of EETs to inflammation, kidney function, and blood pressure regulation in hypertension

have shed light on their potential as a target for therapeutic intervention. Thus, there is a bright future for sEH inhibitors and EET analogs as novel therapies to treat hypertension and stop the progression of CKD to renal failure effectively.

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Disclosures

Dr Imig has patents and patent applications that cover the composition of matter for epoxyeicosatrienoic acid analogs.

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