Angiotensin converting enzyme inhibitors in the treatment of hypertension

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Angiotensin converting enzyme (ACE) catalyses the conversion of angiotensin I (Ang I) to angiotensin II (Ang II). The ACE activity directly related to hypertension as Ang II is the blood pressure regulating hormone. Therefore, ACE inhibitors are a major class of antihypertensive drugs. Captopril, chemical name, was the first orally active ACE inhibitory antihypertensive drug, discovered in 1977. Since then, a number of such drugs have been synthesized. Enzyme-inhibitor bound crystal structural studies reveal a great deal of understanding about the interactions of the inhibitors at the active site of ACE. This can be helpful in the rational design of ACE inhibitors. With the advancement of the combination therapy, it is known that ACE inhibitors having antioxidant activity can be beneficial for the treatment of hypertension. This study describes the development of ACE inhibitors in the treatment of hypertension. Importance of ACE inhibitors having antioxidant activity is also described.

Keywords: Angiotensin converting enzyme, angiotensinogen, hypertension, rennin angiotensin system.

Introduction

BLOOD pressure (BP) is a quantitative term and is highly variable from person to person. The normal BP for an individual should be 120/80 mm Hg. According to a WHO, if BP exceeds 140/90 mm Hg, it is classified as the case of 'high BP' (hypertension)[1]. Several factors are responsible for the increase in BP such as kidney diseases, obesity, insulin resistance, high alcohol intake, high salt intake, ageing, etc. All these effects result in increased BP levels. Genetic factors are also an important cause of hypertension. One of the major causes of hypertension is the malfunctioning of the renin angiotensin system (RAS)[2,3]. RAS consists of a number of peptides that are important in regulation of BP. However, decapeptide angiotensin I (Ang I) and the octapeptide angiotensin II (Ang II) are particularly important as they directly influence BP. Ang I is a prohormone, which is converted to active hormone Ang II by the cleavage of terminal dipeptide (His-Leu)[4]. This reaction is catalysed by a zinc-dependent metalloenzyme known as 'angiotensin converting enzyme' (ACE). Further, ACE is also responsible for the elevation of BP by cleaving the terminal dipeptide (Phe–Arg) of vasodilator hormone bradykinin to its inactive form (bradykinin 1–7, Figure 1)[5,6].

Involvement of RAS in elevation of BP was reported for the first time by Tigerstedt and Bergman[7], who have shown that the saline extract of kidney contains some vasopressor (material that increases BP) activity. It was named 'renin' as it was extracted from kidney. In 1940s, Braun-Mendez and co-workers[8] discovered that renin catalyses the formation of the actual pressor agent, the 'angiotensinogen' (also called hypertensinogen). However, after the separation of two peptide fragments Ang I and Ang II, Skeggs *et al.*[4] discovered that it is not the angiotensinogen, but the peptides that actually elevate BP. They also discovered that the conversion of Ang I to Ang II is catalysed by ACE[9]. However, it took more than a decade for Ng and Vane[2] and Oparil *et al.*[3] to validate that ACE catalyses the conversion of Ang I to Ang II. During this period, ACE was first extracted in its pure form[2,3,10]. ACE exists in the peripheral vasculature, proximal renal tubular cells and the vascular endothelium of the lung. Wong *et al.*[11] and Timmermans *et al.*[12] demonstrated that Ang II acts through two G-protein-coupled receptors, AT_1 and AT_2. The deleterious effects of Ang II (e.g. vasoconstriction and cardiac and vascular hypertrophy) are mediated by the AT_1 receptor, whereas the AT_2 receptor generally mediates opposing effects.

In the classical pathway of RAS (Scheme 1), renin, which is secreted from the juxtaglomerular apparatus[13] of the kidneys in response to a variety of stimuli, acts on the circulating precursor angiotensinogen[14] (23-amino acid peptide) to generate a number of small peptides including the most important decapeptide, Ang I. This peptide has a minor effect on BP. However, cleavage of the C-terminal dipeptide (His-Leu) of Ang I generates the octapeptide hormone Ang II, which interacts through AT_1 and AT_2 receptors. Further, angiotensinase cleaves Ang I or Ang II to smaller peptide fractions[15,16]. Although some of the small peptides (e.g. Ang III) has vasopressor activity, Ang II is the main vasoconstrictor hormone in the RAS system.

In 1926, Petroff[17] discovered that extracts from urine or pancreas contain a hypotensive substance, which was later named as 'kallikrein' by Frey *et al.*[18]. About a decade later, Werle[19] discovered that kallikrein releases a labile vasoactive peptide from plasma protein and was

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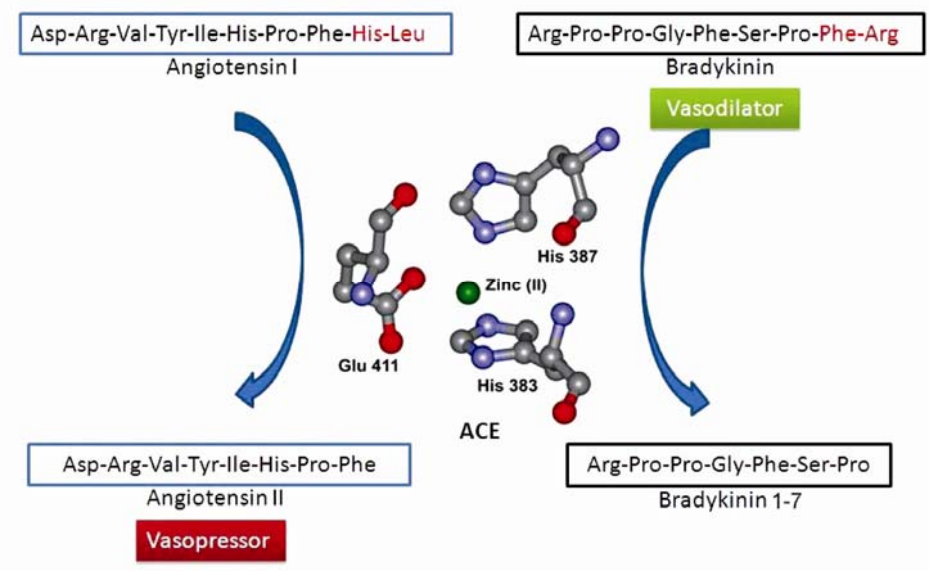
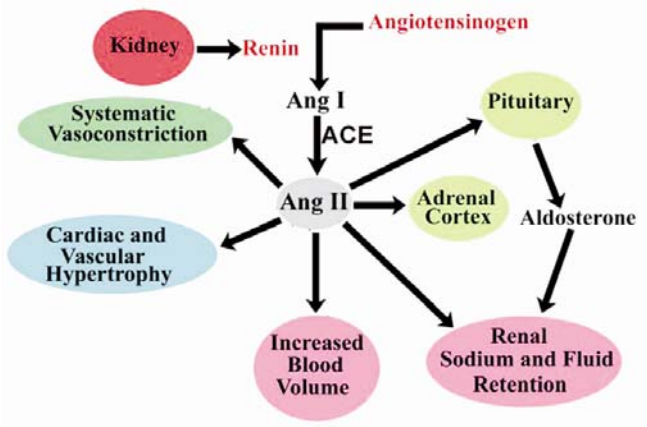


Figure 1. ACE-catalysed conversion of Ang I to Ang II and bradykinin to bradykinin (1–7). Formation of Ang II and depletion of bradykinin concentration result in the elevation of BP.

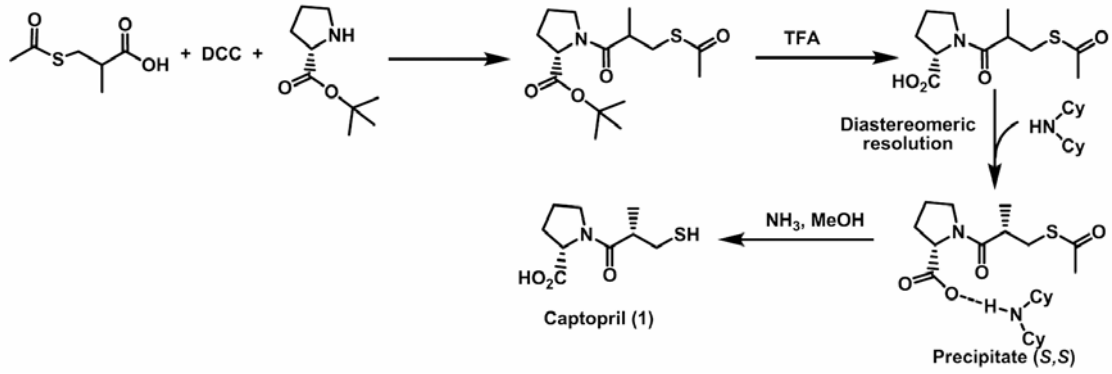


Scheme 1. RAS is responsible for the production of BP regulating hormone Ang II.

identified as kallidin (Lys[1]-bradykinin). The enzyme, kinase II degrades bradykinin to its inactive form[6]. The whole system was named as the 'kallikrein–kinin system' (KKS). Initially, two different research groups worked on the RAS and KKS, possibly being unaware that they were working on identical research problems. The connection was drawn by Erdös *et al.*[20–22] who discovered that kinase II and ACE are the same enzyme. This discovery has not been easily accepted as the Ang II formation is a highly chloride ion-dependent process, whereas bradykinin inactivation is not[23,24]. However, several years later, it was proved that their hypothesis was correct and it was postulated that there are two domains in ACE (N-terminal and C-terminal domains)[25]. The C-terminal domain is highly chloride ion-dependent, which catalyses the conversion of Ang I to Ang II (Figure 1). Although the N-terminal domain active site is similar to that of C-terminal domain, it catalyses the inactivation of bradykinin and is not anion-dependent. The amino acid sequence of ACE was deduced by Soubrier *et al.*[26] from the nucleotide sequence of DNA complementary to ACE and found that it has a HEXXH + E binding motif for the active site zinc(II) binding. The enzyme exists in two isoforms[27,28], somatic ACE (sACE) and testicular ACE (tACE); which are transcribed from the same gene in tissue-specific manner. sACE exists as a single large polypeptide chain of 1277 amino acid residues, whereas tACE exists as a lower mass glycoprotein of 701 amino acid residues. The sACE exists as two homologous domains (N- and C-domain) with two conserved active sites, whereas tACE is a single domain protein. Further, while tACE catalyses the conversion of Ang I to Ang II, sACE catalyses the conversion of bradykinin to bradykinin (1–7).

ACE inhibitors

In the 1960s, scientists studying RAS and KKS realized that inhibition of ACE is important for the treatment of high BP. A breakthrough was achieved in 1965 when Ferreira[29] for the first time described that the snake venom of *Bothrops jararaca* exhibits some bradykinin-potentiating activity. In 1968, Bakhle[30] discovered that the same extract inhibits the formation of Ang II from Ang I *in vitro*. Two years later, Ng and Vane[31] demonstrated the ACE inhibitory effect of the snake venom *in vivo*. The venom extract was first purified at the Squibb Institute for Medical Research and the most potent ACE inhibitor was identified as a nonapeptide (teprotide)[32,33]. This nonapeptide was administered intravenously as an



Scheme 2. Synthesis of captopril as reported by Cushman and Ondetti[35].

antihypertensive drug. However, an oral administration of this peptide was not possible. This led to a search for an oral antihypertensive drug correlated to the structure and activity of snake venom. In 1976, Cushman and Ondetti synthesized Captopril (**1**)[34,35], the first orally active ACE inhibitor. Captopril is a competitive inhibitor of ACE and contains a proline residue for the binding at the enzyme active site and a thiol moiety for coordination to zinc(II). It was approved by the Food and Drug Administration, USA for treatment of human hypertension in 1981. Cushman and Ondetti synthesized captopril by following the procedure given in Scheme 2. Diastereomeric resolution of the optical centre was one of the major steps in the synthesis as it was observed that the compound having (*S*, *S*) configuration exhibits ~3 orders of magnitude better ACE inhibition behaviour than the (*R*, *S*) diastereomer[35]. It was achieved by treating the diastereomer with dicyclohexyl amine in the presence of chloroform and acetonitrile. It should be noted that the (*S*, *S*) diastereomer precipitates first owing to the difference in solubility, leaving the other conformer in solution.

Since the discovery of captopril, several related compounds such as zofenopril (**2**), enalapril (**3**), fosinopril (**4**), lisinopril (**5**), ramipril (**6**), tandolapril (**7**), perindopril (**8**), spirapril (**9**), rentiapril (**10**), alacepril (**11**), benzapril (**12**), quinapril (**13**), moexipril (**14**), cilazapril (**15**) were developed as ACE inhibitors on the basis of structure-based drug design and are successfully used as antihypertensive drugs[36–38]. Most of these inhibitors contain a proline (or derivative) residue as shown in Figure 2.

ACE inhibition activity can be measured either *in vitro* or *in vivo*. *In vitro* studies demonstrate the conversion of Ang I to Ang II with the help of various separation and spectroscopic techniques. Another *in vitro* method for analysing ACE activity involves the conversion of Hip (hippuryl)-His-Leu to hippuric acid and His-Leu peptides as shown in Scheme 3. Hip-His-Leu is a tripeptide analogue of Ang I and, can be synthesized in large quantities. Further, the K_m value observed for the ACE-mediated catalysis for this tripeptide is ~20 times greater than that of Ang I[39]. It should be noted that the ACE activity is very

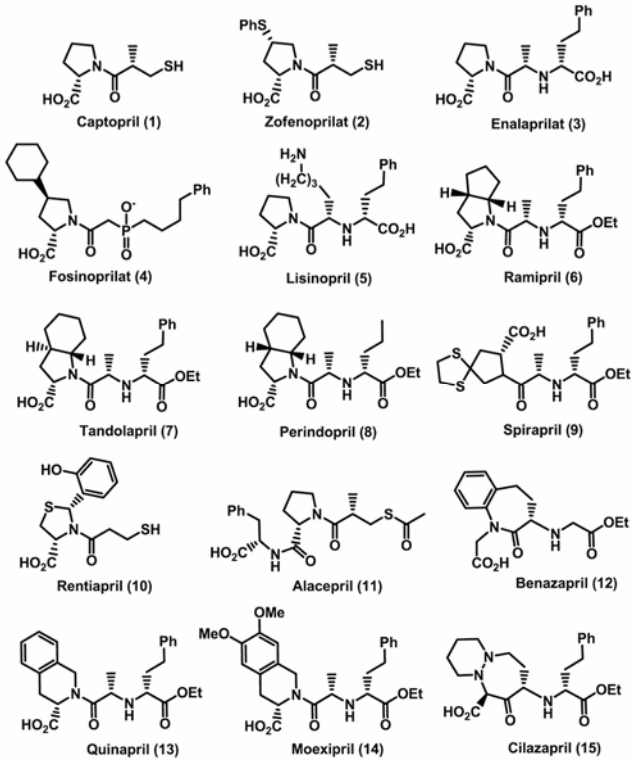
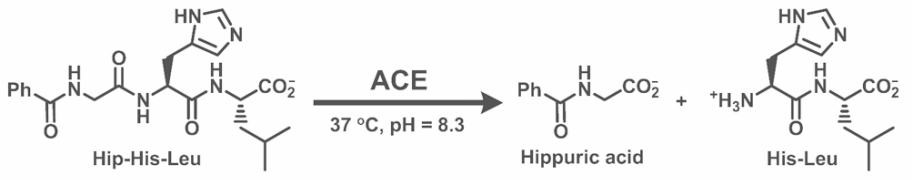


Figure 2. Some of the ACE inhibitors used as antihypertensive drugs.

specific to temperature and pH even under *in vitro* conditions. This enzyme shows optimal activity at 37°C at a pH of 8.3. Radiochemical assays are used as one of the main tools for *in vivo* studies of ACE activity[40].

Binding of ACE inhibitors at the active site

ACE inhibitors can be classified on the basis of their interaction with the active site zinc(II) centre. Inhibitors such as captopril, zofenopril, rentiapril, alacepril bind to the metal centre through the thiol moiety, leading to the formation of zinc(II)-thiolates. Compounds such as enalapril, lisinopril, ramipril, spirapril, etc. interact through



Scheme 3. Hydrolysis of Hip-His-Leu tripeptides by ACE.

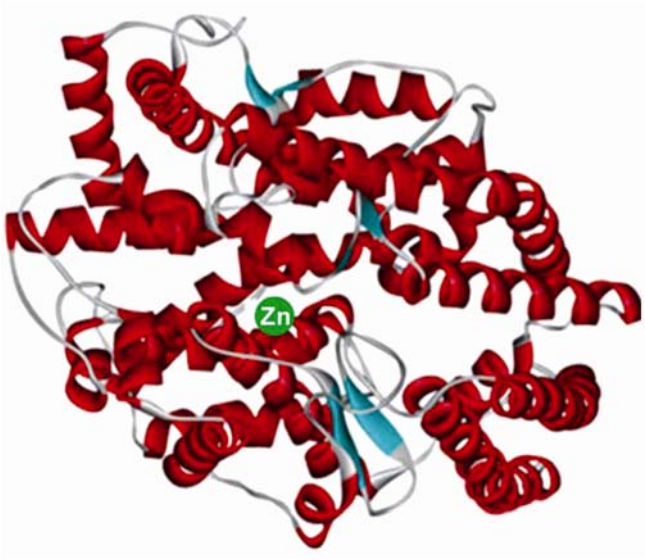


Figure 3. Secondary structure of tACE determined by X-ray crystallographic studies[41].

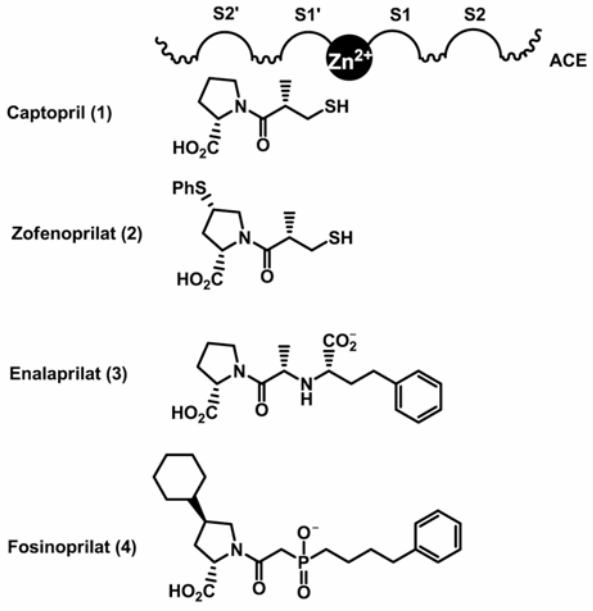


Figure 4. Binding of captopril and its analogues to the ACE active site.

the carboxylate moiety and fosinopril interacts through the phosphate group. In addition to the coordination with the active site metal centre, these inhibitors interact with the binding pockets present at the active site of ACE. Therefore, the side chains and stereochemistry of inhibitors play a crucial role. The structural detail of inhibitor bound lisinopril was first reported by Acharya and co-workers[41]. Since then, a number of ACE-bound inhibitor crystals have been reported[42–46]. From the crystal structure of the enzyme (Figure 3), it was found that the enzyme consists of 27 helices (96% of the total amino acid residues) and six relatively short β-strands. The overall shape is ellipsoid (approx. dimension, $72 \times 52 \times 48$ Å) with a central groove that extends for about 30 Å into the active site and divides the enzyme into two sub-domains. The cavity is covered by four helices and a β-strand. Three of these helices contain charged amino acid residues and restrict the access of larger polypeptides to the active site cleft. Two chloride ions are bound to the interior of the enzyme with a highly ordered active site containing zinc(II) ion. The active site containing zinc(II) is bound to HEXXH + E motif (His 383, His 387 and Glu 411) with a water coordination at the fourth position.

There are four binding subsites (S1, S2, S1′ and S2′) present at the active site of the enzyme. Captopril binds competitively to the Zn(II) centre through the thiolate coordination (Zn–S distance, 2.32 Å). The central carbonyl group between the thiol moiety and the terminal praline residue is positioned by two strong hydrogen bonds from the two histidines (His 513, 2.69 Å; His 353, 2.54 Å). The captopril–ACE complex is further stabilized by another interaction through one of the oxygen atoms of the proline carboxylate with Tyr 520 (2.66 Å), Gln 281 (3.1 Å), and Lys 511 (2.73 Å)[44]. tACE and N- and C-domains of sACE have different amino acid residues at these subsites[44–46]. However, the nature of the amino acid side chains in these subsites are similar in all these active sites. The interaction of inhibitors at these subsites depends on the nature of the side chains. For example, captopril (**1**) interacts with subsite S1′ and S2′ through central carbonyl and the proline residue respectively[44]. Similar interactions were observed in the zofenopril (**2**)–ACE complex. However, in the case of enalapril (**3**) and fosinopril (**4**), there are evidences of additional interactions at subsites S1 and S2 through the aromatic residue[44,45]. For example, in the case of enalapril, the aromatic residue stabilizes the enzyme–inhibitor complex by interacting with the hydrophobic pocket generated by Phe 512 and Val 518 residues. Figure 4 provides a pictorial representation of the ACE–inhibitor interactions.

ACE inhibitors in the treatment of hypertension

In addition to ACE inhibitors, compounds such as diuretics, beta-blockers, Ang II receptor antagonists and calcium channel blockers are used in the treatment of hypertension. Among these compounds, ACE inhibitors are considered as the safest class of antihypertensive drugs[47]. However, a combination of such drugs is considered to be more effective in the treatment of hypertension[47]. For example, combination therapy of two diuretics, diuretic-beta blockers, ACE inhibitor-beta blockers, Ang II receptor antagonists–diuretics, etc. are some of the commonly used drugs in combination therapy. All these different drugs have their own advantages and limitations depending on the nature and cause of hypertension. Recently, it was demonstrated that hypertension is associated with another disease state called oxidative stress[48–52]. 'Oxidative stress' is a condition caused mainly by an imbalance between the production of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damages[53,54]. Small amounts of highly reactive oxidants such as superoxide radical anion ($O_2^{\bullet -}$) hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2), etc. are essential for an organism to perform various metabolic cycles. The cellular concentration of these oxidants is maintained by antioxidants, which undergo oxidation to prevent the oxidation of other molecules. Mammalian enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase and cofactors such as glutathione (GSH) are mainly involved in the natural antioxidant defence mechanism.

Hypertension and oxidative stress

ROS and reactive nitrogen species (RNS) play a crucial role in the pathogenesis of various cardiovascular disease states[48] such as inflammation, ischemia–reperfusion, coronary artery diseases, atherosclerosis, diabetes, hypertension, etc. ROS such as superoxide radical anion ($O_2^{\bullet -}$) react with the nitric oxide radical ($^{\bullet}NO$), thereby decreasing the cellular $^{\bullet}NO$ concentration. The maintenance of the concentration of nitric oxide is important as it is the endothelial-derived relaxing factor[49] and a neurotransmitter. Further, the reaction of these two reactive species generates peroxynitrite (PN, $ONOO^-$)[50], which is considered as both ROS and RNS. It nitrates several tyrosine residues in proteins leading to cardiovascular remodelling. PN also oxidizes arachidonic acid[51,52], a precursor for production of vasodilating hormone prostaglandin.

Ang II can directly modulate vascular cell growth, differentiation and gene expression pathways. Further, Ang II-mediated hypertension is also known to be closely associated with oxidative stress[55,56]. It is well established that there is an overexpression of NADPH oxidase genes owing to overproduction of Ang II, which induces NADPH oxidase enzyme to release excess of $O_2^{\bullet -}$ (refs 57–59). As a result, there is an overproduction of PN, leading to protein nitration. Ang II also contains a tyrosine residue and is susceptible to PN-mediated nitration. Nitration of Ang II leads to a complete loss of its vasoconstricting effect *in vivo*[60]. However, nitration of Ang II leads to endothelial dysfunction and is associated with other cardiovascular complications[48]. Treatment of hypertensive animals/humans with a combination therapy of ACE inhibitors and vitamin C or E reduces their cardiovascular risk factor. Owing to the antioxidant effect of vitamin C and E, which scavenge free radicals in lipid membranes and cytosol[61]. Further, there are also evidences that ACE inhibitors such as captopril exhibit some beneficial effects in the treatment of myocardial ischemia-reperfusion[62,63]. This led to studies on the beneficial effects of sulphur-containing ACE inhibitors. A comparison of the antihypertensive action of ACE inhibitory drugs captopril (**1**), zofenopril (**2**) and fosinopril (**4**) illustrates that zofenopril is a better antihypertensive drug than captopril followed by fosinopril. The better antihypertensive action of zofenopril results from its ability to scavenge ROS[64,65]. Similarly, zofenopril has been shown to be a better antihypertensive drug than enalapril (**3**) owing to its antioxidant activities, although the latter is a better ACE inhibitor. Zofenopril and captopril having a sulphur moiety can readily be oxidized to sulphoxides, and therefore, can provide better protection against oxidative damage.

Recently, we have reported the synthesis and inhibition studies of ACE activity and PN-mediated nitration of peptides by a series of compounds having selenium moiety (compounds **16–19**, Figure 5)[66,67]. It is observed that the selenium analogue of captopril (**16**, IC_{50}: 36.4 ± 1.5 nM) shows ACE inhibition activity similar to that of captopril[66] (IC_{50}: 18.1 ± 1.0 nM). However, selenium compounds are better scavengers of PN than their sulphur

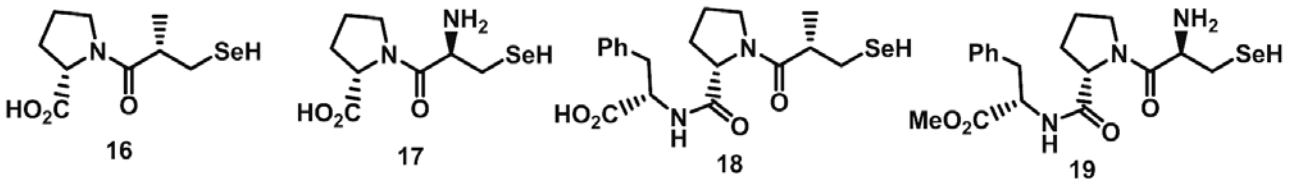


Figure 5. Selenium-containing compounds that exhibit both ACE inhibitory and antioxidant activities.

Table 1. IC_{50} values for inhibition of PN-mediated nitration of Ang II by compounds **1**, **16–19** (refs 66 and 67)

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
1	25.6 ± 0.9	**16**	2.2 ± 0.1
17	4.5 ± 0.2	**18**	6.0 ± 0.5
19	7.0 ± 0.1		

analogues. It should be noted that PN can nitrate the Tyr residues in proteins and peptides. As Ang II contains a Tyr residue, effect of these ACE inhibitors on the inhibition of PN-mediated nitration of Ang II has been studied. The IC_{50} values obtained for the inhibition of PN-mediated nitration of Ang II by these compounds are listed in Table 1. The effect of various substituents on Se–captopril was similar to that of captopril. For example, captopril and Se–captopril are competitive inhibitors of ACE. Similar to captopril, the *S*, *S* isomer of Se–captopril is ~200 times more potent as inhibitor of ACE than the *R*, *S* isomer. These observations indicate that the binding of Se–captopril at the active site of ACE may be similar to that of captopril. Although selenium was considered to be a poison for a long time, it is now considered as an essential trace element in mammals. Selenium is mainly associated with the antioxidant enzymes such as GPx, where selenocysteine is the active site residue. Therefore, detailed pharmacological investigation for these compounds is essential for the development of a new class of combination therapy for hypertensive patients.

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