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PAPER

Antioxidant activity of peptide-based angiotensin converting enzyme inhibitors†

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Angiotensin converting enzyme (ACE) inhibitors are important for the treatment of hypertension as they can decrease the formation of vasopressor hormone angiotensin II (Ang II) and elevate the levels of vasodilating hormone bradykinin. It is observed that bradykinin contains a Ser–Pro–Phe motif near the site of hydrolysis. The selenium analogues of captopril represent a novel class of ACE inhibitors as they also exhibit significant antioxidant activity. In this study, several di- and tripeptides containing selenocysteine and cysteine residues at the N-terminal were synthesized. Hydrolysis of angiotensin I (Ang I) to Ang II by ACE was studied in the presence of these peptides. It is observed that the introduction of L-Phe to Sec–Pro and Cys–Pro peptides significantly increases the ACE inhibitory activity. On the other hand, the introduction of L-Val or L-Ala decreases the inhibitory potency of the parent compounds. The presence of an L-Pro moiety in captopril analogues appears to be important for ACE inhibition as the replacement of L-Pro by L-piperidine 2-carboxylic acid decreases the ACE inhibition. The synthetic peptides were also tested for their ability to scavenge peroxynitrite (PN) and to exhibit glutathione peroxidase (GPx)-like activity. All the selenium-containing peptides exhibited good PN-scavenging and GPx activities.

Introduction

Angiotensin converting enzyme (ACE) inhibitors are a major class of antihypertensive drugs.[1–3] Inhibition of ACE leads to a decrease in the blood pressure due to reduced production of angiotensin II (Ang II), the blood pressure regulating hormone,[4] and elevation in the concentration of vasodilating hormone bradykinin[5] in the blood stream. There are two isoforms of ACE, somatic ACE (sACE), a dimeric enzyme having two identical zinc(II)-containing active sites, and testicular ACE (tACE), a monomeric enzyme having a similar active site. It is known that the N-terminal domain of sACE cleaves the terminal dipeptide of bradykinin, and the C-terminal domain of sACE and tACE produce Ang II. A structural comparison of Ang I and bradykinin indicates that these two substrates contain a conserved Pro–Phe linkage. Recently, we have shown that the introduction of an additional amino acid to captopril analogues (compounds **1–6**, Fig. 1) decreases the inhibition activity of the parent compounds.[6,7b] Although the active site of ACE includes a Phe binding pocket in addition to the Pro binding pocket, the decrease in the activity upon introduction of Phe to captopril analogues is probably due to some minor changes in the orientation of the methyl group of the side chain attached to the Pro moiety.

As bradykinin contains an L-serine (L-Ser) residue attached to the Pro–Phe moiety (Fig. 2), the tripeptides that resemble the Ser–Pro–Phe motif may exhibit better ACE inhibition by mimicking the binding at the active site. In view of this, we have replaced the Ser residue in Ser–Pro–Phe peptide by L-cysteine (L-Cys) or L-selenocysteine (L-Sec) as this replacement may provide a suitable ligand for coordination to the zinc(II) ion at the active site of ACE.[8] In this paper, we report on the synthesis and ACE inhibition activities of peptides **7–13** having Cys or Sec moieties (Fig. 3).

It is known that ACE inhibitors having antioxidant activity are beneficial for the treatment of hypertension as hypertension and oxidative stress are interdependent.[9–12] An enhanced cellular level of Ang II induces the enzyme NADPH oxidase to generate

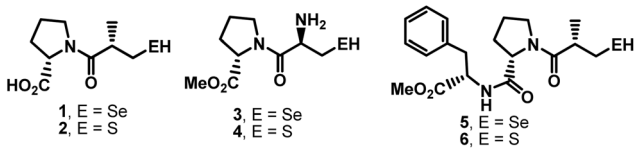

Fig. 1 Captopril (**2**) and peptide analogues reported as ACE inhibitors.[6,7b]

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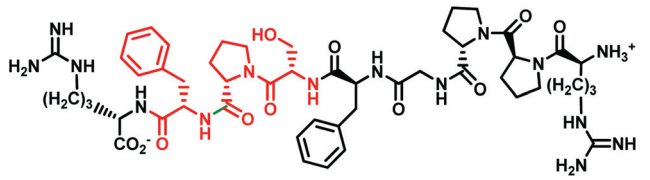


Fig. 2 Structure of bradykinin (1–9) indicating the presence of the Ser–Pro–Phe motif.

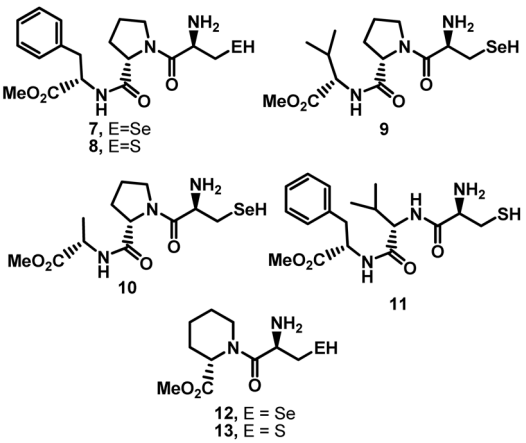
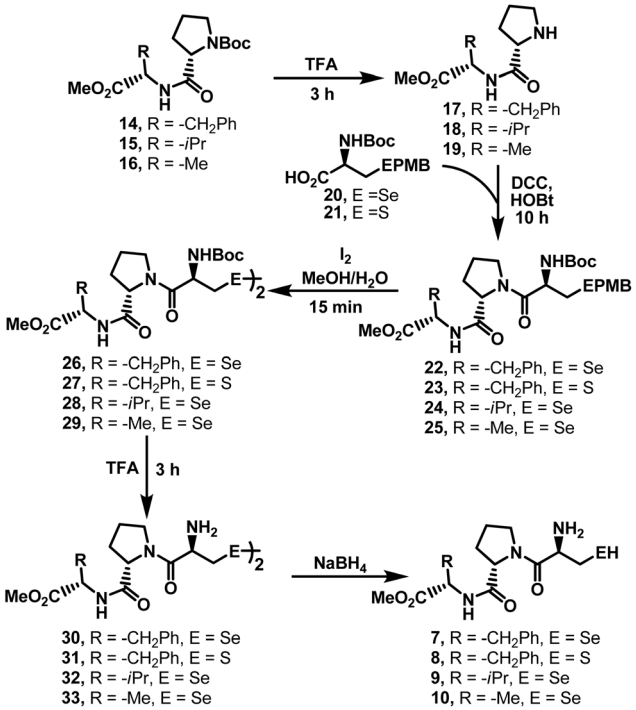
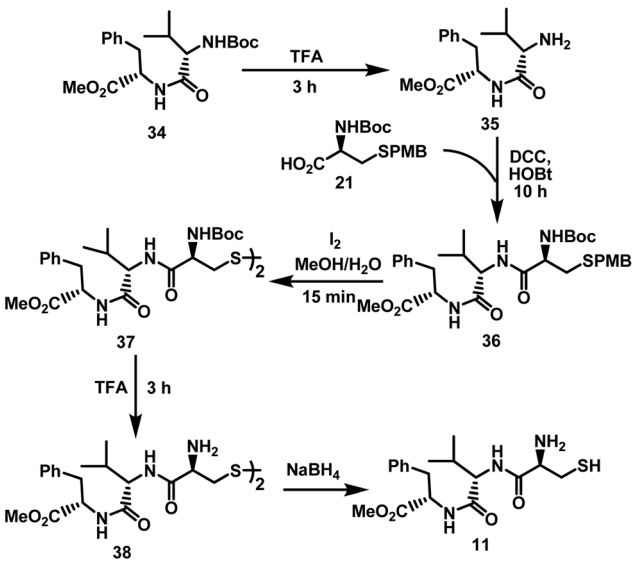


Fig. 3 Di- and tripeptides used as ACE inhibitors and peroxynitrite (PN) scavengers.



Scheme 1 Synthesis of Sec- and Cys-containing tripeptides **7–10**.



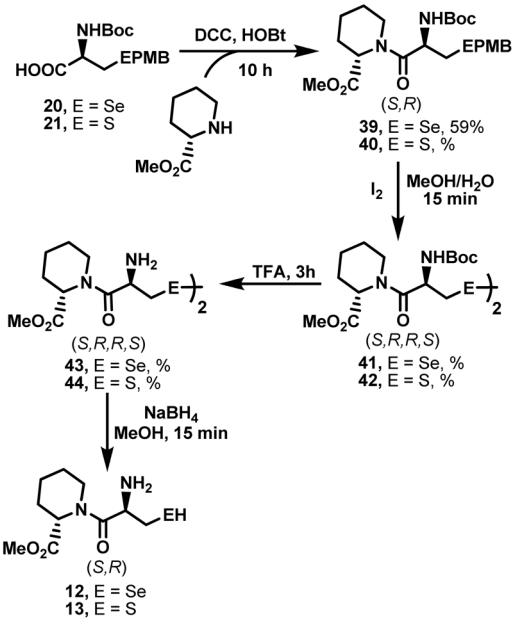
Scheme 2 Synthesis of Cys–Val–Phe tripeptide (**11**).

a superoxide radical anion ($O_2^{\cdot -}$), which reacts with the endothelial derived relaxing factor (EDRF), nitric oxide radical ($^{\cdot}NO$), to produce peroxynitrite (PN). PN is known to cause cellular damage as it can nitrate free or protein tyrosine residues as well as cause lipid peroxidation. Recently, we have shown that the selenium analogues of captopril exhibit both ACE inhibition and PN-scavenging activities.[6,7] To understand the effect of different amino acids on PN-scavenging activity, we have tested compounds **7–13** as inhibitors of PN-mediated nitration of Ang II. In addition to ACE inhibition and PN-scavenging activity, we have also studied the glutathione peroxidase (GPx) activity of the synthetic peptides. GPx is a mammalian enzyme that contains a selenocysteine residue at the active site.[13] This enzyme converts hydroperoxides to water or alcohol by using glutathione (GSH) as a cofactor.[14] The chemistry at the active site of GPx has been studied extensively with the help of various synthetic organoselenium compounds.[15–17] However, it is not known whether ACE inhibitors can exhibit GPx activity.

Results and discussion

The tripeptides **7–10** bearing Sec and Cys residues at the N-terminal were synthesized by solution phase peptide synthesis. These compounds were synthesized by using PMB-protected Sec or Cys as shown in Scheme 1. The initial steps involved the removal of the Boc group from compounds **14–16** by TFA to produce compounds **17–19**. The DCC-mediated coupling of the resulting deprotected dipeptides with PMB-protected Sec (**20**) or Cys (**21**) afforded the tripeptides **22–25**. Cleavage of the PMB group by iodine produced the corresponding diselenides and disulfide (**26–29**) in good yield. Removal of the Boc group by TFA led to the formation of compounds **30–33**, which upon reduction by $NaBH_4$ produced the required selenols and thiols **7–10**.

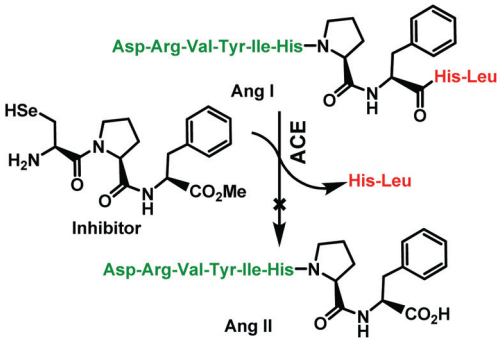
The Cys–Val–Phe–OMe tripeptide (**11**) lacking a Pro moiety was synthesized by following a similar procedure (Scheme 2). TFA-mediated cleavage of the Boc group in compound **34** afforded **35** in nearly quantitative yield. DCC-mediated coupling of the amino group in this peptide with the carboxylic group of PMB-protected Cys (**21**) produced the corresponding tripeptide



Scheme 3 Synthesis of dipeptides **12** and **13** starting from L-piperidine-2-carboxylic acid methyl ester.

36. The usual iodine-mediated removal of the PMB group, removal of the Boc group by TFA and reduction of the disulfide linkage by $NaBH_4$ afforded the Cys–Val–Phe–OMe tripeptide (**11**). It should be noted that the yields of Cys-based tripeptides are slightly higher than those of Sec-based compounds. Furthermore, the thiol groups in the cysteinyl-peptides are more stable in air as compared to the selenol groups in the Sec-based peptides. To understand the importance of the five-membered ring in Pro-based ACE inhibitors, the Sec- and Cys-containing dipeptides **12** and **13** having six-membered rings were synthesized (Scheme 3). DCC-mediated coupling of L-piperidine-2-carboxylic acid methyl ester to the PMB-protected Sec (**20**) and Cys (**21**) afforded the corresponding dipeptides **39** and **40**. The cleavage of the PMB group in **39** and **40** under mild conditions using iodine led to the formation of diselenides **41** and **42**, respectively. The TFA-mediated removal of the Boc group followed by reductive cleavage of the diselenide and disulfide bonds in **43** and **44** by $NaBH_4$ afforded the corresponding selenol **12** and thiol **13**, respectively, in good yield.

The hydrolysis of Ang I to Ang II by ACE was studied in the presence of various inhibitors (Scheme 4). The decrease in the concentration of Ang II with an increase in the concentration of various inhibitors was followed by HPLC. The peak area corresponding to Ang II was obtained during the initial 5–10% of the reaction and, wherever possible, the IC_{50} values (concentration of inhibitors required to inhibit 50% of the enzyme activity) were determined. The reaction mixture was incubated at 37 °C for 30 min prior to analysis. The thiols and selenols were freshly prepared by reducing the corresponding disulfides and diselenides by $NaBH_4$ prior to use and were kept under N_2 atmosphere during the assay. The IC_{50} values obtained for the inhibition of ACE-catalyzed conversion of Ang I to Ang II by the di- and tripeptides are summarized in Table 1. The IC_{50} values for ACE inhibition by compounds **1–6** are included for comparison.[6,7]



Scheme 4 Inhibition of ACE-catalyzed conversion of Ang I to Ang II by peptides.

Table 1 IC_{50} values for the inhibition of ACE by compounds **1–13**

Compound	IC_{50} (nM)	Compound	IC_{50} (nM)
1	36.4 ± 1.5	**8**	3500 ± 100
2	18.1 ± 1.0	**9**	8700 ± 300
3	342 ± 33	**10**	12 700 ± 400
4	6480 ± 640	**11**	50 000[b]
5	4100 ± 330	**12**	6850 ± 400
6	40 000[a]	**13**	40 000[a]
7	183.2 ± 10.6		

[a] Only 35% enzyme activity was inhibited at 40 000 nM inhibitor concentration. [b] Only 25% enzyme activity was inhibited at 50 000 nM inhibitor concentration. Assay conditions: The reactions were carried out in HEPES–HCl buffer (50 mM, pH 8.3) at 37 °C with a final concentration of 50 μM Ang I, 60 mM NaCl and 2 milliunits of ACE in 400 μL reaction mixture.

From Table 1, it is clear that the introduction of L-Phe to Sec-Pro and Cys-Pro peptides increases the ACE inhibitory activity. For example, the IC_{50} value for Sec–Pro–Phe–OMe (compound **7**, IC_{50}: 183.2 ± 10.6 nM) is much lower than that of Sec–Pro–OMe dipeptide (compound **3** IC_{50}: 342 ± 33 nM). A similar change in the activity was observed for the corresponding Cys-containing peptides (compound **8** *vs.* **4**). In contrast, the substitution of L-Phe by L-Val or L-Ala dramatically decreases the inhibitory potency. The IC_{50} value for compound **9** having a Val residue (8700 ± 300 nM) was found to be almost 50 times higher than that of compound **7** (IC_{50}: 183.2 ± 10.6 nM). Similarly, the IC_{50} value for compound **10** having an Ala residue (12 700 ± 400 nM) was found to be almost 70 times higher than that of **7**. Substitution of Pro in compound **8** by Val reduced the inhibitory activity. When compound **11** was used as inhibitor, only 25% inhibition of ACE activity was observed even at 50 μM concentration. These observations clearly indicate that the Pro moiety in captopril analogues is very important for efficient inhibition of ACE. Furthermore, it is observed that the substitution of the five-membered ring of Pro by a six-membered ring of L-piperidine-2-carboxylic acid increases the IC_{50} value. For example, the IC_{50} value observed for compound **12** having a six-membered ring (6850 ± 400 nM) is ~20 times higher than that of compound **3** (342 ± 33 nM). Similarly, when compound **13** was used, only 35% of enzyme activity was inhibited even at 40 μM concentration. In contrast, the Pro-based compound **4**

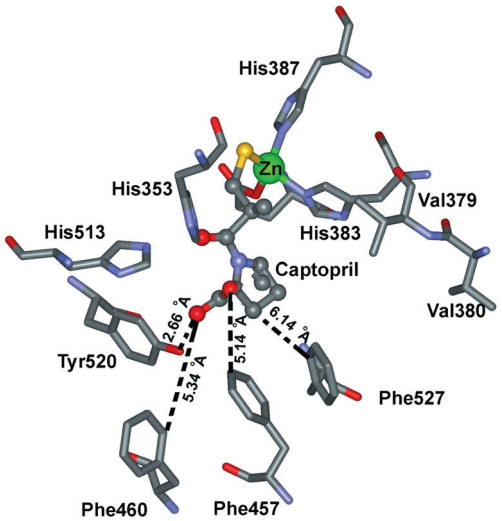
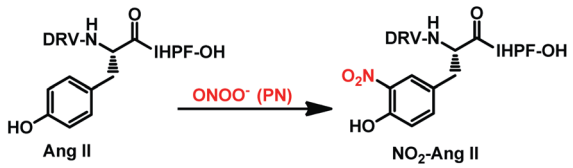


Fig. 4 Crystal structure of captopril bound to the active site of ACE. Captopril binds to ACE through thiolate coordination to Zn(II) and there are additional hydrogen bonding interactions to carbonyl and carboxylate groups of captopril through various amino acid residues present at the active site (PDB code: 1UZF).[2a] A hydrophobic pocket comprising of three Phe residues (457, 460 and 527) may stabilize the binding of tripeptides having Phe residue to ACE active site.



Scheme 5 Peroxynitrite-mediated nitration of Ang II to mono-nitro Ang II.

Table 2 IC_{50} values for inhibition of PN-mediated nitration of Ang II by compounds **7–13** and their corresponding diselenides and disulfides[a]

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
7	7.0 ± 0.1	**30**	6.1 ± 0.3
8	20.2 ± 1.1	**31**	33.7 ± 1.8
9	7.7 ± 0.7	**32**	4.9 ± 0.3
10	9.0 ± 0.8	**33**	3.7 ± 0.3
11	19.6 ± 0.2	**38**	32.4 ± 1.6
12	5.5 ± 0.2	**43**	3.2 ± 0.2
13	14.9 ± 0.8	**44**	37.7 ± 3.0

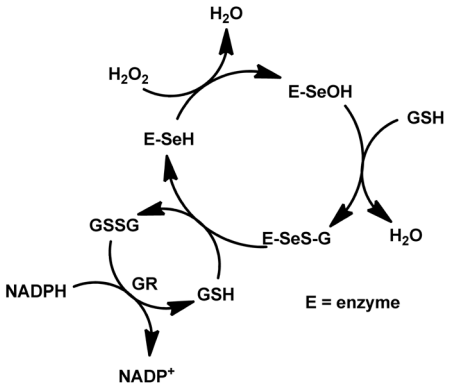
[a] Assay condition: Reactions were carried out in sodium phosphate buffer (100 mM, pH 7.5) at 22 °C with a final concentration of 20 μM Ang II, 300 μM PN and different concentrations of the inhibitors.

inhibited the ACE activity with an IC_{50} value of 6480 ± 640 nM.

The crystal structure of the ACE–captopril adduct shows that the thiol group of the inhibitor interacts with the zinc(II) ion to form a zinc(II)–thiolate complex.[2] The inhibitor is anchored to the central carbonyl group and the Pro carboxylate group by hydrogen bonding. The central carbonyl group of captopril is positioned by two strong hydrogen bonds from the two histidine residues (His353 and His513). The binding of captopril is also stabilized by hydrogen bonding of the Pro carboxylate group with neighboring amino acid residues. The key amino acid residues involved in such hydrogen bonding are Tyr520, Gln281 and Lys511. Introduction of an additional amino acid residue to captopril may lead to a loss of hydrogen bonding interactions between the Pro carboxylate and the polar amino acid residues at the active site. This is probably responsible for the decrease in the inhibitory activity upon introduction of an additional amino acid to the Pro moiety (Fig. 4).

Interestingly, the five-membered ring of the Pro residue appears to be important for a proper orientation of the carboxylate moiety. The substitution of the Pro residue by L-piperidine-2-carboxylic acid drastically decreases the inhibitory potency (Table 1). On the other hand, introduction of a Phe residue to Sec–Pro (**3**) and Cys–Pro (**4**) dipeptides significantly increases the inhibitory potency of the parent compounds. This is probably due to the presence of a free amino group in the Sec or Cys moiety (compounds **7** and **8**), which can stabilize the ACE–inhibitor complex by hydrogen bonding. As the introduction of Tyr or Val drastically decreases the inhibitory potency (compounds **9** and **10**), it can be concluded that there is a binding pocket for the Phe residue at the active site of ACE. This is in agreement with the previous observations.[7,18] Akif *et al*. have shown that the ACE inhibitor RXP380, in which an L-tryptophan residue is present at a position similar to that of the Phe residue in compound **7**, binds to the periphery of subsite S2′ comprising Val379 and Val380 residues in sACE.[18] A close examination of the active site of ACE reveals that there is a hydrophobic pocket (Phe457, Phe460 and Phe527) present at a distance of 4–6 Å from the Pro carboxylate. These Phe residues may stabilize the tripeptides having Sec–Pro–Phe residues and may form a stable enzyme–inhibitor complex. However, to the best of our knowledge, the role of these amino acid residues near the active site has not been studied in detail.

To evaluate the antioxidant activity of di- and tripeptides, we have studied the effect of these compounds on peroxynitrite-mediated nitration of Ang II (Scheme 5). As the formation of 3,5-dinitro-Ang II was also observed in the reaction, only the initial 5–10% of the conversion was followed. During this period, the mononitro-Ang II compound was produced as the major product. The IC_{50} values obtained for the inhibition of PN-mediated nitration of Ang II are summarized in Table 2. From the IC_{50} values, it is evident that the selenium-containing peptides are much better scavengers of PN than the sulfur analogues. In contrast to the ACE inhibition property, the PN-scavenging activity does not depend significantly on the C-terminal amino acid residue. The dipeptide **12** exhibited the highest activity (IC_{50}: 5.5 ± 0.2 μM) among all the selenols and thiols. The IC_{50} value for the Sec-containing tripeptide **7** (7.0 ± 0.1 μM) is comparable to that of **9** (7.7 ± 0.7 μM) and **10** (9.0 ± 0.8 μM) in which the terminal Phe amino acid residue is replaced by Val or Ala, respectively. The selenols were found to be good scavengers of PN, although the IC_{50} values obtained for these compounds were about two times higher than those of the corresponding diselenides. In contrast, the thiols were found to be more efficient than the corresponding disulfides. This is in agreement with our previous report that diselenides can undergo multiple oxidation after reaction with PN.[6,7]



Scheme 6 Proposed catalytic cycle for reduction of hydrogen peroxide by GPx. GR reduces glutathione disulfide to glutathione by using NADPH as cofactor.

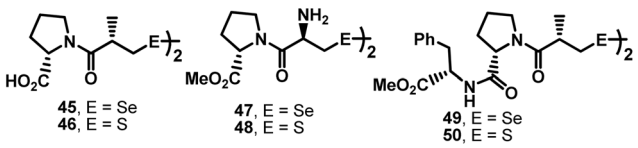


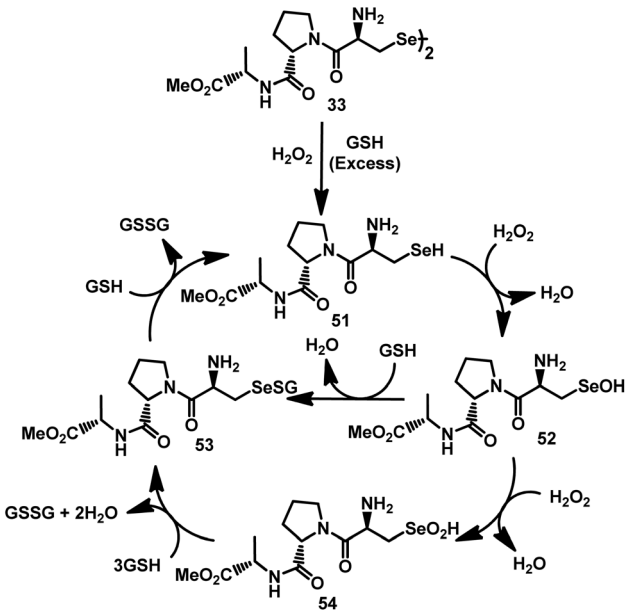
Fig. 5 Diselenides and disulfides used for GPx activity.[6,7b]

It should be noted that the peptides having a Sec or Cys residue inhibit PN-mediated nitration of Ang II with IC_{50} values that are about 10–50 times lower than the concentration of PN in the reaction medium (300 μM). Recently, we reported that organoselenium compounds can catalytically scavenge PN.[19] It has been shown that PN oxidizes the selenides (R_2Se) to the corresponding selenoxides (R_2–Se═O), which can react with nitrite ion (NO_2^-) to produce nitrate (NO_3^-) with a consumption of two molecules of PN in one catalytic cycle. Therefore, we have investigated the reaction of **33** with PN by using mass spectral techniques. It was observed that both the selenium centers in compound **33** undergo two electron oxidation (+2 to +4) immediately after the addition of PN. However, the complete regeneration of the diselenide was not observed even after 2 days. In the presence of reducing agents such as glutathione (GSH), the reduction of selenium centers (+4 to +2) was observed (Fig. S77, ESI†), suggesting that the diselenides may exhibit a redox cycle in the presence of biological thiols. The rapid reduction of the oxidized species by GSH prompted us to investigate the GPx-like antioxidant activity of the peptides as GPx uses GSH as cofactor to catalytically reduce hydroperoxides to alcohols or water. The catalytic cycle of GPx involves the reduction of 1 equiv of peroxide by using two equiv of GSH as cofactor (Scheme 6).[14–17] The GPx-like antioxidant activity of the Cys- and Sec-containing peptides was determined by using the classical glutathione reductase (GR) coupled assay. In brief, a decrease in the concentration of NADPH was followed as a function of time at 340 nm wavelength as NADPH is used as a cofactor by GR to reduce glutathione disulfide (GSSG) to glutathione (GSH) as shown in Scheme 6. The initial rates for the reduction of H_2O_2 by GSH in the presence of compounds **30–33**, **38**, **43–50** (Fig. 5) and ebselen were obtained at identical experimental conditions and are summarized in Table 3.

Table 3 Initial rate for the GPx-like antioxidant activity by the test compounds in the glutathione assay[a]

Compound	Rate (μM min^{-1})	Compound	Rate (μM min^{-1})
30	138.5 ± 2.7	**31**	34.7 ± 2.9
32	165.8 ± 3.9	**33**	199.6 ± 17.4
38	40.8 ± 0.4	**43**	161.8 ± 3.4
44	46.7 ± 3.0	**45**	150.7 ± 0.3
46	36.3 ± 2.3	**47**	157.6 ± 1.4
48	30.8 ± 3.1	**49**	106.8 ± 2.6
50	22.2 ± 0.2	Ebselen	156.3 ± 6.4

[a] Assay conditions: Reactions were carried out in sodium phosphate buffer (100 mM, pH 7.5) at 22 °C with a final concentration of 100 μM catalyst, 4 mM GSH, 0.4 mM NADPH, 1.7 unit GR mL^{-1}, and 5 mM H_2O_2. The initial rates were corrected for the background reaction between peroxide and thiol.



Scheme 7 Proposed catalytic cycle for reduction of hydrogen peroxide by the diselenides.

The GPx activity of most of the diselenides is comparable to that of ebselen, an organoselenium compound used as a standard in GPx model reactions. The initial rate for the reduction of H_2O_2 by compound **33** (199.6 ± 17.4 μM min^{-1}) was found to be higher than that of ebselen (156.3 ± 6.4 μM min^{-1}). The nature of the substituent or ring size does not appear to have any major effect on the GPx activity. For example, the diselenides **43** and **47** having different heterocycles exhibit almost similar GPx activity. In contrast to the diselenides, the disulfides are very poor GPx mimics. To understand the mechanism for the reduction of H_2O_2 by the selenium compounds, we analyzed the different species formed in the reactions by using mass spectrometric techniques. When compound **33** was treated with GSH and H_2O_2, the selenenyl sulfide (**53**), selenol (**51**) selenenic acid (**52**) and seleninic acid (**54**) were observed (Scheme 7 and Fig. S78, S80, ESI†). In this reaction, the formation of GSSG was also observed. A comparison of the reactivity of **33** toward

GSH and H_2O_2 indicates that the reaction with H_2O_2 is faster than that with GSH. This observation suggests that the diselenide may undergo oxidation before reduction by GSH. In the absence of GSH, compound **33** reacted with H_2O_2 to produce several oxidized species. Mass spectral analysis of the products indicated the formation of –Se–(Se═O)– and –(Se═O)–(Se═O)– species. However, when GSH was added, all these species disappeared to produce the selenol, selenenyl sulfide and selenenic–seleninic acids. The selenol could be trapped by treating the reaction mixture with bromopropionic acid (Fig. S84, ESI†) (Fig. 5).

According to the mechanism shown in Scheme 7, the diselenide **33** reacts with H_2O_2 and GSH to generate the selenol **51**, which upon reaction with 1 equiv of H_2O_2 generates the selenenic acid (**52**) with the elimination of a water molecule. The selenenic acid then reacts with 1 equiv of GSH to generate the selenenyl sulfide (**53**) intermediate. As in the case of GPx, the selenenyl sulfide reacts with another equiv of GSH to regenerate the active selenol species. The formation of seleninic acid (**54**) as observed in the mass spectrum can be ascribed to the oxidation of compound **52** in the presence of excess peroxide. It should be noted that the formation of seleninic acid is very common in model reactions and the GPx enzyme itself produces such species under oxidative conditions.[14,20] However, the seleninic acids can be converted to selenenyl sulfides and then to selenols by treating with an excess amount of GSH. In biological systems, where GSH concentration is high, the seleninic acid may lie off the main catalytic pathway. Compound **33** represents the first example of a Pro-based peptide that exhibits GPx activity.[21]

Conclusions

This study on the inhibition of angiotensin converting enzyme (ACE) by Sec–Pro–Phe and Cys–Pro–Phe tripeptides indicate that the presence of a Phe residue at the C-terminal of the Sec–Pro and Cys–Pro dipeptides enhances the inhibitory potency of the parent compounds. Replacement of the terminal Phe residue by aliphatic amino acid residues such as Val or Ala dramatically decreases the inhibition activity. Although Val and Ala are hydrophobic in nature, the inefficient inhibition by compounds **9** and **10** indicates that the hydrophobic pocket at the active site of ACE is specific to aromatic amino acid residues. The presence of Pro residue is crucial for ACE inhibition as the replacement of this residue by Val almost completely abolishes the ACE inhibition potency. It was also observed that the ring size of Pro plays an important role in the enzyme inhibition as the replacement of the five-membered ring in compounds **3** and **4** by a six-membered ring, (*e.g.* compounds **12** and **13**) reduces the ACE inhibition activity by more than 20 times. This is probably due to a different orientation of the C- and N-terminal moieties at the active site. The inhibition of PN-mediated nitration reaction and GPx-like antioxidant activity of these peptides indicate that the selenocysteine-containing peptides may provide better protection against oxidative damage as compared to the cysteine-containing peptides. In contrast to ACE inhibition, the presence of other amino acid residues attached to Cys or Sec does not have any noticeable effect on their antioxidant behavior.

Experimental section

General procedure

Angiotensin converting enzyme (ACE) and captopril were purchased from Sigma-Aldrich Chemical Co. All experiments involving selenols and thiols were carried out under dry and oxygen free nitrogen using standard Schlenk techniques. Column chromatography was performed on glass columns loaded with silica gel or on an automated flash chromatography system (Biotage) by using pre-loaded silica cartridges. ^{1}H (400 MHz), ^{13}C (100.56 MHz), and ^{77}Se (76.29 MHz) NMR spectra were obtained on a Bruker 400 MHz NMR spectrometer. Chemical shifts are cited with respect to $SiMe_4$ as internal (^{1}H and ^{13}C), and Me_2Se as external (^{77}Se) standards. Mass spectral studies were carried out on a Q-TOF micro mass spectrometer or on a Bruker Daltonics 6000 plus mass spectrometer with ESI-MS mode analysis.

Synthesis of 14[22]

To a 100 mL solution of Boc-L-Pro (1.07 g, 5 mmol) in chloroform, DCC (1.54 g, 7.5 mmol) and HOBt (1.15 g, 7.5 mmol) were added at 0 °C. The mixture was stirred for 30 min. 1.35 g (7.5 mmol) of L-Phe-OMe was added and the reaction mixture was allowed to attain room temperature slowly. The mixture was further stirred for 10 h. The precipitate was filtered and the filtrate was washed three times each with 1 M $KHSO_4$ solution, sodium carbonate solution and brine. The organic layer was dried over anhydrous sodium sulfate and solvent was removed under reduced pressure. The compound was purified by flash chromatography (hexane : ethyl acetate; 2 : 1). Yield 1.48 g (74%); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.44 (s, 9H), 1.79–1.95 (m, 3H), 2.98–3.04 (m, 2H), 3.17–3.29 (m, 3H), 3.73 (s, 3H), 4.27–4.30 (dd, 1H), 4.87–4.90 (dd, 1H), 7.09–7.11 (d, 2H), 7.26–7.29 (m, 3H); ^{13}C NMR ($CDCl_3$) δ (ppm): 24.9, 28.7, 31.1, 38.5, 47.4, 52.7, 61.4, 81.2, 127.6, 129.0, 129.7, 136.4, 155.0, 156.1, 172.1; ESI-MS: *m/z* calcd for $C_{20}H_{28}N_2O_5$ $[M + Na]^+$ 399.1896, found 399.3571.

Synthesis of 15[23]

Compound **15** was synthesized following a similar method given for compound **14** using L-Val–OMe in place of L-Phe–OMe. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.74–0.78 (dd, 6H), 1.33 (s, 9H), 1.74–1.78 (m, 3H), 2.01–2.04 (m, 2H), 3.21–3.29 (m, 2H), 3.58 (s, 3H), 4.14–4.17 (dd, 1H), 4.33–4.35 (dd, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm): 17.5, 19.0, 24.6, 28.3, 31.0, 47.0, 52.0, 57.2, 59.6, 61.0, 80.6, 154.6, 155.8, 172.1; ESI-MS: *m/z* calcd for $C_{16}H_{28}N_2O_5$ $[M + Na]^+$ 351.1896, found 351.5418.

Synthesis of 16[24]

Compound **16** was synthesized following a similar method given for compound **14** using L-Ala–OMe in place of L-Phe–OMe. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.39–1.40, (d, 3H), 1.47 (s, 9H), 1.69–1.75 (m, 3H), 2.14–2.18 (m, 1H), 3.45–3.49 (m, 2H), 3.75 (s, 3H), 4.18–4.21 (dd, 1H), 4.54–4.57 (dd, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm): 17.5, 19.0, 24.6, 28.3, 31.0, 47.0, 52.0, 57.2, 61.0, 80.6, 154.6, 155.8, 172.1; ESI-MS: *m/z* calcd for $C_{14}H_{24}N_2O_5$ $[M + Na]^+$ 323.1523, found 322.9257.

Synthesis of 17[22]

To 1.40 g (3.72 mmol) of compound **14** was dissolved in 5 mL chloroform and 14 mL of formic acid was added. The mixture was stirred for 3 h. Excess solvent was evaporated under reduced pressure. Yield 0.84 g (82%); ^{1}H NMR (D_2O) δ (ppm): 1.77–1.98 (m, 3H), 2.15–2.21 (m, 1H), 2.80–2.86 (m, 1H), 3.03–3.18 (m, 3H), 3.59 (s, 3H), 4.04–4.05 (dd, 1H), 4.20–4.22 (dd, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm): 24.6, 30.5, 37.6, 47.5, 53.1, 55.0, 60.1, 127.8, 129.1, 129.5, 135.9, 169.1, 172.0; ESI-MS: *m/z* calcd for $C_{15}H_{21}N_2O_3$ $[M + H]^+$ 277.1552, found 276.9391.

Synthesis of 18[23]

Compound **18** was synthesized by following a similar method given for compound **17** but using compound **15**. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.94–0.96 (dd, 6H), 1.96–2.10 (m, 3H), 2.18–2.22 (m, 1H), 2.44–2.47 (m, 1H), 3.40–3.43 (t, 2H), 3.74 (s, 3H), 4.42–4.45 (dd, 1H), 4.77–4.81 (dd, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm): 17.5, 19.0, 24.6, 28.3, 31.0, 47.0, 52.0, 57.2, 59.6, 61.0, 154.6, 155.8; ESI-MS: *m/z* calcd for $C_{11}H_{20}N_2O_3$ $[M + Na]^+$ 251.1372, found 251.0129.

Synthesis of 19[24]

This compound was synthesized by following a similar method given for the synthesis of **17** but using compound **16**. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.43–1.45 (d, 3H), 2.00–2.16 (m, 3H), 2.44–2.49 (m, 1H), 3.41–3.45 (t, 2H), 3.75 (s, 3H), 4.50–4.54 (dd, 1H), 4.72–4.74 (dd, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm): 16.4, 24.1, 27.9, 30.0, 48.9, 52.4, 59.8, 168.6, 172.8; ESI-MS: *m/z* calcd for $C_9H_{17}N_2O_3$ $[M + H]^+$ 20.11239, found 200.9671.

Synthesis of 20 and 21

Compounds **20** and **21** were synthesized following the literature procedures.[6]

Synthesis of 22

To a 100 mL solution of chloroform, 0.58 g (1.5 mmol) of **20**, 0.46 g (2.25 mmol) DCC and 0.34 g (2.25 mmol) HOBt were added and the mixture was stirred for 20 min at 0 °C. After this, 0.83 g (3 mmol) **17** was added and the reaction mixture was allowed to attain room temperature slowly. The mixture was further stirred for 10 h. The precipitate was filtered and the filtrate was washed three times each with 1 M $KHSO_4$ solution, sodium carbonate solution and brine. The organic layer was dried over anhydrous sodium sulfate and solvent was removed under reduced pressure. The compound was purified by flash chromatography (hexane : ethyl acetate; 1 : 2). Yield 0.59 g (61%). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.44 (s, 9 H), 1.60–1.77 (m, 5H), 2.14–2.18 (m, 1H), 2.56–2.59 (dd, 1H), 2.68–2.70 (dd, 1H), 3.01–3.04 (dd, 1H), 3.12–3.15 (dd, 1H), 3.69 (s, 3H), 3.77 (s, 3H), 3.79 (s, 2H), 4.55–4.57 (m, 2H), 4.79–4.81 (dd, 1H), 6.81–6.83 (d, 2H), 7.06–7.27 (m, 7H); ^{13}C NMR ($CDCl_3$) δ (ppm): 25.2, 27.0, 28.8, 30.2, 33.7, 38.4, 47.9, 51.9, 52.2, 53.8, 55.7, 60.7, 80.6, 114.5, 127.5, 128.1, 129.0, 130.7, 131.1, 136.6, 155.5, 159.1, 171.0, 171.7, 172.2; ^{77}Se NMR ($CDCl_3$) δ (ppm): 222; ESI-MS: *m/z* calcd for $C_{31}H_{41}N_3O_7Se$ $[M + Na]^+$ 670.2007, found 670.2640.

Synthesis of 23

Compound **23** was synthesized by following a similar method given for the synthesis of compound **22** by using compound **21** in place of compound **20**. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.44 (s, 9 H), 1.62–1.65 (m, 1H), 1.73–1.76 (m, 1H), 1.87–1.91 (m, 2H), 2.17–2.20 (m, 2H), 2.51–2.56 (dd, 1H), 2.65–2.70 (dd, 1H), 2.95–2.98 (dd, 1H), 3.11–3.14 (dd, 1H), 3.69 (s, 3H), 3.78 (s, 3H), 3.79 (s, 2H), 4.56–4.58 (m, 2H), 4.79–4.82 (dd, 1H), 6.83–6.85 (d, 2H), 7.09–7.11 (d, 2H), 7.21–7.26 (m, 5H); ^{13}C NMR ($CDCl_3$) δ (ppm): 18.2, 19.6, 25.8, 28.1, 28.8, 31.1, 34.4, 38.3, 52.8, 53.6, 55.7, 59.1, 81.0, 114.6, 127.7, 129.1, 129.7, 130.5, 131.3, 136.2, 155.9, 159.0, 170.7, 171.4, 172.1; ESI-MS: *m/z* calcd for $C_{31}H_{41}N_3O_7S$ $[M + Na]^+$ 622.2563, found 622.3636.

Synthesis of 24

This compound was synthesized by following a procedure similar to the synthesis given for compound **22** but using compound **18** in place of compound **17**. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.83–0.91 (dd, 6H), 1.43 (s, 9H), 1.90–1.93 (m, 2H), 2.09–2.14 (m, 1H), 2.34–2.37 (m, 1H), 2.60–2.66 (m, 1H), 2.76–2.80 (m, 1H), 3.47–0.349 (m, 1H), 3.58–3.60 (dd, 1H), 3.70 (s, 3H), 3.77 (s, 3H), 3.79 (s, 2H), 4.40–4.44 (m, 2H), 4.59–4.61 (d, 1H), 6.80–6.82 (d, 2H), 7.22–7.24 (d, 2H); ^{13}C NMR ($CDCl_3$) δ (ppm): 18.4, 19.6, 25.4, 26.1, 27.9, 28.0, 28.8, 31.5, 48.1, 52.1, 52.6, 55.7, 57.9, 60.6, 80.5, 114.4, 130.6, 131.0, 155.6, 159.0, 171.1, 172.0, 172.6; ^{77}Se NMR ($CDCl_3$) δ (ppm): 224; ESI-MS: *m/z* calcd for $C_{27}H_{41}N_3O_7Se$ $[M + Na]^+$ 622.2007, found 622.1271.

Synthesis of 25

This compound was synthesized by following a procedure similar to the synthesis of compound **22** but using compound **19** in place of **17**. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.23–1.25 (d, 3H), 1.43 (s, 9H), 1.91–1.96 (m, 3H), 2.38–2.41 (m, 1H), 2.67–2.68 (m, 1H), 2.81–2.83 (m, 1H), 3.47–3.49 (m 1H), 3.57–3.59 (m, 1H), 3.71 (s, 3H), 3.78 (s, 3H), 3.79 (s, 2H), 4.46–4.48 (dd, 1H), 4.59–4.63 (m, 2H), 6.80–6.82 (d, 2H), 7.22–7.24 (d, 2H); ^{13}C NMR ($CDCl_3$) δ (ppm): 18.6, 25.3, 26.0, 27.6, 28.8, 48.0, 48.6, 52.1, 52.9, 55.8, 60.5, 80.6, 114.5, 130.6, 131.1, 155.5, 159.0, 170.8, 171.8, 173.6; ^{77}Se NMR ($CDCl_3$) δ (ppm): 220; ESI-MS: *m/z* calcd for $C_{25}H_{37}N_3O_7Se$ $[M + Na]^+$ 594.1694, found 594.2136.

Synthesis of 26

To a 15 mL methanolic solution of **22** (0.32 g, 0.5 mmol), iodine (0.19 g, 0.75 mmol) was added. Water (15 mL) was added to the reaction mixture and stirred for 15 min. To this, 0.5 mL of hydrazine hydrate was added to decompose the excess

iodine. Methanol was removed under reduced pressure and 20 mL of 1 M $KHSO_4$ solution was added. The compound was extracted three times with ethyl acetate and purified by flash chromatography (hexane : ethyl acetate; 1 : 3). Yield 0.22 g (86%);^{1}H NMR ($CDCl_3$) δ (ppm): 1.23–1.25 (m, 4H), 1.41 (s, 9H), 1.87–1.92 (m, 2H), 2.16–2.19 (m, 1H), 3.02–3.04 (dd, 1H), 3.10–3.13 (m, 2H), 3.16–3.20 (m, 1H), 3.56–3.59 (m, 1H), 3.69 (s, 3H), 4.53–4.54 (d, 1H), 4.70–4.80 (m, 2H), 7.11–7.13 (d, 2H), 7.19–7.24 (m, 3H); ^{13}C NMR ($CDCl_3$) δ (ppm): 14.6, 25.2, 28.8, 30.1, 32.9, 38.3, 48.0, 52.8, 53.8, 60.7, 80.7, 127.6, 129.0, 129.7, 136.4, 157.6, 171.1, 171.3, 172.3; ^{77}Se NMR ($CDCl_3$) δ (ppm): 301; ESI-MS: *m/z* calcd for $C_{46}H_{64}N_6O_{12}Se_2$ $[M + Na]^+$ 1073.2818, found 1073.2656.

Synthesis of 27

Compound **27** was synthesized by following a similar method given for compound **26**. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.47 (s, 9H), 1.55–1.58 (m, 1H), 1.78–1.81 (m, 1H), 1.92–1.94 (m, 2H), 2.55–2.60 (m, 2H), 2.69–2.71 (dd, 1H), 3.00–3.02 (dd, 1H), 3.14–3.16 (dd, 1H), 3.45–3.49 (m, 1H), 3.72 (s, 3H), 4.60–4.63 (m, 2H), 4.83–4.85 (dd, 1H), 7.12–7.15 (m, 2H), 7.24–7.32 (m, 3H); ^{13}C NMR ($CDCl_3$) δ (ppm): 25.0, 28.8, 34.4, 36.4, 38.3, 48.0, 52.8, 53.7, 55.8, 60.7, 80.6, 128.9, 129.5, 130.6, 136.7, 159.3, 171.0, 171.6, 172.2; ESI-MS: *m/z* calcd for $C_{46}H_{64}N_6O_{12}S_2$ $[M + Na]^+$ 979.3921, found 980.3397.

Synthesis of 28

This compound was synthesized by following a similar procedure given for compound **26** but using the tripeptide **24**. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.88–0.90 (dd, 6H), 1.23–1.25 (m, 2H), 1.41 (s, 9H), 1.97–2.13 (m, 4H), 2.27–2.29 (m, 1H), 3.20–3.27 (m, 2H), 3.69 (s, 3H), 4.42–4.46 (dd, 1H), 4.59–4.61 (dd, 1H), 4.75–4.79 (m, dd, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm): 18.4, 19.6, 25.5, 28.8, 30.6, 31.5, 32.5, 48.2, 52.6, 52.9, 57.8, 60.8, 80.7, 155.7, 171.1, 171.4, 172.7; ^{77}Se NMR ($CDCl_3$) δ (ppm): 299; ESI-MS: *m/z* calcd for $C_{38}H_{64}N_6O_{12}Se_2$ $[M + Na]^+$ 979.2810, found 979.2982.

Synthesis of 29

This compound was synthesized by following a similar procedure given for compound **26** but using the tripeptide **25**. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.21–1.22 (d, 3H), 1.39 (s, 9H), 1.97–2.02 (m, 3H), 2.05–2.08 (m, 2H), 3.16–3.19 (m, 1H), 3.26–3.29 (m, 1H), 3.69 (s, 3H), 4.45–4.54 (m, 2H), 4.74–4.76 (dd, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm): 18.6, 25.4, 28.8, 30.1, 32.1, 32.8, 48.1, 48.6, 52.9, 60.6, 80.6, 155.7, 171.0, 173.7; ^{77}Se NMR ($CDCl_3$) δ (ppm): 300; ESI-MS: *m/z* calcd for $C_{34}H_{56}N_6O_{12}Se_2$ $[M + Na]^+$ 923.2184, found 923.1868.

Synthesis of 30

Compound **26** (0.20 g, 0.19 mmol) was taken in a round-bottomed flask and 1 mL of trifluoroacetic acid (TFA) was added. The flask was sealed and stirred for 3 h. TFA was evaporated under reduced pressure to obtain the Boc free dipeptide **30**. Yield 0.14 g (86%); ^{1}H NMR (MeOH-d_4) δ (ppm): 0.85–0.89 (m, 4H), 1.85–1.90 (m, 2H), 2.12–2.15 (m, 1H), 2.99–3.01 (dd, 1H), 3.10–3.14 (m, 2H), 3.11–3.13 (m, 1H), 3.56–3.59 (m, 1H), 3.70 (s, 1H), 4.44–4.46 (d, 1H), 4.70–4.75 (m, 2H), 7.12–7.14 (d, 2H), 7.19–7.25 (m, 3H); ^{13}C NMR (MeOH-d_4) δ (ppm): 24.9, 27.7, 29.4, 29.7, 37.3, 51.7, 54.4, 60.5, 78.4, 126.9, 128.5, 129.4, 136.8, 166.4, 172.2, 172.6; ^{77}Se NMR (MeOH-d_4) δ (ppm): 292; ESI-MS: *m/z* calcd for $C_{36}H_{48}N_6O_8Se_2$ $[M + Na]^+$ 875.1762, found 875.1169.

Synthesis of 31

The synthetic procedure followed for the synthesis of compound **31** is similar to the method given for diselenide **30**. ^{1}H NMR (MeOH-d_4) δ (ppm): 1.74–1.82 (m, 4H), 2.58–2.64 (m, 1H), 2.91–2.98 (m, 3H), 3.20–3.26 (m, 2H), 3.55 (s, 3H), 4.08–4.09 (d, 1H), 4.51–4.54 (dd, 1H), 4.86–4.91 (dd, 1H), 6.78–6.80 (d, 2H), 7.08–7.25 (m, 3H); ^{13}C NMR (MeOH-d_4) δ (ppm): 24.7, 29.3, 31.1, 35.2, 37.3, 51.1, 51.6, 54.7, 60.6, 114.1, 128.5, 129.3, 136.8, 159.6, 166.8, 172.1; ESI-MS: *m/z* calcd for $C_{36}H_{48}N_6O_8S_2$ $[M + H]^+$ 757.3053, found 757.2333.

Synthesis of 32

This compound was synthesized by following a similar procedure given for compound **30** but using the tripeptide **28**. ^{1}H NMR (MeOH-d_4) δ (ppm): 0.83–0.85 (dd, 6H), 1.15–1.18 (m, 2H), 1.89–2.01 (m, 5H), 3.35–3.28 (m, 1H), 3.44–3.48 (m, 1H), 3.58 (s, 3H), 4.18–4.20 (dd, 1H), 4.41–4.48 (m, 2H); ^{13}C NMR (MeOH-d_4) δ (ppm): 17.7, 18.6, 25.1, 29.8, 31.6, 37.4, 40.5, 51.7, 54.6, 58.9, 78.5, 167.8, 172.2, 172.4; ^{77}Se NMR (MeOH-d_4) δ (ppm): 292; ESI-MS: *m/z* calcd for $C_{28}H_{48}N_6O_8Se_2$ $[M + H]^+$ 757.1942, found 757.2333.

Synthesis of 33

This compound was synthesized by following a similar procedure given for compound **30** but using the tripeptide **29**. ^{1}H NMR (MeOH-d_4) δ (ppm): 1.21–1.22 (d, 3H), 1.96–2.03 (m, 3H), 2.23–2.27 (m, 2H), 3.25–3.26 (m, 2H), 3.65 (s, 3H), 4.31–4.34 (dd, 1H), 4.43–4.49 (m, 2H); ^{13}C NMR (MeOH-d_4) δ (ppm): 16.2, 24.9, 27.4, 29.5, 29.8, 51.8, 52.4, 60.5, 78.4, 166.4, 172.6, 173.6; ^{77}Se NMR (MeOH-d_4) δ (ppm): 292; ESI-MS: *m/z* calcd for $C_{24}H_{40}N_6O_8Se_2$ $[M + H]^+$ 701.1316, found 701.1487.

Synthesis of 34[25]

To a solution of Boc-L-Val (1.8 g, 8.25 mmol) in chloroform (130 mL), DCC (2.52 g, 12.38 mmol) followed by HOBt (1.89 g, 12.38 mmol) was added at 0 °C and the reaction mixture was stirred for 20 min. To this L-Phe–OMe (2.22 g, 12.38 mmol) was added followed by triethylamine (2.3 mL, 16.5 mmol). The reaction mixture was slowly allowed to attain room temperature and further stirred for 12 h. After the completion of the reaction, the precipitate was filtered and the chloroform layer was washed two times each with 1 M aqueous $KHSO_4$ solution, 1 M aqueous Na_2CO_3 solution and brine

solution successively. Solvent was evaporated under reduced pressure and the compound was purified with column chromatography (pet. ether : ethyl acetate; 3 : 1). Yield:1.66 g (53.1%); ^{1}H NMR ($CDCl_3$) δ: 0.86–0.93 (m, 6H), 1.45 (s, 9H), 2.05–2.13 (m, 1H), 3.11–3.14 (t, 2H), 3.72 (s, 3H), 3.88–3.92 (t, 1H), 4.85–4.90 (q, 1H), 7.10–7.12 (t, 2H), 7.24–7.31 (m, 3H); ^{13}C NMR ($CDCl_3$) δ: 18.2, 19.6, 28.8, 31.4, 38.4, 52.8, 53.6, 60.3, 80.4, 127.7, 129.1, 129.7, 136.1, 171.8, 172.2; ESI-MS: *m/z* calcd for $C_{20}H_{30}N_2O_5$ $[M + Na]^+$ 401.4524, found 400.9930.

Synthesis of 35[25]

To a solution of **34** (1.66 g, 4.4 mmol) in 5 mL chloroform, trifluoroacetic acid (4.4 mL) was added. The reaction mixture was stirred for 4 h. Solvent was evaporated under reduced pressure. Yield:1.03 g (84%); ^{1}H NMR ($CDCl_3$) δ (ppm): 0.87–1.0 (m, 6H), 2.14 (m, 1H), 3.02–3.08 (m, 2H), 3.67 (s, 3H), 3.93 (m, 1H), 4.69–4.71 (d, 1H), 7.09–7.10 (d, 2H), 7.20–7.31 (m, 3H); ^{13}C NMR ($CDCl_3$) δ (ppm): 18.0, 18.1, 30.8, 37.5, 53.0, 54.8, 59.3, 127.9, 129.2, 129.5, 135.6, 171.9; ESI-MS *m/z* calcd for $C_{15}H_{22}N_2O_3$ $[M + H]^+$ 279.1709, found 278.9557.

Synthesis of 36

This compound was synthesized by following a route similar to the synthesis of **22** but using compounds **35** and **21** as starting material. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.83–0.91 (dd, 6H), 1.44 (s, 9H), 2.73–2.75 (dd, 1H), 2.84–2.86 (dd, 1H), 3.08–3.12 (m, 2H), 3.70 (s, 2H), 3.71 (s, 3H), 3.78 (s, 3H), 4.19–4.22 (m, 2H), 4.85–4.87 (dd, 1H), 6.84–6.86 (d, 2H), 7.08–7.10 (d, 2H), 7.22–7.27 (m, 5H); ^{13}C NMR ($CDCl_3$) δ (ppm): 18.1, 19.6, 25.4, 28.8, 31.0, 34.4, 36.5, 38.3, 52.8, 53.6, 55.7, 59.1, 81.0, 114.6, 127.7, 129.1, 129.7, 130.3, 136.1, 155.9, 157.2, 159.3, 170.7, 171.1, 172.1; ESI-MS *m/z* calcd for $C_{31}H_{43}N_3O_7S$ $[M + Na]^+$ 624.2719, found 624.4609.

Synthesis of 37

This disulfide was synthesized by following a method similar to the synthesis of the diselenide **26** but using the tripeptide **36**. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.96–0.98 (dd, 6H), 1.48 (s, 9H), 1.70–1.75 (m, 1H), 1.93–1.97 (m, 1H), 2.11–2.14 (m, 1H), 3.07–3.10 (m, 2H), 3.71 (s, 3H), 4.36–4.38 (dd, 1H), 4.64–4.66 (dd, 1H), 4.88–4.90 (dd, 1H), 7.14–7.16 (d, 2H), 7.24–7.31 (m, 3H); ^{13}C NMR ($CDCl_3$) δ (ppm): 19.1, 19.6, 25.4, 26.0, 28.8, 34.3, 38.3, 52.7, 53.8, 59.6, 80.6, 127.6, 129.1, 129.7, 136.3, 155.8, 171.2, 171.6, 172.3; ESI-MS *m/z* calcd for $C_{46}H_{68}N_6O_{12}S_2$ $[M + Na]^+$ 983.4234, found 983.3778.

Synthesis of 38

This compound was synthesized by following a similar procedure given for compound **30** but using the tripeptide **37.** ^{1}H NMR (MeOH-d_4) δ (ppm): 0.83–0.85 (dd, 6H), 1.71–1.73 (m, 1H), 1.93–1.96 (m, 1H), 2.81–2.84 (m, 1H), 2.96–2.99 (m, 1H), 3.15–3.17 (d, 1H), 3.51 (s, 3H), 4.25 (dd, 1H), 4.38–4.40 (dd, 1H), 4.52–4.54 (dd, 1H), 7.03–7.11 (m, 5H); ^{13}C NMR (MeOH-d_4) δ (ppm): 17.7, 18.6, 25.0, 29.7, 31.5, 37.3, 54.5, 58.8, 78.4, 127.0, 128.6, 129.2, 136.9, 167.7, 172.1, 172.4; ESI-MS *m/z* calcd for $C_{36}H_{52}N_6O_8S_2$ $[M + H]^+$ 761.3366, found 761.3551.

Synthesis of 39

To a solution of **20** (0.79 g, 2.0 mmol) in 120 mL chloroform, DCC (0.62 g, 3 mmol) and HOBt (0.46 g, 3.0 mmol) were added at 0 °C and stirred for 20 min. *S*-Methyl-piperidine-2-carboxylate (0.43 g, 3.0 mmol) was added to this and the mixture was stirred at room temperature for 10 h. The precipitate was filtered and the filtrate was washed three times successively with 1 M $KHSO_4$ solution, 1 M Na_2CO_3 solution and brine. The solvent was evaporated and the compound was purified by column chromatography (hexane : ethyl acetate; 1 : 2). Yield 0.62 g (59%); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.44 (s, 9H), 1.57–1.63 (m, 6H), 2.23–2.26 (d, 1H), 2.59–2.64 (m, 1H), 2.79–2.84 (m, 1H), 3.03–3.06 (t, 1H), 3.69 (s, 3H), 3.76–3.79 (m, 5H), 4.83–4.84 (dd, 1H), 5.33–5.34 (d, 1H), 6.79–6.81 (d, 2H), 7.22–7.24 (d, 2H); ^{13}C NMR ($CDCl_3$) δ (ppm): 21.4, 25.0, 25.6, 26.9, 27.7, 28.8, 40.6, 44.0, 50.6, 52.8, 55.7, 80.2, 114.3, 130.6, 131.4, 155.6, 158.8, 171.3, 171.7; ^{77}Se NMR ($CDCl_3$) δ (ppm) 222; ESI-MS: *m/z* calcd for $C_{23}H_{34}N_2O_6Se$ $[M + Na]^+$ 537.1480, found 537.1052.

Synthesis of 40

To a solution of **21** (0.68 g, 2.0 mmol) in 120 mL chloroform, DCC (0.62 g, 3.0 mmol) and HOBt (0.46 g, 3.0 mmol) were added at 0 °C and stirred for 20 min. *S*-Methyl-piperidine-2-carboxylate (0.43 g, 3.0 mmol) was added to this and the mixture was stirred at room temperature for 10 h. The precipitate was filtered and the filtrate was washed three times each with 1 M $KHSO_4$ solution, 1 M Na_2CO_3 solution and brine. The solvent was evaporated and the compound was purified by column chromatography (hexane : ethyl acetate; 1 : 2). Yield 0.69 g (74%). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.46 (s, 9H), 1.57–1.63 (m, 4H), 1.71–1.75 (m, 2H), 2.24–2.28 (m, 1H), 2.49–2.54 (m, 1H), 2.77–2.82 (m, 1H), 3.03–3.06 (t, 1H), 3.70 (s, 3H), 3.79–3.81 (m, 5H), 4.82–4.84 (dd, 1H), 5.34–5.35 (d, 1H), 6.83–6.85 (d, 2H), 7.26–7.28 (d, 2H); ^{13}C NMR ($CDCl_3$) δ (ppm): 21.3, 25.6, 26.9, 27.7, 28.8, 34.0, 36.2, 43.9, 50.2, 52.8, 55.7, 80.2, 114.3, 130.3, 130.6, 155.8, 159.1, 171.3, 171.7; ESI-MS: *m/z* calcd for $C_{23}H_{34}N_2O_6S$ $[M + Na]^+$ 489.2035, found 489.1072.

Synthesis of 41

This compound was synthesized following a similar method given for compound **26** using compound **39** as the starting material. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.44 (s, 9H), 1.68–1.76 (m, 5H), 2.27–2.30 (d, 1H), 3.21–3.31 (m, 3H), 3.73 (s, 3H), 3.96–3.99 (m, 1H), 4.98–5.01 (dd, 1H), 5.35–5.37 (d, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm): 21.3, 25.5, 26.8, 28.7, 33.1, 40.5, 44.0, 51.0, 52.7, 80.3, 155.5, 170.9, 171.6; ^{77}Se NMR ($CDCl_3$) δ (ppm) 309; ESI-MS: *m/z* calcd for $C_{30}H_{50}N_4O_{10}Se_2$ $[M + Na]^+$ 809.1755, found 809.1743.

Synthesis of 42

Compound **42** was synthesized by following a procedure similar to the synthesis of compound **26** by using compound **40**. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.36 (s, 9H), 1.60–1.68 (m, 5H), 2.19–2.22 (m, 2H), 3.01–3.06 (m, 1H), 3.15–3.20 (t, 1H), 3.65 (s, 3H), 3.91–3.94 (m, 1H), 4.82–4.84 (dd, 1H), 5.28–5.29 (dd, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm): 21.3, 25.5, 26.7, 28.6, 41.7, 43.9, 52.6, 56.6, 80.2, 155.5, 170.8, 171.6; ESI-MS: *m*/*z* calcd for $C_{30}H_{50}N_4O_{10}S_2$ $[M + Na]^+$ 713.2866, found 713.2884.

Synthesis of 43

Compound **43** was synthesized following a procedure similar to the synthesis of **30** but using compound **41** as starting material. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.30–1.33 (m, 1H), 1.52–1.73 (m, 3H), 2.25–2.28 (m, 1H), 3.21–3.31 (m, 2H), 3.44–3.48 (m, 2H), 3.71 (s, 3H), 3.82–3.86 (m, 1H), 4.82–4.84 (dd, 1H), 5.27–5.29 (d, 1H); ^{13}C NMR (MeOH-d_4) δ (ppm): 20.8, 25.1, 26.4, 34.1, 43.8, 51.1, 52.2, 53.1, 167.7, 171.5; ^{77}Se NMR (MeOH-d_4) δ (ppm) 286; ESI-MS: *m*/*z* calcd for $C_{20}H_{34}N_4O_6Se_2$ $[M + Na]^+$ 609.0706, found 609.1763.

Synthesis of 44

This compound was synthesized following a similar method as reported for compound **30** but using compound **42**. ^{1}H NMR (MeOH-d_4) δ (ppm): 1.32–1.35 (m, 1H), 1.69–1.76 (m, 3H), 2.27–2.30 (m, 1H), 3.01–3.07 (m, 1H), 3.28–3.38 (m, 3H), 3.72 (s, 3H), 3.87–3.90 (m, 1H), 4.91–4.94 (dd, 1H), 5.29–5.30 (d, 1H); ^{13}C NMR (MeOH-d_4) δ (ppm): 20.8, 25.1, 26.4, 26.8, 36.3, 43.8, 52.2, 53.2, 167.6, 171.5; ESI-MS: *m*/*z* calcd for $C_{20}H_{34}N_4O_6S_2$ $[M + Na]^+$ 513.1817, found 513.3591.

Synthesis of peroxynitrite (PN)

Peroxynitrite was synthesized by following the literature method with minor modifications.[6]

ACE assay

The assay was performed in 400 μL sample vials and an autosampler was used for sample injection. Ang I and AngII were analyzed by reverse-phase HPLC (Princeton C18 column, 4.6 × 150 mm, 5 μm) with isocratic elution of 50 : 50 MeOH : 0.1% TFA in water. In the ACE inhibition assay, we employed a mixture of 50 μM Ang I, 60 mM sodium chloride, 2 milliunits of ACE in 50 mM HEPES-HCl buffer at pH 8.3 with various concentrations of the inhibitors. The reaction mixture was incubated at 37 °C for 30 min prior to injection. Selenols and thiols were freshly prepared by reducing the diselenides and disulfides by $NaBH_4$ prior to use and were kept under N_2 atmosphere during the assay. The decrease in the formation of Ang II with an increase in the concentration of inhibitor was monitored at 215 nm and the % inhibition was calculated by comparing the peak areas. The inhibition plots were obtained by using Origin 6.1 software utilizing sigmoidal curve fitting and these plots were used for the calculation of IC_{50} values.

Nitration of angiotensin II

High performance liquid chromatography (HPLC) experiments were carried out on a Waters–Alliance system (Milford, MA) consisting of a 2695 separation module, a 2996 photodiode-array detector and a fraction collector. The assays were performed in 1.8 mL sample vials and a built-in autosampler was used for sample injection. The Alliance HPLC System was controlled with EMPOWER software (Waters Corporation, Milford, MA). The nitration assay of Ang II was analyzed by reverse-phase HPLC method (Princeton C18 column, 4.6 × 150 mm, 5 μm) with isocratic ellution of 45 : 55 MeOH: 0.1% TFA in water. In the PN-mediated nitration of Ang II, we employed a mixture containing Ang II (20 μM) and peroxynitrite (300 μM) in sodium phosphate buffer (100 mM) of pH 7.5 without and with increasing concentration of the inhibitor was added to the assay mixture. The reaction mixture was incubated for 5 min before injection. The formation of nitro-Ang II was monitored at the wavelength of 215 nm. The inhibition plots were obtained by using Origin 6.1 software utilizing sigmoidal curve fitting and these plots were used for the calculation of IC_{50} values.

Measurement of GPx activity

GPx activity of the test compounds was carried out by following a literature procedure with minor modifications.[16b] In brief, the reaction mixture contained glutathione (4.0 mM, glutathione disulfide reductase (GR, 1.7 units mL^{-1}), and nicotinamide adenine dinucleotide phosphate (NADPH; 0.4 mM) in 100 mM potassium phosphate buffer of pH 7.5. The samples (100 μM) were added to the reaction mixture at 22 °C, and the reaction was started by addition of H_2O_2 (5 mM). The initial reduction rates (v_0) were calculated from the rate of NADPH oxidation at 340 nm. Each initial rate was measured at least 3 times and calculated from the first 5–10% of the reaction by using the molar extinction coefficient (6.22 mM^{-1} cm^{-1}) for NADPH. The initial rates were corrected for a background reaction between peroxide and glutathione.

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