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Design, synthesis and evaluation of novel 2-hydroxypyrrolobenzodiazepine-5,11-dione analogues as potent angiotensin converting enzyme (ACE) inhibitors

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ABSTRACT

A series of novel 10-substituted 2-hydroxypyrrolobenzodiazepine-5,11-diones designed through structure based rational hybridization approach, synthesized by the cyclodehydration of isotonic anhydride with (2*S*,4*R*)-4-hydroxypyrrolidine-2-carboxylic acid followed by N-substitution, were evaluated as angiotensin converting enzyme (ACE) inhibitors. Among all the new compounds screened (2*R*,11*aS*)-10-((4-bromothiophen-2-yl)methyl)-2-hydroxy-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11*aH*)dione, **5v** (IC_{50}: 0.272 μM) emerged as most active non-carboxylic acid ACE inhibitor with minimal toxicity comparable to clinical drugs Lisinopril, Benazepril and Ramipril. Favorable binding characteristics in docking studies also supported the experimental results.

1. Introduction

Despite of considerable therapeutic advances, cardiovascular disease (CVD) remained responsible for ~27% deaths worldwide, with 80% occurring in developing countries.[1,2] Recent statistics revealed that seven out of ten are suffering from multi-factorial CVD and the numbers are likely to go even higher in coming years.[2] Hypertension (a common quantitative trait under polygenic vascular disorder) is one of the most important treatable factors of CVD.[3] Angiotensin-I converting enzyme (ACE) is a key regulator of blood pressure, due to its critical role in renin–angiotensin–aldosterone system (RAAS)[4] the inhibitors of ACE have emerged as first-line therapy for hypertension in younger patients and second line in geriatric patients.[5,6] Driven by clinical evidence which demonstrates the morbidity and mortality benefits, ACE has become an important target for drug development in treating hypertension. Discovery of Captopril,[7] the first orally active ACE inhibitor, encouraged researchers' worldwide to design and develop ACE inhibitors(using the structure of carboxy peptidase) and were clinically tested.[8] However, these synthetic drugs (Fig. 1) are reported plethora of side effects to say few, renal impairment, hyperkalaemia, dry cough, skin rashes and are not tolerated in patients with long-term treatment.[9] We are now in a unique position to capitalize proven information (the real target molecule) for the design of new and more effective next generation ACE inhibitors[10] with the potential for greater efficacy in controlling hypertension. The natural products generally derived from plants or microbes are essential source for new chemical entities.[11]

They offer promising and amazing chemical diversity, there by inspiring the development of structurally diverse new molecules to play a vital role in drug discovery.[12]

Among the known naturally occurring alkaloids, pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs)[13] isolated from various *Streptomyces* species (a class of biologically active compounds with good pharmacological profile). These natural and synthetic PBDs (Fig. 2) exert anticancer activity through their interaction towards protein sequences to interfere and are currently in clinical development.[14] On the other hand, clinical ACE drugs (Fig. 1) found to possess proline or modified proline type architecture as a critical pharmacophore responsible for exhibiting ACE inhibitor activity.[15–17] With growing interest in use of pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) ring system as a potential pharmacophoric fragment.[16]

We envisaged that manoeuvring PBD with structural features of ACE inhibitors in one molecular frame could deliver a new scaffold for biological evaluation. Herein, we present an efficient synthesis

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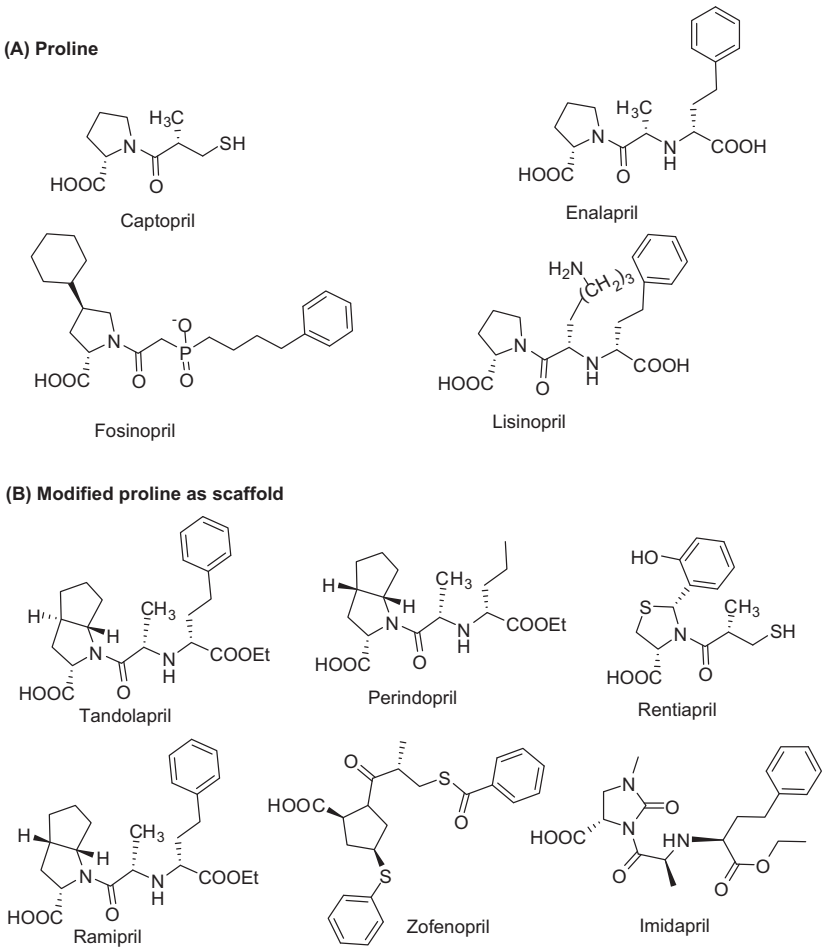


Figure 1. ACE inhibitor drugs having (A) proline; (B) modified proline as scaffold.

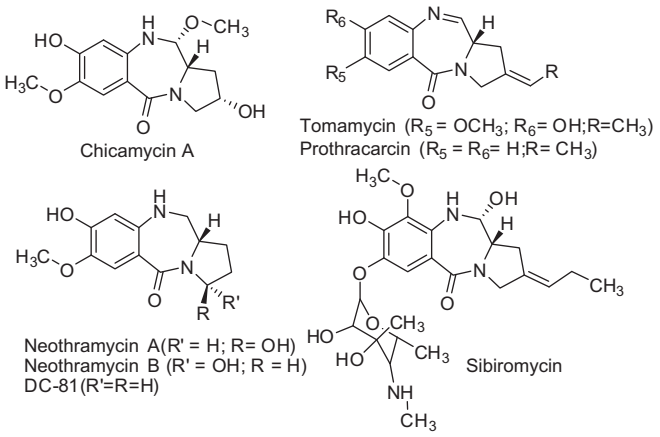


Figure 2. Natural pyrrolo[2,1-*c*][1,4]benzodiazepines.

and bio-evaluation of pyrrolo[2,1-*c*][1,4]benzodiazepine derived proline embodied hybrid heterocycles, designed through molecular hybridization approach. The inclusion of proline unit is due to the architectural similarity with pyrrolo portion in benzodiazepine (Fig. 3). All the new hybrid heterocycles **3** and **5a**–**w** were evaluated for in vitro ACE inhibitor activity using recently developed high-throughput screening method.[18] The ACE inhibition of new analogues was correlated with known reference drugs Lisinopril, Benazepril and Ramipril. In vitro cytotoxicity evaluations and molecular docking studies of lead compounds are also described.

A more holistic approach for design strategy would be through, (i) correlation of the structural features of widely used drugs (Fig. 1); (ii) identification of common pharmacophoric unit(s) and (iii) incorporate them to generate newer scaffolds with potential activity profile.[17] With the fact that, proline is a key pharmacophoric fragment in drugs such as Captopril, Enalapril and Lisinopril, we[18] vision to use this information in designing a newer scaffold. A more recent ACE inhibitor, Benazepril with fused seven member cyclic ring has structural similarity to basic benzodiazepine architecture. This rekindled our curiousness to inculcate a design strategy[15] integrating proline fragment in benzodiazepine architecture with appended liphophilic functionality. The explicit design of new molecules for the present study is depicted in Figure 3.

2. Results and discussion

2.1. Synthesis

Synthetic approach for the preparation of hydroxy pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione **5a**–**w** was presented in Scheme 1.[16] Commercially available isotonic anhydride **1** was taken as starting material. Cyclodehydration of **1** with readily available (2*S*,4*R*)-4-hydroxypyrrolidine-2-carboxylic acid **2** in DMSO resulted (2*R*,11a*S*)-2-hydroxy-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11a*H*)dione **3** in excellent yield. This

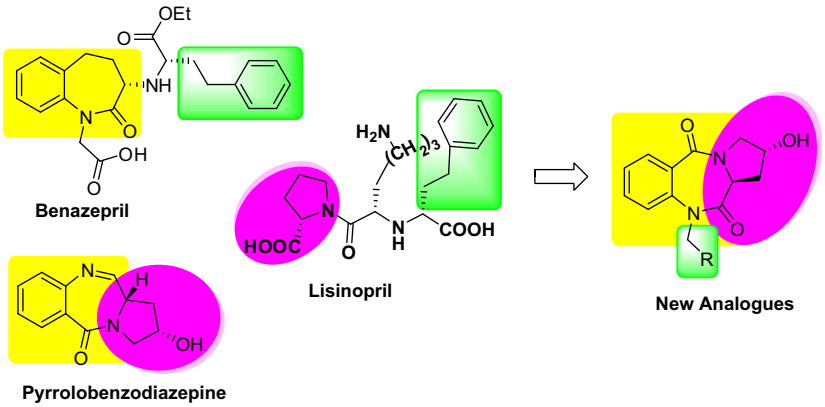
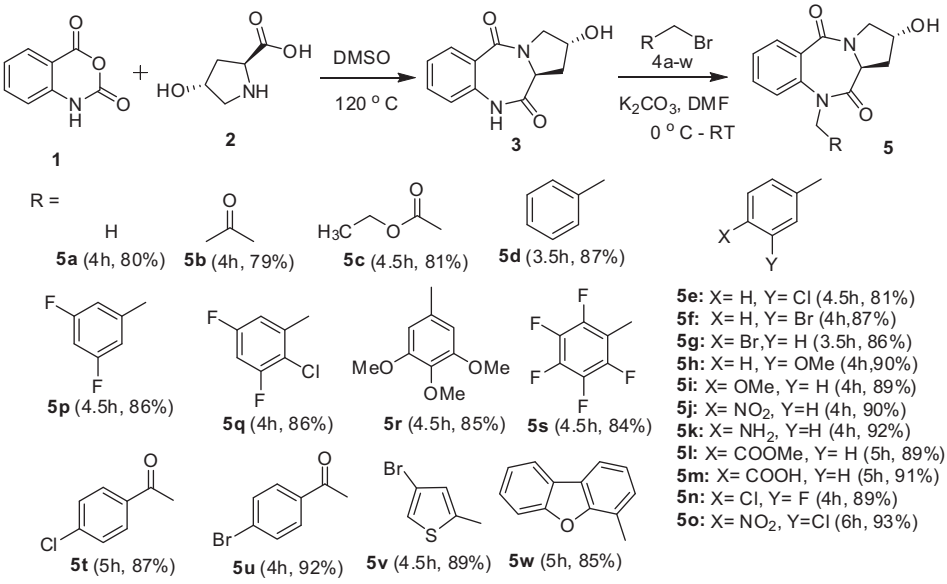


Figure 3. Structure based rational design strategy.



Scheme 1. Synthesis of new 10-substituted hydroxypyrrolo[2,1-*c*][1,4] benzodiazepine-5,11-dione analogues **5a**–**w**.

hydroxy pyrrolobenzodiazepine diones frame unit **3**, was then alkylated with series of alkyl or benzyl bromides **4b**–**w** (methyl iodide in case of **4a**), in the presence of K_2CO_3 in DMF to give 10-substituted (2*R*,11a*S*)-2-hydroxy-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11*aH*)diones **5a**–**w** in excellent yields (81–93%). All of the compounds **5a**–**w** were purified through silica gel column chromatography (HPLC purity >96%) and were fully characterized by IR, ^{1}H NMR, ^{13}C NMR, electron spray ionization (ESI), and high-resolution mass spectral (HRMS) analysis (Supplementary data). Further single crystal X-ray diffraction analysis of **5g** unambiguously confirmed the structure and stereochemistry (Fig. 4).[19]

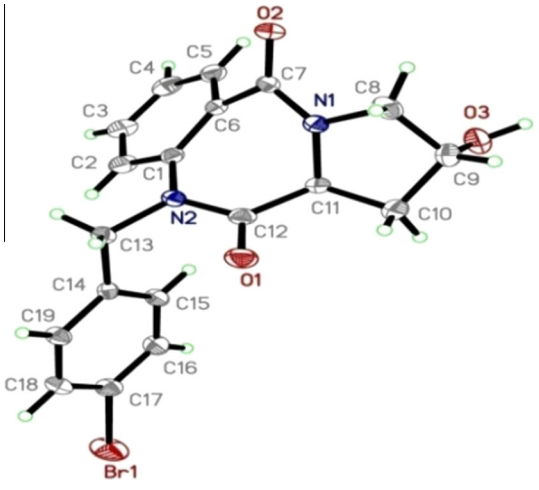


Figure 4. ORTEP representation of **5g** with thermal displacement ellipsoids drawn at the 30% probability.

2.2. Pharmacology

In vitro angiotensin converting enzyme (ACE) inhibition of '24' new analogues **3** and **5a**–**w** were examined using recently developed high-throughput colorimetric screening method.[20] Most of these anti-hypertensive peptides have been characterized by the rabbit lung ACE inhibitor assay, based on the hydrolysis of the synthetic peptide hippuryl–histidyl–leucine (HHL). Angiotensin converting enzyme (ACE) hydrolyses HHL to hippuric acid (HA) and histidyl–leucine (HL). HA released is directly proportional to the ACE activity. In this screening method, the released hippuric acid

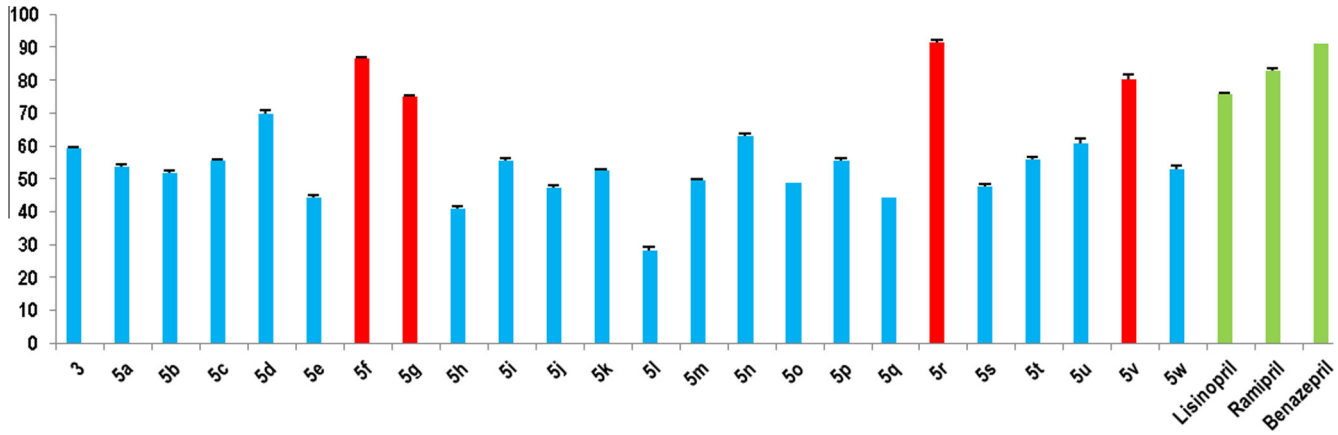


Figure 5. In vitro angiotensin converting enzyme (ACE) inhibition of new analogues **3**, **5a**–**w** and standard drugs.

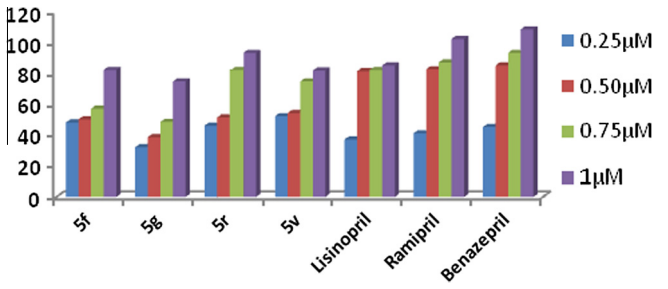


Figure 6. Dose–response curves of synthetic hydroxy pyrrolobenzodiazepine dione derivatives **5f**, **5g**, **5r**, **5v** and standard drugs.

Table 1
MTT assay inhibitory activities (IC_{50}) in μM

Compound	HEK 293[a]	A549[b]
Lisinopril	443	307
Ramipril	414	392
Benazepril	301	358
5f	398	400.4
5g	496	486.7
5r	331	418.7
5v	281	361

[a] HEK 293: Human Embryonic Kidney 293.
[b] A549: Human alveolar basal epithelial cells.

from the substrate hippuryl–histidyl–leucine (HHL) was transformed into yellow colour by mixing with pyridine and benzene sulfonyl chloride. The resulted yellow colour was determined colorimetrically at absorbance 410 nm. The average ACE inhibitor activity was measured in triplicate for new analogues **5a**–**w** and the standard drugs Lisinopril, Benazepril and Ramipril (Fig. 5). The experiment carried out at 1.0 μM concentration of test compounds **3** and **5a**–**w** revealed 28–91% of ACE inhibition activity. Among all the PBD derived analogues, four compounds **5f**–**g**, **5r** and **5v** posses better ACE inhibitory activity (>75%) compared with the other substituted analogues. Among all the substituted PBDs, the trimethoxy derivative **5r** revealed highest ACE inhibition and **5l** has least ACE inhibitory activity. Relating to the reference drugs Lisinopril, Benazepril and Ramipril these four analogues **5f**–**g**, **5r** and **5v** were comparably active with 86%, 75%, 91% and 80% ACE inhibition, respectively. Pyrrolobenzodizepine-5,11-dione **3**, showing 57% ACE inhibition, suggested the importance of PBD as a pharmacophoric fragment in the development of new ACE inhibitors. Also to note that, these three active analogues, **5f**–**g** and **5v** has bromo functionality on phenyl and thiophenyl rings. In case of **5e**, **5p**–**q** and **5s**–**t** having fluoro/chloro groups was resulted in lower ACE inhibition compared to the respective benzyl analogue **5d**. Further, the inhibitory concentration at 50% (IC_{50}) ACE activity for **5f**–**g**, **5r** and **5v** along with reference drugs was calculated from dose–response curves (Fig. 6) obtained by plotting the percentage inhibition verses the concentration.

Evaluation of IC_{50} data for **5f** (0.418 μM), **5g** (0.658 μM), **5r** (0.376 μM) and **5v** (0.272 μM), revealed **5v** as the best lead compound with lowest IC_{50} (0.272 μM). This result also correlate with IC_{50} values of all the three reference drugs, Lisinopril (0.262 μM), Ramipril (0.253 μM) and Benazepril (0.212 μM).

The toxicity evaluation of four most potent ACE inhibitors **5f**–**g**, **5r** and **5v** was assessed by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[21] against HEK 293 (normal human embryonic kidney) and A549 (adeno carcinomic human alveolar basal epithelial) cell lines. The inhibitory activities (IC_{50}) in μM are summarized in Table 1. The compounds **5f**–**g**, **5r** and **5v** evaluated, exhibit higher inhibitory concentration (IC_{50}) at a magnitude of ~1000 times more than the concentration (IC_{50}) of ACE inhibition activity and are considered nontoxic. Under similar conditions, toxicity of new analogues **5f**–**g**, **5r** and **5v** were comparable with the evaluated reference drugs Lisinopril, Benazepril and Ramipril.

2.3. Molecular modelling

To gain insight into the ACE-ligand binding mode, the most potent analogues **5f**–**g**, **5r** and **5v** were docked on to tACE (PDB code: 1O86) using Molegro Virtual Docker (MVD) software.[22] The intermolecular interactions between tACE binding site residues and predicted poses for lead analogue **5v** are shown in Figure 7. Since the first report from Acharya and co-workers on crystal structure of Lisinopril bound–ACE complex,[17,23] a greater understanding of binding site was emerged for the development of new ACE inhibitors. To validate the Molegro Virtual Docker (MVD) software, initially Lisinopril was docked and the results obtained were correlated with reported molecular interactions.[19] Similar to Lisinopril, the new potent analogue **5v** also share interactions at S1, S2, S1′ and S2′ binding subsites at the active site of enzyme. Lisinopril and potent **5v** ligand as the same interactions at Ala 356, His 513

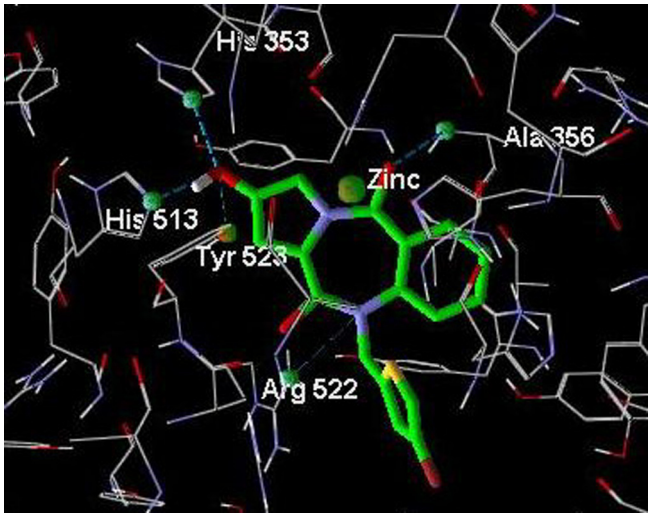


Figure 7. Best predicted binding mode of ligand **5v** at the tACE binding site.

and His 353. Hence, the subsite S2′ plays a major role in tACE binding.

Docking results suggest that the hydroxyl group on pyrrolo residue of **5v** is positioned by strong hydrogen bonds from His513 (2.74 Å), Tyr523 (2.35 Å) and His353(2.69 Å) in S1 and S2′ subsites. The central carbonyl group of phenyl ring also interacts with Ala356 (2.99 Å). These interactions in **5v** bound ACE complex were in close relation with reported ACE-drug complexes of Lisinopril and Captopril.[17,23,25] Also to observe that Arg522 (3.43 Å) in S2 subsite make interaction with 'N' attached to phenyl group in benzodiazepine architecture. The tilted bromo thiophene residue makes additional electrostatic interaction with residues of Glu411 (3.33 Å). Such interaction of new molecules at the subsites depends on the nature of substituent attached and their staking in hydrophobic cavity between Phe512 and Val518 residues in S1 and S1/S2 subsites of ligand bound ACE complex. Such staking of aryl or thiophene substituent was well corroborating with the binding site information reported for ACE bound other drugs, Enalapril and Fosinopril.[24,25] At this point of investigation, it was remarkable to note that almost all the functional groups of **5v** were occupied with either hydrogen bonding or other non-covalent lipophilic interactions and were important in exerting greater affinity towards tACE enzyme and then ACE inhibition activity.

3. Conclusion

In this present study, we report a new series of 10-substituted 2-hydroxy-2,3-dihydro-1*H*-benzo[e]pyrrolo[1,2-*a*][1,4]diaze pine-5,11(10*H*,11*aH*) diones as angiotensin converting enzyme inhibitors. Among them (2*R*,11a*S*)-10-((4-bromothiophen-2-yl)methyl)-2-hydroxy-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11*aH*)dione, **5v** (IC_{50}: 0.272 μM) emerged as most active non-carboxylic acid ACE inhibitor with minimal toxicity comparable to clinical drugs Lisinopril, Benazepril and Ramipril. The structure activity correlation of lead analogue **5v** was also made using molecular docking studies. Docking study revealed that bulky and hydrophobic group substituents affected in vitro activities. The results presented here can lead to generate potential new PBD based non-carboxylic acid ACE inhibitors.

4. Experimental section

Melting points were measured with a Fischer-Johns melting point apparatus and are uncorrected. IR spectra were recorded as neat liquids or KBr pellets and absorptions are reported in cm^{-1}. NMR spectra were recorded on 300 (Bruker) and 500 MHz (Varian) spectrometers in appropriate solvents using TMS as internal standard or the solvent signals as secondary standards and the chemical shifts are shown in δ scales. Coupling constants *J* are expressed in Hertz. ^{13}C NMR spectra were recorded on 75 and 125 MHz spectrometers. High-resolution mass spectra were obtained by using ESI-QTOF mass spectrometry. Reagents and all solvents were analytically pure and were used without further purification. All the experiments were monitored by analytical thin layer chromatography (TLC) performed on silica gel GF254 pre-coated plates. After elution, plate was visualized under UV illumination at 254 nm for UV active materials. Further visualization was achieved by staining with PMA and charring on a hot plate. Silica gel finer than 200 mesh was used for column chromatography. Appropriate names for all the new compounds were given with the help of ChemBioOffice 2010.

4.1. General procedure for preparation of (2*R*,11a*S*)-2-hydroxy-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4] diazepine-5,11(10*H*,11*aH*)-dione (3)

A mixture of 1*H*-benzo[*d*][1,3]oxazine-2,4-dione **1** (5.0 g, 30.6 mmol) and (2*S*,4*R*)-4-hydroxy pyrrolidine-2-carboxylic acid **2** (4.42 g, 33.7 mmol) in DMSO was refluxed for 4 h. After completion (by TLC), the reaction mixture was cooled at room temperature, water (50 mL) was added, solid precipitate thus formed was filtered, washed with water and dried to give the pure (2*R*,11a*S*)-2-hydroxy-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11*aH*)-dione **3** as brown solid. Mp: 223–225 °C $[\alpha]_D^{25}$ + 448 (*c* 1.0, CH_3OH). ^1H NMR (300 MHz, DMSO-d_6) δ 10.29 (s, 1H), 7.89 (d, *J* = 7.74 Hz, 1H), 7.45 (t, *J* = 7.54 Hz, 1H), 7.21 (t, *J* = 7.17 Hz, 1H), 7.13 (d, *J* = 7.93 Hz, 1H), 5.09 (s, 1H), 4.49 (br s, 1H), 4.21 (t, *J* = 6.23 Hz, 1H), 3.82–3.86 (m, 1H), 3.59 (dd, *J* = 4.34 Hz, *J* = 7.93 Hz, 1H), 2.78–2.86 (m, 1H), 2.04–2.17 (m, 1H). HR-MS (ESI) Calcd for $C_{12}H_{12}N_2O_3Na$ $[M+H]^+$: 255.07401. Found 255.07408.

4.2. General procedure for preparation of N-substituted pyrrolobenzodiazepine derivatives 5a–w

(2*R*,11a*S*)-2-Hydroxy-2,3-dihydro-1*H*-benzo[*e*]pyrrole[1,2-*a*][1,4]diazepine-5,11(10*H*,11*aH*)-dione **3** (0.4 g, 1.71 mmol) and K_2CO_3 (0.473 g, 3.43 mmol) in DMF at 0 °C for 30 min was added substituted alkyl bromides **4a**–**w** (2.06 mmol) to the reaction and stirred for 4 h under inert conditions. After completion (by TLC), the reaction mixture was filtered through Buchner funnel and washed with EtOAc. The product was extracted with EtOAc (3 × 30 mL) and cold water (20 mL). Combined organic phase was dried over Na_2SO_4 and evaporated under vacuum. The crude residue thus obtained was further purified by silica gel column chromatography eluted with mixture of ethyl acetate and hexane.

4.2.1. (2*R*,11a*S*)-2-Hydroxy-10-methyl-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11*aH*)-dione 5a

Brown syrup. $[\alpha]_D^{25}$ + 186.3 (*c* 1.0, CH_3OH). ^1H NMR (300 MHz, CD_3OD) δ 7.82 (dd, *J* = 1.51 Hz, *J* = 6.23 Hz, 1H), 7.62 (t, *J* = 6.98 Hz, 1H), 7.43 (d, *J* = 8.12 Hz, 1H), 7.35 (t, *J* = 7.93 Hz, 1H), 4.48–4.54 (m, 1H), 4.25 (dd, *J* = 5.66 Hz, *J* = 2.26 Hz, 1H), 3.57–3.72 (m, 2H), 3.39 (s, 3H), 2.81–2.90 (m, 2H), 2.00–2.09 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ 171.1, 167.9, 142.1, 133.7, 130.8, 129.8, 126.8, 123.5, 69.7, 57.4, 54.7, 36.5, 35.8. IR (Neat) 3428, 2923, 2853, 1675, 1629, 1467, 1423, 1384,769 cm^{-1}. HR-MS (ESI) Calcd for $C_{13}H_{15}N_2O_3$ $[M+H]^+$: 247.10772. Found 247.10773.

4.2.2. (2*R*,11a*S*)-2-Hydroxy-10-(2-oxopropyl)-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11*aH*)-dione 5b

Light yellow syrup. $[\alpha]_D^{25}+267.3$ (*c* 1.0, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$) δ 7.87 (d, *J* = 3.30 Hz, 1H), 7.47 (t, *J* = 8.30 Hz, 1H), 7.29 (t, *J* = 7.55 Hz, 1H), 7.03 (d, *J* = 8.30 Hz, 1H), 4.86 (d, *J* = 18.1 Hz, 1H), 4.59–4.63 (m, 1H), 4.30–4.34 (m, 1H), 4.19 (d, *J* = 18.1 Hz, 1H), 3.84–3.89 (m, 1H), 3.61–3.67 (m, 1H), 2.83–2.95 (m, 2H), 2.28 (s, 3H), 1.97–2.16 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.0, 168.9, 165.8,132.4, 130.3, 129.0,126.2, 121.8, 69.0,59.4, 55.7, 54.0, 34.9, 29.6. IR (KBr) 3414, 2923, 2854, 1728, 1629, 1464, 1417, 1214, 766 cm^{-1}. HR-MS (ESI) Calcd for $C_{15}H_{17}N_2O_4$ $[M+H]^+$: 289.1188. Found 289.1193.

4.2.3. Ethyl 2-((2*R*,11a*S*)-2-hydroxy-5,11-dioxo-2,3,11,11*a*-tetrahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepin-10-(5*H*)-yl) acetate 5c

Mp: 195–197 °C $[\alpha]_D^{25}+360.0$ (*c* 1.0, CH_3OH). ^1H NMR (500 MHz, DMSO-d_6) δ 7.86 (d, *J* = 7.99 Hz, 1H), 7.55 (t, *J* = 6.99 Hz, 1H), 7.34 (t, *J* = 6.99 Hz, 1H), 7.26 (d, *J* = 7.99 Hz, 1H), 4.57 (d, *J* = 17.9 Hz, 1H), 4.49 (br s, 1H), 4.41 (d, *J* = 17.9 Hz, 1H), 4.29 (t, *J* = 6.99 Hz, 1H), 4.19 (q, *J* = 7.99 Hz, *J* = 7.99 Hz, 2H), 3.74 (d, *J* = 12.9 Hz, 1H), 3.57 (dd, *J* = 4.99 Hz, *J* = 6.99 Hz, 1H), 2.80–2.84 (m, 2H), 2.00–2.09 (m, 1H), 1.26 (t, *J* = 7.99 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6) δ 167.9, 167.3, 164.0, 138.1, 131.0, 128.8, 128.3, 124.8, 121.0, 66.9, 60.1, 54.4, 52.6, 49.9, 33.7, 12.8. IR (KBr): 3349, 2990, 1752, 1681, 1571, 1466, 1375, 1202, 1080, 761, 590 cm^{-1}. HR-MS (ESI) Calcd for $C_{16}H_{19}N_2O_5$ $[M+H]^+$: 319.12885. Found 319.12873.

4.2.4. (2*R*,11a*S*)-10-Benzyl-2-hydroxy-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11*aH*)-dione 5d

Mp: 180–182 °C $[\alpha]_D^{25}+324.3$ (*c* 1.0, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$) δ 7.86 (d, *J* = 6.42 Hz, 1H), 7.42 (t, *J* = 6.42 Hz, 1H), 7.13–7.31 (m, 7H), 5.17 (d, *J* = 15.67 Hz, 1H), 5.00 (d, *J* = 15.67 Hz, 1H), 4.65–4.68 (m, 1H), 4.36 (t, *J* = 6.23 Hz, 1H), 3.89–3.95 (m, 1H), 3.67 (dd, *J* = 4.53 Hz, *J* = 4.53 Hz, 1H), 2.96–3.06 (m, 2H), 2.12–2.20 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6) δ 168.0, 164.4, 138.4, 135.9, 131.0, 129.1, 128.8, 127.6, 126.2, 125.6, 124.8, 121.3, 67.4, 55.0, 52.9, 50.9, 34.1. IR (KBr) 3401, 2925, 1676, 1630, 1464, 1385, 1219, 1092, 771 cm^{-1}. HR-MS (ESI) Calcd for $C_{19}H_{19}N_2O_3$ $[M+H]^+$: 323.1395. Found 323.1398.

4.2.5. (2*R*,11a*S*)-10-(3-Chlorobenzyl)-2-hydroxy-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11*aH*)-dione 5e

Mp: 110 °C $[\alpha]_D^{25}+387.8$ (*c* 1.0, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 7.86 (d, *J* = 7.74 Hz, 1H), 7.47 (t, *J* = 7.17 Hz, 1H), 7.16–7.32 (m, 5H), 7.03 (d, *J* = 6.98 Hz, 1H), 5.12 (d, *J* = 15.8 Hz, 1H), 5.04 (d, *J* = 15.8 Hz, 1H), 4.55 (br s, 1H), 4.35 (t, *J* = 6.04 Hz, 1H), 3.79–3.83 (m, 1H), 3.62 (dd, *J* = 4.91 Hz *J* = 7.55 Hz, 1H), 2.87–2.96 (m, 2H), 2.08–2.16 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ 170.7, 167.8, 140.6, 140.6, 135.5, 133.8, 131.2, 131.1, 130.9, 128.5, 128.3, 127.4, 126.4, 124.3, 69.3, 57.6, 54.7, 52.0, 35.8. IR (KBr) 3418, 2930, 2096, 1644, 1601, 1463, 1384, 1218, 1164, 1078, 959, 856, 771 cm^{-1}. HR-MS Calcd for $C_{19}H_{18}N_2ClO_3$ $[M+H]^+$: 357.1005. Found 357.1017.

4.2.6. (2*R*,11a*S*)-10-(3-Bromobenzyl)-2-hydroxy-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11*aH*)-dione 5f

Mp: 171–173 °C $[\alpha]_D^{25}+268.0$ (*c* 1.0, CH_3OH). ^1H NMR (500 MHz, DMSO-d_6) δ 7.80 (d, *J* = 8.27 Hz, 1H), 7.49 (t, *J* = 8.27 Hz, 1H), 7.28–7.35 (m, 4H), 7.17 (t, *J* = 8.27 Hz, 1H), 7.07 (d, *J* = 7.24 Hz, 1H), 5.04–5.15 (m, 3H), 4.48–4.50 (m, 1H), 4.34 (t, *J* = 6.20 Hz, 1H), 3.70 (dd, *J* = 3.10 Hz, *J* = 9.31 Hz, 1H), 3.58 (dd, *J* = 4.13 Hz, *J* = 7.24 Hz, 1H), 2.82–2.87 (m, 1H), 2.04–2.09 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6) δ 167.5, 163.5, 138.2, 137.6, 130.6, 128.8, 128.6, 128.5, 128.3, 128.1, 124.4, 123.8, 120.9, 66.6, 54.3, 52.2, 49.6, 33.5. IR (KBr) 3418, 2962, 1677, 1602, 1471, 1384, 1218, 1149, 1086, 950, 867, 764, 669 cm^{-1}. HR-MS (ESI) Calcd for $C_{19}H_{18}N_2O_3Br$ $[M+H]^+$: 401.0500. Found 401.0497.

4.2.7. (2*R*,11a*S*)-10-(4-Bromobenzyl)-2-hydroxy-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11*aH*)-dione 5g

Mp: 150–152 °C $[\alpha]_D^{25}+556.0$ (*c* 1.0, CH_3OH). ^1H NMR (300 MHz, DMSO-d_6) δ 7.85 (d, *J* = 7.74 Hz, 1H), 7.46 (t, *J* = 8.12 Hz, 1H), 7.39 (d, *J* = 8.30 Hz, 2H), 7.27 (q, *J* = 7.74 Hz, *J* = 10.1 Hz, 2H), 7.03 (d, *J* = 8.30 Hz, 2H), 4.99–5.07 (m, 2H), 4.55 (br s, 1H), 4.33 (t, *J* = 7.55 Hz, 1H), 3.81 (d, *J* = 12.46 Hz, 1H), 3.60 (dd, *J* = 4.72 Hz, *J* = 7.55 Hz, 1H), 2.82–2.96 (m, 2H), 1.99–2.16 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6) δ 168.4, 164.7, 138.6, 135.6, 131.5, 131.0, 129.5, 129.3, 128.1, 125.4, 121.8, 120.2, 67.7, 55.4, 53.3, 50.7, 34.5. IR(KBr) 3399, 3007, 2939, 1678, 1632, 1572, 1464, 1391, 1214, 986, 872, 757 cm^{-1}. HR-MS (ESI) Calcd for $C_{19}H_{18}N_2O_3Br$ $[M+H]^+$: 401.04953. Found 401.04961.

4.2.8. (2*R*,11a*S*)-2-hydroxy-10-(3-methoxybenzyl)-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11*aH*)-dione 5h

Mp: 102–105 °C $[\alpha]_D^{25}+355.3$ (*c* 1.0, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$) δ 7.85 (d, *J* = 6.23 Hz, 1H), 7.41 (t, *J* = 6.61 Hz, 1H), 7.17–7.28 (m, 3H), 6.67–6.77 (m, 3H), 5.14 (d, *J* = 15.8 Hz, 1H), 4.98 (d, *J* = 15.8 Hz, 1H), 4.63–4.68 (m, 1H), 4.35 (t, *J* = 6.04 Hz, 1H), 3.90 (d, *J* = 11.3 Hz, 1H), 3.74 (s, 3H), 3.67 (dd, *J* = 4.53 Hz, *J* = 8.12 Hz, 1H), 2.95–3.05 (m, 2H), 2.11–2.19 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9, 165.7, 159.8, 139.6, 138.3, 132.1, 130.3, 129.7, 126.1, 122.2, 118.9, 112.7, 112.2, 69.2, 56.0, 55.1, 53.9, 52.5, 35.1. IR 3401, 2929, 1675, 1463, 1385, 1260, 1219, 1158, 1049, 771 cm^{-1}. HR-MS (ESI) Calcd for $C_{20}H_{21}N_2O_4$ $[M+H]^+$: 353.14958. Found 353.14977.

4.2.9. (2*R*,11a*S*)-2-hydroxy-10-(4-methoxybenzyl)-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11*aH*)-dione 5i

Mp: 124–126 °C $[\alpha]_D^{25}+433.3$ (*c* 1.0, CH_3OH). ^1H NMR (500 MHz, $CDCl_3$) δ 7.78 (br s, 1H), 7.34 (t, *J* = 6.99 Hz, 1H), 7.15–7.18 (m, 2H), 6.98 (d, *J* = 7.99 Hz, 2H), 6.72 (d, *J* = 8.99 Hz, 2H), 4.95 (s, 2H), 4.58 (br s, 1H), 4.24 (t, *J* = 6.99 Hz, 1H), 3.80 (d, *J* = 12.9 Hz, 1H), 3.67 (s, 3H), 3.60 (d, *J* = 12.9 Hz, 1H), 2.87–2.96 (m, 2H), 2.03–2.08 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.8, 165.8, 158.8, 139.6, 132.1, 130.3, 129.7, 128.7, 128.2, 126.1, 122.4, 114.1, 69.3, 56.1, 55.1, 53.9, 52.0, 35.0. IR (KBr) 3402, 2933, 1676, 1572, 1464, 1295, 1246, 1092, 956, 769 cm^{-1}. HR-MS (ESI) Calcd for $C_{20}H_{21}N_2O_4$ $[M+H]^+$: 353.1501. Found 353.1517.

4.2.10. (2*R*,11a*S*)-2-Hydroxy-10-(4-nitrobenzyl)-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11*aH*)-dione 5j

Mp: 80–82 °C $[\alpha]_D^{25}+425.3$ (*c* 1.0, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 8.17 (d, *J* = 9.06 Hz, 2H), 7.85 (d, *J* = 7.55 Hz, 1H), 7.46 (t, *J* = 6.79 Hz, 1H), 7.27–7.33 (m, 3H), 7.16 (d, *J* = 8.30 Hz, 1H), 5.18 (s, 2H), 4.63–4.68 (m, 1H), 4.39 (t, *J* = 7.55 Hz, 1H), 3.66 (dd, *J* = 4.53 Hz, *J* = 7.55 Hz, 1H), 3.90–3.95 (m, 1H), 2.93–3.01 (m, 2H), 2.14–2.22 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.0, 165.6, 147.2, 144.1, 139.1, 132.4, 130.6, 129.5, 127.5, 126.5, 124.0, 121.9, 69.0, 56.0, 54.1, 52.1, 34.9. IR (KBr)

3401, 1679, 1632, 1519, 1463, 1385, 1346, 1219, 1092, 771 cm^{-1}. HR-MS (ESI) Calcd for $C_{19}H_{18}N_3O_5$ $[M+H]^+$: 368.1246. Found 368.1258.

4.2.11. (2*R*,11a*S*)-10-(4-Aminobenzyl)-2-hydroxy-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11a*H*)-dione 5k

Mp: 95–97 °C $[\alpha]_D^{25}$ + 480.0 (*c* 1.0, CH_3OH). ^1H NMR (300 MHz, CD_3OD) δ 7.74 (dd, *J* = 1.51 Hz, *J* = 6.60 Hz, 1H), 7.48–7.57 (m, 2H), 7.27–7.32 (m, 1H), 6.81 (d, *J* = 8.49 Hz, 2H), 6.54 (d, *J* = 8.49 Hz, 2H), 5.34 (d, *J* = 15.1 Hz, 2H), 4.79 (d, *J* = 15.1 Hz, 1H), 4.52–4.58 (m, 1H), 4.33 (dd, *J* = 5.47 Hz, *J* = 2.64 Hz, 1H), 3.57–3.72 (m, 2H), 2.87–2.96 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6) δ 168.2, 164.8, 145.5, 138.7, 131.1, 129.2, 127.3, 125.0, 121.9, 114.3, 67.7, 55.3, 53.0, 50.8, 34.3. IR (KBr) 3357, 2933, 1671, 1628, 1465, 1396, 1249, 1052, 956, 715, 620 cm^{-1}. HR-MS (ESI) Calcd for $C_{19}H_{20}N_3O_3$ $[M+H]^+$: 338.1504 found: 338.1514.

4.2.12. Methyl 4-(((2*R*,11a*S*)-2-hydroxy-5,11-dioxo-2,3,11,11*a*-tetrahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepin-10(5*H*)-yl)methyl)benzoate 5l

$[\alpha]_D^{25}$ + 262.2 (*c* 1.0, CH_3OH). ^1H NMR (300 MHz, CD_3OD) δ 7.84 (d, *J* = 8.30 Hz, 2H), 7.77 (dd, *J* = 1.88, *J* = 6.42 Hz, 1H), 7.48–7.54 (m, 1H), 7.40 (d, *J* = 8.30 Hz, 1H), 7.26–7.31 (m, 1H), 7.19 (d, *J* = 8.30 Hz, 2H), 5.37 (d, *J* = 16.24 Hz, 1H), 5.04 (d, *J* = 16.2 Hz, 1H), 4.51–4.57 (m, 1H), 4.37 (dd, *J* = 5.6 Hz, *J* = 2.45 Hz, 1H), 3.82 (s, 3H), 3.59–3.75 (m, 2H), 2.83–2.96 (m, 2H), 2.04–2.13 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9, 166.6, 165.7, 141.9, 139.4, 132.2, 130.4, 130.0, 129.4, 129.2, 126.6, 126.2, 122.0, 69.0, 56.0, 54.0, 52.5, 52.0, 34.9. IR (KBr) 3413, 2952, 1719, 1680, 1463, 1389, 1281, 1218, 1019, 770, 714 cm^{-1}. HR-MS (ESI) Calcd for $C_{21}H_{21}N_2O_5$ $[M+H]^+$: 381.14450. Found 381.14462.

4.2.13. 4-(((2*R*,11a*S*)-2-Hydroxy-5,11-dioxo-2,3,11,11*a*-tetrahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepin-10(5*H*)-yl)methyl)benzoic acid 5m

$[\alpha]_D^{25}$ + 442.0 (*c* 1.0, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 7.96 (d, *J* = 8.12 Hz, 2H), 7.89 (d, *J* = 6.98 Hz, 1H), 7.42 (t, *J* = 9.8 Hz, 1H), 7.17–7.31 (m, 4H), 5.22 (d, *J* = 16.0 Hz, 1H), 5.05 (d, *J* = 16.0 Hz, 1H), 4.56–4.59 (m, 1H), 4.37 (t, *J* = 7.55 Hz, 1H), 3.83–3.88 (m, 1H), 3.58–3.71 (m, 2H), 2.91–2.99 (m, 1H), 2.10–2.18 (m, 1H). ^{13}C NMR (75 MHz,CD_3OD) δ 170.7, 167.9, 163.9, 143.5, 140.6, 133.8, 131.1, 130.9, 128.1, 127.4, 124.2, 69.8, 57.6, 54.8, 52.3, 35.8. IR (KBr) 3403, 2954, 1683, 1466, 1421, 1393, 1251, 1108, 1053, 987, 843, 764 cm^{-1}. HR-MS (ESI) Calcd for $C_{20}H_{18}N_2O_5Na$ $[M+Na]^+$; 389.1113. Found 389.1108.

4.2.14. (2*R*,11a*S*)-10-(4-Chloro-3-fluorobenzyl)-2-hydroxy-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11a*H*)-dione 5n

$[\alpha]_D^{25}$ + 249.7 (*c* 1.0, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$) δ 7.88 (dd, *J* = 1.70 Hz, *J* = 6.04 Hz, 1H), 7.43–7.34 (m, 1H), 7.27–7.34 (m, 2H), 7.17 (d, *J* = 8.30 Hz, 1H), 6.98 (dd, *J* = 2.07 Hz, *J* = 7.74 Hz, 1H), 6.88 (dd, *J* = 1.32 Hz, *J* = 6.98 Hz, 1H), 5.11 (d, *J* = 15.6 Hz, 1H), 4.95 (d, *J* = 15.6 Hz, 1H), 4.64–4.69 (m, 1H), 4.36 (dd, *J* = 6.04 Hz, *J* = 1.88 Hz, 1H), 3.88–3.94 (m, 1H), 3.67 (dd,, *J* = 4.53 Hz, *J* = 8.12 Hz, 1H), 2.96–3.04 (m, 2H), 2.11–2.21 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.0, 165.6, 159.7, 139.3, 137.8, 132.3, 130.9, 130.5, 129.6, 126.4, 123.0, 121.9, 115.4, 115.1, 69.1, 56.0, 54.0, 51.9. IR (KBr) 3384, 2927, 1628, 1490, 1464, 1423, 1060, 764 cm^{-1}. HR-MS (ESI) Calcd. for $C_{19}H_{17}N_2O_3ClF$ $[M+H]^+$: 375.09062. Found 375.09062.

4.2.15. (2*R*,11a*S*)-10-(3-Chloro-4-nitrobenzyl)-2-hydroxy-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11a*H*)-dione 5o

Mp: 81–83 °C $[\alpha]_D^{25}$ + 321.3 (*c* 1.0, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$) δ 7.87 (dd, *J* = 2.26 Hz, *J* = 6.04 Hz, 1H), 7.72 (d, *J* = 1.51 Hz, 1H), 7.47–7.52 (m, 2H), 7.27–7.34 (m, 2H), 7.16 (d, *J* = 7.55 Hz, 1H), 5.10 (s, 2H), 4.62–4.68 (m, 1H), 4.37 (dd, *J* = 6.04 Hz, *J* = 2.26 Hz, 1H), 3.90 (d, *J* = 13.5 Hz, 1H), 3.65 (dd, *J* = 4.53 Hz, *J* = 8.30 Hz, 1H), 2.87–3.01 (m, 2H), 2.11–2.22 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.0, 165.6, 159.7, 156.4, 139.3, 137.7, 132.3, 130.9, 130.5, 129.6, 126.4, 123.1, 121.9, 115.4, 69.1, 56.0, 54.0, 51.9, 35.0. IR (KBr) 3390, 2929, 1679, 1631, 1535, 1463, 1389, 1250, 1049, 756 cm^{-1}. HR-MS (ESI) Calcd for $C_{19}H_{16}N_3O_5ClNa$ $[M+Na]^+$: 424.06707. Found 424.06598.

4.2.16. (2*R*,11a*S*)-10-(3,5-Difluorobenzyl)-2-hydroxy-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11a*H*)-dione 5p

$[\alpha]_D^{25}$ + 269.6 (*c* 1.0, CH_3OH). ^1H NMR (300 MHz, CD_3OD) δ.7.81 (dd, *J* = 1.70 Hz, *J* = 6.23 Hz, 1H), 7.55–7.60 (m, 1H), 7.43 (d, *J* = 7.55 Hz, 1H), 7.32–7.38 (m, 1H), 6.70–6.81 (m, 3H), 5.33 (d, *J* = 16.05 Hz, 1H), 5.03 (d, *J* = 16.05 Hz, 1H), 4.52–4.58 (m, 1H), 4.41 (dd, *J* = 5.66 Hz, *J* = 2.45 Hz, 1H), 3.70–3.76 (m, 1H), 3.63 (dd, *J* = 4.72 Hz, *J* = 7.74 Hz, 1H), 2.84–2.98 (m, 2H), 2.05–2.14 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ 170.8, 167.8,166.3, 166.1, 143.0, 140.5, 133.9, 131.0, 127.5, 124.1, 111.2, 110.9, 103.5, 69.8, 57.6, 54.8, 51.9, 35.8. IR (KBr) 3392, 2932, 1678, 1628, 1463, 1391, 1118, 992, 760 cm^{-1}. HR-MS (ESI) Calcd for $C_{19}H_{17}O_3N_2F_2$ $[M+H]^+$: 359.12018. Found 359.12025.

4.2.17. (2*R*,11a*S*)-10-(2-Chloro-3,5-difluorobenzyl)-2-hydroxy-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11a*H*)-dione 5q

Mp: 161–163 °C $[\alpha]_D^{25}$ + 248.4 (*c* 1.0, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (dd, *J* = 1.54 Hz, *J* = 6.04 Hz, 1H), 7.42–7.48 (m, 1H), 7.34 (d, *J* = 8.30 Hz, 1H), 7.22 (t, *J* = 7.55 Hz, 1H), 6.90–6.97 (m, 1H), 6.78–6.88 (m, 1H), 5.80 (d, *J* = 15.10 Hz, 1H), 4.94 (d, *J* = 15.10 Hz, 1H), 4.62–4.68 (m, 1H), 4.26 (dd, *J* = 5.28 Hz, *J* = 3.02 Hz, 1H), 3.85–3.90 (m, 1H), 3.65 (dd, *J* = 4.53 Hz, *J* = 7.55 Hz, 2H), 2.94–3.04 (m, 1H), 2.09–2.16 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.2, 165.5, 158.7, 156.1, 137.5, 131.6, 130.1, 126.3, 122.5, 116.6, 166.3, 114.6, 114.3, 69.0, 56.2, 42.4, 34.9. IR (KBr) 3393, 2928,1681,1635,1471, 1421, 1388, 1237, 1095, 761 cm^{-1}. HR-MS (ESI) Calcd for $C_{19}H_{15}O_3N_2ClF_2Na$ $[M+Na]^+$: 415.06315. Found 415.06210.

4.2.18. (2*R*,11a*S*)-2-Hydroxy-10-(3,4,5-trimethoxybenzyl)-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11a*H*)-dione 5r

$[\alpha]_D^{25}$ + 264.3 (*c* 1.0, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, *J* = 7.74 Hz, 1H), 7.46 (t, *J* = 7.74 Hz, 1H), 7.25–7.29 (m, 2H), 6.30 (s, 2H), 5.17 (d, *J* = 15.6 Hz, 1H), 4.97 (d, *J* = 15.6 Hz, 1H), 4.66 (br s, 1H), 4.36 (t, *J* = 7.7 Hz, 1H), 3.92 (d, *J* = 12.8 Hz, 1H), 3.78 (s, 3H), 3.76 (s, 6H), 3.62–3.71 (m, 1H), 2.96–3.05 (m, 2H), 2.11–2.19 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.0, 165.7, 153.3, 139.4, 132.3, 132.1, 130.3, 129.9, 126.2, 122.5, 103.5, 69.1, 60.7, 56.0, 53.9, 52.1, 35.0. IR (KBr) 3430, 2936, 1675, 1634, 1462, 1423, 1330, 1219, 1125, 772 cm^{-1}. HR-MS (ESI) Calcd for $C_{22}H_{25}N_2O_6$ $[M+H]^+$: 413.1712 found: 413.1729.

4.2.19. (2*R*,11a*S*)-2-Hydroxy-10-(perfluorobenzyl)-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11a*H*)-dione 5s

$[\alpha]_D^{25}$ + 294.2 (*c* 1.0, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$) δ 7.80 (m, 1H), 7.50–7.56 (m, 1H), 7.30 (dd, *J* = 7.55 Hz, *J* = 6.04 Hz, 2H), 5.75 (d, *J* = 15.1 Hz, 1H), 4.84 (d, *J* = 15.1 Hz, 1H), 4.61–4.67 (m,

1H), 4.25 (dd, *J* = 5.28 Hz, *J* = 2.26 Hz, 1H), 3.85–3.90 (m, 1H), 3.63 (dd, *J* = 4.53 Hz, *J* = 8.30 Hz, 1H), 2.87–3.00 (m, 2H), 2.08–2.17 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.0, 165.3, 137.6, 132.1, 130.5, 130.2, 126.7, 122.1, 109.5, 69.0, 56.0, 53.8, 39.6, 34.8. IR (KBr) 3392, 2927, 1698, 1631, 1505,1463, 1249, 1124, 1040, 997, 955, 764, 713, 609 cm^{-1}. HR-MS (ESI) Calcd for $C_{19}H_{14}N_2O_3F_5$ $[M+H]^+$: 413.09191. Found 413.09193.

4.2.20. (2*R*,11a*S*)-10-(2-(4-Chlorophenyl)-2-oxoethyl)-2-hydroxy-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4] diazepine-5,11(10*H*,11a*H*)-dione 5t

Mp: 141–143 °C $[\alpha]_D^{25} + 374.7$ (*c* 1.0, CH_3OH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87–7.91 (m, 3H), 7.43–7.47 (m, 3H), 7.28 (t, *J* = 6.9 Hz, 1H), 7.09 (d, *J* = 7.9 Hz, 1H), 5.47 (d, *J* = 17.9 Hz, 1H), 4.77 (d, *J* = 17.9 Hz, 1H), 4.59 (br s, 1H), 4.34 (t, *J* = 6.99 Hz, 1H), 3.87 (d, *J* = 13.99 Hz, 1H), 3.63 (dd, *J* = 3.99 Hz, *J* = 3.99 Hz, 1H), 2.87–2.95 (m, 2H), 2.09–2.16 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.0, 169.0, 165.8, 140.5, 139.6, 132.8, 132.4, 130.3, 129.4, 129.2, 126.2, 121.9, 69.0, 56.2, 55.8, 54.0, 34.9. IR (KBr) 3388, 3012, 2934, 1699, 1466, 1420, 1222, 1095, 984, 764 cm^{-1}. HR-MS (ESI) Calcd for $C_{20}H_{18}N_2O_4Cl$ $[M+H]^+$: 385.0955. Found 385.0950.

4.2.21. (2*R*,11a*S*)-10-(2-(4-Bromophenyl)-2-oxoethyl)-2-hydroxy-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diaz epine-5,11(10*H*,11a*H*)-dione 5u

Mp: 159–161 °C $[\alpha]_D^{25} + 421.3$ (*c* 1.0, CH_3OH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80–7.86 (m, 3H), 7.61 (d, *J* = 8.68 Hz, 2H), 7.40–7.46 (m, 1H), 7.24–7.29 (m, 1H), 7.08 (d, *J* = 7.74 Hz, 1H), 5.47 (d, *J* = 17.5 Hz, 1H), 4.75 (d, *J* = 17.5 Hz, 1H), 4.53–4.62 (m, 1H), 4.27–4.32 (m, 1H), 3.85–3.90 (m, 1H), 3.60 (dd, *J* = 4.53 Hz, *J* = 7.93 Hz, 1H), 2.83–2.91 (m, 2H), 2.04–2.13 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6) δ 191.2, 167.7, 164.0, 138.2, 132.2, 130.9, 130.6, 128.6, 128.4, 128.1, 127.3, 124.5, 120.9, 66.8, 54.6, 54.3, 52.4, 33.6. IR (KBr) 3337, 3086, 2935, 1707, 1677, 1620, 1461, 1418, 1383, 1302, 1117, 1095, 988, 765 cm^{-1}. HR-MS (ESI) Calcd for $C_{20}H_{17}N_2O_4BrNa$ $[M+Na]^+$: 451.02639. Found 451.02524.

4.2.22. (2*R*,11a*S*)-10-((4-Bromothiophen-2-yl)methyl)-2-hydroxy-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11a*H*)-dione 5v

$[\alpha]_D^{25} + 261.6$ (*c* 1.0, CH_3OH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (dd, *J* = 1.51 Hz, *J* = 6.04 Hz, 1H), 7.48–7.54 (m, 1H), 7.28–7.35 (m, 2H), 7.11 (d, *J* = 1.51 Hz, 1H), 6.88 (s, 1H), 5.20 (d, *J* = 15.8 Hz, 1H), 5.05 (d, *J* = 15.8 Hz, 1H), 4.63–4.69 (m, 1H), 4.29 (dd, *J* = 6.04 Hz, *J* = 2.26 Hz, 1H), 3.86–3.91 (m, 1H), 3.66 (dd, *J* = 4.53 Hz, *J* = 8.30 Hz, 1H), 2.96–3.04 (m, 2H), 2.10–2.20 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.8, 165.5, 141.0, 139.1, 132.3, 130.5, 129.7, 128.8, 126.5, 123.1, 122.1, 109.0, 69.2, 56.0, 54.0, 48.1, 34.9. IR (KBr) 3355,3101,2926,1642,1462,1247,1093,983,762 cm^{-1}. HR-MS (ESI) Calcd for $C_{17}H_{16}N_2O_3BrS$ $[M+H]^+$: 407.00595. Found 407.00578.

4.2.23. (2*R*,11a*S*)-10-(Dibenzo[*b*,*d*]furan-4-ylmethyl)-2-hydroxy-2,3-dihydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*,11a*H*)-dione 5w

$[\alpha]_D^{25} + 192.0$ (*c* 1.0, CH_3OH). ^{1}H NMR (300 MHz, CD_3OD) δ 7.90 (d, *J* = 7.74 Hz, 1H), 7.80 (dd, *J* = 2.07, *J* = 4.53 Hz, 1H), 7.65 (dd, *J* = 1.32 Hz, *J* = 6,42 Hz, 1H), 7.41–7.57 (m, 4H), 7.14–7.32 (m, 4H), 5.90 (d, *J* = 15.6 Hz, 1H), 5.20 (d, *J* = 15.6 Hz, 1H), 4.56–4.63 (m, 1H), 4.37 (dd, *J* = 5.28 Hz, *J* = 2.83 Hz, 1H), 3.68 (d, *J* = 4.53 Hz, 2H), 2.92–3.00 (m, 2H), 2.05–2.14 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ 170.6, 167.7, 157.3, 154.9, 140.3, 133.4, 131.1, 130.6, 128.4, 127.7, 127.1, 125.6, 125.0, 124.5, 124.1, 123.9, 121.7, 121.0, 112.8, 69.8, 57.7, 54.7, 47.6, 35.8. IR (KBr) 3403, 2925, 2854, 1677, 1633, 1463, 1186, 755 cm^{-1}. HR-MS (ESI) Calcd for $C_{25}H_{21}O_4N_2$ $[M+H]^+$: 413.14958. Found 413.14947.

5. X-ray crystallography

X-ray data for the compounds were collected at room temperature using a Bruker Smart Apex CCD diffractometer with graphite monochromatic MoKα radiation (λ = 0.71073 Å) with ω-scan method. Preliminary lattice parameters and orientation matrices were obtained from four sets of frames.

Integration and scaling of intensity data were accomplished using SAINT program. The structure was solved by direct methods using SHELXS97 and refinement was carried out by full-matrix least-squares technique using SHELXL97. Anisotropic displacement parameters were included for all non-hydrogen atoms. The atom C12 of 5 g is disordered over two sites (C12/C121) and the site-occupation factors refined to 0.708(7) and 0.292(7). EADP and DFIX constraints were applied to the disordered atoms. The O bound H atoms in 5 g were located in a difference density map and refined isotropically. All other H atoms were located in difference Fourier maps and subsequently geometrically optimized and allowed for as riding atoms, with C–H = 0.93–0.97 Å, with U_{iso}(H) = 1.5 U_{eq}(C) for methyl H or 1.2 U_{eq}(C). The methyl groups were allowed to rotate but not to tip. The absolute configuration of the procured material was known in advance and was confirmed by unambiguous refinement of the absolute structure parameter.

Crystal data for **5g**: $C_{19}H_{17}BrN_2O_3$, *M* = 401.26, colorless plate, 0.12 × 0.06 × 0.06 mm^3, monoclinic, space group $P2_1$ (No. 4), *a* = 5.7253 (6), *b* = 12.3763 (13), *c* = 11.9108 (13) Å, β = 94.641 (2)°, *V* = 841.21(15) Å^3, *Z* = 2, D_c = 1.584 g/cm^3, F_{000} = 408, CCD Area Detector, MoKα radiation, λ = 0.71073 Å, *T* = 294(2) K, $2\theta_{max}$ = 50.0°, 7355 reflections collected, 2836 unique (R_{int} = 0.0246). Final *GooF* = 1.046, *R1* = 0.0252, *wR2* = 0.0647, *R* indices based on 2683 reflections with $I > 2\sigma(I)$ (refinement on F^2), 230 parameters, 1 restraint. μ = 2.465 mm^{-1}. Absolute structure parameter = 0.048(8).

6. ACE inhibition assay

ACE inhibition assay was performed using the colorimetric method. 1 g of rabbit lung acetone powder (Sigma–Aldrich, USA, Cat. No. L0756) was homogenized with 10 mL of 0.05 M sodium borate buffer (pH 8.2) containing 0.3 M NaCl and 0.5% Triton X-100 at 4 °C followed by centrifugation at 15,000 rpm for 60 min at 4 °C. Supernatant was used as a source of ACE enzyme for this assay. ACE activity was assayed by monitoring the release of Hippuric acid (HA) from the hydrolysis of hippuryl–histidyl–leucine (HHL) (Sigma–Aldrich, USA, Cat No. H1635). The enzyme reaction was started by adding 7 µl of ACE enzyme, 12.5 µl of 0.05 M sodium borate buffer (pH 8.2) containing 0.3 M NaCl and 50 µl of 5 mM substrate (HHL) followed by incubation at 37 °C for 30 min. The reaction was arrested by the addition of 0.1 mL of 1 M HCl. After stopping the reaction, 0.2 mL of pyridine (SD Fine chemical, India) was added followed by 0.1 mL of benzene sulphonyl chloride (SD Fine chemical, India). The solution was mixed by inversion for 1 min and cooled on ice. The yellow color developed was measured at 410 nm. The decreased concentration of HA in the test reaction compared with the control reaction was expressed as percentage inhibition and calculated from the equation: Inhibition% = $100 - [A_{Test}/A_{Control}] \times 100$, where A_{Test} = absorbance of test reaction and $A_{Control}$ = absorbance of control reaction. The therapeutic drug Lisinopril, Benazepril and Ramipril was used as standard. Depending on the inhibition assay of all synthesized derivatives, we selected effective derivatives **5f**, **5g**, **5r** and **5v** for measuring IC_{50}, concentration which inhibit 50% of enzyme activity. IC_{50} value was measured by plotting the percentage inhibition versus the serial concentrations of the chosen derivatives.

7. In vitro 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay

Cytotoxicity activity of active compounds **5f**, **5g**, **5r** and **5v** were studied by means of a colorimetric microculture assay (MTT).[6] It was measured by reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase to insoluble coloured formazan product, using Dulbecco's modified Eagle's medium (DMEM, Sigma, USA) for normal HEK 293 cells and A549 cancer cells. 1×10^4 cells/well were seeded in 100 μl DMEM, supplemented with 10% FBS in each well of 96-well micro culture plates and incubated for 24 h at 37 °C in a CO_2 incubator. The desired concentrations of the compounds/complexes were made and added to the wells with respective vehicle control. After 24 h of incubation, add 10 μl MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (5 mg/mL) was added to each well and the plates were further incubated for 4 h. Then the supernatant from each well was carefully removed, formazon crystals were dissolved in 100 μl of DMSO and absorbance at 540 nm wavelength was recorded. Cell survival rate was determined by comparing the absorbance value of treated cells with that in the control cells. 50% effective concentration (EC_{50}) values were decided by probit analysis.

8. Molecular docking studies

Molegro Virtual Docker (MVD, Ver: 2012.5.0.0) is a program for determining the most conformations of how a ligand will bind to an enzyme site. The identification of ligand binding modes is done iteratively by evaluating a number of candidate solutions (ligand conformations) and estimating the energy of their interactions with the enzyme. The highest scoring solutions are returned for further analysis. MVD performs flexible ligand docking, so the optimal geometry of the ligand will be determined during the docking. Docking studies of active ligands **5f**, **5g**, **5r** and **5v** were performed using this software. Crystal structure of t-ACE (PDB ID: 1O86) along with the co-factor (Zinc) was downloaded from RCSB Protein Data Bank. Structure of tACE (residues 37-625) adopts an overall ellipsoid shape with central groove that extends for about 30 Å into molecules and divides the protein into two sub domains. Zinc is an important catalytic component of ACE, bound at the active site. The boundaries of the groove are provided by helices α and β strands. Structure of tACE—lisinopril complex used to locate the active site of the molecules. tACE contains 27 helices. Structure of tACE revealed the location of two buried chloride ions seperated by 20.3 Å. All the structures of new compounds **5f**, **5g**, **5r** and **5v** were prepared by using Chem Draw and saved to mol file format. These structures were further converted into mol2 file format by using Marvin Sketch. MVD was used to perform computational studies, cavity prediction, assigning bond orders, structure refinement, defining the active binding sites of the t-ACE and structure preparation. Residues involved in hydrogen bonding interaction with ligands are as follows: Glu 162, Gln 281, Ala 354, His 353, Tyr 520, Ala 356, Tyr 523, Arg 522, His 513, Lys 511. As most of the ligands are surrounded by the above residues, it can be concluded that these residues are highly conserved and plays an important functional roles. Interaction of the ligands with these residues might be changing the active site structure, which leads to inhibition of enzyme. These ligands bind with high affinity against tACE and show low free energy.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.05.031.

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