Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 3685–3691

BIOORGANIC & MEDICINAL CHEMISTRY

Novel Peptidomimics as Angiotensin-Converting Enzyme Inhibitors: A Combinatorial Approach

Manju Bala,[a] M. A. Qadar Pasha,[b] D. K. Bhardwaj[c] and Santosh Pasha[a,*]

[a]*Peptide Research Laboratory, Centre for Biochemical Technology (CSIR), Delhi University Campus, Mall Road, Delhi-110007, India*
[b]*Biochemistry and Diagnostics Laboratory, Centre for Biochemical Technology (CSIR), Delhi University Campus, Mall Road, Delhi-110007, India*
[c]*Department of Chemistry, University of Delhi, Delhi-110007, India*

Received 23 January 2002; accepted 11 April 2002

Abstract—One of the efficient mode of treatments of chronic hypertension and cardiovascular disorders has been to restrain the formation of angiotensin-II by inhibiting the action of angiotensin-converting enzyme (ACE) on angiotensin-I. A number of ACE inhibitors (ACEIs) have been put to therapeutic use during the last two decades. The efforts continue towards achieving superior molecules or drugs with improved affinities, better bioavailability and thus long duration of action with minimum side effects. The present work evolves around similar objectives. In order to understand the mode of interaction of inhibitors with the active site of the enzyme and subsequently to have lead compounds as possible inhibitors the novel dipeptidomimics and tripeptidomimics have been designed and synthesized using combinatorial chemistry approach. A Focussed library of 10 di- and tri-peptides, eight dipeptidomemics and forty tripeptidomemics was generated. The pharmacophoric heterocyclic moieties and the amino acids have been selected to have affinities with the S_1, $S_{1'}$, and $S_{2'}$ subsites of the active site of the enzyme. ACE inhibition studies clearly demonstrated the structural-activity relationships within these classes of peptidomimics. The dipeptidomimics interacted only with $S_{1'}$ and $S_{2'}$ subsites, whereas the tripeptidomemics had additional interaction with S_1 subsite, which accounted for their significant ACE inhibition potencies. The in-vitro screening of these peptidomimics have resulted in identification of four promising tripeptidomimics 34[2-benzimidazolepropionyl-Val-Trp], 35[5hydroxytryptophanyl-Val-Trp], 40[2-benzimidazolepropionyl-Ile-Trp] and 45[2-benzimidazolepropionyl-Lys-Trp] with IC_{50} values in micromolar concentrations.

Introduction

Angiotensin-converting enzyme (EC 3.4.15.1, ACE) is an important enzyme of the renin–angiotensin–aldosterone system. It converts inactive decapeptide angiotensin-I (AI) to biologically active octapeptide angiotensin-II (AII), which raises blood pressure by vasoconstriction as well as by triggering the formation of sodium and water retaining steroidal hormone, aldosterone, in the human body.[1–3] Increased serum ACE levels have been associated with hypertension and hypertension-related target organ disorders such as congestive heart failure, left ventricular hypertrophy, acute myocardial infarction and as well as in some nephrological and pulmonary disorders.[4–6] Because of its clinical relevance, various research groups have been actively engaged and have highlighted different approaches in the synthesis and development of new compounds as potential ACE inhibitors for the above-mentioned disorders..[7–12] In this context, we took advantage of the combinatorial approach for the synthesis and screening of suitably designed novel peptidomimics as ACE inhibitors (Fig. 1).

The field of peptidomimics[13] is progressing at a rapid pace and is now offering solutions to the age-old issues of bioavailability and oral activity.[14] The integration of rational designing process with combinatorial methods offers a powerful strategy for the generation and optimization of peptidomimics as pharmaceutical leads for the ever-expanding development of drug pipeline thereby accelerating the drug discovery process.[15–20] Traditionally, the heterocyclics are known to constitute a rich reservoir of molecular diversity and are the crucial pharmacophoric components in the biologically active materials which have evolved up to now.[21] In the

*Corresponding author. Tel.: +91-11-766-6156; fax: +91-11-766-7471; e-mail: spasha@cbt.res.in

0968-0896/02/$ - see front matter
PII: S0968-0896(02)00166-9

present work, therefore, suitable selected heterocyclic moieties with selected amino acids and dipeptide motifs were incorporated for designing the library of dipeptidomimics and tripeptidomimics.

The library of novel peptidomimics was designed on the basis of structural–activity relationship studies (SAR studies) of the existing ACEIs and their multiple interactions with the active site of ACE. In the active site of ACE, the tetrahedrally co-ordinated zinc is proposed to be surrounded by three hydrophobic subsites designated S_1, S_1 and S_2.[22–24] ACE belongs to thermolysin family of zinc metallopeptidases that contains the HExxH consensus sequence.[25] Site directed mutagenesis experiments,[26] revealed that, the two histidines from this consensus sequences are involved in zinc chelation in the active site of ACE, whereas glutamate is involved in the catalysis of its substrate. One glutamate from the enzyme and one water molecule from the surroundings also co-ordinate with zinc.

The structural–activity relationship studies of the existing ACEIs have indicated the preference of aromatic and cyclic amino acid residues in P_2 in ACEIs position to interact with S_2 subsites of ACE, therefore phenylalanine, tyrosine and tryptophan, and cyclic imino acid proline were selected for this position.[27] Reports have suggested that S_1 subsite of ACE constitute hydrophobic domain and could efficiently be occupied by linear or cyclic moieties,[28] therefore valine, isoleucine and lysine were selected for ACEIs. The linear side chains in these residues limit, to some extent, the freedom of the main chain to induce the conformational constraint in the molecule, thus enabling it to interact efficiently with the active site of the enzyme.[29] The heterocyclic moieties (Fig. 2) such as pyroglutamine, Pyr (**1**); 2-oxo-4-thiazolidine carboxylic acid, OTC (**2**); nipecotic acid, NIP (**3**); 2-bornaneacetic acid, NBA (**4**); 5-mercapto-1-tetrazoleacetic acid, MTA (**5**); 5-methoxy-1-indanone-3-acetic acid, MIA (**6**); 5-methoxy-2-methyl-3-indoleacetic acid, MMI (**7**); (*S*)-(+) 2-oxo-4-phenyl-3-oxazolidineacetic acid, OPA (**8**); 2-benzimidazolepropionic acid, BPA (**9**); 5-hydroxy-L-tryptophan, HTP (**10**); 4-(2-thienyl) butyric acid, TBA (**11**); 1,2-dithiolane-3-pentanoic acid, DTP (**12**) were selected wherein the hydrophobic side chains as well as the specific functional groups were proposed to interact with the S_1 subsite and zinc, respectively, located in the enzyme's active site. The selected amino acids and heterocyclic moieties were incorporated to design a library of dipeptidomimics and tripeptidomimics. The library was comprised of two sets of dipeptidomimics (Sets A and B) consisting of eight compounds (Table 1); and nine sets of tripeptidomimics (Sets C–K) consisting of 40 tripeptidomimics along with 10 parent peptides (Table 1). The desired library of these designed peptidomimics were synthesized using combinatorial chemistry with an aim to understand their mode of interaction with the active site of the enzyme and subsequently to have lead compounds with improved affinities, better bio-availabities and thus long duration of action with minimum side effects.

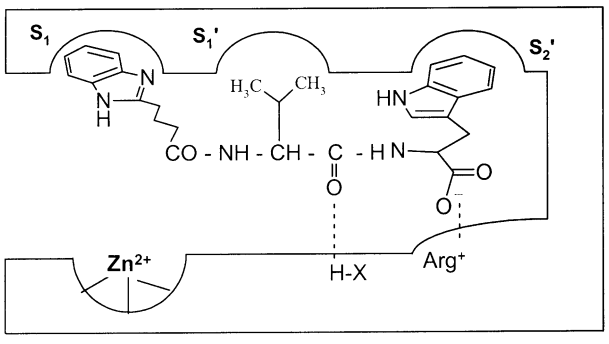

Figure 1. Proposed model of binding of 34 with the active site of ACE.

The designed compounds were synthesised on solid support employing multiple parallel synthesis technique of combinatorial chemistry.[30] The general motif for the dipeptidomimics and tripeptidomimics can be represented as H-AA_1 and H-AA_1-AA_2, respectively, where H is the selected heterocyclic moiety and AA denotes amino acid moiety.

Results

The active site of angiotensin-converting enzyme as discussed consists of tetra-coordinated zinc surrounded by three hydrophobic subsites–S_1, S_1 and S_2. Based on this model and the SAR studies of the existing ACE inhibitors, nine dipeptides, **21**, **32**, **38**, **43**, **49**, **55**, **60**, **65** and **68**, as shown in Table 1, were selected. These scaffolds were then linked with selected heterocyclic moieties to generate structural diversity to explore the ACE active site and as well as to find possible angiotensin-converting enzyme inhibitors. For this purpose, a combinatorial library of eight dipeptidomimics, 40 tripeptidomimics and 10 peptides categorized into 11 sets was used. These were assessed by enzyme kinetics for their ACE activity inhibition efficiencies. All synthetic peptides/peptidomimics were first tested at a fixed concentration of 25 μM as shown in Table 1. This also provided comparison of the ACE inhibition potencies of the compounds within each individual set. The peptidomimic(s) showing maximum ACE activity inhibitions from each set were selected. The selected peptidomimics were then statistically compared (Table 2) to identify those showing over-all maximum ACE activity inhibition potency. IC_{50} of these peptidomimics was determined as shown in Table 2.

Inhibition studies at 25 μM concentration showed that in Set A of the dipeptido-mimics (**13**–**17**), dipeptidomimic **16** had maximum efficiency to inhibit ACE activity (24.06%, $p < 0.001$). The inhibition potency of dipeptidomimics **13**, **14**, **15** and **17** was less ($p < 0.01$) in comparison to **16**; whereas, in Set B of the dipeptidomimics (**18**–**20**), no ACE activity inhibition was observed. These observations indicated that the designed dipeptidomimic model was not suitable to interact with the subsites of ACE and, therefore, this structural motif was not considered further.

The experiments with the tripeptidomimics were more encouraging. In the isoleucyl phenyalanine (**21**) derived

tripeptidomimics (Set C), HTP-*Ile-Phe* (**30**), showed maximum ACE inhibition (39.63%). In valinyl tryptophan (**32**)-derived peptidomimics (Set D) BPA-Val-Trp (**34**) and HTP-Val-Trp (**37**) showed significant ACE inhibition efficiency (66.68%, $p<0.001$ and 46.51%, $p<0.01$, respectively). In isoleucyl tryptophan (**38**) derived tripeptidomimics (Set E), BPA-*Ile-Trp* (**40**) exhibited maximum ACE inhibition (52.03%, $p<0.05$). In the lysyl tryptophan (**43**) derived tripeptidomimics (Set F), BPA-*Lys-Trp* (**45**), TBA-*Lys-Trp* (**47**) and DTP-*Lys-Trp* (**48**) showed significant ACE inhibitions (44.63%, $p<0.001$; 36.24%, $p<0.001$ and 33.56%, $p<0.001$, respectively). In the lysyl proline (**49**)-derived tripeptidomimics (Set G), HTP-*Lys-Pro* (**52**), TBA-*Lys-Pro* (**53**) and DTP-*Lys-Pro* (**54**) showed significant ACE inhibitions (34.93%, $p<0.001$; 36.24%, $p<0.001$ and 40.44%, $p<0.001$, respectively).

However, the tripeptidomimics derived from 55 (Set H), **60** (Set I), **65** (Set J) and **68** (Set K) with ACE activity inhibitions in the range of 6.04–15.99% were not as potent as their respective parent peptides, thereby showing that the constrained moieties, heterocyclic moieties and unusual amino acids linked to these parent dipeptides are not suitable.

The 11 promising tripeptidomimics, **30**, **34**, **35**, **37**, **40**, **45**, **47**, **48**, **52**, **53** and **54**, selected from the above experiments were studied for their comparative ACE inhibitory potencies. The results are presented in Table 2. It was inferred from this study that four peptidomimics **34**, **35**, **40** and **45** had significant ACE inhibitory potencies ($p<0.001$, $p<0.01$, $p<0.01$ and $p<0.01$, respectively). These four peptidomimics were further studied at different concentrations such as 12.5, 6.25 and 3.17 μM to determine their IC_{50} concentrations (Table 2).

Discussion

The ACE inhibition experiments have explored the mode of interactions of these synthetic novel peptidomimics as possible inhibitors with the active site of the enzyme. These structure–activity relationships have clearly demonstrated that dipeptidomimics interact only with S_1 and S_2 subsites of the enzyme, whereas in tripeptidomimics, the additional interaction with S_1 subsite of ACE accounts for their significant ACE inhibition potencies. Among the different tripeptidomimics screened, a significant preference for a trypto-

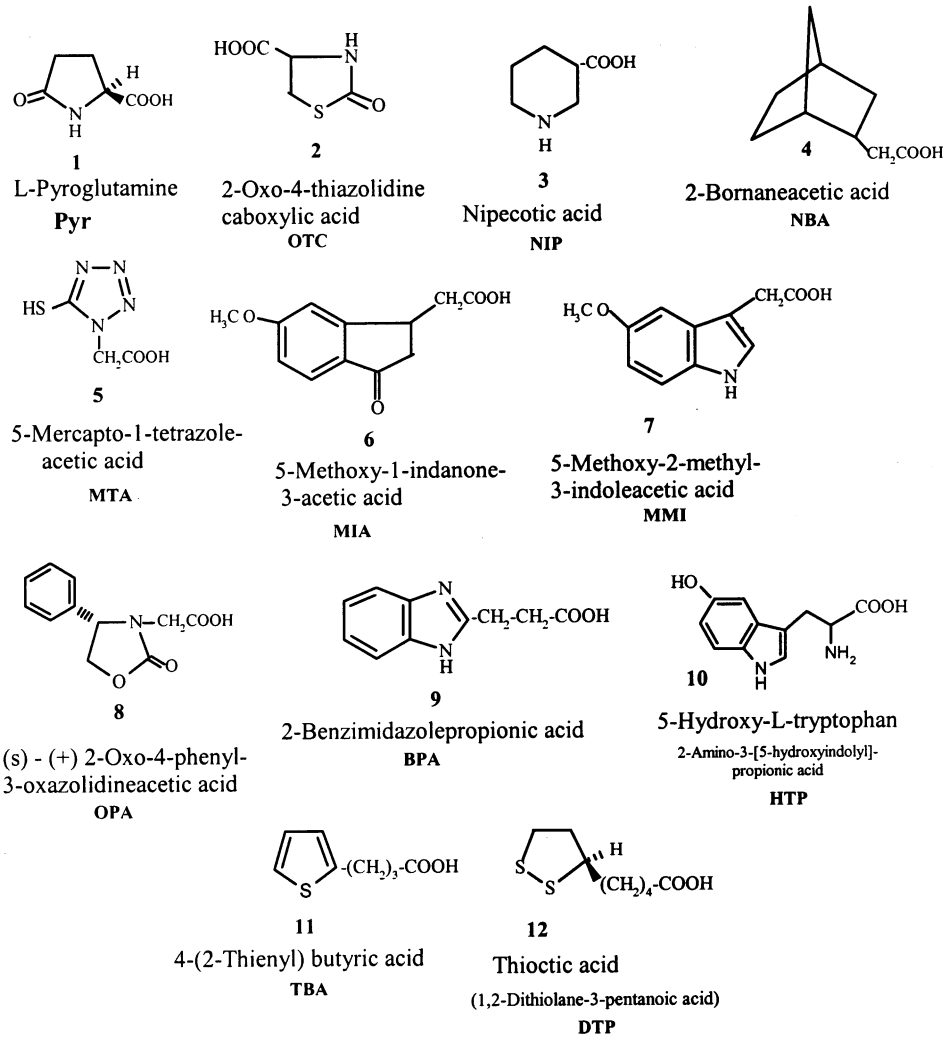

Figure 2. Heterocyclic moieties.

Table 1. ACE inhibition studies of di and tri peptidomimics

Compounds	No.	m/z[b]	HPLC profile[e] (rt,%)	ACE activity[a] (Units/L)	ACE inhibition (%)	P value
Set A						
MTA-*Ile*	13	—	4.1 (91.6)	130.0±2.1	2.25	NS[f]
MTA-*Pro*	14	—	5.4 (75.7)	118.0±4.6	11.28	<0.01
MTA-*His*	15	—	5.8 (76.4)	119.0±4.6	10.53	<0.01
MTA-*Tyr*	16	—	4.6 (95.2)	101.0±4.6	24.06	<0.001
MTA-*Trp*	17	—	5.7 (90.1)	119.0±1.7	10.53	<0.01
Set B						
MTA-*Phe*	18	307.0	6.2 (94.2)	139.0±6.5	Nil	
NBA-*Phe*	19	302.3	4.0 (89.6)	135.0±2.7	Nil	
MIA-*Phe*	20	367.3	6.1 (87.5)	133.0±3.0	Nil	
Set C						
Ile-Phe[h]	21	278.4	6.9 (95.2)	92.7±5.6	31.53	<0.05
Phe-*Ile-Phe*	22	447.6[c]	4.2 (89.5)	84.2±3.6	37.83	<0.001[g]
Pyr-*Ile-Phe*	23	389.5	3.1 (71.6)	113.4±3.7	16.23	
OTC-*Ile-Phe*	24	407.0	6.5 (82.1)	135.4±3.0	Nil	
NIP-*Ile-Phe*	25	390.2	7.1 (92.1)	108.0±2.1	20.26	<0.001[g]
MTA-*Ile-Phe*	26	421.5	7.3 (72.7)	135.0±6.0	Nil	
MMI-*Ile-Phe*	27	481.2	5.9 (85.9)	135.4±4.0	Nil	
MIA-*Ile-Phe*	28	503.8	3.9 (94.8)	135.2±2.1	Nil	
OPA-*Ile-Phe*	29	—	4.3 (87.4)	135.4±4.0	Nil	
HTP-*Ile-Phe*	30	481.6	2.2 (99.2)	81.7±5.6	39.63	<0.01
BPA-*Ile-Phe*	31	498.8[d]	3.8 (89.2)	115.9±4.0	14.41	<0.001[g]
Set D						
Val-Trp[h]	32	303.5	3.1 (94.2)	90.5±2.1	33.02	
Pyr-*Val-Trp*	33	445.8	5.6 (87.9)	101.7±6.3	24.77	<0.05[g]
BPA-*Val-Trp*	34	492.7[c]	5.4 (89.1)	45.5±4.0	66.68	<0.001
HTP-*Val-Trp*	35	503.8	6.3 (96.4)	72.3±9.4	46.51	<0.01
TBA-*Val-Trp*	36	453.7	6.1 (98.4)	90.5±9.3	33.02	NS[f]
DTP-*Val-Trp*	37	491.8	7.1 (97.2)	81.0±3.0	40.00	<0.05
Set E						
Ile-Trp[h]	38	318.4	3.7 (92.7)	70.8±1.8	47.15	
Pyr-*Ile-Trp*	39	430.2	3.1 (84.1)	81.7±5.6	39.02	<0.01[g]
BPA-*Ile-Trp*	40	489.2	2.6 (95.8)	64.3±1.8	52.03	<0.05
HTP-*Ile-Trp*	41	519.6	2.5 (91.8)	80.7±1.8	39.83	<0.01[g]
TBA-*Ile-Trp*	42	457.6	3.0 (93.2)	106.8±1.8	20.32	<0.01[g]
Set F						
Lys-Trp[h]	43	368.7[d]	2.7 (94.8)	95.4±3.9	28.86	
Pyr-*Lys-Trp*	44	445.1	3.0 (89.5)	115.2±3.1	14.09	<0.05[g]
BPA-*Lys-Trp*	45	546.9[d]	2.9 (83.5)	74.2±1.9	44.63	<0.001
HTP-*Lys-Trp*	46	534.0	2.5 (91.2)	96.3±7.4	27.74	<0.05[g]
TBA-*Lys-Trp*	47	457.7	2.8 (85.5)	85.5±4.1	36.24	<0.001
DTP-*Lys-Trp*	48	549.1	3.7 (86.8)	89.1±2.7	33.56	<0.001
Set G						
Lys-Pro[h]	49	—	3.1 (90.5)	106.0±4.6	22.06	
Pyr-*Lys-Pro*	50	354.5	5.5 (93.4)	105.2±4.2	14.09	<0.05[g]
BPA-*Lys-Pro*	51	—	6.8 (84.2)	106.0±4.6	22.06	NS[f]
HTP-*Lys-Pro*	52	457.7	2.9 (91.9)	88.5±2.1	34.93	<0.001
TBA-*Lys-Pro*	53	379.4	2.9 (83.5)	90.0±3.0	36.24	<0.001
DTP-*Lys-Pro*	54	433.4	6.9 (85.1)	81.0±3.0	40.44	<0.001
Set H						
Ile-Pro[h]	55	—	3.0 (95.1)	92.6±1.8	30.89	
Pyr-*Ile-Pro*	56	—	5.3 (96.6)	116.6±8.7	13.01	<0.001[g]
BPA-*Ile-Pro*	57	408.1	3.4 (89.0)	116.6±5.0	13.01	<0.001[g]
HTP-*Ile-Pro*	58	438.6	3.3 (81.5)	103.5±2.1	22.76	<0.05[g]
DTP-*Ile-Pro*	59	459.1[d]	5.3 (95.9)	118.8±6.7	11.38	<0.001[g]
Set I						
Val-His[h]	60	—	2.9 (90.5)	113.0±1.7	16.72	
Pyr-*Val-His*	61	—	2.8 (75.6)	—		
BPA-*Val-His*	62	431.5	5.3 (95.5)	127.5±2.1	6.04	<0.001[g]
HTP-*Val-His*	63	451.2	3.0 (94.0)	—		
TBA-*Val-His*	64	438.7[d]	3.5 (85.5)	114.0±3.0	15.99	NS[f]
Set J						
Ile-His[h]	65	—	3.4 (91.6)	—		

(*continued on next page*)

Table 1 (*continued*)

Compounds	No.	m/z[b]	HPLC profile[e] (rt,%)	ACE activity[a] (Units/L)	ACE inhibition (%)	P value
Pyr-*Ile-His*	66	379.7	3.2 (97.4)	113.0±6.5	16.73	NS[f]
HTP-*Ile-His*	67	472.5	3.3 (97.2)	114.0±3.0	15.99	<0.01
Set K						
Lys-His[h]	68	—	3.0 (89.7)	—		<0.01
Pyr-*Lys-His*	69	397.5	3.5 (95.2)	114.0±3.0	15.99	<0.001
HTP-*Lys-His*	70	534.0[d]	2.9 (91.4)	—		<0.01

[a]Compounds were assayed against purified angiotensin-converting enzyme with activity 135 Units/L using a spectrophotometric assay with FAPGG as the substrate.
[b]Mass analysed and confirmed by MALDI-TOF.
[c]Molecular mass + sodium ion.
[d]Molecular ion + potassium ion.
[e]HPLC: C_{18} isocratic water/acetonitrile (3:7) 0.05% TFA.
[f]Non-significant ACE inhibition.
[g]Observed ACE inhibition was less than the reference (parent) peptide.
[h]Reference for the statistical comparison of the ACE inhibition potencies of peptidomimics of a particular set.
—Insoluble under present experimental conditions.

phanyl residue as AA_2 ($P_{2'}$ position) was observed as it is evident from Table 2. Tripeptidomimics **34**, **35**, **40** and **45** showed significant ACE activity inhibition potencies. Tryptophan at this position putatively interacts and blocks the hydrophobic S_2 subsite of the enzyme through its imidazole nucleus present in the side chain (Fig. 1). Furthermore, the free carboxylic group of tryptophan residue interacts with the side chain of the arginine residue in the active site of ACE. The investigation of the structure of these peptidomimics has further revealed that valinyl, isoleucyl and lysyl residues at AA_1 position have preference for the S_1 subsite of ACE where these amino acid residues are proposed to interact efficiently through their hydrophobic aliphatic side chains to efficiently block the ACE active site. Among the various heterocyclics selected, 2-benzimidazolepropionic acid (BPA, **9**) is found to be the most appropriate for linking to a suitable dipeptide. It was noteworthy that acetic acid (C_2) derived heterocyclics, 5-mercapto-1-tetrazoleacetic acid (MTA, **5**), 5-methoxy-1-indanone-3-acetic acid (MIA, **6**), 5-methoxy-2-methyl-3-indoleacetic acid (MMI, **7**) and (*S*)-(+)2-oxo-4-phenyl-3-oxazolidineacetic acid (OPA, **8**) and butyric acid (C_4) derived heterocyclic, 4-(2-thienyl)butyric acid (TBA, **11**), when incorporated into the peptidomimics **26**, **27**, **28**, **29**, **36** and **64** showed no significant ACE inhibition potencies, whereas propionic acid (C_3) derived heterocyclics, 2-benzimidazole-propionic acid (BPA) **9**, 5-hydroxy-L-tryptophan (HTP, **10**) and pentanoic (C_5) acid-derived heterocyclics, 1,2-dithiolane-3-pentanoic acid (DTP, **12**), when incorporated into the peptidomimics, showed significant ACE activity inhibitions. However, C_3-derived heterocyclic containing tripeptidomimics **34**, **35**, **40** and **45** showed more inhibitory potencies than C_5-derived heterocyclic containing tripeptidomimics **37** and **48**. This can be attributed to the orientation of C_3 chain which exerts the desirable conformational constraint on the rest of the tripeptidomimic molecule. This conformational constraint enables the peptidomimics **34**, **35**, **40** and **45** to interact with the tetra co-ordinated zinc and also with the three hydrophobic subsites—S_1, S_1 and S_2—as needed to efficiently inhibit ACE activity with the IC_{50} concentrations as 6.2, 12.5, 12.5 and 25.0 μM, respectively.

Table 2. Comparative effects of selected peptidomimics on ACE activity inhibition

Peptidomimics selected from individual sets	ACE activity[a] (Units/L)	ACE inhibition (%)	p value	IC_{50} (μM)
HTP-*Ile-Phe* (**30**)	81.7±5.6	39.70	<0.05	
BPA-*Val-Trp* (**34**)	45.5±4.0	66.42	<0.001	6.2
HTP-*Val-Trp* (**35**)	72.3±9.4	46.64	<0.01	12.5
DTP-*Val-Trp* (**37**)	81.0±3.1	40.22	<0.05	
BPA-*Ile-Trp* (**40**)	64.3±1.8	52.55	<0.01	12.5
BPA-*Lys-Trp* (**45**)	74.2±1.9	45.23	<0.01	25.0
TBA-*Lys-Trp* (**47**)	85.5±4.1	36.90	<0.05	
DTP-*Lys-Trp* (**48**)	89.1±2.7	34.69	NS[b]	
HTP-*Lys-Pro* (**52**)	88.5±2.1	34.24	NS[b]	
TBA-*Lys-Pro* (**53**)[c]	90.0±3.0	33.58		
DTP-*Lys-Pro* (**54**)	80.2±2.1	40.81	<0.05	

[a]Peptidomimics were assayed against purified angiotensin-converting enzyme with activity 135.5 Units/L using a spectrophotometric assay with FAPGG as the substrate.
[b]Non-significant inhibition.
[c]Reference peptidomimic for comparing the ACE inhibition of other selected peptidomimics.

Conclusion

The results from the present studies indicate a useful role of these novel peptidomimics in effectively inhibiting the angiotensin-converting enzyme activity. In view of these encouraging results on the structure–activity relationships, efficient ACE inhibition can be obtained by the compounds with judiciously chosen moieties, optimized for S_1, S_1 and S_2 subsite domains of the enzyme. Á more detailed in-vivo and in-vitro pharmacological evaluation of these conformationally constrained peptidomimics and their related analogues will be presented in near future. The structural approach of the ACE inhibitors developed in the present work can be used with further suitable modifications in the hetrocyclic moiety with different combinations of amino acids and dipeptides or tripeptides to obtain leads to

potentiate ACE activity inhibition in basic as well as clinical research applications. With the advent of pharmacogenomics, the present approach may yield fruitful results.

Experimental

Protected amino acids were obtained from Novabiochem. Heterocyclic moieties—pyruglutamine, Pyr (**1**); 2-oxo-4-thiazolidine carboxylic acid, OTA (**2**); nipecotic acid, NIP (**3**); 2-bornaneacetic acid, NBA (**4**); 5-mercapto-1-tetrazoleacetic acid, MTA (**5**); 5-methoxy-1-indanone-3-acetic acid, MIA (**6**); 5-methoxy-2-methyl-3-indoleacetic acid, MMI (**7**); (*S*)-(+) 2-oxo-4-phenyl-3-oxazolidineacetic acid, OPA (8); 2-benzimidazolepropionic acid, BPA (**9**); 5-hydroxy-L-tryptophan, HTP (**10**); 4-(2-thienyl) butyric acid, TBA (11); 1,2-dithiolane-3-pentanoic acid, DTP (**12**)—were purchased from Aldrich Chemical Co. All reagents were obtained from Sigma Chemical Co. CH_2Cl_2, HPLC grade and DMF were obtained from Merck. DMF was double distilled prior to use.

Synthesis of peptidomimics

All peptidomimics were synthesised by combinatorial chemistry employing a multiple parallel synthesis techchnique[31] on a solid support using automatic multiple peptide synthesiser (ACT 348⋆ model, Advanced Chemtech, Louisville, KY, USA). The support used in the present work was 2-chlorotrityl chloride resin[32] to which the first amino acid was linked by diisopropylethylamine (DIPEA) method.[33] The other moieties were linked to the resin using the standard chemistry of fluorenylmethoxy carbonyl (Fmoc) amino acids and 1-hydroxy benzotriazole (HOBt)/diisopropyl carbodiimide (DIPCD) activation method. After the complete assembly, the peptidomimics were cleaved from the resin using a dichloromethane/trifluoroethanol mixture (8:2).[34] Deprotection of the trifluoroacetic acid-labile group was achieved by trifluoroacetic acid.[35] The peptidomimics were purified by gel filtration method on LH-20 using methanol as eluant. The purity of the peptidomimics were assessed on semi-preparative reverse phase HPLC (Waters, India) with 70% acetonitrile containing 0.05% trifluoroacetic acid (Table 1). The molecular mass of the synthetic peptidomimics were confirmed by MALDI-Tof (Kratos Analytical) (Table 1).

Purification of bovine kidney ACE

ACE was purified from bovine kidney by the method of Bull et al.[36] with slight modifications. The enzyme was solubilised from the kidney tissues with 1% Nonidet P-40 solution in 5.0 mM Tris–HCl buffer (pH = 8.5). The enzyme was released into the crude extract from the tissue membranes by a sonicator (W-385, Heat systems-Ultrasonic) equipped with a$^{3/4}$-in horn using 10 pulses of 15 s each.The enzyme in the extract was purified by affinity chromatography. For this purpose, the inhibitor, lisinopril, was attached to sepharose CL-6B matrix through a 12-atom spacer, 1,4-butanediol diglycidyl ether. Elution was carried out with 10 μM of lisinopril in the buffer. The enzyme, having more affinity towards freely available lisinopril readily dissociated from the lisinopril linked to sepharose. The lisinopril from the enzyme was removed by extensive dialysis against 1.0 mM EDTA solution. The enzyme was refolded to its active conformation by providing zinc ions in the second dialysis against 1.0 mM zinc acetate.

In vitro inhibition studies of ACE activity

The library of peptidomimic compounds was evaluated for their ACE inhibition potencies by determining the ACE activity using a spectrophotometric method.[37] In this assay, ACE hydrolyses synthetic substrate *N*-[3-(2-furyl) acryloyl]-L-phenyl-alanylglycyglycine (FAPGG) to *N*-[3-(2-furyl) acryloyl]-L-phenylalanine (FAP) and glycylglycine (GG). The assay mixture in a volume of 105 μL contained 50 mM Tris buffer (pH = 8.0), 300 mM sodium chloride, 0.5 mM FAPGG, 2.5 μL of ACE (135 Units/L) and 2.5 μL of various known concentrations of peptidomimics. It was incubated for 30 min at 37 °C. The reaction was monitored at 340 nm over a period of 15 min.

Statistical analysis

The experimental data of the ACE inhibition studies was compared by *analysis of variance (ANOVA) using Kruskal–Wallis one-way nonparametric analysis*. Subsequently comparisons by multiple range tests were carried out wherever applicable. Significance level was set as $p \leq 0.05$.

Acknowledgements

The work was supported in part by the Council of Scientific and Industrial Research (CSIR) and the University Grants Commission (UGC). The authors would like to thank Mr Rajbir Singh, Department of Biostatistic, All India Institute of Medical Sciences, New Delhi, India for the statistical analysis of the data. Particular acknowledgment is due to M/s Lupin Pharmaceutical Industry, Mandideep, India for providing lisinopril for the studies.

References and Notes

1. Erdös, E. G.; Skidgel, R. A. *Biochem. Soc. Trans.* **1985**, *13*, 42.
2. Erdös, E. G. *Hypertension* **1990**, *16*, 363.
3. Corvol, P.; Michaud, A.; Soubrier, F.; Williams, T. A. *J. Hypertension* **1995**, *13* (Suppl. 3), S3.
4. Wood, J. M. *J. Cardiovas. Pharmacol.* **1990**, *16* (Suppl. 4), S1.
5. Sumida, H.; Yasue, H.; Matsuyama Yosimura, M.; Hokimoto, S.; Mizuno, Y. *Am. J. Cardiol* **1999**, *84*, 774.
6. Antonios, T. F. T.; MacGregor, G. A. *J. Hypertension* **1995**, *13* (Suppl. 3), S11.
7. Cushman, D. W.; Cheung, H. S.; Sabo, E. S.; Ondetti, M. A. *Biochemistry* **1977**, *16*, 5484.

8. Patchett, A. A.; Harris, E.; Tristram, E. W.; Wyvratt, M. J.; Wu, H. T.; Taub, D.; Peterson, E. R.; Ikeler, T. J.; ten Brocke, J.; Payne, L. J.; Ondeyka, D. L.; Thorsett, E. D.; Greenlee, W. J.; Lohr, N. S.; Hoffsommer, R. D.; Joshua, H.; Ruyle, W. V.; Rothrock, J. W.; Aster, S. D.; Maycock, A. L.; Robinson, F. M.; Hirschmann, R.; Sweet, C. S.; Ulm, E. H.; Gross, D. M.; Vassil, T. C.; Stone, C. A. *Nature (London)* **1980**, *288*, 280.
9. Smith, E. M.; Swiss, G. F.; Neustadt, B. R.; Gold, E. H.; Sommer, J. A.; Brown, A. D.; Chiu, P. J. S.; Moran, R.; Sybertz, E. J.; Baum, J. *J. Med. Chem.* **1988**, *31*, 875.
10. Robl, J. A.; Cimarusti, M. P.; Simpkins, L. M.; Brown, B.; Ryono, D. E.; Bird, J. E.; Asad, M. M.; Schaeffer, T. R.; Trippodo, N. C. *J. Med. Chem.* **1996**, *39*, 494.
11. Robl, J. A.; Sulsky, R.; Sieber-McMaster, E.; Ryono, D.E; Cimarusti, M. P.; Simpkins, L. M.; Karanewsky, D. S.; Chao, S.; Asad, M. M.; Fox, M.; Smith, P. L.; Trippodo, N. C. *J. Med. Chem.* **1999**, *42*, 305.
12. Coric, P.; Tarcaud, S.; Meudal, H.; Roques, B. P.; Fournie-Zaluski, M.-C. *J. Med. Chem.* **1996**, *39*, 1210.
13. Ripka, A. S.; Rich, D. H. *Curr. Opin. Chem. Biol.* **1998**, *2*, 441.
14. Beelay, N. *TIBTECH* **1994**, *12*, 213.
15. Weber, L. *Curr. Opin. Chem. Biol.* **2000**, *4*, 295.
16. Hogan, J. C., Jr. *Nat. Biotech.* **1997**, *15*, 328.
17. Murphy, M. M.; Schullek, J. R.; Gordon, E. M.; Gallop, M. A. *J. Am. Chem. Soc.* **1995**, *117*, 7029.
18. Campbell, D. A.; Bermak, J. C. *J. Am. Chem. Soc.* **1995**, *116*, 6039.
19. Lenz, G. R.; Nash, H. M.; Jindal, S. *Drug Dis. Tech.* **2000**, *5*, 145.
20. Gallop, M. A.; Barret, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233.
21. Gordon, E. M. *Curr. Opin. Biotech.* **1995**, *6*, 624.
22. Jiang, W.; Bond, J. S. *FEBS* **1992**, *312*, 110.
23. Williams, T. A.; Corvol, P.; Soubrier, F. *J. Biol. Chem.* **1994**, *269*, 29430.
24. Cushman, D. W.; Cheung, H. S.; Sabo, E. S.; Ondetti, M. A. *Biochemistry* **1977**, *16*, 5484.
25. Ondetti, M. A.; Rubin, B.; Cushman, D. W. *Science* **1977**, *196*, 441.
26. Petrillo, E. W.; Ondetti, M. A. *Med. Res. Rev.* **1982**, *2*, 1.
27. Cheung, H. S.; Wang, F. L.; Ondetti, M. A.; Sabo, E. F.; Cushman, D. W. *J. Biol. Chem.* **1980**, *255*, 401.
28. Wyvratt, M. J.; Patchett, A. A. *Med. Res. Rev.* **1985**, *5*, 483.
29. Ladner, R. C. *TIBTECH* **1995**, *13*, 426.
30. Houghten, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 5131.
31. Spaller, M. R.; Burger, M. T.; Fardis, M.; Barlett, P. A. *Curr. Opin. Chem. Biol.* **1997**, *1*, 47.
32. Barlos, K.; Chatzi, O.; Gatos, D.; Stravropoulos, G. *Int. J. Pept. Protein. Res.* **1991**, *37*, 513.
33. Barlos, K.; Chatzi, O.; Gatos, D.; Stavropoulos, G. *Int. J. Pep. Protein. Res.* **1991**, *37*, 513.
34. Barlos, K.; Gatos, D.; Kutsogianni, S.; Papaphotiou, G.; Poulos, C.; Tsegenidis, T. *Int. J. Pept. Protein Res.* **1991**, *38*, 562.
35. Pearson, D. A.; Blanchette, M.; Baker, M.; Guindon, C. A. *Tetrahedron Lett.* **1989**, *30* (21), 2739.
36. Bull, H. G.; Thornberry, N. A.; Cordes, E. H. *J. Biol. Chem.* **1985**, *260*, 2963.
37. Bala, M.; Gupta, S.; Pasha, M. A. Q. *Clin. Biochem.* **2000**, *33*, 687.