Bioorganic & Medicinal Chemistry Letters 19 (2009) 4364–4366

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Effect of varying chain length between P_1 and $P_{1'}$ position of tripeptidomimics on activity of angiotensin-converting enzyme inhibitors

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ARTICLE INFO

Article history:
Received 13 March 2009
Revised 12 May 2009
Accepted 20 May 2009
Available online 27 May 2009

Keywords:
ACE Inhibitors
LUDI
Tripeptidomimics
Docking
Discovery studio

ABSTRACT

One of the efficient modes 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. The efforts continue towards achieving superior molecules or drugs with improved affinities, better bioavailability and thus to achieve long duration of action with minimum side effects. Previously, we reported a library of tripeptidomimics of Ornithyl–Proline (Orn–Pro) conjugated with various unnatural amino acids and carboxylic acid derived heterocyclics based on the SAR studies of existing ACE inhibitors. Their synthesis and screening for possible inhibitors of angiotensin-converting enzyme (ACE) revealed that increase in the backbone chain length by one carbon atom results in a sudden decrease in their activity. Therefore, in the present study heterocycles with different chain length were introduced to interact with the hydrophobic S_2 sub-site of ACE and screened for their in vitro ACE inhibition activity. Further, their binding interaction with C-domain of somatic ACE was also determined. Docking and consequent LUDI scores showed good correlation with binding of these molecules in the active site of ACE. Results suggest that heterocycles with C_3 chain length are more appropriate for the effective binding of the tripeptidomimics within the active site of ACE.

Angiotensin-I converting enzyme (ACE, 1 EC 3.4.15.1) is a zinc metallopeptidase that has received considerable attention because of its pivotal role in blood pressure regulation by catalyzing the proteolysis of angiotensin-I to the vasopressor angiotensin-II.[1–4] ACE inhibitors are widely used to treat cardiovascular diseases, including high blood pressure, heart failure, coronary artery disease and kidney failure.[5]

Several ACE inhibitors have been developed during the last two decades[6–10,4,11–13] but the use of ACE inhibitors is fraught with many side effects like dry cough, skin irritation, angioedema and other problems particularly multiple dosing due to less bioavailability.[14] In our previous study, to overcome the problem of limited bioavailability, a new class of ACE inhibitors, termed as peptidomimics[15,16] consisting of nonpeptide moieties like unnatural amino acids or carboxylic acid derived heterocyclics were conjugated with the peptidic moieties and thus a library of tripeptidomimics was successfully designed and synthesized. The results of their biological evaluation showed that increase in the backbone chain length of the tripeptidomimics by one carbon atom results in a sudden decrease in their activity.[17] These findings encouraged us to make further efforts to explore the desired backbone chain length of the tripeptidomimics.

Therefore, in further continuation of our previous study, library of compounds containing different derivatives of thiophene and indole rings was designed and synthesized. In the designing of tripeptidomimics, usual amino acid that is, Proline was kept at $P_{2'}$ position while $P_{1'}$ position was occupied by unusual amino acid that is, Ornithine. At the $P_{2'}$ position, the $-COO^-$ group of Pro is likely to interact with positively charged side chain of Arg (or Lys) in the $S_{2'}$ sub-site of ACE while Orn was placed at the $P_{1'}$ position to interact with the $S_{1'}$ sub-site of ACE. At the P_1 position, thiophene and indole ring containing heterocycles with different chain length like thiophene-3-carboxylic acid (TCA, 1), thiophen-3-yl acetic acid (TAA, 2), 3-(thiophen-3-yl) propanoic acid (TPA, 3), 4-(thiophen-3-yl) butanoic acid (TBA, 4), 1H-indole-3-carboxylic acid (ICA, 5), 1H-indol-3-yl acetic acid (IAA, 6), 3-(1H-indol-3-yl) propanoic acid (IPA, 7) and 4-(1H-indol-3-yl) butanoic acid (IBA, 8) were incorporated to interact with the S_1 subsite and Zn^{2+} ion located in the enzyme's active site (Fig. 1). In the present study, the tripeptidomimics were further synthesized and screened for their in vitro ACE inhibition activity according to the previously described method.[17] Further, binding interactions of these compounds within the active site of ACE was also determined by using CDOCKER program of the Discovery Studio.

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doi:10.1016/j.bmcl.2009.05.079

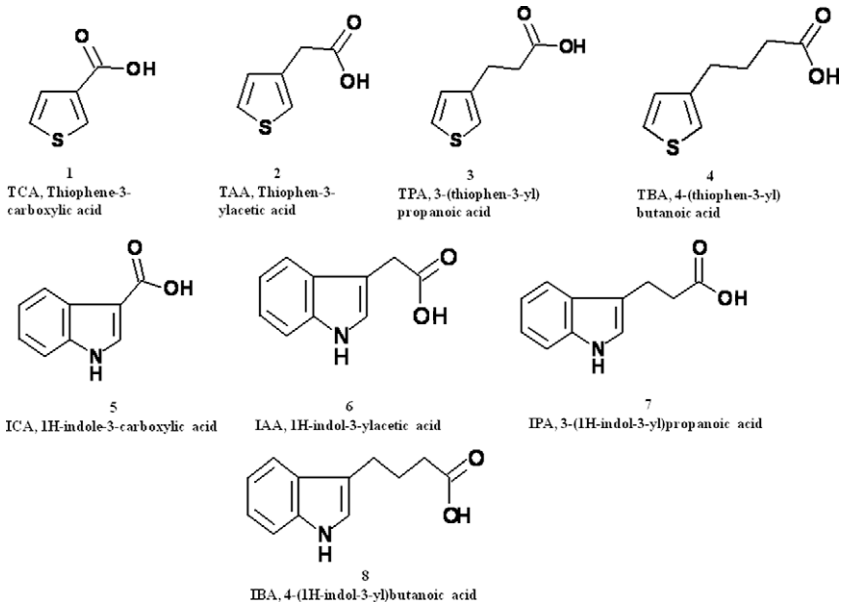


Figure 1. Heterocyclic moieties incorporated at the P_1 position of the tripeptidomimics.

Somatic ACE is comprised of two functionally active domains C-domain and N-domain, each of which is catalytically active and functionally independent.[18] The C-domain is primarily involved in blood pressure regulation whereas the N-domain is far more specific for the hemo-regulatory tetrapeptide AcSDKP, which controls hematopoietic stem cell differentiation and proliferation.[19] Recently, it is also demonstrated that the inhibition of one domain (C-domain) may be necessary and sufficient for the treatment of certain cardio renal diseases.[5] Therefore, in the present study, docking of the designed tripeptidomimics was done only with the C-domain of somatic ACE that is, with testicular ACE.

The three-dimensional structures of the aforementioned compounds were constructed using Builder module of the Accelrys, DS Studio 2.0 (Accelrys, San Diego CA). The structures were energy minimized using steepest descent followed by conjugate gradient to achieve a final derivative of 0.001. The crystal structures of human angiotensin-converting enzyme complexed with inhibitor lisinopril was retrieved from the RCSB Protein Data Bank (PDB code: 1O86). All bound waters and ligands were removed from the protein and the polar hydrogens were added to the proteins.

Docking was performed using CDOCKER program of the DS. Using a CHARMm-based MD simulation scheme; CDOCKER docks ligands in a receptor binding site. Briefly a binding site of 8 Å radii was defined and Random Conformation Dynamics steps were fixed to 1000. After a simulated annealing step the final minimization was allowed to full potential and resultant 10 poses were stored for further evaluation. Scoring was provided as –CDOCKER energy and finally docked poses were then scored using LUDI module for calculation of Ludi Energy Estimate 3. Ludi energy estimate 3 is related to the K_i by following expression:

$$\text{Score} = 100 \log K_i,$$

where K_i is given by the expression

$$\Delta G = -RT \ln(K_i)$$

where ΔG is an empirical function involving contributions from binding energy, H-bond, unperturbed ionic interaction, lipophilic interaction, rotational degrees of freedom and aromatic–aromatic interaction.

Figure 1 shows designed derivatives of the lead molecules containing thiophene and indole moieties.

At position P_1 of the lead structure, long lipophilic alkyl side chains were introduced to interact with the hydrophobic S_2 subsite. After energy minimization, the lowest energy position in the active site for the generated lead molecule was determined by the CDOCKER and scored by the score-3 function in LUDI module. The calculated LUDI scores and interaction energy of each molecule are given in Table 2.

The mode of action of lead molecule with the active site of tACE is shown in Figure 2. After flexible ligand docking and subsequent molecular dynamics simulated annealing studies, carboxyl group at the C-terminal form H-bonds with the residues Tyr 520 (2.35 Å), Lys 511 (1.78 Å) and Gln 281 (2.09 Å). Interaction of –NH group between the P_1 and $P_{1'}$ position of tripeptidomimic and Tyr 523 (2.56 Å) was also observed. At P_1 position, heterocycle with alkyl side chain was interacting with the S_1 sub-site. As it is well known, that H-bond stabilizes the docking complex and plays an important role for structure and function of biological molecules, especially for the enzyme catalytic reactions.[20] Greater num-

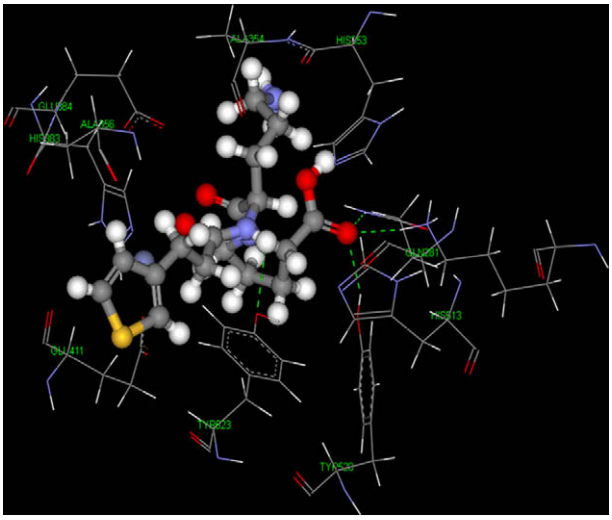
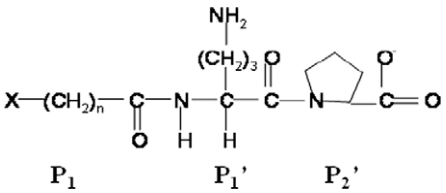


Figure 2. The mode of action of lead molecule TPA-Orn–Pro with the active site of tACE.

Table 1
In vitro activity of tripeptidomimics as angiotensin-converting enzyme inhibitors



S.No.	Tripeptidomimics	ACE activity[a] (mU/ml)	ACE inhibition (mU/ml)	ACE inhibition (%)	*P*-value
1	TCA-Orn–Pro (X = thiophene, *n* = 0)	10.20 ± 0.06	3.25	23.74	<0.0001
2	TAA–Orn–Pro (X = thiophene, *n* = 1)	09.17 ± 0.13	4.28	35.64	<0.001
3	TPA-Orn–Pro (X = thiophene, *n* = 2)	03.11 ± 0.11	10.34	72.67	<0.01
4	TBA-Orn–Pro (X = thiophene, *n* = 3)	08.20 ± 0.11	5.25	37.23	<0.001
5	ICA-Orn–Pro (X=indole, *n* = 0)	09.32 ± 0.15	4.13	27.71	<0.0001
6	IAA–Orn–Pro (X = indole, *n* = 1)	08.61 ± 0.14	4.84	38.51	<0.001
7	IPA-Orn–Pro (X = indole, *n* = 2)	06.85 ± 0.07	6.60	59.74	<0.0001
8	IBA-Orn–Pro (X = indole, *n* = 3)	09.69 ± 0.16	3.76	33.82	<0.0001

[a] Tripeptidomimics were assayed against rabbit lung angiotensin-converting enzyme with activity 13.46 ± 0.03 mU/ml of assay volume with synthetic substrate Hip-His-Leu.

Table 2
Calculated interaction energies and LUDI scores of the tripeptidomimics

S.No.	Tripeptidomimics	Interaction energy (kcal/mol)	Ludi scores
1	TCA-Orn–Pro	52.551	408
2	TAA–Orn–Pro	54.391	486
3	TPA-Orn–Pro	57.667	753
4	TBA-Orn–Pro	34.381	311
5	ICA-Orn–Pro	53.323	393
6	IAA–Orn–Pro	44.181	400
7	IPA-Orn–Pro	54.336	663
8	IBA-Orn–Pro	36.257	328

ber of hydrogen bonds in the complex suggests its effective interaction with the active site of tACE and thus contributes to their better inhibition potency.[21]

In vitro inhibition of ACE for these tripeptidomimics was evaluated by a synthetic substrate of ACE, Hip-His-Leu, using the spectrophotometric method. The ACE inhibitory activity of the tripeptidomimics TCA-Orn–Pro, TAA–Orn–Pro, TPA-Orn–Pro, TBA-Orn–Pro, ICA-Orn–Pro, IAA–Orn–Pro, IPA-Orn–Pro and IBA-Orn–Pro were less than 50% that is, 3.25 mU/ml (23.74%, $P < 0.0001$), 4.28 mU/ml (35.64%, $P < 0.001$), 10.34 mU/ml (72.67%, $P < 0.01$), 5.25 mU/ml (37.23%, $P < 0.001$), 4.13 mU/ml (27.71%, $P < 0.0001$), 4.84 mU/ml (38.51%, $P < 0.001$), 6.60 mU/ml (59.74%, $P < 0.0001$) and 3.76 mU/ml (33.82%, $P < 0.0001$), respectively. The ACE activity inhibition values and statistically analyzed *P*-values of these tripeptidomimics are represented in Table 1.

In vitro results revealed that tripeptidomimics TCA-Orn–Pro, TAA–Orn–Pro, TBA-Orn–Pro, ICA-Orn–Pro, IAA–Orn–Pro and IBA-Orn–Pro were showing less than 50% of inhibition potency at a concentration of 25 μM suggesting that the appropriate substitution at the P_1 position was important for the ACE inhibitory activity of the designed tripeptidomimics. Tripeptidomimics TBA-Orn–Pro and IBA-Orn–Pro were less active because the alkyl side chain at position P_1 was too long to be accommodated by the hydrophobic S_1 subsite while tripeptidomimics ICA-Orn–Pro, IAA-Orn–Pro, TCA-Orn–Pro and TAA–Orn–Pro were less potent because of their too short alkyl side chain to interact firmly with the hydrophobic region, although they were able to enter the active site. These results suggests that heterocycle with C_3 chain length are appropriate for the effective binding within the active site of ACE.

The overall trend for the interaction energy and LUDI scores (Table 2) was in good qualitative agreement with the ACE inhibitory activities in vitro. All of the results gave us the suggestion to further design and synthesize their derivatives.

The structural approach of the ACE inhibitors developed in the present work can be used with further suitable modifications at the various positions keeping C_3 chain length between P_1 and $P_{1'}$ position of the tripeptidomimics constant to obtain leads to potentiate ACE activity inhibition in basic as well as clinical research applications.

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