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Short communication

Angiotensin-I-converting enzyme inhibitory peptides: Chemical feature based pharmacophore generation

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ABSTRACT

A validated 3D pharmacophore model was generated for a series of ACE inhibitory peptides, which consisted of five features (two hydrophobic functions, two hydrogen bond acceptors, and a negative ionizable function). The built model was able to correctly predict the activity of known ACE inhibitors. The model was then used as query to search 3D databases of peptides. Three novel peptides (I, II and III) were synthesized and biologically evaluated *in vitro*. It appears that the *in vitro* activity of peptides I, II and III was consistent with their molecular modeling results. Our results provided confidence for the utility of the pharmacophore model to retrieve novel ACE inhibitory peptides with desired biological activity by virtual screening.

1. Introduction

Angiotensin-I-converting enzyme (ACE) plays an important role in the regulation of hypertension. ACE catalyzes the conversion of decapeptide (angiotensin I) to the potent vasoconstring octapeptide (angiotensin II). Inhibition of ACE activity leads to a decrease in the concentration of angiotensin II and consequently reduces blood pressure [1]. Synthesizing ACE inhibitors have been used extensively in the treatment of hypertension in humans, such as captopril, enalapril, alacepril and lisinopril [2–7]. However, studies have shown that the aforementioned ACE inhibitors have a number of side effects, including the inducement of coughing, taste disturbances and skin rashes [8]. Natural ACE inhibitory peptides can control hypertension and have minimum adverse side effects. As a result, interest has increased in identifying foods as potential natural sources of ACE inhibitory peptides [9].

Chemical feature based pharmacophore model may serve as a guide in identifying potential natural ACE inhibitory peptides. Recently, Ismail MA et al. generated an ACE inhibitors pharmacophore model from the biologically active conformation of Lisinopril [10]. This model can be used to evaluate the activities of compounds using compare/fit process. However, the estimated IC_{50} values could not be yielded. In 2008, we constructed a pharmacophore for ACE inhibitory peptides using Catalyst/HypoGen program. This model allows for direct estimation of IC_{50} values of the tested compounds. However, the Δcost of this model is 40.71, which means that the hypothesis is deemed a 75%–90% statistical probability [11].

Though many different natural ACE inhibitory peptides had been identified, to the best of our knowledge, there is little information available regarding the highly predictive pharmacophore for such kind of compounds up to date. This study aims to construct more reliable chemical feature based pharmacophore models for ACE inhibitory peptides. This model may serve as a guide in identifying selective natural ACE inhibitory peptides.

2. Materials and methods

2.1. Generation of pharmacophore models

This study was performed using the software package Catalyst. Based on the published literature, we collected a set of ACE inhibitory peptides as the training set to generate pharmacophore models (Fig. 1) [12–17]. To ensure the statistic relevance of the calculated

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model, the training set contained 28 peptides together with their activity values and the range of *in vitro* ACE inhibitory activity (IC_{50} value) spanned 6 orders of magnitude (0.32–9200 μM). The peptides were built and were minimized to the closest local minimum using the CHARMm force field. Diverse conformational models for each peptide were generated using an energy constraint of 20 kcal/mol. The maximum number of conformers of each molecule was specified to be 255 to ensure the maximum coverage of the conformational space. In this study, the chemical features used were hydrogen bond acceptor (A), general hydrophobic features (H) and ionizable negative feature (N) [18].

2.2. Peptide synthesis

The peptides were obtained from Shanghai Hanhong Chemical Co., LTD (Shanghai, China). Briefly, the peptides were synthesized by conventional solid-phase chemistry. High performance liquid chromatographic analysis and purification were carried out.

2.3. Biological testing

The ACE inhibitory activity was assayed by the modified method of Cushman and Cheung [19]. Briefly, 40 μl of ACE inhibitory peptide solution, 40 μl of 100 mM sodium borate buffer containing 300 mM sodium chloride (pH 8.3) and 50 μl of 5 mM hippuryl-His-Leu hydrate solution were mixed. After 5 min of pre-incubation at 37 °C, 10 μl of ACE dissolved in distilled water (0.1 U/ml) was added to initiate the reaction, and the reaction was incubated at 37 °C for 30 min. Then the reaction was terminated by addition of 200 μl of 1 M HCl. 40 μl of distilled water was used as control. The inhibition ratio was calculated as follows:

$$I\% = \frac{A - B}{A} \times 100\%$$

(A: the peak area of hippuric acid in control sample; B: the peak area of hippuric acid in the sample containing ACE inhibitory peptide).

2.4. Molecular docking

Human testicular ACE (tACE) in complex with captopril (PDB code: 1UZF) was used as a template for docking experiments with the peptides [20]. The eHiTS software package was used for flexible docking. Active site detection was carried out using the "*-complex*" parameter with high accuracy. The program automatically detected the ligand in the complex and selected the part of enzyme within

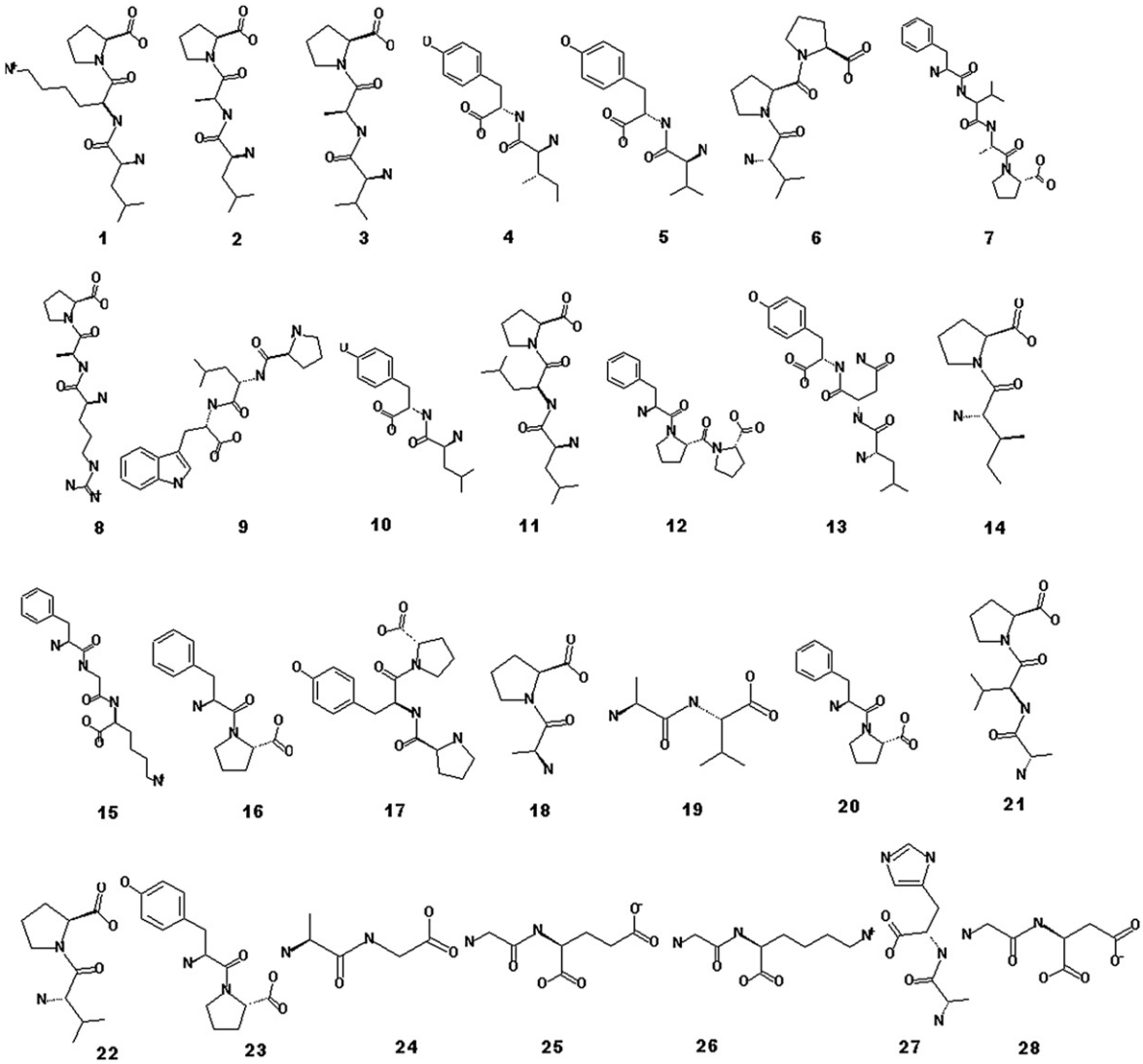


Fig. 1. Molecular structures of the 28 training set compounds.

Table 1
Information of statistical significance and predictive power for top 10 hypotheses as a result of automated HypoGen pharmacophore generation process.[a]

Hypothesis no.	Total cost	Δcost	RMS deviation	Correlation (*r*)
1	117.330	60.873	0.85299	0.937584
2	118.876	59.327	0.91750	0.927368
3	124.000	54.203	1.07715	0.898486
4	125.808	52.395	1.16930	0.878741
5	126.225	51.978	1.13502	0.886631
6	126.313	51.890	1.15128	0.883047
7	127.056	51.147	1.18379	0.875781
8	128.506	49.697	1.21671	0.868323
9	128.546	49.657	1.19156	0.874273
10	128.557	49.646	1.19647	0.873134

[a] Null cost of 10 top-scored hypotheses is 178.203. Fixed cost value is 106.662. Configuration cost is 11.3565.

a 7 Å residues around the ligand to be the active site. The peptide was then docked to the active site using a higher accuracy mode of docking (accuracy set to 6) and further scored with eHiTS Score, which is included in the eHiTS software package.

3. Results and discussion

Continuing our investigation in this field and in an attempt to identify novel ACE inhibitory peptides, 3D pharmacophore models for ACE inhibitory peptides were constructed using a training set. The best hypothesis has quantitatively predictive ability and then was helpful to estimate the activity potential of new lead peptides identified by virtual screening. Moreover, the resulting pharmacophore model could serve as a guide for the rational design of high potent ACE inhibitory peptides.

3.1. 3D pharmacophore generation

Based on the training set presented in Fig. 1, we constructed the chemical feature based pharmacophore models for ACE inhibitory peptides. The characteristics, such as the cost value, the root mean square (RMS) and the regression coefficient r, of the 10 hypotheses with the lowest cost values were listed in Table 1. The total fixed cost of the run was 106.662, and the cost of the null hypothesis was 178.203. The statistical relevance of the various hypotheses obtained was assessed on the basis of their cost values relative to that of the null hypothesis and their *r* values. The first hypothesis (Hypo1) was characterized by the highest cost difference (60.873), lowest RMS error (0.853), and the best correlation coefficient *r* (0.938). Hypo1 displayed five chemical features: one negative ionizable feature (N), two hydrogen bond acceptor features (A1 and A2) and two hydrophobic features (H1 and H2), which were in agreement with other results [10]. Fig. 2 showed the Hypo1 mapping with the most active peptide leu-lys-pro ($IC_{50} = 0.32$ nM) in the training set. Compare to the pharmacophore model constructed by Ismail et al., this model allows for direct estimation of IC_{50} values of compounds. Moreover, the Δcost of this model is 60.873, which means that the hypothesis is deemed a 90% statistical probability. By contrast, the pharmacophore for ACE inhibitory peptides we constructed in 2008 is deemed a 75%–90% statistical probability. It means that this model is more reliable.

3.2. Validation of the pharmacophore model

Hypotheses are believed to be statistically relevant when the overall hypothesis cost is close to the fixed and far away from null cost values. If the Δcost is over 60 bits then the hypothesis is deemed a 90% statistical probability. The Δcost of Hypo1 was 60.873. Moreover, values of RMS error and correlation coefficient *r* of Hypo1 were 0.853 and 0.938, respectively. These output parameters determine the quality of Hypo1.

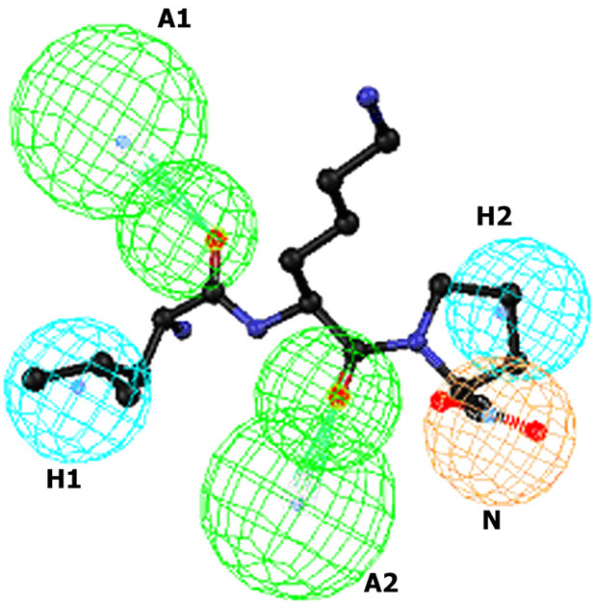


Fig. 2. The Hypo1 mapping with training set peptide leu-lys-pro. Pharmacophore features are color-coded: green for hydrogen bond acceptor features (A1 and A2), light blue for hydrophobic features (H1 and H1) and orange for ionizable negative feature (N). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Furthermore, Hypo1 had a reliable ability to predict the activities of training set compounds (Table 2). Most of compounds were

Table 2
Experimental activity and the estimated activity for the training set peptides based on the top ranked hypothesis.

	Peptide	Measured IC_{50} (μM)[a]	Estimated IC_{50} (μM)	Error factor[b]
1	LKP	0.32	1.7	+5.4
2	nLAP	0.7	1.4	+2
3	VAP	2	4.2	+2.1
4	IY	3.7	17	+4.6
5	VY	7	15	+2.1
6	VPP	9	18	+2
7	FVAP	10	10	+1
8	RAP	16	40	+2.5
9	PLW	36	25	−1.4
10	LY	44	49	+1.1
11	LLP	57	14	−4
12	FPP	78	16	−4.9
13	LNY	81	32	−2.5
14	IP	150	330	+2.2
15	FGK	160	37	−4.3
16	FP	210	160	−1.3
17	PYP	220	35	−6.2
18	AP	270	340	+1.3
19	AV	300	210	−1.4
20	FP	320	160	−2
21	AVP	340	110	−3
22	VP	420	390	−1.1
23	YP	720	330	−2.2
24	AG	2500	9700	+3.9
25	GE	5400	9700	+1.8
26	GK	5400	9300	+1.7
27	AH	9000	9700	+1.1
28	GD	9200	9700	+1.1

[a] References [12–17].
[b] The error factor is computed as the ratio of the measured activity to the activity estimated by the hypothesis or the inverse if estimated is greater than measured.

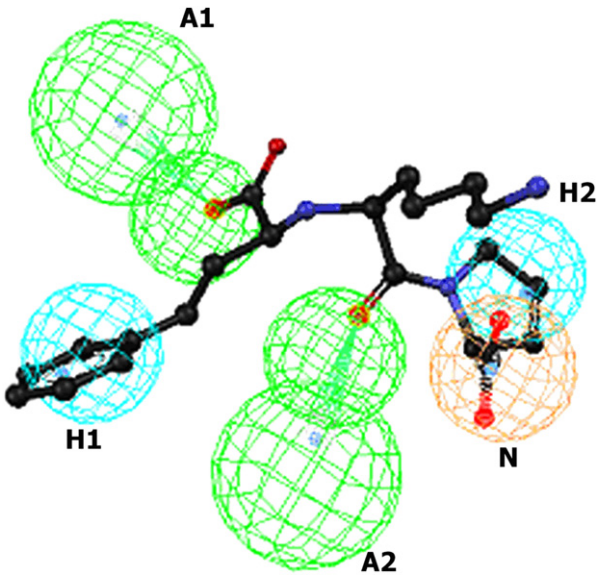
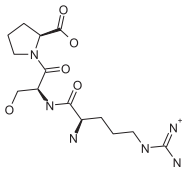
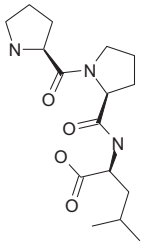
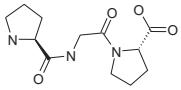


Fig. 3. The Hypo1 mapping with the crystal structure of compound Lisinopril. Pharmacophore features are color-coded: green for hydrogen bond acceptor features (A1 and A2), light blue for hydrophobic features (H1 and H1) and orange for ionizable negative feature (N). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Experimental activity and the estimated activity for the 3 peptide hits based on the top ranked hypothesis.

	Peptide	Structure	Measured IC_{50} (μM)	Estimated IC_{50} (μM)	Error factor
I	RSP		247.74	300	+1.21
II	PPL		427.15	510	+1.17
III	PGP		97.28	72	−0.74

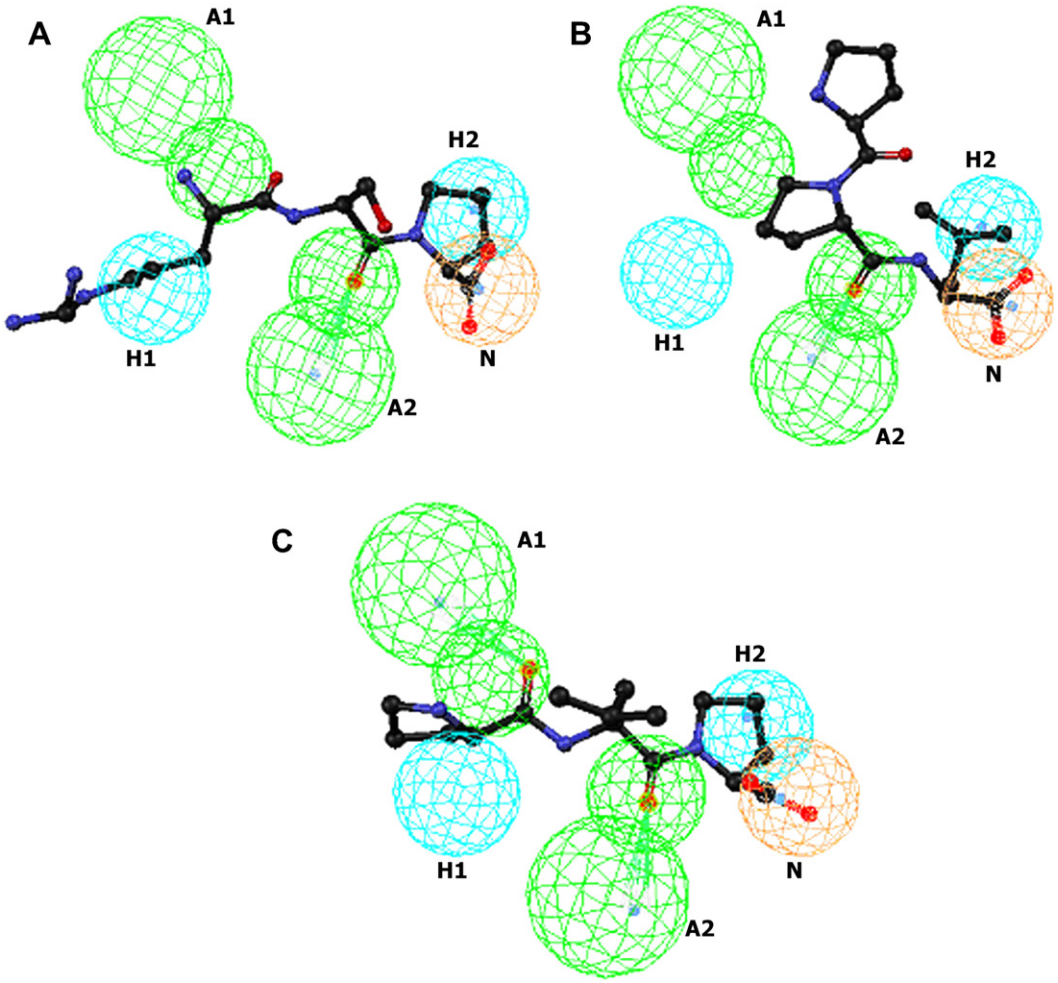


Fig. 4. The Hypo1 mapping with lead peptides I (A), II (B) and III (C). Pharmacophore features are color-coded: green for hydrogen bond acceptor features (A1 and A2), light blue for hydrophobic features (H1 and H1) and orange for ionizable negative feature (N). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

predicted appropriately. We further used the crystal structure of Lisinopril to validate model Hypo1, and it allowed proper mapping of all the features of the generated hypothesis. The estimated activity (2.4 nM) of Lisinopril was similar to the measured one (2.1 nM) with a relative error of −0.875. This result was a significant confirmation for the reliability of our pharmacophore model. The mapping of the crystal structure of Lisinopril onto the Hypo1 was represented in Fig. 3.

In addition, Hypo1 was used as a query to retrieve potential of novel peptide hits from database so that the reliability of Hypo1 was assessed. Using our in-house database of food-derived peptides, a virtual screening is performed. The pharmacophore captured 12 hits from database with more than two thousands peptides. Here we presented only three candidate structures (I, II and III) derived from the milk casein for being further investigated. Our results indicated that the estimated activities of 3 peptides (I, II and III) were 300 μM, 510 μM and 72 μM, respectively (Table 3). Moreover, the synthesis and biological evaluation of the peptides were carried out to verify the calculated results. The experimental results showed that the measured IC_{50} values of peptides I, II and III were 247.74 μM, 427.15 μM and 97.28 μM, respectively (Table 3). It showed that the order of the biological activities of lead peptides were consistent with that of theoretically calculated ones, that is III > I > II. At the same time, the results indicated that the activities of the peptides could be predicted correctly, which indicated that the pharmacophore model built in this paper was powerful and reliable enough to search and predict novel ACE peptide inhibitors.

3.3. Pharmacophore model serving as a guide in rational design of ACE peptide inhibitors

Hypo1 can be used to guide the rational design of novel ACE peptide inhibitors. Generally, more active peptides map well to all the features of the hypothesis, and peptides showing low activities

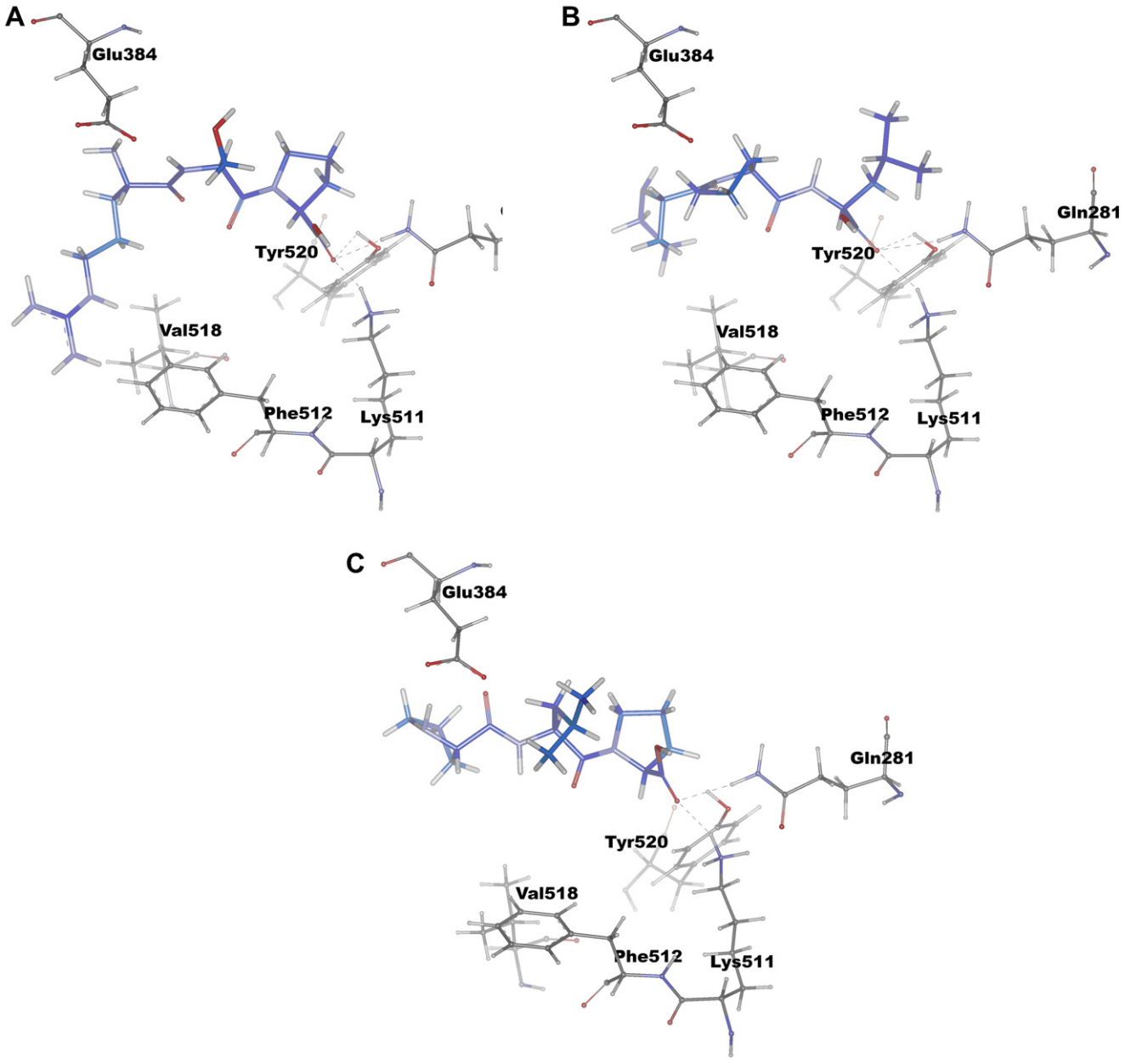


Fig. 5. Binding of lead peptide I (A), II (B) and III (C) to the active site of ACE. The hydrogen bonds are represented by black lines.

map poorly to the hypothesis. Among the five features of the Hypo1, most of the less active compounds such as peptide 14, 18, 19, 22, 24, 25, 27 and 28 were unable to map the H1 and A1 features, as shown by the presence of an asterisk in the mapping column of the output file of catalyst run (see Supporting information). The mapping of the peptides I, II and III onto the Hypo1 was represented in Fig. 4. It is worth noting that three peptides did not map the H1 feature. The results also indicated that peptides I and II were unable to map the A1 feature well. We speculated that ACE inhibitory potency might be improved by introduction of functionality in these lead peptides, which might map to the H1 and A1 features of the Hypo1.

Moreover, we presented the study of the binding modes between three lead peptides and ACE. It is well known that the binding site of ACE included Gln281, His353, Glu384, Lys511, Phe512, Lys513, Val518 and Tyr520. They play an essential role in ACE-substrate interactions. Those amino acid residues usually involved in the binding of the peptides. Peptides I, II and III were then docked into the ACE. As expected, three lead peptides bound to the substrate-binding site of ACE with binding modes very similar to what had been observed in the Lisinopril-ACE crystal complex. However, due to the orientation of N-termini regions, peptides I, II and III became too far from Phe512 and Val518 to form hydrophobic interactions (Fig. 5). Moreover, three lead peptides did not form additional hydrogen bond with Glu384. These docking results agree with the previously reported less active character of three tested peptides proposed by pharmacophore model. This docking result further validated the robustness of the pharmacophore. Therefore this validated pharmacophore model could be recommended for further studies.

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Appendix. Supporting information

Supporting information associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2011.05.007.

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