Application of Bioinformatics in Search for Cleavable Peptides of SARS-CoV M^{pro} and Chemical Modification of Octapeptides

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Abstract: According to the "distorted key" theory as elaborated in a review article years ago (Chou, K.C.: ***Analytical Biochemistry***, 1996, **233**, 1-14), the knowledge of the cleavable peptides by SARS-CoV M^{pro} (severe acute respiratory syndrome coronavirus main proteinase) can provide very useful insights on developing drugs against SARS. In view of this, the softwares, ZCURVE_CoV 1.0 and ZCURVE_CoV 2.0 (http://tubic.tju.edu.cn/sars/), developed recently for SARS-Coronavirus are used to analyze the 36 complete SARS-Coronavirus RNA sequences in the gene bank NCBI (http://www.ncbi.nlm.nih.gov/) from different sources for protein coding genes, and to search for the cleavage sites of SARS-CoV M^{pro} in polyproteins pp1a and pp1ab. A total of 396 cleavage points are found in the 36 SARS-Coronavirus and 11 cleavable octapeptides abstracted from the 396 cleavage sites. The statistical distributions of amino acids for the cleavable octapeptides at the subsites R4, R3, R2, R1, R1′, R2′, R3′ and R4′ are calculated. The cleavage-specific positions are on R2, R1 and R1′, and the positions R3 and R4 are featured by some certain specificity for SARS-CoV M^{pro}. The structural characters of amino acid residues around the cleavage-specific positions are discussed. Two most promising octapeptides, *i.e.*, NH_2-ATLQ↓ AIAS-COOH and NH_2-ATLQ↓ AENV-COOH, are selected to be the candidates for chemical modification, converting into the inhibitors of SARS-CoV M^{pro}. A possible strategy to convert a cleavable octapeptide by SARS enzyme into a drug candidate against SARS is elucidated.

Key Words: SARS, Coronavirus, Gene Identification, SARS-CoV M^{pro}, Octapeptide, Peptide bond modification, Inhibitor, Distorted key theory.

I. INTRODUCTION

After three decades of rapid progress, accurate gene identification tools for prokaryotic genomes are available through web service all over the world, such as GeneMark.hmm [1] and Glimmer [2]. It is possible that once we know the DNA or RNA sequence of a virus species, we know all the genomes and the coding proteins of the virus by using the gene identification software. Currently, bioinformatics has become a powerful tool for novel drug discovery (see, e.g., a recent review by Chou [3]).

The newly found virus, called SARS-coronavirus, is the leading hypothesis for the cause of SARS (severe acute respiratory syndrome) [4,5]. It is also known that the process of cleaving the SARS-CoV polyproteins by a special proteinase, the so-called SARS-Coronavirus main proteinase (CoV M^{pro}), is the key step for the transcription and replication of SARS-CoV [6-8]. The functional importance of the M^{pro} in the viral life cycle not only suggests that this proteinase is the culprit of SARS, but also makes it an attractive target for developing drugs directly against the new disease [6-8]. Anand *et al*. [6] suggested that the rhinovirus $3C^{pro}$ inhibitor AG7088 could well serve as a starting point for anti-SARS drug based on the theoretical homology model of SARS CoV M^{pro}. Chou *et al*. [7] found the fitting problem of AG7088 to the binding pocket of SARS CoV M^{pro}, and they suggested its derivative KZ7088 as a better starting point. Sirois *et al.* [9] did virtual screening for SARS-CoV protease based on KZ7088 pharmacophore points. On the other hand, it is also a very promising approach to find inhibitors against SARS-CoV M^{pro} by search for the cleavage sites in proteins by the SARS enzyme, just like the case for finding peptide inhibitors against HIV (human immunodeficiency virus) protease [7,10,11].

Currently, in gene bank NCBI (National Center for Biotechnology Information) RefSeq project (http://www.ncbi.nlm.nih.gov/) there are at lest 162 complete SARS-Coronavirus RNA sequences from different sources all over the world. Based on the RNA sequences of SARS-Coronavirus and with the help of gene identification tools, the polyprotein chain ORFs 1a and 1b are coded, and some major structural proteins, including spike protein (S), small envelope protein (E), membrane protein (M), and nucleocapsid protein (N), are found, and also 5 to 6 putative proteins with unknown functions are predicted [12,13]. These discoveries from bioinformatics are greatly helpful for the structure-based design of anti SARS drugs.

II. GENE IDENTIFICATION OF SARS-CoV

Currently, most algorithms for gene identification in prokaryotic genomes, such as GeneMark.hmm [1] and

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Glimmer [2], are based on either the higher-order Markov chain model, or the hidden Markov chain model, in which thousands of parameters have to be trained thru known homologous species. The large number of parameters may result in less adaptability, especially for the virus species with small genomes and less available training data, such as SARS-Coronavirus.

ZCURVE_CoV 1.0 [12] and ZCURVE_CoV 2.0 [13] were designed specially for the gene analysis of coronavirus family, in which the parameters were trained based on a SARS-Coronavirus Toronto 2 strain (TOR2, NC_004718) as well as six known non-SARS-Coronavirus, i.e., avian infectious bronchitis virus (IBV, NC_001451), bovine coronavirus (BCoV, NC_003045), human coronavirus 229E (HCoV-229E, NC_002645), murine hepatitis virus (MHV, NC_001846), porcine epidemic diarrhea virus (PEDV, NC_003436), and transmissible gastroenteritis virus (TGEV, NC_002306). Besides, ZCURVE_CoV 1.0 [12] was also designed for the gene recognition of protein-coding genomes of SARS-Coronavirus. In this study, the software is applied to the gene sequences of 36 SARS-Coronavirus strains, which are selected from gene bank NCBI (http://www.ncbi.nlm.nih.gov) with higher sequenced quality. Table **1** lists the annotation results of the 12 protein-coding genes of SARS-CoV strain Beijing 01 (BJ01, AY278488). Polyproteins ORF1a and ORF1b are connected by a ribosomal frameshift site, which is believed to occur at the conserved 'slippery sequence', UUUAAAC [12,13]. It results in the translation of an ORF1a protein and a carboxyl-extended ORF1ab frameshift protein, which are also known as replicase polyproteins pp1a and pp1ab [6,13]. Because of the ribosomal frameshift site in replicase gene, the length of polyprotein pp1a is 4377 a.a. (amino acid) and the length of pp1ab is 4377+2695=7072 a.a.

SARS-CoV main proteinase (M^{pro}) is initiated by the enzyme's own autolytic cleavage from pp1a and pp1ab [14,15]. In turn, the polyproteins pp1a and pp1ab are cleaved by SARS-CoV M^{pro} at no less than 11 conserved sites and the resulted peptides mediate all the functions required for viral replication and transcription [6]. The functional importance of SARS-CoV main proteinase in the viral life cycle has made it an attractive target in the structure-based drug design against SARS [6-8].

III. SEARCHING FOR SARS-COV M^{PRO} CLEAVAGE SITES

A key step in finding peptide inhibitors against a proteinase is to search for the peptides cleavable by the enzyme and identify their cleavage sites. Many efforts have been made in this regard (see, e.g., [10,16-29] as well as a comprehensive review [11] in this area). The underlying principle is the same for finding peptide inhibitors against SARS-Coronavirus main proteinase [7]. ZCURVE_CoV 2.0 is designed for the prediction of proteinase cleavage sites in polyproteins of coronaviruses by SARS-CoV M^{pro} and by papain-like assistant proteinase [13]. The parameters used in ZCURVE_CoV 2.0 are trained according to 77 cleavage sites of CoV M^{pro}, which are annotated by NCBI RefSeq project (http://www.ncbi.nlm.nih.gov) from 6 non-SARS-Coronaviruses and 1 SARS-Coronavirus mentioned in section II.

There are big differences between SARS-Coronavirus and other three group coronaviruses. The amino acid sequence of SARS-CoV M^{pro} displays 40% and 44% identity, respectively, to HCoV M^{pro} and TGEV M^{pro} of the group I coronaviruses. The identity levels are 50% and 49%, respectively, between SARS-CoV M^{pro} and the corresponding proteinases in group II coronaviruses, MHV M^{pro} and BCoV M^{pro}. SARS-CoV M^{pro} shares only 39% sequence identity with IBV M^{pro}, the only member in group III coronaviruses [6]. Therefore, SARS-Coronavirus is thought to be the group IV coronavirus [12]. Although the amino acid sequence

Table 1. The Prediction Results of Putative Protein Coding Genes of SARS-CoV Strain BJ01 (AY278488)

No	Start	Stop	Length (bp)	Length (a.a.)	Frame	Z-score	Feature
1	265	13398	13134	4377	+1	0.1985	ORF 1a
2	13398	21485	8088	2695	+3	0.1596	ORF 1b
3	21492	25259	3768	1255	+3	0.1593	S protein
4	25268	26092	825	274	+2	0.0914	Sars274
5	26117	26347	231	76	+2	0.1921	E protein
6	26398	27063	666	221	+1	0.1342	M protein
7	27074	27265	192	63	+2	0.2740	Sars63
8	27273	27641	369	122	+3	0.0437	Sars122
9	27638	27772	135	44	+2	0.0388	Sars44
10	27779	27898	120	39	+2	0.1071	Sars39
11	27864	28118	255	84	+3	0.1979	Sars84
12	28120	29388	1269	422	+1	0.0276	N protein

identity between SARS-Coronavirus and other coronaviruses is limited, the cleavage sites of CoV M^{pro} in polyproteins pp1a and pp1ab and the catalytic pattern are highly conservative in coronavirus family [15].

In order to find the cleavage sites of SARS-CoV M^{pro} in polyproteins pp1a and pp1ab, ZCURVE_CoV 2.0 is applied to 36 gene sequences of SARS-Coronavirus strains. A total of 396 cleavage sites are detected from the polyproteins pp1a and pp1ab of the 36 SARS-Coronavirus strains. The statistical distribution of amino acid residues surrounding the cleavage site $R_1 \downarrow R_1$ are calculated, and the results are shown in the Fig. **1**. The statistical distribution of amino acid residues surrounding 66 M^{pro} cleavage sites of 6 non-SARS-Coronavirus strains taken from [13] is shown in Fig. **1** (A), and that surrounding 396 M^{pro} cleavage sites of the 36 SARS-Coronavirus strains is shown in Fig. **1** (B).

As shown in Fig. **1**, the cleavage specificities of M^{pro} between SARS-Coronavirus and non-SARS-Coronaviruses are basically the same. The M^{pro} cleavage specificities are the amino acid residues on the positions R_2, R_1 and $R_{1'}$. The position R_1 is invariably occupied by amino acid residue Gln (Q). Amino acid residue Leu (L) has the largest probability on position R_2. However, on position $R_{1'}$, for non-SARS-Corona-viruses the order is S>A>N; while for SARS-Coronavirus, the order is A>S>G=N. It is interesting to see from Fig. **1** (B) and Table 2 that the positions R_3 and R_4 show certain cleavage specificities for SARS-CoV M^{pro}: on position R_4, amino acid residue Ala (A) has the largest probability (36.4%); while on position R_3 amino acid residue Thr (T) has the largest probability (36.4%). Listed in Table **2** are the percentage probabilities of amino acids on the cleavage specific positions of SARS-CoV M^{pro} calculated from the 36 SARS-Coronaviruses.

IV. SELECTION OF OCTAPEPTIDES FOR INHIBITOR

Table **3** lists 11 cleavable peptides according to the cleavage sites in polyproteins pp1a and pp1ab of SARS-CoV strain Toronto 02 (TOR2, NC_004718) predicted using ZCURVE_CoV 2.0. The cleavage sites are indicated by symbol "↓", and on each side of cleavage site 6 amino acids residues are shown. The protease-susceptible sites in a cleavable peptide usually extend to an octapeptide region [10,11]. Although the protein being cleaved contains amino acid residues much more than 8, in most case only an octapeptide fits in the active region of a protease and the cleavage site is always on the peptide bond between R_1 and $R_{1'}$. Therefore, our attention is focused on the cleavability of octapeptides.

According to the "distorted key" theory as illustrated by Fig. **2** of a comprehensive review paper by Chou [11], a competitive inhibitor for SARS-CoV M^{pro} needs to meet the following two conditions: (1) it has the competitive affinity with its acceptor, SARS-CoV M^{pro}, and (2) it resists the cleavage by SARS-CoV M^{pro}. Because the octapeptides taken from the cleavage sites in pp1a and pp1ab are actually cleavable by SARS-CoV M^{pro}, they must fit the catalytic cleft of SARS-CoV M^{pro} very well (see Fig. **2a** of [11]) and form strong combination with their receptor. The 11 cleavable octapeptides in Table **3** have strong affinity with

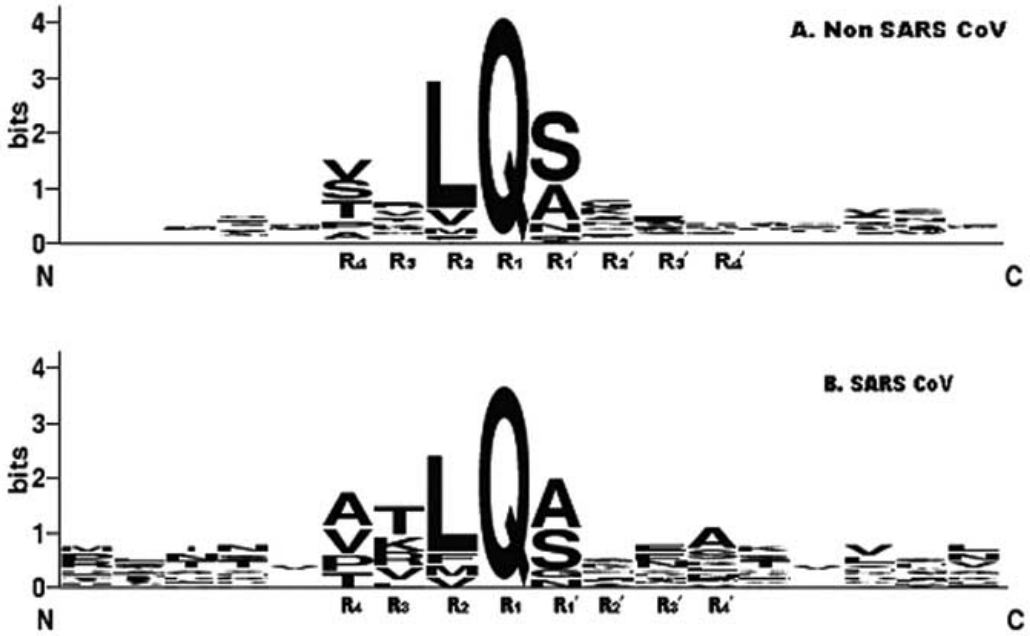

Fig. (1). The cleavage specificities of M^{pro} of non-SARS-Coronavirus and SARS-Coronaviruses. The statistical data are converted to logo presentations in which the size of an amino acid is proportional to its conservation at specific position and the sampling size. The amino acid conservation is measured in bits of information plotted on a vertical axis whose upper limit is determined by the diversity of 20 natural amino acids expressed as a logarithm of 2 [36]. (A) The statistical distribution of amino acid residues surrounding the 66 cleavage sites of non-SARS-Coronaviruses are used; (B) The statistical distribution of amino acid residues surrounding the 396 cleavage sites of SARS-Coronaviruses are used.

Table 2. The Percentage Probabilities of Amino Acids on the Cleavage Specific Positions of SARS-CoV M^{pro}

R4	A: 36.4%	V: 27.3%	P: 18.2%	T: 18.2%
R3	T: 36.4%	K: 18.2%	R: 18.2%	V: 18.2%
R2	L: 72.7%	F: 9.1%	M: 9.1%	V: 9.1%
R1	Q: 100%	___	___	___
	A: 45.5%	S: 36.4%	G: 9.1%	N: 9.1%

SARS-CoV M^{pro} and satisfy the first condition for a competitive inhibitor. Therefore, the 11 octapeptides in Table **3** could well serve as the good starting point for inhibitor design against SARS-Coronavirus main proteinase. Actually, it has been observed *via* the tube test [30] that the octapeptide NH-AVLQ↓SGFR-COOH (OP1) in Table **3** shows high inhibiting activity against SARS-Coronavirus with $EC50=2.7x10^{-2}$ mg/L and no toxicity to cells.

A careful observation on Tables **2** and **3** will find that, for the octapeptides OP4 and OP9, the amino acids A, T, L, Q and A have the highest statistical probabilities at the positions R_4, R_3, R_2, R_1 and $R_{1'}$. Accordingly, octapeptides NH_2-ATLQ↓AIAS-COOH (OP4) and NH_2-ATLQ↓AENV-COOH (OP9) may be two of the best candidates for inhibitor of SARS-CoV M^{pro}.

A cleavable octapeptide does not meet the second condition for inhibitor of SARS-CoV M^{pro} because it has a scissile peptide bond to be cleaved by main proteinase. However, a cleavable octapeptide could become an effective inhibitor after some proper chemical modification. For a detailed discussion about this, see Fig. **2b** of a review [11] as well as some relevant papers [31,32]. It can be seen from Table **3** that the position R_1 is unchangeably occupied by glutamine (Q), that the amino acids at position R_2, such as L, M, F, and V, all have a strong hydrophobic side chain, and that the amino acids on position $R_{1'}$, such as A, S and G, bear a small side chain. Such a structural pattern is very favorable not only for the chemical modification to change a cleavable octapeptide to a non-cleavable one, but also for conducting the non-peptide inhibitor design because the ligand-receptor interaction is extremely sensitive to small change in chemical structure on the cleavage site of inhibitor.

The development of peptides as clinically useful drugs is greatly limited by their poor metabolic stability and low bioavailability, which is partially due to their inability to readily crossing through membrane barriers such as the intestinal and blood-brain barriers. Systematic chemical modification strategies that convert peptides into drugs are an attractive research topic in current medicinal chemistry [33]. For developing peptide inhibitors against proteinase, the chemical modification should be focused on the scissile peptide bond between R_1 and $R_{1'}$ that is cleavable by SARS-CoV M^{pro}. The quantum chemical study shows that after the peptide bond CO=NH between R_1 and $R_{1'}$ is replaced by a single bond, such as CH_2–NH, CF_2–NH, or CO–CH_2, the cleavage of such a modified octapeptide by SARS-CoV M^{pro} is difficult or impossible [31]. This is supported by the fact

Table 3. Eleven Cleavable Peptides Taken from Polyproteins pp1a and pp1ab of SARS-CoV, TOR2 (NC_004718)*

No.	R6	R5	R4	R3	R2	R1		R1'	R2'	R3'	R4'	R5'	R6'
OP1	T	S	A	V	L	Q	_	S	G	F	R	K	M
OP2	S	G	V	T	F	Q	_	G	K	F	K	K	I
OP3	K	V	A	T	V	Q	_	S	K	M	S	D	V
OP4	N	R	**A**	**T**	**L**	**Q**	_	**A**	I	A	S	E	F
OP5	S	A	V	K	L	Q	_	N	N	E	L	S	P
OP6	A	T	V	R	L	Q	_	A	G	N	A	T	E
OP7	R	E	P	L	M	Q	_	S	A	D	A	S	T
OP8	P	H	T	V	L	Q	_	A	V	G	A	C	V
OP9	N	V	**A**	**T**	**L**	**Q**	_	**A**	E	N	V	T	G
OP10	T	F	T	R	L	Q	_	S	L	E	N	V	A
OP11	F	Y	P	K	L	Q	_	A	S	Q	A	W	Q

*Shown in bold-face type are the amino acids A, T, L, Q, and A at the positions R_4, R_3, R_1 and R1' of octapeptides OP4 and OP9; these amino acids have the largest statistical probabilities in Table 2.

that, after the peptide bond CO=NH of an octapeptide cleavable by renin is replaced by a single bond CH_2–NH, the octapeptide has become strongly resistant to the enzyme hydrolysis although its affinity to the enzyme is increased [34,35].

V. DISCUSSION AND CONCLUSION

In this study we present a bioinformatical approach and a practical example that directly links the genome search to drug finding. The input data in this approach are the RNA sequences of SARS-Coronaviruses, and the output are the cleavable octapeptides, which can be considered as promising candidates for modification to become effective inhibitors against SARS-CoV M^{pro} .

Two octapeptides NH_2-ATLQ↓AIAS-COOH and NH_2-ATLQ↓AENV-COOH have been found. They are selected from 11 cleavable octapeptides of SARS-CoV M^{pro}, and are the best starting point, for further modification according to the "distorted key" theory [10,11], leading to an effective inhibitor of SARS-CoV M^{pro}. The chemical modification strategy that replaces the scissile peptide bond by a strong single bond [31] may convert a cleavable octapeptide by SARS enzyme into a drug candidate for the therapeutic treatment against SARS.

NOTE

An interesting relevant paper [37] has been seen during the process of proof. In that paper the cellular automata images are used to identify the difference between SARS coronaviruses and non-SARS coronaviruses.

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REFERENCES

[1] Besemer, J.; Borodovsky, M. *Nucleic Acids Res.*, **1999**, *27*, 3911-3920.
[2] Salzberg, S. L.; Delcher, A. L.; Kasif, S.; White, O. *Nucleic Acids Res.*, **1998**, *26*, 544-548.
[3] Chou, K. C. *Current Medicinal Chemistry*, **2004**, *11*, 2105-2134.
[4] Ksiazek, T. G.; Erdman, D.; Goldsmith, C. S.; Zaki, S. R.; Peret, T.; Emery, S.; Tong, S.; Urbani, C.; Comer, J. A.; Lim, W. *The New England Journal of Medicine*, **2003**, *348*, 033801911.
[5] Shortridge, K. F. *Am. J. Respir. Crit. Care Med.*, **2003**, *168*, 1416-1420.
[6] Anand, K.; Ziebuhr, J.; Wadhwani, P.; Mesters, J. R.; Hilgenfeld, R. *Science*, **2003**, *300*, 1763-1767.
[7] Chou, K. C.; Wei, D. Q.; Zhong, W. Z. *Biochem. Biophys. Res. Comm.*, **2003**, *308*, 148-151.
[8] Yang, H.; Yang, M.; Ding, Y.; Liu, Y.; Lou, Z.; Zhou, Z.; Sun, L.; Mo, L.; Ye, S.; Pang, H.; Gao, G. F.; Anand, K.; Bartlam, M.; Hilgenfeld, R.; Rao, Z. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 13190-13195.
[9] Sirois, S.; Wei, D. Q.; Du, Q.; Chou, K. C. *J. Chem. Inf. Comput. Sci.*, **2004**, *44*, 1111-1122.
[10] Chou, K. C. *Journal of Biological Chemistry*, **1993**, *268*, 16938-16948.
[11] Chou, K. C. *Analytical Biochemistry*, **1996**, *233*, 1-14.
[12] Chen, L. L.; Ou, H. Y.; Zhang, R.; Zhang, C. T. *Biochem. Biophys. Res. Commu.*, **2003**, *307*, 382-388.
[13] Gao, F.; Ou, H. Y.; Chen, L. L.; Zheng, W. X.; Zhang, C. T. *FEBS Letters*, **2003**, *553*, 451-456.
[14] Ziebuhr, J.; Snijder, E. J.; Gorbalenya, A. E. *J. Gen. Virol.*, **2000**, *81*, 853-879.
[15] Hegyi, A.; Ziebuhr, J. *J. Gen. Virol.*, **2002**, *83*, 595-599.
[16] Chou, J. J. *Journal of Protein Chemistry*, **1993**, *12*, 291-302.
[17] Chou, J. J. *Biopolymers*, **1993**, *33*, 1405-1414.
[18] Elhammer, A. P.; Poorman, R. A.; Brown, E.; Maggiora, L. L.; Hoogerheide, J. G.; Kezdy, F. J. *Journal of Biological Chemistry*, **1993**, *268*, 10029-10038.
[19] Chou, K. C. *Protein Science*, **1995**, *4*, 1365-1383.
[20] Chou, K. C.; Tomasselli, A. L.; Reardon, I. M.; Heinrikson, R. L. *PROTEINS: Structure, Function, and Genetics*, **1996**, *24*, 51-72.
[21] Thompson, T. B.; Chou, K. C.; Zheng, C. *Journal of Theoretical Biology*, **1995**, *177*, 369-379.
[22] Chou, K. C.; Jones, D.; Heinrikson, R. L. *FEBS Letters*, **1997**, *419*, 49-54.
[23] Chou, K. C.; Tomasselli, A. G.; Heinrikson, R. L. *FEBS Letters*, **2000**, *470*, 249-256.
[24] Cai, Y. D.; Lin, S.; Chou, K. C. *Peptides*, **2003**, *24*, 159-161.
[25] Cai, Y. D.; Chou, K. C. *Advances in Engineering Software*, **1998**, *29*, 119-128.
[26] Cai, Y. D.; Yu, H.; K.C.Chou *Journal of Protein Chemistry*, **1998**, *17*, 607-615.
[27] Yang, Z. R.; Chou, K. C. Journal of Chemical Information and Computer Sciences, **2003**, *43*, 1748-1753.
[28] Yang, Z. R.; Chou, K. C. *Bioinformatics*, **2004**, *20*, 735-741.
[29] Yang, Z. R.; Chou, K. C. *Bioinformatics*, **2004**, *20*, 903-908.
[30] Gan, Y. R.; Huang, Y. D.; Huang, H. *Peptides*, **2004**, in press.
[31] Du, Q. S.; Wang, S. Q.; Wei, D. Q.; Zhu, Y.; Guo, H.; Sirois, S.; Chou, K. C. *Peptides*, **2004**, *25*, 1857-1864.
[32] Schulz, G. E.; Schirmer, R. H. *Principles of Protein Structure, Chapter 2, pp.17-18*; Springer-Verlag: New York, 1985.
[33] Adessi, C.; Soto, C. *Curr. Med. Chem.*, **2002**, *9*, 963-978.
[34] Szelke, M.; Leckie, B. J.; Tree, M.; Brown, A.; Grant, J.; Hallett, A.; Hughes, M.; Jones, D. M.; Lever, A. F. *Hypertension*, **1982**, *4*, 59.
[35] Venkatesan, N., Kim, B. H. *Curr. Med. Chem.*, **2002**, *9*, 2243-2270.
[36] Schneider, T. D.; Stephens, R. M. *Nucleic Acids Res.*, **1990**, *18*, 6097-6100.
[37] Wang, M., Yao, J. S., Huang, Z. D., Xu, Z. J., Liu, G. P., Zhao, H. Y., Wang, X. Y., Yang, J., Zhu, Y. S., Chou, K. C. A new nucleotide-composition based fingerprint of SARS-CoV with visualization analysis. *Medicinal Chemistry*, **2005**, *1*, 39-47.

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