甘肽氧化酶、还原酶和转移酶类均无明显作用. Hed诱导肝金属硫蛋白50倍，并同时增加肝脏锌(80 %)和铜(30 %)含量. Hed对超氧化物歧化酶，蔗酮还原酶无明显影响，大剂量时降低过氧化氢酶. Hed还增加肝脏抗坏血酸含量20 %，但对维生素E含量无明显影响. 结论：Hed的保肝作用至少在某一方面是由于诱导肝脏非酶类的抗氧化损伤物质.

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Binding conformers searching method for ligands according to the structures of their receptors and its application to thrombin inhibitors[1]

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KEY WORDS molecular conformation; phosphonopeptides; thrombin receptor; ligands; structure-activity relationship; molecular mechanics

AIM: To develop a method of finding binding conformers for ligands according to the three-dimensional structures of their receptors. **METHODS:** Combining the systematic search method of ligand with the molecular docking approach of ligand fitting into its receptor, we developed a binding conformer searching method for ligands. **RESULTS:** The binding conformers of phosphonopeptidyl thrombin inhibitors were recognized. The binding (interaction) energies between these inhibitors and thrombin were calculated with molecular mechanical method. **CONCLUSION:** Both of the total binding energies and steric binding energies have good correlations with the inhibitory activities of these thrombin inhibitors, demonstrating that our approach is reasonable. It can also be used to explain the inhibition mechanism of thrombin interacting with these inhibitors.

The interaction of ligands with their receptor macromolecules are central to all of biological processes, because that tells us the binding fashion of the ligands to their receptors, and from the interaction fashions, one can design novel ligands which might bind to the receptors tightly[1]. Nowadays, there is accumulating evidence that ligand-receptor non-covalent interactions can be modeled and simulated with relatively simple molecular mechanical approaches[2]. Among these approaches, the key difference, of course, is the sampling problem, since one should, in principle, consider the many alternative conformational states of the macromolecule, the ligands, and the complexes of them[2]. But how to recognize binding (bioactive) conformer of a ligand when which interacts with the receptor is still an unsolved problem, especially for the ligand with a large number of freedoms of flexibility. Our present approach attempts to go one step further to solve this kind of problem.

In this approach, we combined the systematic search method of ligand with the molecular docking approach[3] of ligand fitting into its receptor to try to find the binding conformer on the basis of the 3D structure of the active site of a receptor. Once the binding conformer of a ligand has been found, one can perform the calculation of the ligand interacting with the receptor, and design more potent molecules to bind to the receptor.

Thrombin, a trypsin-like serine protease, is the final enzyme in the blood coagulation cascade[4], and is an ideal target for the development of an anticoagulant protease inhibitors[5]. Modeled on the "fibrinogen-like" sequence *D*-Phe-Pro-Arg, a

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series of phosphonopeptides (*D*-Ala-Pro-Aa^P ($OPh)_2$) as the thrombin inhibitors were synthesized and assayed[6]. With the binding conformational search method, we recognized the binding conformer of these phosphorus-containing peptidomimetics, and calculated the interactions between these inhibitors and thrombin. The calculation results can be used to explain the inhibitory mechanism of the phosphonopeptides satisfactorily, which tested and verified the resonableness and reliability of our binding conformational search approach.

METHODS

Our computational paradigm is that: first we perform a systematic conformational search for an isolated ligand with the subroutine SEARCH of SYBYL 6.1[7], and save the sampled conformers into a molecular spreadsheet (MSS) table (other conformational search methods, such as random search or molecular dynamics, can be used to local the conformations of the ligand); second, bring the conformers one by one from the MSS and dock them into the active site of a receptor; third, merge the abstracted conformers onto receptor and calculate the total energies of the complexes of the ligand-receptor ($E_{complex}$); the fourth step is to calculate the binding energy (E_{bind}) of each conformer with following formula:

$$E_{bind} = E_{complex} - E_{ligand} - E_{receptor} \quad (1)$$

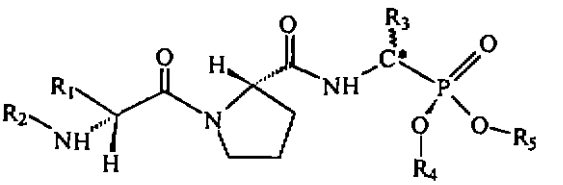
where E_{ligand} is the conformational total energy of the ligand, and $E_{receptor}$ is the energy of the receptor. All above energies are calculated with the routine MAXIMIN2 encoded in SYBYL 6.1[7]; the final step is to save above binding energies, E_{bind}, back into the MSS table, and using the statistical tools of MSS to find the conformer having the lowest binding energy, which is the probable binding conformer of the ligand.

With SYBYL programming Language (SPL)[8] we convert above computational paradigm into a computer programme, named BCSPL (binding conformation search program for ligand), and implemented this program into the interface of SYBYL.

RESULTS AND DISCUSSION

We applied the above approach to recognize the binding conformers of these phosphonopeptides according to the three-dimensional structure of active site of thrombin[9] and calculated the interactions between these inhibitors and human α-thrombin. The calculation result gave a good explanation for the structure-activity relationship of the above phosphonopeptidyl thrombin inhibitors.

The structural formulae of these phosphonopeptides are shown in Fig 1.



	R_1	R_2	R_3	R_4, R_5
1	$PhCH_2$	H	*n*-pentyl (R)	Ph
2	Ph_2CH	H	*n*-pentyl (R)	Ph
3	$PhCH_2$	H	3-methoxypropyl (R)	Ph
4	Ph_2CH	H	3-methoxypropyl (R)	Ph
5	$PhCH_2$	PhCOO	*n*-pentyl (R)	Ph
6	$PhCH_2$	H	*n*-pentyl (R)	H
7	Ph_2CH	H	*n*-pentyl (R)	H
8	Ph_2CH	H	*n*-pentyl (S)	H

Fig 1. Structure formulae of phosphonopeptides.

Binding conformers With above BCSPL program, we recognized the binding conformers of the 8 phosphonopeptides. The probable binding conformers of these 8 inhibitors of thrombin are shown in Fig 2, their alignments in the active site of thrombin are shown in Fig 3, and the structural mode of the complexes of inhibitor-thrombin is shown in Fig 4.

Interaction mode From the optimized structures of phosphonopeptide-thrombin complexes (Fig 4), we found that the interaction mode of phosphonopeptides to thrombin is similar to that of PPACK[9]. Like that of PPACK in PPACK-thrombin complex, these inhibitors run antiparallel to Ser214-Gly216 and are stabilized by very favorable interactions with thrombin. The substituents of D-Aa lie in the hydrophobic pocket of thrombin formed by His57, Tyr60A, Trp60D, Leu99, Ile174, and Trp215, which is the apolar binding site of thrombin[9]. The Pro residue also lies within this apolar site. The n-pentyl or methoxypropyl is located in the S1 site of thrombin in an extended conformation and interacts with the Ala190, Gly216 and Gly219 by hydrophobic interactions. The $PO(OPh)_2$ group of some of these inhibitors lies in a hydrophobic and apolar

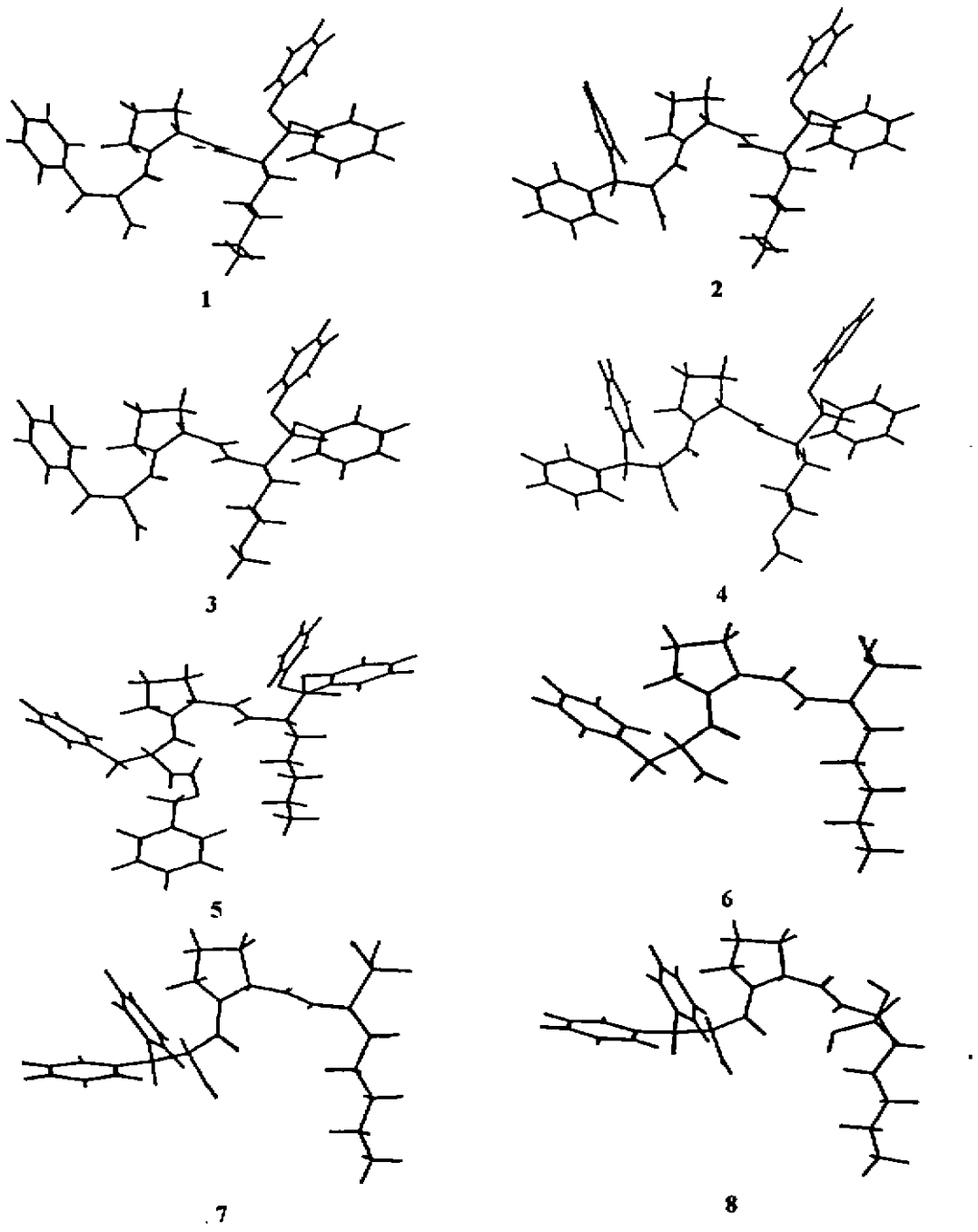


Fig 2. Binding conformations of the title inhibitors.

pocket formed by Ala55, His57, Cys58, Gly193, Asp194, Ser195, and Ser214, and interacts with these residues using hydrophobic and aploar interactions.

The hydrogen-bond interaction fashion between these inhibitors and thrombin and the hydrogen-bond lengths are shown in Fig 5.

From Fig 5, we can see that for all of the 8 inhibitors, the amino or imino group of D-Aa hydrogen bonds to the carbonyl oxygen of Gly216, while the carboxyl group forms a hydrogen bond with the hydrogen of amide group in Gly216. For

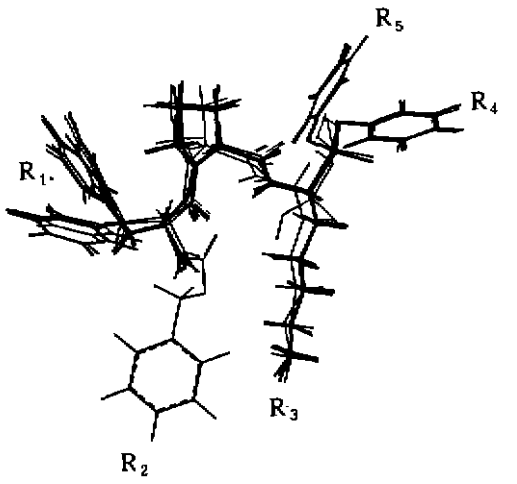


Fig 3. Alignment of binding conformers in thrombin active site.

compounds 1—6, phosphoryl oxygen forms a triplet hydrogen-bond with the two NH groups in Gly193 and Ser195, and HG of Ser195, respectively, and the phosphoroso-oxygen of R_4 hydrogen bonds to HG atom of Ser195. For compound 5, there is an another hydrogen bond between the hydrogen of NH group in Gly219 and carboxylate oxygen in R_2 substituent. For compound 6, besides above hydrogen bonds formed with thrombin, other 3 hydrogen bonds exist between these compound and thrombin: phosphoroso-hydrogen of R_4 bonds to OG of Ser195; phosphoroso-hydrogen of R_4 bonds to NE2 of His57; and phosphoroso-hydrogen of R_5 bonds to OE1 of Gly193. For compounds 7, the hydrogen bonds formation is similar to compound 6, excepting there is no hydrogen bond between phosphoryl oxygen and NH of Ser195. From our modeling results, we found that absolute configuration of the inhibitor can affect the hydrogen bonds formation. When compound 7 from its R configuration (for C * atom) converted to its S configuration, compound 8, the hydrogen bond number was decreased dramatically (Tab 1). This probably is one of the reasons that why the activity of S configuration of phosphonopeptides is lower than that of R configuration[6].

Tab 1. The inhibitory activity of phosphonopeptides to thrombin[a].

Compound	IC_{50}	$-\lg IC_{50}$	K_i
1	–	–	1.70
2	0.00094	9.0269	0.48
3	0.012	7.9208	3.10
4	0.0024	8.6189	>31.0
5	0.019	7.7212	>98.0
6	4.40	5.3565	2.30
7	0.082	6.0862	–
8	0.75[b]	5.8751[b]	35.0[b]

[a]Activity values are from reference 11.
[b]These values corresponds 1:1 ratio of diastereomers.

Structure-activity relationship The biological activities, IC_{50}, $-\lg IC_{50}$ and K_i are listed in Tab 1. The conformational energies (E_{cf}) corresponding to the binding conformers of the 8 phosphonopeptidyl thrombin inhibitors, the binding energies of these inhibitors with human α-thrombin (E_{bind}), the global minimum energies (E_{min}), and the energy differences (ΔE) between Ecf and Emin are listed in Tab 2.

Tab 2. Calculation energy (kcal·mol^{-1}) of phosphonopeptides[a].

Compound	E_{bind}			E_{cf}	E_{min}	ΔE
	E_{bind}^{total}	E_{bind}^{steric}	E_{bind}^{elec}			
1	−187.666	−178.574	−9.092	15.3385	4.796	10.5425
2	−196.078	−186.986	−9.092	25.8532	5.793	20.0602
3	−183.477	−176.715	−6.762	16.4900	5.422	11.0680
4	−187.381	−180.320	−7.062	23.7749	6.313	17.4619
5	−206.692	−200.247	−6.715	30.3381	1.658	28.6807
6	−135.590	−118.883	−16.707	9.6151	5.009	4.6061
7	−141.376	−125.894	−15.482	17.8482	6.304	11.5442
8	−111.821	−109.223	−2.597	34.2988	8.433	25.8658

[a] E_{bind}^{total} is the total binding energy, E_{bind}^{steric} is steric binding energy, E_{bind}^{elec} is the electrostatic binding energy, E_{cf} is the conformational energy of the binding conformer, E_{min} is the global minimum energy of the inhibitors.

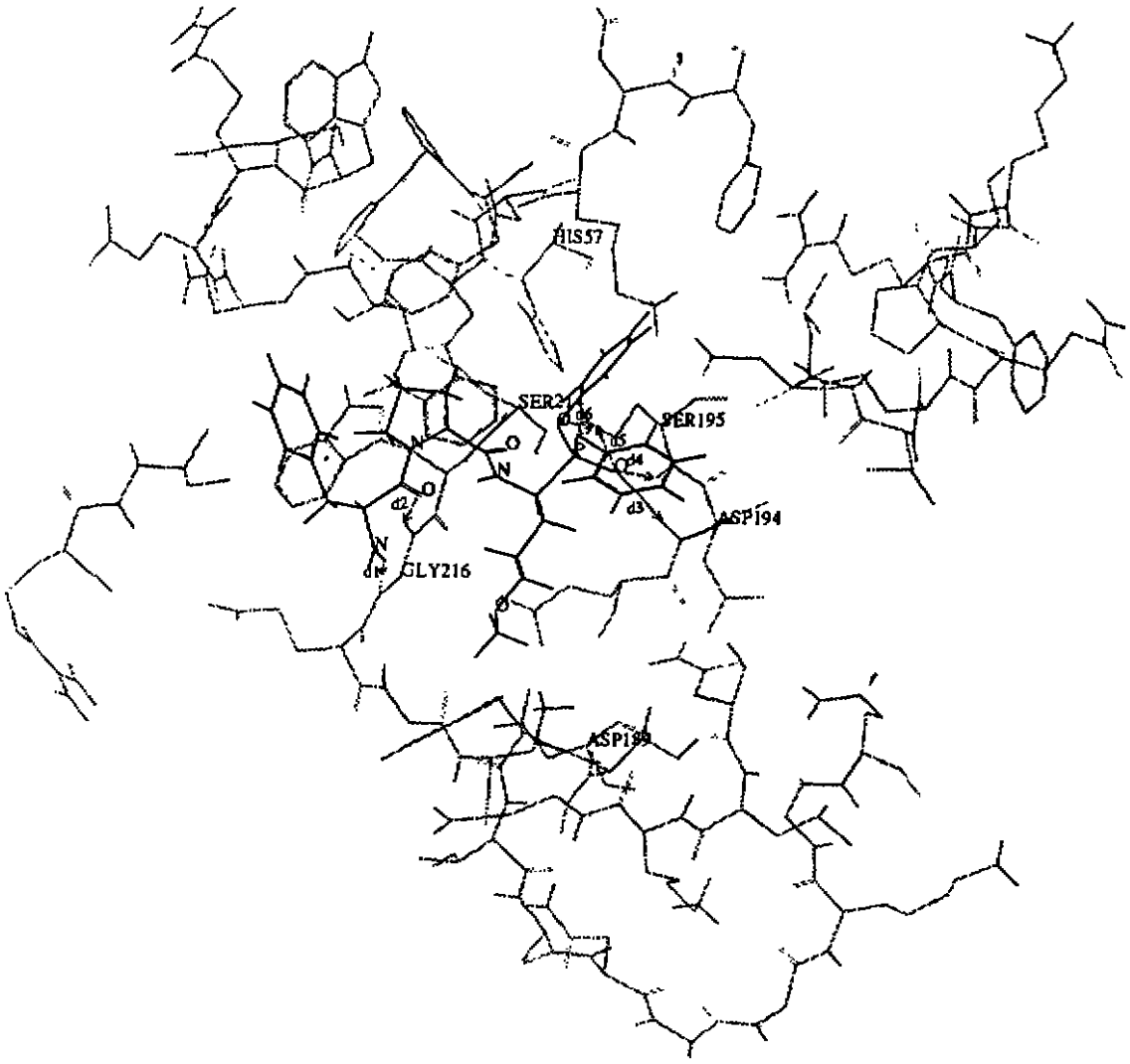


Fig 4. The complex of molecule 1 and thrombin, showing only the main residues of the active site. The arrow lines represent the hydrogen bonds between the inhibitor and thrombin.

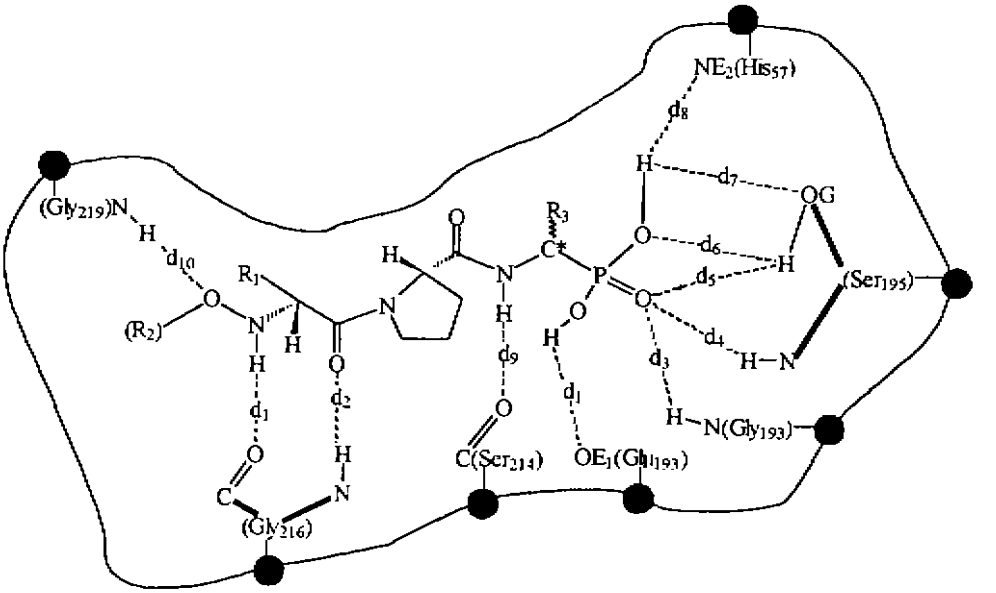
From Tab 1 and Tab 2, we can find that the biological activities, $-\lg IC_{50}$, have good correlations with the total binding energies and steric binding energies of phosphonopeptides with thrombin, respectively (Fig 6). With partial least squares (PLS) method[10], we got the regression equations between the biological activities, $-\lg IC_{50}$, and the binding energies, E_{bind}, of the inhibitors interacting with thrombin. They are shown in equations (2) - (3a), respectively.

$$-\lg IC_{50} = -0.126 - 0.043E_{bind}^{total}$$
$$n = 6,\ r^2 = 0.786,\qquad (2)$$
$$F(n1 = 1,\ n2 = 4;\ \alpha = 0.019) = 14.694,$$
$$s = 0.746$$

$$-\lg IC_{50} = -2.138 - 0.057E_{bind}^{total}$$
$$n = 5,\ r^2 = 0.979,\qquad \text{(Omit compound 5) (2a)}$$
$$F(n1 = 1,\ n2 = 3;\ \alpha = 0.001) = 141.851,$$
$$s = 0.267$$

$$-\lg IC_{50} = 1.223 - 0.038E_{bind}^{steric}$$
$$n = 6,\ r^2 = 0.792,\qquad (3)$$
$$F(n1 = 1,\ n2 = 4;\ \alpha = 0.018) = 15.189,$$
$$s = 0.736$$

$$-\lg IC_{50} = -0.255 - 0.049E_{bind}^{steric}$$
$$n = 5,\ r^2 = 0.971,\qquad \text{(Omit compound 5) (3a)}$$
$$F(n1 = 1,\ n2 = 3;\ \alpha = 0.002) = 101.148,$$
$$s = 0.315$$



	d_1	d_2	d_3	d_4	d_5	d_6	d_7	d_8	d_9	d_{10}	d_{11}
1	0.1538	0.2362	0.2612	0.2529	0.1861	0.1420	-	-	-	-	-
2	0.1521	0.2357	0.2634	0.2568	0.1871	0.1447	-	-	-	-	-
3	0.1364	0.2337	0.2703	0.2416	0.1777	0.1406	-	-	-	-	-
4	0.1862	0.2436	0.2782	0.2391	0.1699	0.1408	-	-	-	-	-
5	0.1600	0.2365	0.2568	0.2565	0.1925	0.1423	-	-		0.2464	-
6	0.1526	0.2359	0.2527	0.2636	0.1951	0.1029	0.1832	0.1255	0.2729	-	-
7	0.1718	0.2832	0.2544	-	0.1980	0.0976	0.1822	0.1261	0.2669	-	-
8	0.1699	0.2438	-	-	-	-	-	-	-	-	0.2256

Fig 5. Hydrogen-bond network and their lengths (nm) of inhibitor-thrombin complexes.

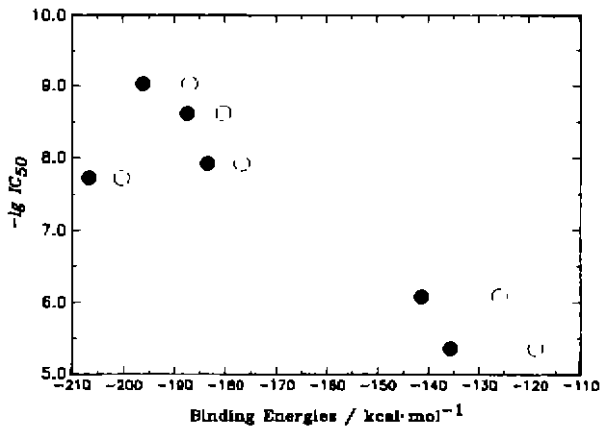


Fig 6. Correlation between binding energies (● = total binding energies, ○ = binding energies) of the title inhibitors and their activities.

Equations (2) – (3a) indicate that the activity of the phosphonopeptides against thrombin is in inverse proportion to the binding energies between the inhibitors and thrombin, both for total binding energies and steric binding energies. But the electrostatic binding energy has no correlation with the activity of these inhibitors, which can be seen from Tab 2. This indicates that the steric interactions, which may include van der Waals interaction, hydrophobic interaction and hydrogen interaction, are important for these inhibitors to bind to thrombin. The reason may be that the active site, $S_1 - S_3$, of thrombin is formed by a series of hydrophobic residues, therefore, hydrophobic interaction is the driven force of these

inhibitors binding to thrombin, and then these compounds adjust their conformation and orientation to fit the active site of thrombin in favourable interactions. On the other hand, the variable tendency of the activities of the phosphonopeptides in pace with the total and steric binding energies indicates that the binding conformers of these inhibitors are probably reasonable, and this conclusion was tested and verified by the comparative molecular field analysis (CoMFA), this result will be published in another paper.

From Tab 2 and Fig 6, we found that the orders of both total binding energies and steric binding energies of the inhibitors are in agreement with that of their activities except compound 5. This can be illustrated by the difference of the conformational energy and the global minimum energy (ΔE). We can consider the overall binding process of inhibitor interacting with its receptor with two steps: the first step is that the flexible inhibitor is locked in its binding conformation to fit the active site structure of the receptor, which will cost some energy; the second step is the inhibitor binds to the receptor at the state of its binding conformer, which will release some energy. Normally, the binding energy is the sum of above two kinds of energies. From Tab 2, we can see that the difference of the conformational energy and the global minimum energy of compound 5 is the largest among these inhibitors. This indicates that the addition of substituent group, R_2, at the amide nitrogen of D-Aa increases the flexibility of compound 5, which increases the energy requirement from its minimum conformer to convert to its binding conformer, and deduces the activity. Regressing the activities with the binding energies without compound 5 gave a more good regression result [see equations (2a) and (3a)].

The activity of compound 8 is dramatically lower than that of its antimer compound 7. This can be explained by the interaction mode of the inhibitor to thrombin. From Fig 5, we can see that the configuration conversion decreased the hydrogen bond formation ability of compound 8 with thrombin, which reduced its ability to interact with Ser195, His57 and Gly193 (Fig 5), and therefore the compound can not form covalent enzyme-inhibitor complex with thrombin. This might be the reason that why the activity of the S configuration for atom C^* (Fig 1) of the phosphonopeptides is lower than that of R configuration.

Inhibition mechanism According to pharmacological test[6], phosphonopeptides presumably formed covalent enzyme-inhibitor complexes, and thought that the interaction between these inhibitors and thrombin involved two mechanisms: the direct dephosphonylation from the serine residue on thrombin to release active enzyme and, speculatively, the hydrolysis of the phenyl ester group to give a more stable enzyme-inhibitor complex. Our calculation result is in accordance with the above suggestion. From Fig 5, we can see that the oxygen atoms of phosphonyl group in the diphenylphosphonates (compounds 1 – 5) form hydrogen bonds with Gly193 and Ser195, The hydrogen bond lengths of d_5 (about 0.185 nm) and d_6 (about 0.15 nm) is shorter than that of the normal hydrogen bond length (from 0.25 nm to 0.29 nm) in protein, which indicates that the diphenylphosphonates have formed transition state complexes with thrombin. These hydrogen bond interactions weaken the bond P=O, which makes it easy for the diphenylphosphonates to be hydrolyzed by water. The hydrolyzed products of diphenylphosphonates bind to thrombin with covalent interaction, which can be seen from the hydrogen bond formation of compounds 6 and 7. For compounds 6 and 7, three more hydrogen bonds are formed between phosphoroso-hydrogen of R_4 and OG of Ser195; phosphoroso-hydrogen of R_4 and NE2 of His57; and phosphoroso-hydrogen of R_5 and OE1 of Gly193. The hydrogen bond lengths of d_5 and d_6 become shorter than that of biphenyl ester, which indicates that the phosphoric acid inhibitor may react with thrombin to form covalent enzyme-inhibitor complex. Accordingly, we conclude three-step process is involved in the inhibition of thrombin by diphenylphosphonates. The probable first step is the recognition of diphenylphosphonates by thrombin with the hydrophobic and hydrogen-bonding interactions; the second step is the hydrolysis of the diphenylphosphonates catalyzed by Ser195 and Gly193 to produce phosphoric acid

analogs; the finally step is the formation of covalent complexes between phosphonic acid and thrombin. The reason why the activities of phosphonic acid analogs are lower than the corresponding ester analogs is that the two phenyl groups of ester analogs increase the hydrophobic interaction to these inhibitors interacting with thrombin, and the hydrophobic interaction may be, as described above, the driven force for the inhibitors bind to thrombin. This can also be seen when comparing the $(Ph)_2CH$ substituted compounds and $PhCH_2$ substituted compounds, such as compounds 1 and 2. The $(Ph)_2CH$ and $PhCH_2$ groups lie in the hydrophobic pocket (S_3 site) of thrombin, and the hydrophobility of $(Ph)_2CH$ is larger than that of $PhCH_2$ group, therefore, the activity of the former substituted analogs is higher than that of the later.

CONCLUSION

The binding conformation of a ligand is not always corresponding to its global minimum conformer. Therefore, many methods have been suggested to recognize the binding conformer for the ligand, such as Active Analogs Approach (AAA)[11], and DISCO[12]. However, all these methods are based on the pharmacophoric concept for small molecules and not based on receptor site. Even if the three-dimensional structure of a receptor site is known, it is difficult to find the binding conformers for the flexible ligands. For this reason, we suggest this new approach of finding binding conformers for the flexible ligands according to the structure of receptor site.

With our conformational search approach BCSPL, we found the probable binding conformers of phosphonopeptidyl thrombin inhibitors, and calculated the binding energies of these compounds interacting with thrombin. The binding energies have a linear correlation with their inhibitory activities, $-\lg IC_{50}$. The binding mode of the inhibitors interacting with thrombin were suggested based on the calculation results, which can give a reasonable explanation for the interaction fashion of the phosphonopeptides with thrombin.

All these factors indicate that our approach is reasonable and reliable. Moreover the calculation structure of the ligand-receptor complex can be used as initial structure to perform further accurate theoretical calculation with more sophisticated methods, such as molecular dynamics[13] or free energy perturbation (FEP) approach[14].

REFERENCES

1 Colman PM. Structure-based drug design
Curr Opin Struct Biol 1994; 4: 868 - 74.

2 Kollman PA.
Theory of macromolecule-ligand interactions
Curr Opin Struct Biol 1994; 4: 240 - 5.

3 Blaney JM, Dixon JC.
A good ligand is hard to find: automated docking methods.
Persp Drug Discov Design 1993; 1: 301 - 19.

4 Lorand E, Konishi K.
Activation of the fibrin-stabilizing factor of plasma by thrombin
Arch Biochem Biophys 1964; 105: 58 - 67

5 Tapparelli C, Metternich R, Ehrhardt C, Cook NS
Synthesis low-molecular weight thrombin inhibitors: molecular design and pharmacological profile.
Trends Pharmacol Sci 1993; 141: 366 - 76.

6 Cheng L, Goodwin CA, Scully MF, Kakkar VV, Claeson G.
Substrate-related phosphonopeptides, a new class of thrombin inhibitors. Tetrahedron Lett 1991; 49: 7333 - 6.

7 SYBYL [Computer programme]. Version 6.1.
St Louis (MO): Tripos Associates, 1994

8 SYBYL programming language (SPL).
[Computer programming language for SYBYL software].
St Louis (MO): Tripos Associates, 1994.

9 Bode W, Mayr I, Baumann U, Huber R, Stone SR, Hfsteenge J. The refined 1.9 Å crystal structure of human α-thrombin: interaction with *D*-Phe-Pro-Arg chloromethylketone and significance of the Tyr-Pro-Pro-Trp insertion segment.
EMBO J 1989; 8: 3467 - 5

10 Cramer RD III
Partial least squares (PLS) strengths and limitations.
Presp Drug Discov Des 1993; 1: 269 - 78.

11 Marshall GR, Barry CD, Bosshard HE, Dammkoehler RA, Dunn DA. The conformational parameter in drug design. In: Olson EC, Christofersen RE, editors. The American Chemical Society Symposium; vol 112. Washington DC: American Chemical Society, 1979; 205 - 26.

12 Martin YC, Bures MG, Danaher EA, DeLazzer J, Lico I, Pavlik PA. A fast new approach to pharmacophore mapping and its application to dopaminergic and benzodiazepin agonists.
J Comput-Aided Mol Des 1993; 7 (1): 83 - 102

13 Brook III CL. Methodological advances in molecular dynamics simulations of biological systems.
Curr Opin Struct Biol 1995; 5: 211 - 5.

14 Kollman PA. Free energy calculations: applications to chemical and biochemical phenomena
Chem Rev 1993; 93: 2395 - 417.

36-44

根据受体三维结构搜寻配体活性构象的方法及在凝血酶抑制剂中的应用[1]

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关键词 分子构象；膦酰肽；凝血酶受体；配体；结构-活性关系；分子力学

目的：发展一种根据受体三维结构搜寻配体活性构象的方法. 方法：结合系统构象搜寻方法和配体—受体分子对接(Dock)方法，我们发展了一种根据受体三维结构搜寻配体小分子活性构象的方法. 结果：应用这一方法，我们搜寻出了膦酰肽类凝血酶抑制剂的活性构象，由搜寻结果，我们又用分子力学方法，计算了膦酰肽类抑制剂和凝血酶的结合能. 结论：计算结果表明，这些凝血酶抑制剂和凝血酶的结合能与其抑制活性之间有很好的相关性，这说明我们计算方法的可靠性. 同时，计算结果能很好地解释膦酰肽类凝血酶抑制剂的作用机理.

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Pharmacokinetics of recombinant human granulocyte colony-stimulating factor in rabbits and mice

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KEY WORDS granulocyte colony-stimulating factor; gel chromatography; pharmacokinetics

AIM: To study the pharmacokinetics of recombinant human granulocyte colony-stimulating factor (rhGCSF) in rabbits and mice. **METHODS**: ^{125}I-rhGCSF was prepared by iodogen method and determined by size exclusive HPLC (SEHPLC). **RESULTS**: Concentration-time curves after iv ^{125}I-rhGCSF in rabbits were best fitted with 2-compartment open model. The α and terminal elimination $T_{\frac{1}{2}}$ were 0.25 - 0.33 and 3.2 - 4.6 h, respectively. AUC increased with doses, and Cl_s and K_{10} were similar. T_{peak} was 0.59 ± 0.25 h after sc, and elimination $T_{\frac{1}{2}}$ was similar to that after iv. The bioavailability after sc was 1.0. In mice the highest level was found in renal system, the next was bile-enteric system. Levels in lymph nodes, bone marrow, and spleen were approximately equal to or slightly lower than that in plasma, while the levels in brain, fat, and muscles were the lowest. About 68 %-86 % were recovered in urine and feces. **CONCLUSION**: Pharamcokinetics of ^{125}I-rhGCSF in rabbits and mice provided a useful index for clinical trial.

Granulocyte colony-stimulating factor (GCSF) is a 18.8 kDa protein that stimulates the proliferation of bone marrow precursor cells and their differentiation into granulocyte colonies. The bacterially synthesized recombinant human GCSF (rhGCSF) supports the formation of granulocyte colonies from precursor cells. In this paper the pharmacokinetic profile of rhGCSF[1] was studied.

MATERIALS AND METHODS

Iodogen, synthesized by Prof LI De-Yu of the Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences. $Na^{125}I$, 0.74 TBq · L^{-1} (Amersham, radiochemical purity 99.4 %, specific activity 577.2 TBq/g of iodine). rhGCSF was produced by Associate Professor CHEN Hui-Peng (batch number 9403, purity >98 %, 0.83 g · L^{-1} in 0.60 mL) in our Institute of Radiation Medicine. Sephacryl S-200 HR (Pharmacia); Protein-Pak 125 column, Waters, packed by Dalian Elite Scientific Instruments Co. ID

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