Polymer 49 (2008) 3346–3351

Contents lists available at ScienceDirect

Polymer

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

Theoretical study on potency and selectivity of novel non-peptide inhibitors of matrix metalloproteinases MMP-1 and MMP-3

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ARTICLE INFO

Article history:
Received 11 September 2007
Received in revised form 1 March 2008
Accepted 15 May 2008
Available online 22 May 2008

Keywords:
MMP
Inhibitors
Modeling

ABSTRACT

By means of molecular modeling and docking studies, two novel non-peptide inhibitors (pyrogallic acid and myricetin) with a new zinc binding group (ZBG) have been evaluated as inhibitors of MMP-1 and MMP-3. The differences in binding affinities for MMP-1 and MMP-3 have been rationalized, and the results are consistent with the experiments of Fang et al. The density functional theory (DFT) method B3LYP/6-31G* has also been employed to characterize the interactions between ZBG of pyrogallic acid and the catalytic zinc ion in MMP-1. Our results may be useful for further research in the structure-based design of inhibitors with improved potency and selectivity.

1. Introduction

Matrix metalloproteinases (MMPs) are a family of structurally related, zinc- and calcium-dependent, neutral endopeptidases, which regulate a variety of biological processes [1]. The excess synthesis and expression of these proteins result in the accelerated matrix degradation associated with a series of diseases such as cancer, arthritis, and other diseases [2,3]. Therefore, developing MMP inhibitors may provide a new treatment for these diseases, especially for cancer [4,5].

As a rule, the requirement for a molecule to be an effective inhibitor of the MMPs is a zinc binding group (ZBG), at least one functional group which provides a hydrogen bond interaction with the enzyme backbone, and one or more groups which undergo effective van der Waals interaction with the enzyme subsites [6]. The MMP inhibitors containing different ZBGs, such as hydroxamic acid [7–9], carboxylic acid [10–15], thiol [16–20], phosphonic acid [21] and phosphinic acid [22], have been investigated for long. Recently, a class of non-peptide MMP inhibitors with a new ZBG have been found on experiments by Fang et al. [23], while the intrinsic structures of the complexes formed by inhibitors and MMPs are unclear. In the present study, pyrogallic acid and myricetin, two non-peptide inhibitors with the new ZBG, have been simulated to interact with MMP-1 and MMP-3, respectively, to reveal the binding mode, which will help to improve the potency and selectivity of the inhibitors.

2. Theory and methods

All the molecule simulations are performed on the SGI O3900 workstations using InsightII software package developed by Accelrys [24]. The Extensible and Systematic Force Field (ESFF) is used for energy minimization and docking simulations. Quantum chemistry calculations are carried out using Gaussian 03 [25].

2.1. Modeling the initial structures of MMPs

The starting structures, MMP-1 (PDB code 1HFC) and MMP-3 (PDB code 1SLN), are obtained from the Protein Data Bank (www.rcsb.org). The complexes selected for the whole research merely contain the catalytic domains based on the criteria that the enzymatic activity and substrate specificity of MMP catalytic domains are reported to be similar to full-length activated MMPs [26]. The Builder module is used to modify the PDB files. The first step is to extract ligand from the complex in each PDB file and hydrogen atoms are added to the enzymes at the condition of pH 7.5, which corresponds to the environment of testing the IC_{50} values on experiments [23]. And then, to release any internal strain in the crystal structure, we fix the secondary structure and perform an energy minimization of 300 steps of conjugate gradient with a convergence value of 0.05 kcal mol^{-1} $Å^{-1}$, in which an explicit solvent model TIP3P water [27] is used and 5 Å thickness of water layer is added to MMP-1 and MMP-3, respectively.

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doi:10.1016/j.polymer.2008.05.026

2.2. Docking ligand to MMPs

Affinity is a suite of programs for automatically docking a ligand to a receptor by a combination of Monte Carlo type and Simulated Annealing procedure [28]. After the energy minimization, the potentials and partial charges are calculated and assigned to the MMPs according to InsightII, however, the formal charges are assigned artificially with a value of +2.0 e to the catalytic zinc ion. The active sites which are movable with the ligand during the docking process should be defined before the affinity procedure, while the other atoms of the receptor are held rigid. The residues included in the active sites consisting of six subsites, named S1′–S3′ (prime site) and S1–S3 (nonprime site), are selected exactly according to the Chemical Review of Mark Whittaker et al. [29] to ensure the accuracy of the docking results. Three dimensional structures of the two inhibitors, pyrogallic acid and myricetin, are constructed by the Builder module and optimized through Gaussian 03, and are shown in Fig. 1a and b, respectively. After all of the above are done, automatic molecular dockings are performed without any constraints. The docked complexes are selected for further research by interacting energy and geometrical matching quality.

2.3. Quantum chemistry calculations

In order to characterize the interactions between ZBG and the catalytic zinc ion more accurately, quantum chemistry calculations are employed. The quantum chemistry calculation method B3LYP, a density functional theory (DFT) type of calculation approach based on a hybrid function, has shown to be the most accurate density functional method, and it gives good or better geometries and energies for the first-row transition metal complexes [30]. In our study, B3LYP is selected to optimize the docked complex at the basis of level of 6-31G*. Frequency calculation is then carried out to verify the rationality of the optimized structure.

3. Results and discussion

3.1. Potency

3.1.1. Potency of inhibitors with MMP-1

So as to explain the potency of inhibitors with MMP-1, comparison of the complexes formed by pyrogallic acid (p-MMP1) and myricetin (m-MMP1) is performed (Fig. 2). In p-MMP1 (Fig. 2b), the ZBG is the O1 atom on one side hydroxyl, which is the same to that in m-MMP1 (d). The O1–Zn distances in p-MMP1 and m-MMP1 are both 2.59 Å (Fig. 2), while the O3–Zn distances are 2.79 and 4.94 Å for p-MMP1 and m-MMP1, respectively (data not shown). The similar distances, 2.59 and 2.79 Å in p-MMP1, attract our more attention to the problem that whether the pyrogallic acid binds to MMP-1 in a monodentate or a bidentate manner. A quantum chemistry calculation using Gaussian 03 is employed to rationalize the alternative binding manner below. The hydrogen bond interaction which is critical to the stability of complex in docking is investigated. The pyrogallic acid forms three hydrogen bonds with MMP-1, H6 with O of Ala184, O5 with H of Ala184, and H4 with O of Glu219 from left to right (Fig. 2b). While in m-MMP1 (Fig. 2d), six hydrogen bonds are formed, which are from left to right H6 with O of Asn180, O7 with H of Leu181, H8 with O of Gly179, H12 with each of the three N atoms of side chain of Arg214. The number of hydrogen bonds in m-MMP1 is more than that in p-MMP1, thus the hydrogen bond interactions contribute more to the stability of m-MMP1 than that of p-MMP1.

What else results in the different potency is the large substituent at C13 atom in myricetin while only a hydrogen atom at the corresponding position in pyrogallic acid. The substituent called P1′ in myricetin occupies the shallow S1′ subsite of MMP-1 (Fig. 2d). The P1′, which points to Arg214 with the hydroxyl (O11 and H12), not only forms three hydrogen bonds with Arg214, but also has strong electrostatic interaction with Arg214, which contributes a lot to the inhibiting ability. The detailed explanation of interaction between P1′ and S1′ subsites of MMP-1 will be discussed in Section 3.2 below.

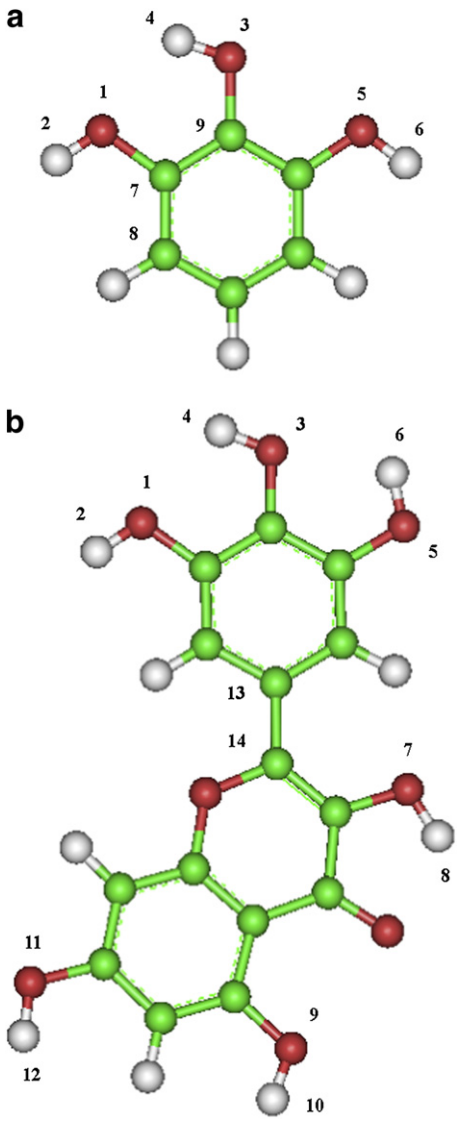

Fig. 1. Structures of two inhibitors displayed in Ball-and-Stick model: (a) pyrogallic acid; (b) myricetin.

Furthermore total interaction energy, calculated by means of the Affinity module of InsightII software package and used to access the binding ability, is employed to confirm our analysis. Table 1 shows that the total interaction energies of p-MMP1 and m-MMP1 are −77.07 and −108.39 kcal mol^{-1}, respectively. This also indicates that myricetin binds to MMP-1 more tightly than pyrogallic acid, which is consistent with the activity order (IC_{50}) obtained from the experiments of Fang et al. [23], of which the IC_{50} values are 1.01 and 2.57 μM, respectively (Table 2).

3.1.2. Potency of inhibitors with MMP-3

Reasons for the difference in potency on MMP-3 will be discussed in comparison of complexes formed by pyrogallic acid (p-MMP3) and myricetin (m-MMP3) (Fig. 3). The distances between ZBG O atom and catalytic zinc ion are 2.61 and 2.68 Å for pyrogallic acid and myricetin, respectively (Fig. 3b and d). It seems that the pyrogallic acid interacts with MMP-3 more strongly than

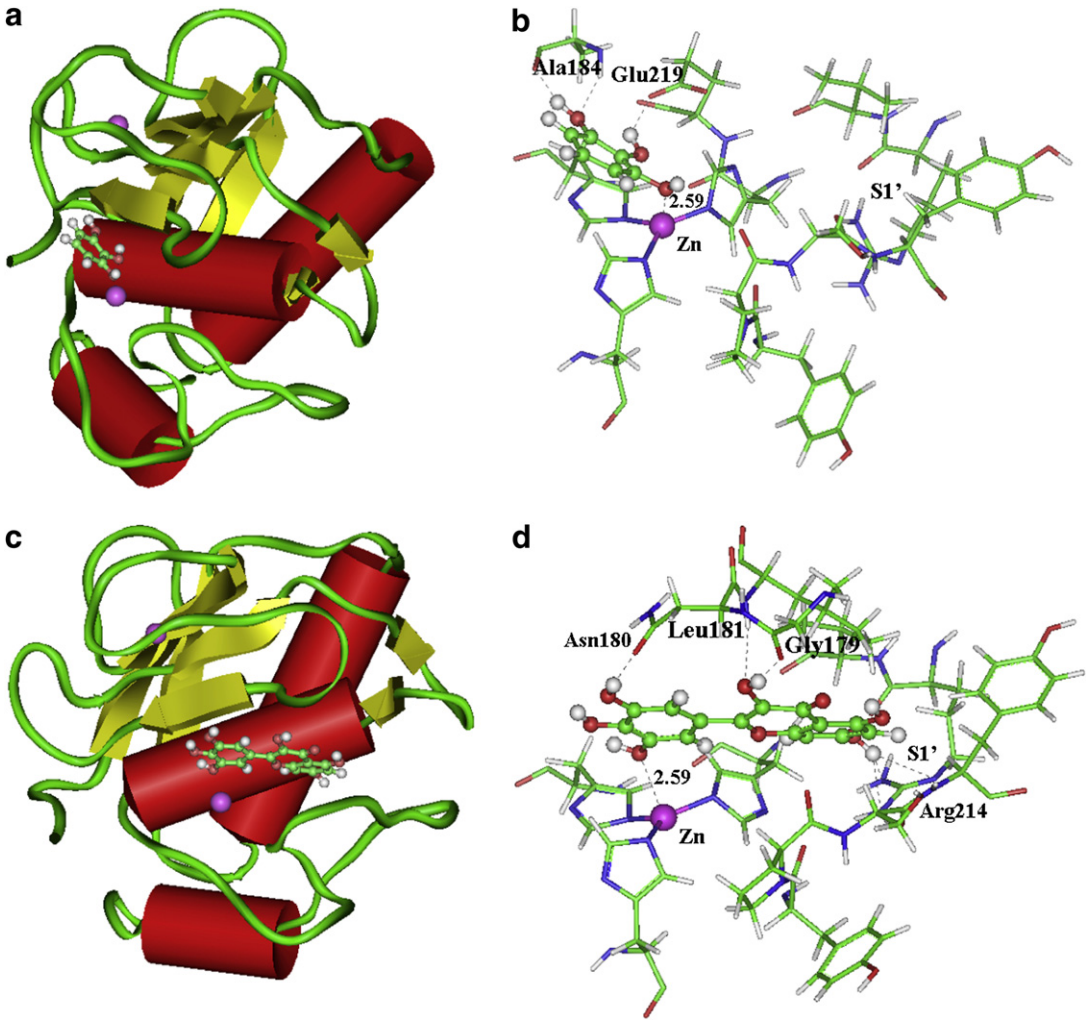

Fig. 2. Secondary structure representation of complexes, the zinc ions are shown as purple spheres: (a) p-MMP1; (c) m-MMP1. Detailed representation of inhibitors and MMP-1 active site, the distance between oxygen and catalytic zinc is given by green line while the other lines are the hydrogen bonds: (b) p-MMP1; (d) m-MMP1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

myricetin for pyrogallic acid has shorter distance, 2.61 Å, than myricetin, 2.68 Å; however, the fact is opposite. This is because the relative long distances (more than 2.60 Å) should correspond to relative weak interaction, so that the other interactions in complexes will be more important in determining the potency with MMP-3.

First, the number of hydrogen bonds formed in p-MMP3 and m-MMP3 is three for both. Pyrogallic acid forms three hydrogen bonds, H2 with O of Glu219, H4 with O of Pro238, and H6 with O of Pro238 from left to right (Fig. 3b, residue number is obtained according to that for MMP-1). Myricetin forms three hydrogen bonds too, while with different residues, which are from left to right H4 with O of Ala182, H2 with O of Ala182, and O1 with H of Ala182 (Fig. 3d, residue number is obtained according to that for MMP-1). The same number of hydrogen bonds indicates that the contribution of hydrogen bonds to the potency on MMP-3 is almost the same.

Secondly, the P1′ substituent at C13 atom of myricetin, which has inserted into S1′ subsite of MMP-3, interacts with the residues of S1′ subsite (detailed explanation see Section 3.2 below), which should contribute to the inhibiting ability a lot and compensate for the potency on MMP-3. The total interaction energies of m-MMP3 and p-MMP3, −74.56 and −52.37 kcal mol^{-1}, respectively, shown in Table 1, give direct certifications to the binding affinities that myricetin owns better potency on MMP-3 than pyrogallic acid, which is in agreement with the inhibiting activities with the IC_{50} values, 4.18 and 12.47 μM, respectively (Table 2) [23].

3.2. Selectivity

Selectivity that is important in minimizing undesirable side effects during long-term medical treatment has been the focus in

Table 1
Total interaction energies of pyrogallic acid and myricetin with MMP-1 and MMP-3

Inhibitor	Total interaction energy (kcal mol^{-1})	
	MMP-1	MMP-3
Pyrogallic acid	−77.07	−52.37
Myricetin	−108.39	−74.56

Table 2
The IC_{50} values of pyrogallic acid and myricetin with MMP-1 and MMP-3

Inhibitor	IC_{50} (μM)	
	MMP-1	MMP-3
Pyrogallic acid	2.57	12.47
Myricetin	1.01	4.18

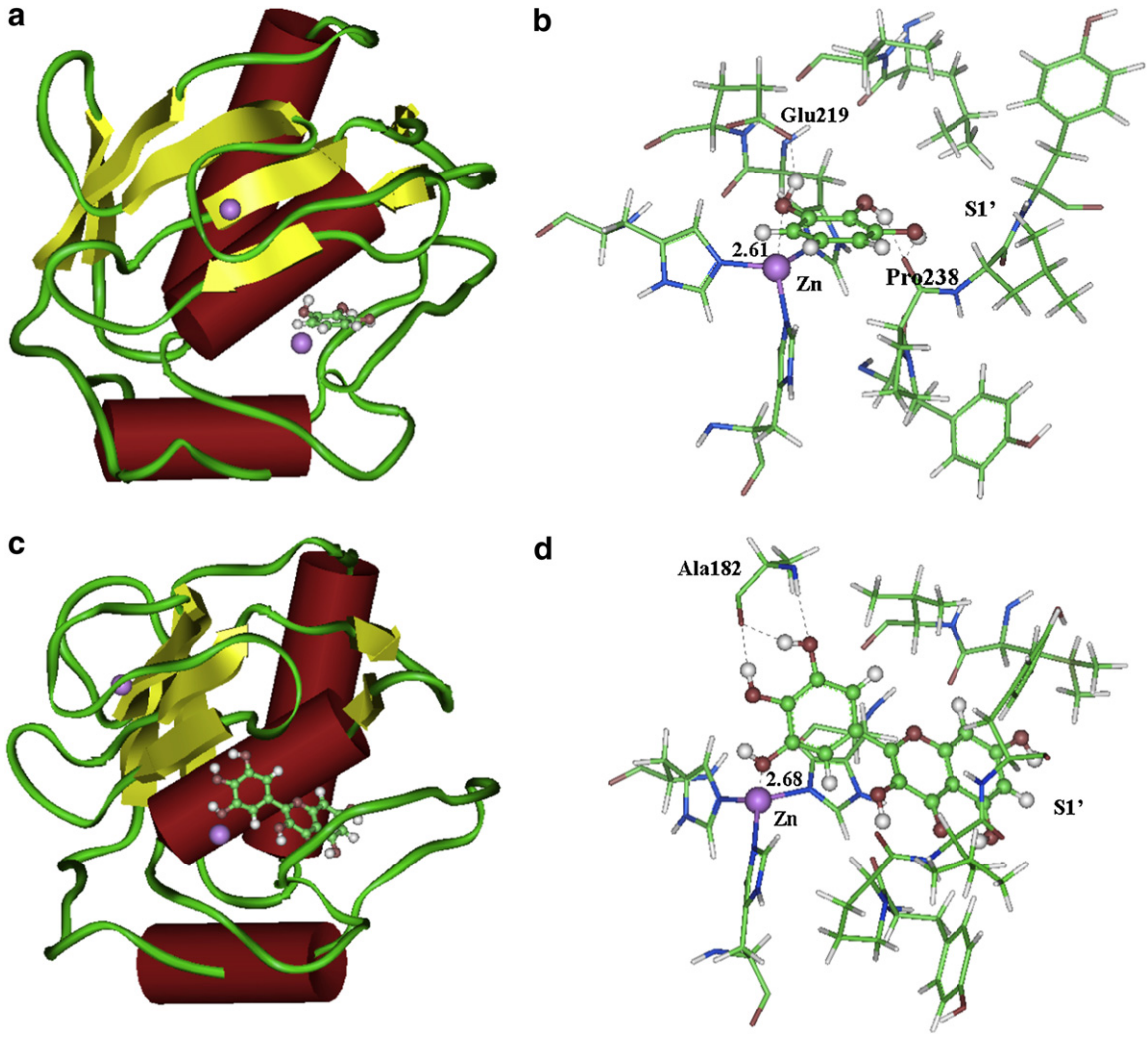

Fig. 3. Secondary structure representation of complexes, the zinc ions are shown as purple spheres: (a) p-MMP3; (c) m-MMP3. Detailed representation of inhibitors and MMP-3 active site, the distance between oxygen and catalytic zinc is given by green line while the other lines are the hydrogen bonds: (b) p-MMP3; (d) m-MMP3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

drug design for long [31–33]. With the aim at exploring inhibitors with both high selectivity and potency, only the selectivity of myricetin will be discussed, for myricetin owns better potency on both MMP-1 and MMP-3 than pyrogallic acid. So what have determined the selectivity of myricetin attract us to pay attention to the intrinsic structures of m-MMP1 and m-MMP3.

The first is the different ZBG atoms in m-MMP1 (Fig. 2d) and m-MMP3 (Fig. 3d). Myricetin binds to the catalytic zinc with O1 atom in MMP-1, but with O5 atom in MMP-3, which may be a factor of the final different conformations and indicate that myricetin can bind to MMPs with either of the two side hydroxyls of the three adjacent.

Secondly, the hydrogen bonds should have more effects on binding affinity of myricetin with MMP-1 than MMP-3, with the number six and three, respectively, as mentioned above.

Thirdly, the interactions between P1′ of myricetin and S1′ subsite of both MMPs also give the reasons for different selectivity of myricetin. Table 3 lists the energies between myricetin and key residues of S1′ subsite of MMP-1 and MMP-3, most of which total contributions are more than 2 kcal mol^{-1}. In m-MMP1, Arg214 has the most effect on the interaction between P1′ and S1′, in which the electrostatic energy prevails with −10.82 kcal mol^{-1} compared to E_{vdw} −0.56 kcal mol^{-1}. Pro238 and Ser239 also contribute a lot to the binding affinity with −6.31 and −6.13 kcal mol^{-1}, respectively, wherein the van der waals energies are dominant. In m-MMP3, Tyr237 and Tyr240 contribute the most with −9.69 and −8.10 kcal mol^{-1}, and the electrostatic energy prevails in the former while the van der waals energy in the latter. Total energies between P1′ and S1′ subsites of MMP-1 and MMP-3 are −33.48 and −28.99 kcal mol^{-1}, while −22.10 and −24.62 kcal mol^{-1} except the contributions of Arg214 in MMP-1 and Leu214 in MMP-3, respectively. Therefore we think that Arg214 in MMP-1 compensates for the binding affinity between P1′ and S1′ subsites and the different interactions between myricetin and residue214, which is Arg214 in MMP-1 while Leu214 in MMP-3, may be the major reason

Table 3
The total energy (E_{total}), van der waals energy (E_{vdw}), and electrostatic energy (E_{ele}) between myricetin and key residues of S1′ pockets of MMP-1 and MMP-3 (kcal mol^{-1})

MMP-1				MMP-3			
Residue	E_{vdw}	E_{ele}	E_{total}	Residue	E_{vdw}	E_{ele}	E_{total}
Arg214	−0.56	−10.82	−11.38	Leu214	−3.31	−1.06	−4.37
Val215	−3.26	−0.48	−3.74	Val215	−3.42	−0.15	−3.57
Tyr237	−1.83	−0.64	−2.47	Tyr237	−4.20	−5.49	−9.69
Pro238	−3.96	−2.35	−6.31	Pro238	−3.11	2.40	−0.71
Ser239	−4.08	−2.05	−6.13	Leu239	−1.89	−0.66	−2.55
Tyr240	−3.51	0.06	−3.45	Tyr240	−9.61	0.51	−8.10

for better selectivity. The total interaction energies of m-MMP1 and m-MMP3 are −108.39 and −74.56 kcal mol^{-1} (Table 1) and also conclude that myricetin is more selective on MMP-1 than MMP-3, which are consistent with inhibiting order with the IC_{50} values, 1.01 and 4.18 μM for, respectively (Table 2).

Through observing the four complexes, p-MMP1, p-MMP3, m-MMP1 and m-MMP3, two important points are found: the first is the new ZBG is a relatively weak binding group because of the long distances between the ZBG O atom and the catalytic zinc ion, most of which are almost more than 2.60 Å while the general is from around 1.90 to 2.20 Å. The second is that when one side hydroxyl binds to MMP acting as the ZBG, the other side hydroxyl will form at least one hydrogen bond with MMP, for example, in MMP-1, when O1 atom of one side hydroxyl in pyrogallic acid binds to catalytic zinc ion, O5 and H6 of the other side hydroxyl form two hydrogen bonds with the enzyme; when O1 atom of one side hydroxyl in myricetin binds to catalytic zinc ion, H6 atom of the other side hydroxyl forms a hydrogen bond with the enzyme. The similar mode happens in MMP-3 too. So we infer that the two-side-hydroxyl mode may be conserved in the complexes of this kind.

3.3. Quantum chemistry calculation

3.3.1. Interaction characteristics between ZBG and catalytic zinc ion of MMP

Typical zinc coordination sphere in MMPs is made up of three His residues and one exogenous ligand [34]. Thus the p-MMP1, used in the quantum calculation, from the docking results, is intercepted and modeled by the mode which consists of a catalytic zinc ion, three imidazoles, and an inhibitor. The frequency calculation result of the optimized complex shows that no imaginary frequency exists, which indicates that the structure is in the state of true minimum energy. We have encountered a problem whether pyrogallic acid binds to the catalytic zinc ion in a monodentate or bidentate manner from the result of docking simulation above. The geometry parameters of the quantum calculation can give an explanation to that. As shown in Fig. 4, p-MMP1 adopts little distorted tetrahedral structure with three angles of O1–Zn10–N11, O1–Zn10–N12 and O1–Zn10–N13 98.7°, 98.9°, and 112.0°, respectively. These are close to that of standard tetrahedral configuration (109.5°) and indicate that pyrogallic acid binds to MMP-1 in a monodentate manner.

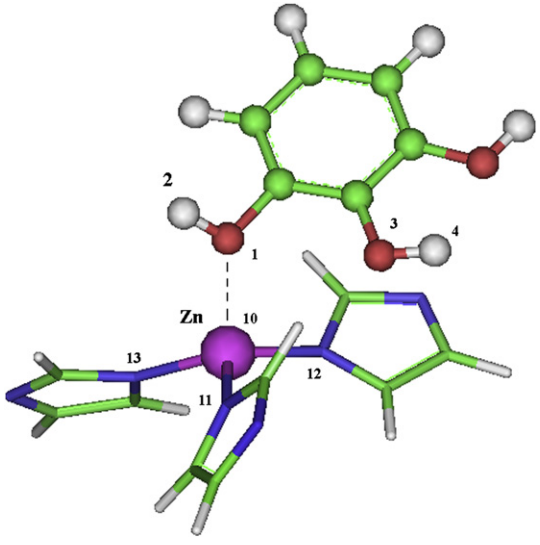

Fig. 4. Structure of p-MMP1 complex optimized through Gaussian 03.

3.3.2. Charge population analysis

The Natural Bond Orbital (NBO) method which uses natural atomic orbital to calculate the atomic charge is applied to the Charge Population analysis instead of the Mulliken method which is dependent on the basis set when calculating the atomic charge. We regard pyrogallic acid as a group for clear discussion and the group charge is the summation of atomic charges of all of the atoms that compose pyrogallic acid. The calculation results show that the group charge is 0.0 Q/e in free state while 0.374 Q/e in complex of p-MMP1. The group charge increases in the complex indicating the electrons have transferred from pyrogallic acid to the zinc ion which is a strong certification that the new ZBG can interacts with the catalytic zinc ion of MMPs.

4. Conclusion

By means of molecular modeling and docking simulations, two novel inhibitors have been evaluated on the inhibiting ability of MMP-1 and MMP-3, and the reasons for the differences in potency and selectivity, respectively, have been demonstrated. Myricetin owns better potency than pyrogallic acid on both MMP-1 and MMP-3 mainly due to the P1′ substituent interacting with S1′ subsite of MMPs. Both pyrogallic acid and myricetin are more selective on MMP-1 than MMP-3 with approximately 5-fold and 4-fold, respectively (Table 2). The improvement of pyrogallic acid, which introduces a P1′ substituent to pyrogallic acid, is a good beginning for increasing the potency and almost keeping the selectivity. From the four complexes, we infer that the new ZBG is a relatively weak binding group and the two-side-hydroxyl mode may be conserved in the complexes of this kind. The quantum chemistry calculations show that the ZBG can interact with MMPs and inhibitors bind to MMPs in a monodentate manner. All the results mentioned above may be helpful in the structure-based design of MMP inhibitors with improved potency and selectivity.

Acknowledgements

This work was supported by the Natural Science Foundation of China, Key Projects in the National Science & Technology Pillar Program, and Specialized Research Fund for the Doctoral Program of Higher Education of China (Grant Nos. 20573042, 2006BAE03B01, and 20070183046).

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