Send Orders of Reprints at reprints@benthamscience.net

1242 *Current Topics in Medicinal Chemistry,* **2013**, *13*, **1242-1253**

Metallo-β-Lactamases: Structural Features, Antibiotic Recognition, Inhibition, and Inhibitor Design

Jing-Fang Wang[1,2,4,*] and Kuo-Chen Chou[3,4]

[1]*Key Laboratory of Systems Biomedicine (Ministry of Education), Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai 200240, China;* [2]*Shanghai Center for Bioinformation Technology, 1278 Keyuan Road, Shanghai 201203, China;* [3]*Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia;* [4]*Gordon Life Science Institute, 53 South Cottage Road, Belmont, Massachusetts 02478, United States of America*

Abstract: Owing to their ability in destroying or slowing down the growth of bacteria, antibiotics have been widely used to treat the bacterial infections. However, because of the long-term and irresponsible use of antibiotics, resistance to antibiotics has become a serious problem directly threatening the public health worldwide. To fight against and resist β-lactam antibiotics, bacteria usually employed β-lactamases, especially the metallo-β-lactamases, to hydrolyze the C-N bond of the β-lactam ring so as to inactivate the antibiotics. In this minireview, we are to summarize the structural features of the metallo-β-lactamases, as well as their antibiotic binding modes and resistance mechanisms, in hopes that the discussion and analysis presented in this paper can stimulate new strategies to overcome the resistance problem and find novel inhibitors against the metallo-β-lactamases.

Keywords: β-Lactamase; antibiotics; resistance; new Delhi metallo-β-lactamase.

INTRODUCTION

Before the early 20th century, treatment for infections was the major challenge for medicinal chemistry. Many ancient people such as Chinese, Egyptians and Greeks used specially selected mold and plant materials and their extracts to treat infections on the basis that some antibiotics might be contained therein. Nowadays, antibiotics are widely used to treat infections caused by bacteria because they can destroy or slow down the growth of bacteria [1]. However, due to the long-term and irresponsible use of antibiotics, some bacterial strains have become increasingly resistant to the regular antibiotics. According to the European Center for Disease Prevention and Control (ECDC), the antibiotic resistance problem has become a serious public health threat worldwide. In a statement issued in 19th Nov. 2012, the ECDC informed that an estimated 25,000 people die each year in the European Union from antibiotic-resistant bacterial infections.

As one of the most commonly prescribed drugs to treat the bacterial infections, β-lactam antibiotics can inhibit transpeptidase involved in cell wall biosynthesis [2,3]. β-Lactam antibiotics are so-named because they share a core structure, β-lactam ring, in which the nitrogen atom is attached to the β-carbon relative to the carbonyl [4]. To fight against β-lactam antibiotics, bacteria have developed some strategies to resist the aforementioned antibiotics. The most effective strategy is to involve a family of enzymes, β-lactamases [3], which can hydrolyze the C-N bond to render the antibiotic inactive. Bacterial β-lactamases can be grouped into two types: serine β-lactamases and metallo β-lactamases [5]. The former employs an active serine as a nucleophile, and the latter uses zinc ions to effect β-lactam cleavage [6]. Unlike the serine β-lactamases, metallo-β-lactamases show strong resistance to the clinical antibiotics, and cannot be effectively inactivated by the conventional inhibitors [7].

The first metallo-β-lactamase was identified from a *Bacillus cereus* strain in 1966 [8,9]. By now metallo-β-lactamases are found in at least 20 strains, such as *Aeromonas hydrophila*, *Stenotrophomonas maltophilia*, and *Pseudomonas aeruginosa* [10-12]. Since then, a number of experimental and theoretical attempts have been made to get the structural insight into the metallo-β-lactamase and their resistance mechanism against antibiotics.

During the last decade, lots of progresses have been made in structure-based cheminformatics that are closely associated with the targets of medicinal chemistry, such as QSAR [13-18], molecular modeling and docking [19-32], as well as in biomedical cheminformatics such as identifying signal propagation during colorectal cancer progression [33], identifying nucleosomes [34], identifying recombination spots of DNA [35], identifying antimicrobial peptides and their functional types [36], predicting secretory proteins of malaria parasite [37], identifying HIV cleavage sites in proteins [38,39], identifying colorectal cancer related genes [40], predicting signal peptides [41], predicting protein subcellular locations for the systems with multi-labels [42-44], identifying DNA binding proteins [45], predicting proteases and their types [46], predicting antimicrobial peptides [36], identify nuclear receptors and their types [47], predicting GPCRs and their types [48], classifying hepatocellular cir-

*Address correspondence to this author at the Key Laboratory of Systems Biomedicine (Ministry of Education), Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai 200240, China; Tel: and Fax: (86) 21 3420-7344; E-mails: jfwang@gordonlifescience.org and jfwang8113@gmail.com

1873-5294/13 $58.00+.00

rhosis and carcinoma [49], identifying anatomical therapeutic chemical (ATC) drugs classification [50], deciphering the effects of gene deletion on yeast longevity [51], predicting cysteine S-nitrosylation sites [52], and a series of powerful web-server predictors listed in Table 3 of [53] and references of [54]. These cheminformatics tools can generate many useful data for which it would be otherwise time-consuming and costly to obtain by experiments alone. Actually, these data, combined with the information derived from the structural bioinformatics tools (see, e.g., [55,56]) can timely provide very useful insights for both medicinal chemistry research and drug development. In this minireview, we are to summarize the recent advances with cheminfrmatics on the metallo-β-lactamases, especially the New Delhi metallo-β-lactamase [57], which can make bacteria resistant to a broad range of β-lactam antibiotics. Particularly, we shall focus on the general structure features, antibiotic recognition and inhibition, as well as the novel inhibitor design against these enzymes.

STRUCTURAL FEATURES OF METALLO-β-LACTAMASE

In a pioneer paper published in 1980 by Amber [58], metallo-β-lactamases were formally categorized from serine β-lactamases. At that time, only 2 transferable types of metallo-β-lactamases had been studied; they were CcrA from *Bacteroides fragilis* and IMP-1 from *Pseudomonas aeruginosa*. So far more than 20 metallo-β-lactamases have been identified from different organisms. Based on the sequence identity and structural features, these enzymes can be categorized into the following three subgroups [59]. The subgroup 1 (also called B1) contains those enzymes that posses the key zinc coordinating residues of 3 histidines and 1 cysteine, i.e., IMP-1 from *Serratia marcescens* [60], Bc1I from *Bacillus cereus* [8], and VIM-2 from *Pseudomonas aeruginosa* [61]. The subgroup 2 (also called B2) contains those that posses an asparagines instead of the histidine at the first position of the principal zinc-binding motif NXHXD, i.e., SFH-1. The subgroup 3 (also called B3) contains those that are functionally represented as a tetramer. See Table **1** for the detailed information.

According to the crystal structure studies [62-73], metallo-β-lactamases (especially the B1 subgroup) shares a common αβ/βα sandwich fold with two β-sheets at the core and four α-helices at the external faces, as shown in Fig. **1**. The active site is located at the bottom of a solvent-accessible groove bounded by some loops, which is also the interface between domains. In this common structure for the B1 subgroup metallo-β-lactamases, there are about 6 residues in the active site that coordinate with one or two zinc ions, which are thought to be essential for the catalytic mechanism of the metallo-β-lactamases. Besides metallo-β-lactamases, some other enzymes (referred to non-β-lactamases) also have such αβ/βα sandwich folding structures, i.e., glyoxalase II, arylsulfatase, cyclase/dihydrase [74]. The major differences in the folding structures between the non-β-lactamases and metallo-β-lactamases are the metal ions and the residues that bridge the metal ions. Different from metallo-β-lactamases, the non-β-lactamases usually can bind a diverse set of metal ions such as zinc, iron, and manganese [75]. Even if the non-β-lactamases coordinate zinc, the residue that bridges the metal ions is usually an aspartate.

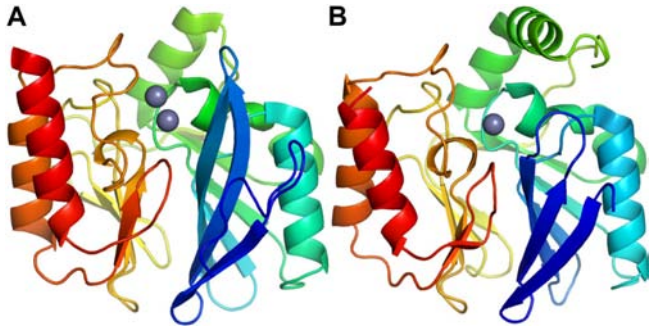

Fig. (1). Schematic illustration of the αβ/βα sandwich folding structures of (A) di-zinc, and (B) mono-zinc metallo-β-lactamases. The backbone structures were shown in rainbow cartoons and the zinc ions were labeled as spheres. The graphics were generated from the PDB files with 1KO3 for the di-zinc enzyme and 1X8G for the mono-zinc enzyme, respectively.

In the crystal structure of B1 subgroup of metallo-β-lactamases, 2 zinc atoms (labeled as Zn1 and Zn2 in this paper) were detected. The Zn1 binding site is a tetragonal geometry by the imidazole groups of 3 histidine residues, the so-called the 3H site (Fig. **2A**). Thus, the Zn1 is also called the tetrahedrally coordinated zinc ion. The Zn2 binding site is composed of the side chains of an aspartate, a cysteine and a histidine, and two additional water molecules, the so-called the DCH site (Fig. **2B**). So, the Zn2 is also called a trigonal bipyramidally coordinated zinc ion. Both the 3H site and DCH site are conserved among the B1 subgroup enzymes, which are thus used to distinguish metallo-β-lactamases as B1 subgroup. Additionally, the enzymes in the B1 subgroup are usually active having one zinc ion in the active site with an increased activity on the binding of the second zinc [76], while those in the B2 subgroup are only active when having a single zinc ion in the active site with the second zinc binding inhibiting turnover [77]. The enzymes in the B3 subgroup can only perform hydrolysis when both zinc ions are coordinated in the active site [78].

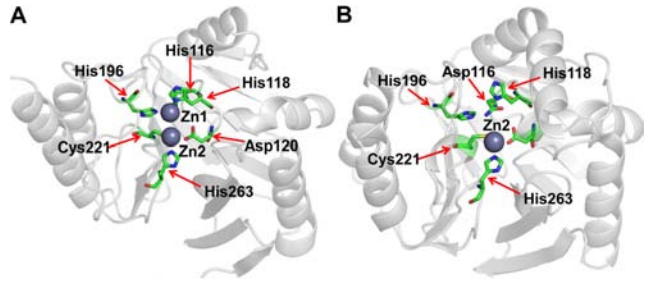

Fig. (2). Close view of the zinc ion binding sites for (A) di-zinc and (B) mono-zinc metallo-β-lactamases. The Zn1 binding site is a tetragonal geometry by the imidazole groups of 3 histidine residues, the so-called the 3H site. The Zn2 binding site, also called the DCH site, is composed of the side chains of an aspartate, a cysteine and a histidine. The graphics were generated from the PDB files with 1ZNB for the di-zinc enzyme and 1X8G for the mono-zinc enzyme, respectively

Table 1. The Detailed Information for All the Chromosomally Encoded Metallo-β-Lactamases.

No.	Name	Organism	Subgroup	Ref.
1	Bce 170	Alkalophilic *Bacillus* spp.	B1	8
2	Bla2	*Bacillus anthracis*	B1	39
3	Bc1I-5/B/6	*Bacillus cereus*	B1	41
4	Bc1I-569/H	*Bacillus cereus*	B1	8
5	CGB-1	*Chryseobacterium gleum*	B1	37
6	IND-1	*Chryseobacterium indologenes*	B1	36
7	IND-2, 2a, 3, 4	*Chryseobacterium indologenes*	B1	38
8	BlaB	*Chryseobacterium meningosepticum*	B1	45
9	BlaB2, BlaB3	*Chryseobacterium meningosepticum*	B1	50
10	BlaB4-8	*Chryseobacterium meningosepticum*	B1	37
11	JOHN-1	*Flavobacterium johnsoniae*	B1	43
12	MUS-1	*Myroides odoratimimus*	B1	42
13	TUS-1	*Myroides odoratus*	B1	42
14	VIM-2	*Pseudomonas aeruginosa*	B1	17
15	SPM-1	*Pseudomonas aeruginosa*	B1	28
15	IMP-1	*Seratia marcescens*	B1	16
16	CphA	*Aeromonas hydrophilia*	B2	24
17	ImiS	*Aeromonas veronii*	B2	49
18	AsbM1	*Aeromonas veronii*	B2	51
19	SFH-1	*Serratia fonticola*	B2	46
20	Mb11B	*Caulobacter crescentus*	B3	47
21	CAU-1	*Caulobacter crescentus*	B3	40
22	GOB-1-7	*Chryseobacterium meningosepticum*	B3	38
23	THIN-B	*Janthinobacterium lividium*	B3	44
24	FEZ-1	*Legionella gormanii*	B3	27
25	L1a	*Stenotrophomonas maltophilia*	B3	48
26	L1-BlaS	*Stenotrophomonas maltophilia*	B3	12
27	L1c, L1d, L1e	*Stenotrophomonas maltophilia*	B3	35

ANTIBIOTIC RECOGNITION AND INHIBITION

Metallo-β-lactamases, especially the B1 subgroup enzymes, can recognize a wide range of the β-lactam antibiotics, i.e., penicilllin, ampicillin, carbenicillin, zalocillin, piperacillin and ticarcillin. Summarized in Table **2** are the steady-state kinetic parameters for IMP-1, VIM-1 and SMP-1 against a wide range of the β-lactam antibiotics, which raise a big question on why there are so many variations in binding and hydrolysis of the β-lactam antibiotics with similar enzymes. To address this question, we have to study and understand the binding modes of the β-lactam antibiotics in metallo-β-lactamases and their catalytic mechanisms. The crystal structures from different species showed that the substrate (referred to the β-lactam antibiotics) can bind to several conformations of the metallo-β-lactamases leading to productive interactions [79-95]. In the crystal structure of the L1 enzyme from *Stenotrophomonas maltophilia* [96], Ser223 and the tetrahedrally coordinated zinc ion polarizes the carbonyl group of the β-lactam ring of the substrate, forming an oxyanion hole to facilitate hydrolysis (Fig. **3**). The trigonal bipyramidally coordinated zinc ion may interact with the nitrogen to position the substrate in the correct orientation for the nucleophilic attack.

Table 2. The Steady-State Kinetic Parameters for IMP-1, VIM-1 and SPM-1 Against a Wide Range of β-Lactam Antibiotics.

B-Lactams	IMP-1			VIM-1			SPM-1		
	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($\mu M^{-1}s^{-1}$)	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($\mu M^{-1}s^{-1}$)	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($\mu M^{-1}s^{-1}$)
Ampicillin	950	200	4.8	37	917	0.04	117	72	1.6
Cefepime	7	11	0.66	549	145	3.8	18	18	1
Cefotaxime	1.3	4	0.35	169	247	0.68	16	9	1.9
Cefoxitin	16	8	2	26	131	0.2	8	2	4
Ceftazidime	8	44	0.18	60	794	0.076	28	46	0.6
Cefuroxime	8	37	0.22	324	42	7.7	37	4	8.8
Cephalothin	48	21	2.4	281	53	5.3	43	4	11.7
Imipenem	46	38	1.2	2.0	1.5	1.3	33	37	1
Meropenem	50	10	0.12	13	48	0.27	63	281	0.22
Nitrocefin	63	27	2.3	95	17	5.6	0.53	4	0.12
Penicilllin	320	520	0.62	29	841	0.034	108	38	2.8
Tazobactam	>1,000	>3.98	0.0039	5.3	337	0.016	0.6	3	0.2
Ticarcillin	1.1	740	0.0015	452	1,117	0.41	—	<0.35	—

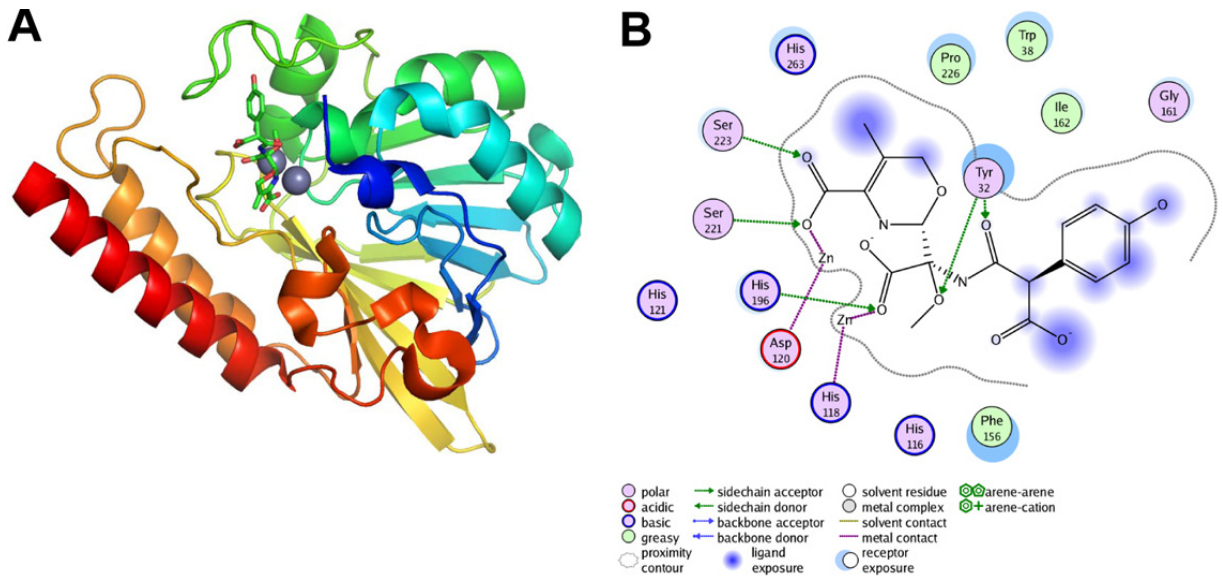

Fig. (3). Binding modes of β-lactam antibiotic substrate in the metallo-β-lactamases. (A) The overview of the moxalactam binding mode in the Stenotrophomonas maltophilia L1 enzyme. The protein structure is shown in rainbow cartoons with the substrate in stick models and the zinc ions in spheres. (B) Detailed information for the interactions between the substrate moxalactam and Stenotrophomonas maltophilia L1 enzyme.

As mentioned above, the metal ions in the metallo-β-lactamases are essential for the catalytic mechanism of the β-lactam antibiotics. The enzymes in the B1 and B3 subgroups of the metallo-β-lactamases can utilize two zinc ions, a tetrahedrally coordinated zinc ion and a trigonal bipyramidally coordinated zinc ion, to hydrolyze the β-lactam antibiotics, while the ones in the B2 subgroup are only activated with a single zinc ion. This observation gives an indication that there should be two possible catalytic mechanisms.

Many recent studies have indicated that computational or cheminformatics approaches, such as structural bioinformatics [55], predicting drug-target interaction [97], molecular docking [56], and protein cleavage site prediction [39] can timely provide very useful information and insights for drug development and hence are widely welcome by science community. Particularly, molecular docking studies can provide useful information for in-depth understanding some subtle action mechanisms at the molecular biology level,

such as the marvelous allosteric mechanism revealed recently by the NMR observations on the M2 proton channel of influenza A virus [98,99]. They can also provide useful insights to stimulate drug developments as demonstrated by a series of recent studies [17,22,26,31,55,56,100-107]). Also, as is well known, the structures of biomacromolecules such as proteins and DNA are not static but in a dynamic state with low-frequency internal motion [108-113]. Many marvelous biological functions in proteins and DNA and their profound dynamic mechanisms, such as switch between active and inactive states [114,115], cooperative effects [116], allosteric transition [117,118], intercalation of drugs into DNA [119], and assembly of microtubules [120], can be revealed by studying their internal motions [121]. Actually, the functions of low-frequency internal motions in biomacromolecules and cells have also been used for medical treatments (see, e.g., [122-124]). Therefore, to really understand the action mechanism of metallo-β-lactamases, we should consider not only the static structures concerned but also the dynamical information obtained by simulating their internal motions or dynamic process. Accordingly, to provide an in-depth understanding of the catalytic mechanism of the metallo-β-lactamases, multiple computational and theoretical approaches (i.e., molecular modeling [107,125-129], flexible docking [130-135] and molecular dynamics simulation [107,130,131,134,136-142]) have been applied to study the crystal structures and the substrate binding features of the metallo-β-lactamases, in order for providing atomic insights into the conformational dynamics during the catalytic processes [107,127,130,143,144].

For the di-zinc metallo-β-lactamases that include the B1 and B3 subgroup enzymes, the bridging water molecule (colored in green in Fig. **4**) acts as a reaction nuleophile in β-lactam hydrolysis by the metallo-β-lactamases [145]. The bridging water molecule can make a re-face attack upon the carbonyl carbon of the substrate with a carbonyl oxygen atom ligated to Zn1 and the carboxylate in the five-member ring of the substrate bound to Zn2, as shown in Fig. **4A** [145,146]. Such structure is stabilized by both the zinc atoms and the residues between the metal ions. Subsequently, the nucleophilic attach on the carbonyl carbon occurs, resulting in a tetrahedral intermediate (Fig. **4B**) [146,147]. The tetrahedral intermediate will transfer to an anionic nitrogen intermediate by the nitrogen protonation (Fig. **4C**). Finally, the functional lactam ring of the β-lactam substrates will be cleaved (Fig. **4D**).

For the mono-zinc metallo-β-lactamases that include the B2 subgroup enzymes, a non-zinc bound water molecule was found to act as the nucleophile. In the initial nucleophilic addition step, this water molecule can attack the carbonyl carbon of the β-lactam substrates with the help of Asp120 (Fig. **5A**), resulting in the cleavage of the C-N bond in the lactam ring (Fig. **5B**) [148]. The anionic nitrogen part of the substrate is then stabilized by the zinc ion to form an enzyme-intermediate complex, with the carbonyl part and the residue His118 as well as the non-zinc bound water together to form tetrahedral structure (Fig. **5B**) [149]. This tetrahedral structure will be broken to release a water molecule, which can further protonate the anionic nitrogen (Fig. **5C**), and the functional lactam ring of the β-lactam substrates will finally be cleaved (Fig. **5D**) [150].

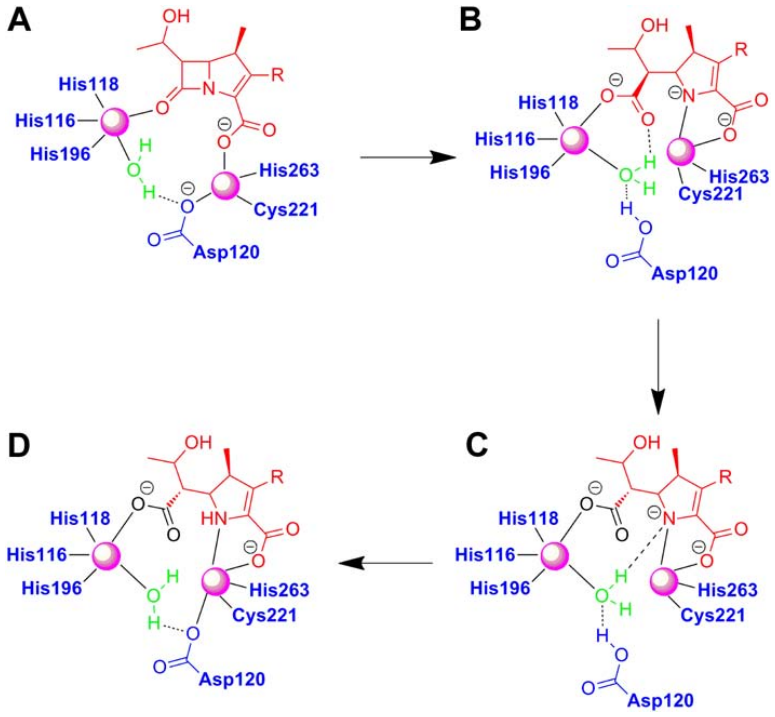

Fig. (4). Schematic illustration to show the catalytic mechanism for the di-zinc metallo-β-lactamases. The bridging water molecule acts as the nucleophile to attack the carbonyl carbon in the lactam ring of the substrate. After the C-N bond cleavage, the intermediate structure is stabilized by the zinc ions (shown in pink balls), which will convert into an anionic nitrogen intermediate and further be released with the functional lactam ring cleft.

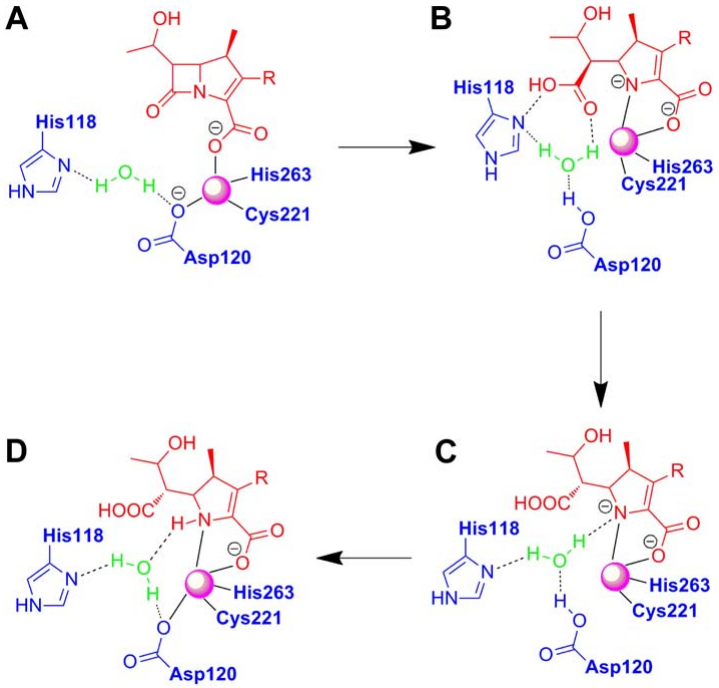

Fig. (5). Schematic illustration of the catalytic mechanism for the mono-zinc metallo-β-lactamases. Non-zinc bound water acts as the nucleophile to attack the carbonyl carbon in the lactam ring of the substrate with the help of Asp120. After the C-N bond cleavage, the anionic nitrogen part of the substrate is stabilized by the zinc ion (shown in a pink ball) to form an enzyme-intermediate complex, which will be further protonated by a water molecule. The functional lactam ring of the substrate is then cleft.

Table 3. The Detailed Information Obtained by Experiments for the Metallo-β-Lactamases.

Inhibitor type	Compound name	Target	Affinity/μM	Ref.
Biphenyl tetrazole	L161, L189	CcrA	IC_{50} = 0.30	96
Cysteinyl peptide	D-phenylalanine derivatives	BcII	K_i = 3.0	97
Penicillin derivatives	Penicillinate sulfone	L1	IC_{50} = 0.10	98
Thioester derivatives	Morpholinoethanesulfonic acid	CcrA	K_i = 23	103
	SB217782/8018	L1	IC_{50} < 1.9	100
	SB214572	L1	IC_{50} = 2	100
	Biphenylmethyl derivatives	IMP-1	IC_{50} < 0.01	96
		CcrA	IC_{50} = 180	96
Tiol	Mercaptoacetic acid	IMP-1	K_i = 0.23	109
	Mercaptopropionic acid	IMP-1	K_i = 0.19	109
	2'-Mercaptoethyl-derivatives	BcII	K_i = 70	99
	Thiobenzoate derivatives	IMP-1	IC_{50} < 0.01	115
		CcrA	IC_{50} = 180	115
	2-*para*-Thiomandelic acid	BcII	K_i = 0.21	106
	Quinoline C45H	IMP-1	IC_{50} = 1.2	108
		VIM-2	IC_{50} = 1.1	108
	Thioacid	BcII	K_i = 96	119

(Table 3) contd….

Inhibitor type	Compound name	Target	Affinity/μM	Ref.
Tricyclic derivatives	SB238569	BcII	K_i = 79	111
		IMP-1	K_i = 17	111
		CcrA	K_i = 3.4	111
	2S-3S disubstitute	IMP-1	IC_{50} > 0.21	114
Trifluoromethyl alcohol	D-Alanine derivatives	L1	K_i = 1.5	112
		BcII	K_i = 300	112

INHIBITOR DESIGN AGAINST METALLO-β-LACTAMASE

Owing to the significant resistance against the β-lactam antibiotics, many good attempts have been made to design inhibitors against all the β-lactamases, including metallo-β-lactamases. One of the most successful cases is the combination of amoxicillin-clavulanate for the class A β-lactamases, which can form a stable covalent intermediate with the class A β-lactamases [151]. However, such combinations are rare for the metallo-β-lactamases due to the following reasons. The active site architectures for different metallo-β-lactamases from different organisms are different, which is not propitious to designing a single inhibitor efficacious against metallo-β-lactamases [74]. The metallo-β-lactamases have very broad activities and do not form stable reaction intermediates during their catalytic processes, making it hard to copy the inhibition mode of the β-lactam-like compounds (i.e., clavulanate) [152].

By now, a number of structurally disparate compounds have been designed and proved to be potential inhibitors against the metallo-β-lactamases, such as biphenyl tetrazoles [153,154], cysteinyl peptides [155], penicillin derivatives [156], thioester derivatives [157-160], tiol derivatives [2,161-167], tricyclic natural products [168], trifluoromethyl alcohols [169], sulfonyl hydrazones [170], succinic acid derivatives [171], mecraptocarboxylates [168,172], 1-β-methylcarbapenem [173,174], cefotetan [175], and thioxocephalosporins [176]. The detailed information for these kinds of potential inhibitors and their affinities are summarized in Table **3**.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENT

This work was sponsored by the grants from National Natural Science Foundation of China (No. 31200547), Doctoral Program Foundation of Institutions of Higher Education of China (No. 20090073120079 and 20110073120078), China Postdoctoral Science Foundation (No.20110490068), as well as the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry.

REFERENCES

[1] von Nussbaum, F.; Brands, M.; Hinzen, B.; Weigand, S.; Habich, D. Antibacterial natural products in medicinal chemistry--exodus or revival? *Angew Chem Int Ed Engl*, **2006**, *45*, 5072-5129.

[2] Bounaga, S.; Laws, A. P.; Galleni, M.; Page, M. I. The mechanism of catalysis and the inhibition of the Bacillus cereus zinc-dependent beta-lactamase. *Biochem J*, **1998**, *331 (Pt 3)*, 703-711.

[3] Drawz, S. M.; Bonomo, R. A. Three decades of beta-lactamase inhibitors. *Clin Microbiol Rev*, **2010**, *23*, 160-201.

[4] Felici, A.; Amicosante, G.; Oratore, A.; Strom, R.; Ledent, P.; Joris, B.; Fanuel, L.; Frere, J. M. An overview of the kinetic parameters of class B beta-lactamases. *Biochem J*, **1993**, *291 (Pt 1)*, 151-155.

[5] Fisher, J. F.; Meroueh, S. O.; Mobashery, S. Bacterial resistance to beta-lactam antibiotics: compelling opportunism, compelling opportunity. *Chem Rev*, **2005**, *105*, 395-424.

[6] Bush, K. Metallo-beta-lactamases: a class apart. *Clin Infect Dis*, **1998**, *27 Suppl 1*, S48-53.

[7] Page, M. I. The reactivity of beta-lactams, the mechanism of catalysis and the inhibition of beta-lactamases. *Curr Pharm Des*, **1999**, *5*, 895-913.

[8] Hussain, M.; Carlino, A.; Madonna, M. J.; Lampen, J. O. Cloning and sequencing of the metallothioprotein beta-lactamase II gene of Bacillus cereus 569/H in Escherichia coli. *J Bacteriol*, **1985**, *164*, 223-229.

[9] Sabath, L. D.; Abraham, E. P. Zinc as a cofactor for cephalosporinase from Bacillus cereus 569. *Biochem J*, **1966**, *98*, 11C-13C.

[10] Laraki, N.; Franceschini, N.; Rossolini, G. M.; Santucci, P.; Meunier, C.; de Pauw, E.; Amicosante, G.; Frere, J. M.; Galleni, M. Biochemical characterization of the Pseudomonas aeruginosa 101/1477 metallo-beta-lactamase IMP-1 produced by Escherichia coli. *Antimicrob Agents Chemother*, **1999**, *43*, 902-906.

[11] Massidda, O.; Rossolini, G. M.; Satta, G. The Aeromonas hydrophila cphA gene: molecular heterogeneity among class B metallo-beta-lactamases. *J Bacteriol*, **1991**, *173*, 4611-4617.

[12] Sanschagrin, F.; Dufresne, J.; Levesque, R. C. Molecular heterogeneity of the L-1 metallo-beta-lactamase family from Stenotrophomonas maltophilia. *Antimicrob Agents Chemother*, **1998**, *42*, 1245-1248.

[13] Dea-Ayuela, M. A.; Perez-Castillo, Y.; Meneses-Marcel, A.; Ubeira, F. M.; Bolas-Fernandez, F.; Chou, K. C.; Gonzalez-Diaz, H. HP-Lattice QSAR for dynein proteins: Experimental proteomics (2D-electrophoresis, mass spectrometry) and theoretic study of a Leishmania infantum sequence. *Bioorg Med Chem*, **2008**, *16*, 7770-7776.

[14] Du, Q. S.; Huang, R. B.; Wei, Y. T.; Du, L. Q.; Chou, K. C. Multiple Field Three Dimensional Quantitative Structure-Activity Relationship (MF-3D-QSAR). *J. Comput. Chem.*, **2008**, *29*, 211-219.

[15] Prado-Prado, F. J.; Gonzalez-Diaz, H.; de la Vega, O. M.; Ubeira, F. M.; Chou, K. C. Unified QSAR approach to antimicrobials. Part 3: First multi-tasking QSAR model for Input-Coded prediction, structural back-projection, and complex networks clustering of antiprotozoal compounds. *Bioorganic & Medicinal Chemistry*, **2008**, *16*, 5871-5880.

[16] Prado-Prado, F. J.; Martinez de la Vega, O.; Uriarte, E.; Ubeira, F. M.; Chou, K. C.; Gonzalez-Diaz, H. Unified QSAR approach to antimicrobials. 4. Multi-target QSAR modeling and comparative multi-distance study of the giant components of antiviral drug-drug complex networks. *Bioorg Med Chem*, **2009**, *17*, 569-575.

[17] Wei, H.; Wang, C. H.; Du, Q. S.; Meng, J.; Chou, K. C. Investigation into adamantane-based M2 inhibitors with FB-QSAR. *Medicinal Chemistry*, **2009**, *5*, 305-317.

[18] Du, Q. S.; Huang, R. B.; Wei, Y. T.; Pang, Z. W.; Du, L. Q.; Chou, K. C. Fragment-Based Quantitative Structure-Activity Relationship (FB-QSAR) for Fragment-Based Drug Design. *J. Comput. Chem.*, **2009**, *30*, 295-304.

[19] Liu, L.; Ma, Y.; Wang, R. L.; Xu, W. R.; Wang, S. Q.; Chou, K. C. Find novel dual-agonist drugs for treating type 2 diabetes by means of cheminformatics. *Drug Design, Development and Therapy*, **2013**, open access to scientific and medical research.

[20] Chou, K. C. Insights from modelling three-dimensional structures of the human potassium and sodium channels. *Journal of Proteome Research*, **2004**, *3*, 856-861.

[21] Chou, K. C. Insights from modelling the tertiary structure of BACE2. *Journal of Proteome Research*, **2004**, *3*, 1069-1072.

[22] Ma, Y.; Wang, S. Q.; Xu, W. R.; Wang, R. L.; Chou, K. C. Design novel dual agonists for treating type-2 diabetes by targeting peroxisome proliferator-activated receptors with core hopping approach. *PLoS One*, **2012**, *7*, e38546.

[23] Chou, K. C. Insights from modeling the 3D structure of DNA-CBF3b complex. *Journal of Proteome Research*, **2005**, *4*, 1657-1660.

[24] Du, Q. S.; Wang, S. Q.; Chou, K. C. Analogue inhibitors by modifying oseltamivir based on the crystal neuraminidase structure for treating drug-resistant H5N1 virus. *Biochem Biophys Res Comm*, **2007**, *362*, 525-531.

[25] Chou, K. C.; Jones, D.; Heinrikson, R. L. Prediction of the tertiary structure and substrate binding site of caspase-8. *FEBS Lett.*, **1997**, *419*, 49-54.

[26] Du, Q. S.; Huang, R. B.; Wang, S. Q.; Chou, K. C. Designing inhibitors of M2 proton channel against H1N1 swine influenza virus. *PLoS ONE*, **2010**, *5*, e9388.

[27] Chou, K. C.; Tomasselli, A. G.; Heinrikson, R. L. Prediction of the Tertiary Structure of a Caspase-9/Inhibitor Complex. *FEBS Lett.*, **2000**, *470*, 249-256.

[28] Chou, K. C. The convergence-divergence duality in lectin domains of the selectin family and its implications. *FEBS Lett.*, **1995**, *363*, 123-126.

[29] Wang, S. Q.; Du, Q. S.; Chou, K. C. Study of drug resistance of chicken influenza A virus (H5N1) from homology-modeled 3D structures of neuraminidases. *Biochem Biophys Res Comm*, **2007**, *354*, 634-640.

[30] Chou, K. C. Coupling interaction between thromboxane A2 receptor and alpha-13 subunit of guanine nucleotide-binding protein. *Journal of Proteome Research*, **2005**, *4*, 1681-1686.

[31] Li, X. B.; Wang, S. Q.; Xu, W. R.; Wang, R. L.; Chou, K. C. Novel Inhibitor Design for Hemagglutinin against H1N1 Influenza Virus by Core Hopping Method. *PLoS One*, **2011**, *6*, e28111.

[32] Chou, K. C.; Howe, W. J. Prediction of the tertiary structure of the beta-secretase zymogen. *Biochem. Biophys. Res. Commun.*, **2002**, *292*, 702-708.

[33] Jiang, Y.; Huang, T.; Lei, C.; Gao, Y. F.; Cai, Y. D.; Chou, K. C. Signal propagation in protein interaction network during colorectal cancer progression. *BioMed Research International, openly accssible at http://www.hindawi.com/journals/bmri/2013/287019/*, **2013**, *2013*, 1-9.

[34] Chen, W.; Lin, H.; Feng, P. M.; Ding, C.; Zuo, Y. C.; Chou, K. C. iNuc-PhysChem: A Sequence-Based Predictor for Identifying Nucleosomes via Physicochemical Properties. *PLoS ONE*, **2012**, *7*, e47843.

[35] Chen, W.; Feng, P. M.; Lin, H.; Chou, K. C. iRSpot-PseDNC: identify recombination spots with pseudo dinucleotide composition. *Nucleic Acids Res.*, **2013**, *41*, e68.

[36] Xiao, X.; Wang, P.; Lin, W. Z.; Jia, J. H.; Chou, K. C. iAMP-2L: A two-level multi-label classifier for identifying antimicrobial peptides and their functional types. *Anal. Biochem.*, **2013**, *436*, 168-177.

[37] Lin, W. Z.; Fang, J. A.; Xiao, X.; Chou, K. C. Predicting Secretory Proteins of Malaria Parasite by Incorporating Sequence Evolution Information into Pseudo Amino Acid Composition via Grey System Model. *PLoS One*, **2012**, *7*, e49040.

[38] Chou, K. C. A vectorized sequence-coupling model for predicting HIV protease cleavage sites in proteins. *J. Biol. Chem.*, **1993**, *268*, 16938-16948.

[39] Chou, K. C. Review: Prediction of human immunodeficiency virus protease cleavage sites in proteins. *Anal. Biochem.*, **1996**, *233*, 1-14.

[40] Li, B. Q.; Huang, T.; Liu, L.; Cai, Y. D.; Chou, K. C. Identification of colorectal cancer related genes with mRMR and shortest path in protein-protein interaction network. *PLoS ONE*, **2012**, *7*, e33393.

[41] Chou, K. C.; Shen, H. B. Signal-CF: a subsite-coupled and window-fusing approach for predicting signal peptides. *Biochem Biophys Res Comm*, **2007**, *357*, 633-640.

[42] Chou, K. C.; Wu, Z. C.; Xiao, X. iLoc-Hum: Using accumulation-label scale to predict subcellular locations of human proteins with both single and multiple sites. *Molecular Biosystems*, **2012**, *8*, 629-641.

[43] Lin, W. Z.; Fang, J. A.; Xiao, X.; Chou, K. C. iLoc-Animal: A multi-label learning classifier for predicting subcellular localization of animal proteins *Molecular BioSystems*, **2013**, *9*, 634-644.

[44] Chou, K. C. Some Remarks on Predicting Multi-Label Attributes in Molecular Biosystems. *Molecular Biosystems*, **2013**, *9*, 1092-1100.

[45] Lin, W. Z.; Fang, J. A.; Xiao, X.; Chou, K. C. iDNA-Prot: Identification of DNA Binding Proteins Using Random Forest with Grey Model. *PLoS ONE*, **2011**, *6*, e24756.

[46] Chou, K. C.; Shen, H. B. ProtIdent: A web server for identifying proteases and their types by fusing functional domain and sequential evolution information. *Biochem. Biophys. Res. Comm.*, **2008**, *376*, 321-325.

[47] Wang, P.; Xiao, X.; Chou, K. C. NR-2L: A Two-Level Predictor for Identifying Nuclear Receptor Subfamilies Based on Sequence-Derived Features. *PLoS ONE*, **2011**, *6*, e23505.

[48] Xiao, X.; Wang, P.; Chou, K. C. GPCR-2L: Predicting G protein-coupled receptors and their types by hybridizing two different modes of pseudo amino acid compositions. *Molecular Biosystems*, **2011**, *7*, 911-919.

[49] Huang, T.; Wang, J.; Cai, Y. D.; Yu, H.; Chou, K. C. Hepatitis C virus network based classification of hepatocellular cirrhosis and carcinoma. *PLoS ONE*, **2012**, *7*, e34460.

[50] Chen, L.; Zeng, W. M.; Cai, Y. D.; Feng, K. Y.; Chou, K. C. Predicting Anatomical Therapeutic Chemical (ATC) classification of drugs by integrating chemical-chemical interactions and similarities. *PLoS ONE*, **2012**, *7*, e35254.

[51] Huang, T.; Zhang, J.; Xu, Z. P.; Hu, L. L.; Chen, L.; Shao, J. L.; Zhang, L.; Kong, X. Y.; Cai, Y. D.; Chou, K. C. Deciphering the effects of gene deletion on yeast longevity using network and machine learning approaches. *Biochimie*, **2012**, *94*, 1017-1025.

[52] Xu, Y.; Ding, J.; Wu, L. Y.; Chou, K. C. iSNO-PseAAC: Predict cysteine S-nitrosylation sites in proteins by incorporating position specific amino acid propensity into pseudo amino acid composition *PLoS ONE*, **2013**, *8*, e55844.

[53] Chou, K. C.; Shen, H. B. Review: recent advances in developing web-servers for predicting protein attributes (doi: 10.4236/ns.2009.12011). *Natural Science*, **2009**, *2*, 63-92 (openly accessible at http://www.scirp.org/journal/NS/)

[54] Chou, K. C. Some remarks on protein attribute prediction and pseudo amino acid composition (50th Anniversary Year Review). *J. Theor. Biol.*, **2011**, *273*, 236-247.

[55] Chou, K. C. Review: Structural bioinformatics and its impact to biomedical science. *Current Medicinal Chemistry*, **2004**, *11*, 2105-2134.

[56] Chou, K. C.; Wei, D. Q.; Zhong, W. Z. Binding mechanism of coronavirus main proteinase with ligands and its implication to drug design against SARS. (Erratum: ibid., 2003, Vol.310, 675). *Biochem Biophys Res Comm*, **2003**, *308*, 148-151.

[57] Yong, D.; Toleman, M. A.; Giske, C. G.; Cho, H. S.; Sundman, K.; Lee, K.; Walsh, T. R. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella

pneumoniae sequence type 14 from India. *Antimicrob Agents Chemother*, **2009**, *53*, 5046-5054.

[58] Ambler, R. P. The structure of beta-lactamases. *Philos Trans R Soc Lond B Biol Sci*, **1980**, *289*, 321-331.

[59] Garau, G.; Garcia-Saez, I.; Bebrone, C.; Anne, C.; Mercuri, P.; Galleni, M.; Frere, J. M.; Dideberg, O. Update of the standard numbering scheme for class B beta-lactamases. *Antimicrob Agents Chemother*, **2004**, *48*, 2347-2349.

[60] Osano, E.; Arakawa, Y.; Wacharotayankun, R.; Ohta, M.; Horii, T.; Ito, H.; Yoshimura, F.; Kato, N. Molecular characterization of an enterobacterial metallo beta-lactamase found in a clinical isolate of Serratia marcescens that shows imipenem resistance. *Antimicrob Agents Chemother*, **1994**, *38*, 71-78.

[61] Poirel, L.; Naas, T.; Nicolas, D.; Collet, L.; Bellais, S.; Cavallo, J. D.; Nordmann, P. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-beta-lactamase and its plasmid- and integron-borne gene from a Pseudomonas aeruginosa clinical isolate in France. *Antimicrob Agents Chemother*, **2000**, *44*, 891-897.

[62] Borra, P. S.; Leiros, H. K.; Ahmad, R.; Spencer, J.; Leiros, I.; Walsh, T. R.; Sundsfjord, A.; Samuelsen, O. Structural and computational investigations of VIM-7: insights into the substrate specificity of vim metallo-beta-lactamases. *J Mol Biol*, **2011**, *411*, 174-189.

[63] Carfi, A.; Duee, E.; Galleni, M.; Frere, J. M.; Dideberg, O. 1.85 A resolution structure of the zinc (II) beta-lactamase from Bacillus cereus. *Acta Crystallogr D Biol Crystallogr*, **1998**, *54*, 313-323.

[64] Concha, N. O.; Janson, C. A.; Rowling, P.; Pearson, S.; Cheever, C. A.; Clarke, B. P.; Lewis, C.; Galleni, M.; Frere, J. M.; Payne, D. J.; Bateson, J. H.; Abdel-Meguid, S. S. Crystal structure of the IMP-1 metallo beta-lactamase from Pseudomonas aeruginosa and its complex with a mercaptocarboxylate inhibitor: binding determinants of a potent, broad-spectrum inhibitor. *Biochemistry*, **2000**, *39*, 4288-4298.

[65] Concha, N. O.; Rasmussen, B. A.; Bush, K.; Herzberg, O. Crystal structure of the wide-spectrum binuclear zinc beta-lactamase from Bacteroides fragilis. *Structure*, **1996**, *4*, 823-836.

[66] Docquier, J. D.; Benvenuti, M.; Calderone, V.; Stoczko, M.; Menciassi, N.; Rossolini, G. M.; Mangani, S. High-resolution crystal structure of the subclass B3 metallo-beta-lactamase BJP-1: rational basis for substrate specificity and interaction with sulfonamides. *Antimicrob Agents Chemother*, **2010**, *54*, 4343-4351.

[67] Fabiane, S. M.; Sohi, M. K.; Wan, T.; Payne, D. J.; Bateson, J. H.; Mitchell, T.; Sutton, B. J. Crystal structure of the zinc-dependent beta-lactamase from Bacillus cereus at 1.9 A resolution: binuclear active site with features of a mononuclear enzyme. *Biochemistry*, **1998**, *37*, 12404-12411.

[68] Garau, G.; Bebrone, C.; Anne, C.; Galleni, M.; Frere, J. M.; Dideberg, O. A metallo-beta-lactamase enzyme in action: crystal structures of the monozinc carbapenemase CphA and its complex with biapenem. *J Mol Biol*, **2005**, *345*, 785-795.

[69] Garcia-Saez, I.; Docquier, J. D.; Rossolini, G. M.; Dideberg, O. The three-dimensional structure of VIM-2, a Zn-beta-lactamase from Pseudomonas aeruginosa in its reduced and oxidised form. *J Mol Biol*, **2008**, *375*, 604-611.

[70] Garcia-Saez, I.; Hopkins, J.; Papamicael, C.; Franceschini, N.; Amicosante, G.; Rossolini, G. M.; Galleni, M.; Frere, J. M.; Dideberg, O. The 1.5-A structure of Chryseobacterium meningosepticum zinc beta-lactamase in complex with the inhibitor, D-captopril. *J Biol Chem*, **2003**, *278*, 23868-23873.

[71] Garcia-Saez, I.; Mercuri, P. S.; Papamicael, C.; Kahn, R.; Frere, J. M.; Galleni, M.; Rossolini, G. M.; Dideberg, O. Three-dimensional structure of FEZ-1, a monomeric subclass B3 metallo-beta-lactamase from Fluoribacter gormanii, in native form and in complex with D-captopril. *J Mol Biol*, **2003**, *325*, 651-660.

[72] Murphy, T. A.; Catto, L. E.; Halford, S. E.; Hadfield, A. T.; Minor, W.; Walsh, T. R.; Spencer, J. Crystal structure of Pseudomonas aeruginosa SPM-1 provides insights into variable zinc affinity of metallo-beta-lactamases. *J Mol Biol*, **2006**, *357*, 890-903.

[73] Ullah, J. H.; Walsh, T. R.; Taylor, I. A.; Emery, D. C.; Verma, C. S.; Gamblin, S. J.; Spencer, J. The crystal structure of the L1 metallo-beta-lactamase from Stenotrophomonas maltophilia at 1.7 A resolution. *J Mol Biol*, **1998**, *284*, 125-136.

[74] Daiyasu, H.; Osaka, K.; Ishino, Y.; Toh, H. Expansion of the zinc metallo-hydrolase family of the beta-lactamase fold. *FEBS Lett*, **2001**, *503*, 1-6.

[75] Campos-Bermudez, V. A.; Gonzalez, J. M.; Tierney, D. L.; Vila, A. J. Spectroscopic signature of a ubiquitous metal binding site in the metallo-beta-lactamase superfamily. *J Biol Inorg Chem*, **2010**, *15*, 1209-1218.

[76] Davies, R. B.; Abraham, E. P. Metal cofactor requirements of beta-lactamase II. *Biochem J*, **1974**, *143*, 129-135.

[77] Hernandez Valladares, M.; Felici, A.; Weber, G.; Adolph, H. W.; Zeppezauer, M.; Rossolini, G. M.; Amicosante, G.; Frere, J. M.; Galleni, M. Zn(II) dependence of the Aeromonas hydrophila AE036 metallo-beta-lactamase activity and stability. *Biochemistry*, **1997**, *36*, 11534-11541.

[78] Bebrone, C. Metallo-beta-lactamases (classification, activity, genetic organization, structure, zinc coordination) and their superfamily. *Biochem Pharmacol*, **2007**, *74*, 1686-1701.

[79] Avison, M. B.; Higgins, C. S.; von Heldreich, C. J.; Bennett, P. M.; Walsh, T. R. Plasmid location and molecular heterogeneity of the L1 and L2 beta-lactamase genes of Stenotrophomonas maltophilia. *Antimicrob Agents Chemother*, **2001**, *45*, 413-419.

[80] Bellais, S.; Leotard, S.; Poirel, L.; Naas, T.; Nordmann, P. Molecular characterization of a carbapenem-hydrolyzing beta-lactamase from Chryseobacterium (Flavobacterium) indologenes. *FEMS Microbiol Lett*, **1999**, *171*, 127-132.

[81] Bellais, S.; Naas, T.; Nordmann, P. Genetic and biochemical characterization of CGB-1, an Ambler class B carbapenem-hydrolyzing beta-lactamase from Chryseobacterium gleum. *Antimicrob Agents Chemother*, **2002**, *46*, 2791-2796.

[82] Bellais, S.; Poirel, L.; Leotard, S.; Naas, T.; Nordmann, P. Genetic diversity of carbapenem-hydrolyzing metallo-beta-lactamases from Chryseobacterium (Flavobacterium) indologenes. *Antimicrob Agents Chemother*, **2000**, *44*, 3028-3034.

[83] Chen, Y.; Succi, J.; Tenover, F. C.; Koehler, T. M. Beta-lactamase genes of the penicillin-susceptible Bacillus anthracis Sterne strain. *J Bacteriol*, **2003**, *185*, 823-830.

[84] Docquier, J. D.; Pantanella, F.; Giuliani, F.; Thaller, M. C.; Amicosante, G.; Galleni, M.; Frere, J. M.; Bush, K.; Rossolini, G. M. CAU-1, a subclass B3 metallo-beta-lactamase of low substrate affinity encoded by an ortholog present in the Caulobacter crescentus chromosome. *Antimicrob Agents Chemother*, **2002**, *46*, 1823-1830.

[85] Lim, H. M.; Pene, J. J.; Shaw, R. W. Cloning, nucleotide sequence, and expression of the Bacillus cereus 5/B/6 beta-lactamase II structural gene. *J Bacteriol*, **1988**, *170*, 2873-2878.

[86] Mammeri, H.; Bellais, S.; Nordmann, P. Chromosome-encoded beta-lactamases TUS-1 and MUS-1 from Myroides odoratus and Myroides odoratimimus (formerly Flavobacterium odoratum), new members of the lineage of molecular subclass B1 metalloenzymes. *Antimicrob Agents Chemother*, **2002**, *46*, 3561-3567.

[87] Naas, T.; Bellais, S.; Nordmann, P. Molecular and biochemical characterization of a carbapenem-hydrolysing beta-lactamase from Flavobacterium johnsoniae. *J Antimicrob Chemother*, **2003**, *51*, 267-273.

[88] Rossolini, G. M.; Condemi, M. A.; Pantanella, F.; Docquier, J. D.; Amicosante, G.; Thaller, M. C. Metallo-beta-lactamase producers in environmental microbiota: new molecular class B enzyme in Janthinobacterium lividum. *Antimicrob Agents Chemother*, **2001**, *45*, 837-844.

[89] Rossolini, G. M.; Franceschini, N.; Riccio, M. L.; Mercuri, P. S.; Perilli, M.; Galleni, M.; Frere, J. M.; Amicosante, G. Characterization and sequence of the Chryseobacterium (Flavobacterium) meningosepticum carbapenemase: a new molecular class B beta-lactamase showing a broad substrate profile. *Biochem J*, **1998**, *332 (Pt 1)*, 145-152.

[90] Saavedra, M. J.; Peixe, L.; Sousa, J. C.; Henriques, I.; Alves, A.; Correia, A. Sfh-I, a subclass B2 metallo-beta-lactamase from a Serratia fonticola environmental isolate. *Antimicrob Agents Chemother*, **2003**, *47*, 2330-2333.

[91] Simm, A. M.; Higgins, C. S.; Pullan, S. T.; Avison, M. B.; Niumsup, P.; Erdozain, O.; Bennett, P. M.; Walsh, T. R. A novel metallo-beta-lactamase, Mbl1b, produced by the environmental bacterium Caulobacter crescentus. *FEBS Lett*, **2001**, *509*, 350-354.

[92] Walsh, T. R.; Hall, L.; Assinder, S. J.; Nichols, W. W.; Cartwright, S. J.; MacGowan, A. P.; Bennett, P. M. Sequence analysis of the L1 metallo-beta-lactamase from Xanthomonas maltophilia. *Biochim Biophys Acta*, **1994**, *1218*, 199-201.

[93] Walsh, T. R.; Neville, W. A.; Haran, M. H.; Tolson, D.; Payne, D. J.; Bateson, J. H.; MacGowan, A. P.; Bennett, P. M. Nucleotide and amino acid sequences of the metallo-beta-lactamase, ImiS, from Aeromonas veronii bv. sobria. *Antimicrob Agents Chemother*, **1998**, *42*, 436-439.

[94] Woodford, N.; Palepou, M. F.; Babini, G. S.; Holmes, B.; Livermore, D. M. Carbapenemases of Chryseobacterium (Flavobacterium) meningosepticum: distribution of blaB and characterization of a novel metallo-beta-lactamase gene, blaB3, in the type strain, NCTC 10016. *Antimicrob Agents Chemother*, **2000**, *44*, 1448-1452.

[95] Yang, Y.; Bush, K. Biochemical characterization of the carbapenem-hydrolyzing beta-lactamase AsbM1 from Aeromonas sobria AER 14M: a member of a novel subgroup of metallo-beta-lactamases. *FEMS Microbiol Lett*, **1996**, *137*, 193-200.

[96] Spencer, J.; Read, J.; Sessions, R. B.; Howell, S.; Blackburn, G. M.; Gamblin, S. J. Antibiotic recognition by binuclear metallo-beta-lactamases revealed by X-ray crystallography. *J Am Chem Soc*, **2005**, *127*, 14439-14444.

[97] He, Z.; Zhang, J.; Shi, X. H.; Hu, L. L.; Kong, X.; Cai, Y. D.; Chou, K. C. Predicting drug-target interaction networks based on functional groups and biological features. *PLoS ONE*, **2010**, *5*, e9603.

[98] Schnell, J. R.; Chou, J. J. Structure and mechanism of the M2 proton channel of influenza A virus. *Nature*, **2008**, *451*, 591-595.

[99] Pielak, R. M.; Jason R. Schnell, J. R.; Chou, J. J. Mechanism of drug inhibition and drug resistance of influenza A M2 channel. *Proceedings of National Academy of Science, USA*, **2009**, *106*, 7379-7384.

[100] Du, Q. S.; Wang, S.; Wei, D. Q.; Sirois, S.; Chou, K. C. Molecular modelling and chemical modification for finding peptide inhibitor against SARS CoV Mpro. *Anal. Biochem.*, **2005**, *337*, 262-270.

[101] Huang, R. B.; Du, Q. S.; Wang, C. H.; Chou, K. C. An in-depth analysis of the biological functional studies based on the NMR M2 channel structure of influenza A virus. *Biochem. Biophys. Res. Comm.*, **2008**, *377*, 1243-1247.

[102] Du, Q. S.; Huang, R. B.; Wang, C. H.; Li, X. M.; Chou, K. C. Energetic analysis of the two controversial drug binding sites of the M2 proton channel in influenza A virus. *J. Theor. Biol.*, **2009**, *259*, 159-164.

[103] Wang, S. Q.; Du, Q. S.; Huang, R. B.; Zhang, D. W.; Chou, K. C. Insights from investigating the interaction of oseltamivir (Tamiflu) with neuraminidase of the 2009 H1N1 swine flu virus. *Biochem. Biophys. Res. Commun.*, **2009**, *386*, 432-436.

[104] Cai, L.; Wang, Y.; Wang, J. F.; Chou, K. C. Identification of Proteins Interacting with Human SP110 During the Process of Viral Infections. *Medicinal Chemistry*, **2011**, *7*, 121-126.

[105] Liao, Q. H.; Gao, Q. Z.; Wei, J.; Chou, K. C. Docking and Molecular Dynamics Study on the Inhibitory Activity of Novel Inhibitors on Epidermal Growth Factor Receptor (EGFR). *Medicinal Chemistry*, **2011**, *7*, 24-31.

[106] Wang, J. F.; Chou, K. C. Insights from modeling the 3D structure of New Delhi metallo-beta-lactamase and its binding interactions with antibiotic drugs. *PLoS ONE* **2011**, *6*, e18414.

[107] Wang, J. F.; Chou, K. C. Insights into the mutation-induced HHH syndrome from modeling human mitochondrial ornithine transporter-1. *PLoS One*, **2012**, *7*, e31048.

[108] Chou, K. C.; Chen, N. Y. The biological functions of low-frequency phonons. *Scientia Sinica*, **1977**, *20*, 447-457.

[109] Chou, K. C. Identification of low-frequency modes in protein molecules. *Biochem. J.*, **1983**, *215*, 465-469.

[110] Martel, P. Biophysical aspects of neutron scattering from vibrational modes of proteins. *Prog Biophys Mol Biol*, **1992**, *57*, 129-179.

[111] Chou, K. C. Low-frequency vibration of DNA molecules. *Biochem. J.*, **1984**, *221*, 27-31.

[112] Chou, K. C. Low-frequency motions in protein molecules: beta-sheet and beta-barrel. *Biophys. J.*, **1985**, *48*, 289-297.

[113] Chou, K. C.; Maggiora, G. M.; Mao, B. Quasi-continuum models of twist-like and accordion-like low-frequency motions in DNA. *Biophys. J.*, **1989**, *56*, 295-305.

[114] Chou, K. C. Biological functions of low-frequency vibrations (phonons). III. Helical structures and microenvironment. *Biophys. J.*, **1984**, *45*, 881-889.

[115] Wang, J. F.; Chou, K. C. Insight into the molecular switch mechanism of human Rab5a from molecular dynamics simulations. *Biochem. Biophys. Res. Commun.*, **2009**, *390*, 608-612.

[116] Chou, K. C. Low-frequency resonance and cooperativity of hemoglobin. *Trends Biochem. Sci.*, **1989**, *14*, 212-213.

[117] Chou, K. C. The biological functions of low-frequency phonons. 4. Resonance effects and allosteric transition. *Biophysical Chemistry*, **1984**, *20*, 61-71.

[118] Chou, K. C. The biological functions of low-frequency phonons: 6. A possible dynamic mechanism of allosteric transition in antibody molecules. *Biopolymers*, **1987**, *26*, 285-295.

[119] Chou, K. C.; Mao, B. Collective motion in DNA and its role in drug intercalation. *Biopolymers*, **1988**, *27*, 1795-1815.

[120] Chou, K. C.; Zhang, C. T.; Maggiora, G. M. Solitary wave dynamics as a mechanism for explaining the internal motion during microtubule growth. *Biopolymers*, **1994**, *34*, 143-153.

[121] Chou, K. C. Review: Low-frequency collective motion in biomacromolecules and its biological functions. *Biophysical Chemistry*, **1988**, *30*, 3-48.

[122] Gordon, G. Extrinsic electromagnetic fields, low frequency (phonon) vibrations, and control of cell function: a non-linear resonance system. *Journal of Biomedical Science and Engineering (JBiSE)*, **2008**, *1*, 152-156 (open accessible at http://www.srpublishing.org/journal/jbise/).

[123] Gordon, G. Designed Electromagnetic Pulsed Therapy: Clinical Applications. *J. Cell. Physiol.*, **2007**, *212*, 579-582.

[124] Madkan, A.; Blank, M.; Elson, E.; Chou, K. C.; Geddis, M. S.; Goodman, R. Steps to the clinic with ELF EMF (doi:10.4236/ns.2009.13020). *Natural Science* **2009**, *1*, 157-165 (openly accessible at http://www.scirp.org/journal/NS/).

[125] Zeng, Q. K.; Du, H. L.; Wang, J. F.; Wei, D. Q.; Wang, X. N.; Li, Y. X.; Lin, Y. Reversal of coenzyme specificity and improvement of catalytic efficiency of Pichia stipitis xylose reductase by rational site-directed mutagenesis. *Biotechnol Lett*, **2009**, *31*, 1025-1029.

[126] Wang, J. F.; Wei, D. Q.; Li, L.; Zheng, S. Y.; Li, Y. X.; Chou, K. C. 3D structure modeling of cytochrome P450 2C19 and its implication for personalized drug design. *Biochem Biophys Res Commun*, **2007**, *355*, 513-519.

[127] Wang, J. F.; Wei, D. Q.; Chen, C.; Li, Y.; Chou, K. C. Molecular modeling of two CYP2C19 SNPs and its implications for personalized drug design. *Protein Pept Lett*, **2008**, *15*, 27-32.

[128] Wang, J. F.; Chou, K. C. Insights from modeling the 3D structure of New Delhi metallo-beta-lactamse and its binding interactions with antibiotic drugs. *PLoS One*, **2011**, *6*, e18414.

[129] Wang, J. F.; Chou, K. C. Molecular modeling of cytochrome P450 and drug metabolism. *Curr Drug Metab*, **2010**, *11*, 342-346.

[130] Wang, J. F.; Chou, K. C. Insight into the molecular switch mechanism of human Rab5a from molecular dynamics simulations. *Biochem Biophys Res Commun*, **2009**, *390*, 608-612.

[131] Li, J.; Wei, D. Q.; Wang, J. F.; Yu, Z. T.; Chou, K. C. Molecular dynamics simulations of CYP2E1. *Med Chem*, **2012**, *8*, 208-221.

[132] He, J.; Wei, D. Q.; Wang, J. F.; Chou, K. C. Predicting protein-ligand binding sites based on an improved geometric algorithm. *Protein Pept Lett*, **2011**, *18*, 997-1001.

[133] Chen, Q.; Zhang, T.; Wang, J. F.; Wei, D. Q. Advances in human cytochrome p450 and personalized medicine. *Curr Drug Metab*, **2011**, *12*, 436-444.

[134] Wang, J. F.; Chou, K. C. Insights from studying the mutation-induced allostery in the M2 proton channel by molecular dynamics. *Protein Eng Des Sel*, **2010**, *23*, 663-666.

[135] Tang, B.; Gong, K.; Wang, J. F.; Li, Y. X.; Wei, D. Q. The structure of phospholamban and its MD simulations. *Chinese Sci Bull*, **2010**, *55*, 1619-1624.

[136] Zhang, L. S.; Wang, S. Q.; Xu, W. R.; Wang, R. L.; Wang, J. F. Scaffold-Based Pan-Agonist Design for the PPARalpha, PPARbeta and PPARgamma Receptors. *PLoS One*, **2012**, *7*, e48453.

[137] Wang, J. F.; Chou, K. C. Recent advances in computational studies on influenza a virus M2 proton channel. *Mini Rev Med Chem*, **2012**, *12*, 971-978.
[138] Wang, J. F.; Yan, J. Y.; Wei, D. Q.; Chou, K. C. Binding of CYP2C9 with diverse drugs and its implications for metabolic mechanism. *Med Chem*, **2009**, *5*, 263-270.
[139] Ping, J.; Wang, Y. J.; Wang, J. F.; Li, X.; Li, Y. X.; Hao, P. Negatively cooperative binding properties of human cytochrome P450 2E1 with monocyclic substrates. *Curr Drug Metab*, **2012**, *13*, 1024-1031.
[140] Lian, P.; Wei, D. Q.; Wang, J. F.; Chou, K. C. An allosteric mechanism inferred from molecular dynamics simulations on phospholamban pentamer in lipid membranes. *PLoS One*, **2011**, *6*, e18587.
[141] Li, J.; Wei, D. Q.; Wang, J. F.; Li, Y. X. A negative cooperativity mechanism of human CYP2E1 inferred from molecular dynamics simulations and free energy calculations. *J Chem Inf Model*, **2011**, *51*, 3217-3225.
[142] Gu, H.; Chen, H. F.; Wei, D. Q.; Wang, J. F. Molecular dynamics simulations exploring drug resistance in HIV-1 protease. *Chinese Sci Bull*, **2010**, *55*, 2677-2683.
[143] Wang, J. F.; Wei, D. Q.; Chou, K. C. Drug candidates from traditional chinese medicines. *Curr Top Med Chem*, **2008**, *8*, 1656-1665.
[144] Guo, X.; Wang, J. F.; Zhu, Y.; Wei, D. Q. Recent Progress on Computer-Aided Inhibitor Design of H5N1 Influenza A Virus. *Curr Comput Aided Drug Des*, **2010**, *6*, 139-146.
[145] Wang, Z.; Fast, W.; Benkovic, S. J. On the mechanism of the metallo-beta-lactamase from Bacteroides fragilis. *Biochemistry*, **1999**, *38*, 10013-10023.
[146] Park, H.; Brothers, E. N.; Merz, K. M., Jr. Hybrid QM/MM and DFT investigations of the catalytic mechanism and inhibition of the dinuclear zinc metallo-beta-lactamase CcrA from Bacteroides fragilis. *J Am Chem Soc*, **2005**, *127*, 4232-4241.
[147] Crowder, M. W.; Spencer, J.; Vila, A. J. Metallo-beta-lactamases: novel weaponry for antibiotic resistance in bacteria. *Acc Chem Res*, **2006**, *39*, 721-728.
[148] Wu, S.; Xu, D.; Guo, H. QM/MM studies of monozinc beta-lactamase CphA suggest that the crystal structure of an enzyme-intermediate complex represents a minor pathway. *J Am Chem Soc*, **2010**, *132*, 17986-17988.
[149] Bebrone, C.; Delbruck, H.; Kupper, M. B.; Schlomer, P.; Willmann, C.; Frere, J. M.; Fischer, R.; Galleni, M.; Hoffmann, K. M. The structure of the dizinc subclass B2 metallo-beta-lactamase CphA reveals that the second inhibitory zinc ion binds in the histidine site. *Antimicrob Agents Chemother*, **2009**, *53*, 4464-4471.
[150] Simona, F.; Magistrato, A.; Dal Peraro, M.; Cavalli, A.; Vila, A. J.; Carloni, P. Common mechanistic features among metallo-beta-lactamases: a computational study of Aeromonas hydrophila CphA enzyme. *J Biol Chem*, **2009**, *284*, 28164-28171.
[151] Miller, L. A.; Ratnam, K.; Payne, D. J. Beta-lactamase-inhibitor combinations in the 21st century: current agents and new developments. *Curr Opin Pharmacol*, **2001**, *1*, 451-458.
[152] Toney, J. H. Metallo-beta-lactamase inhibitors: could they give old antibacterials new life? *Curr Opin Investig Drugs*, **2003**, *4*, 115-116.
[153] Toney, J. H.; Cleary, K. A.; Hammond, G. G.; Yuan, X.; May, W. J.; Hutchins, S. M.; Ashton, W. T.; Vanderwall, D. E. Structure-activity relationships of biphenyl tetrazoles as metallo-beta-lactamase inhibitors. *Bioorg Med Chem Lett*, **1999**, *9*, 2741-2746.
[154] Toney, J. H.; Fitzgerald, P. M.; Grover-Sharma, N.; Olson, S. H.; May, W. J.; Sundelof, J. G.; Vanderwall, D. E.; Cleary, K. A.; Grant, S. K.; Wu, J. K.; Kozarich, J. W.; Pompliano, D. L.; Hammond, G. G. Antibiotic sensitization using biphenyl tetrazoles as potent inhibitors of Bacteroides fragilis metallo-beta-lactamase. *Chem Biol*, **1998**, *5*, 185-196.
[155] Bounaga, S.; Galleni, M.; Laws, A. P.; Page, M. I. Cysteinyl peptide inhibitors of Bacillus cereus zinc beta-lactamase. *Bioorg Med Chem*, **2001**, *9*, 503-510.
[156] Buynak, J. D.; Chen, H.; Vogeti, L.; Gadhachanda, V. R.; Buchanan, C. A.; Palzkill, T.; Shaw, R. W.; Spencer, J.; Walsh, T. R. Penicillin-derived inhibitors that simultaneously target both metallo- and serine-beta-lactamases. *Bioorg Med Chem Lett*, **2004**, *14*, 1299-1304.
[157] Payne, D. J.; Du, W.; Bateson, J. H. beta-Lactamase epidemiology and the utility of established and novel beta-lactamase inhibitors. *Expert Opin Investig Drugs*, **2000**, *9*, 247-261.
[158] Payne, D. J.; Bateson, J. H.; Gasson, B. C.; Khushi, T.; Proctor, D.; Pearson, S. C.; Reid, R. Inhibition of metallo-beta-lactamases by a series of thiol ester derivatives of mercaptophenylacetic acid. *FEMS Microbiol Lett*, **1997**, *157*, 171-175.
[159] Hammond, G. G.; Huber, J. L.; Greenlee, M. L.; Laub, J. B.; Young, K.; Silver, L. L.; Balkovec, J. M.; Pryor, K. D.; Wu, J. K.; Leiting, B.; Pompliano, D. L.; Toney, J. H. Inhibition of IMP-1 metallo-beta-lactamase and sensitization of IMP-1-producing bacteria by thioester derivatives. *FEMS Microbiol Lett*, **1999**, *179*, 289-296.
[160] Fitzgerald, P. M.; Wu, J. K.; Toney, J. H. Unanticipated inhibition of the metallo-beta-lactamase from Bacteroides fragilis by 4-morpholineethanesulfonic acid (MES): a crystallographic study at 1.85-A resolution. *Biochemistry*, **1998**, *37*, 6791-6800.
[161] Siemann, S.; Clarke, A. J.; Viswanatha, T.; Dmitrienko, G. I. Thiols as classical and slow-binding inhibitors of IMP-1 and other binuclear metallo-beta-lactamases. *Biochemistry*, **2003**, *42*, 1673-1683.
[162] Scrofani, S. D.; Chung, J.; Huntley, J. J.; Benkovic, S. J.; Wright, P. E.; Dyson, H. J. NMR characterization of the metallo-beta-lactamase from Bacteroides fragilis and its interaction with a tight-binding inhibitor: role of an active-site loop. *Biochemistry*, **1999**, *38*, 14507-14514.
[163] Mollard, C.; Moali, C.; Papamicael, C.; Damblon, C.; Vessilier, S.; Amicosante, G.; Schofield, C. J.; Galleni, M.; Frere, J. M.; Roberts, G. C. Thiomandelic acid, a broad spectrum inhibitor of zinc beta-lactamases: kinetic and spectroscopic studies. *J Biol Chem*, **2001**, *276*, 45015-45023.
[164] Kurosaki, H.; Yasuzawa, H.; Yamaguchi, Y.; Jin, W.; Arakawa, Y.; Goto, M. Detection of a metallo-beta-lactamase (IMP-1) by fluorescent probes having dansyl and thiol groups. *Org Biomol Chem*, **2003**, *1*, 17-20.
[165] Jin, W.; Arakawa, Y.; Yasuzawa, H.; Taki, T.; Hashiguchi, R.; Mitsutani, K.; Shoga, A.; Yamaguchi, Y.; Kurosaki, H.; Shibata, N.; Ohta, M.; Goto, M. Comparative study of the inhibition of metallo-beta-lactamases (IMP-1 and VIM-2) by thiol compounds that contain a hydrophobic group. *Biol Pharm Bull*, **2004**, *27*, 851-856.
[166] Goto, M.; Takahashi, T.; Yamashita, F.; Koreeda, A.; Mori, H.; Ohta, M.; Arakawa, Y. Inhibition of the metallo-beta-lactamase produced from Serratia marcescens by thiol compounds. *Biol Pharm Bull*, **1997**, *20*, 1136-1140.
[167] Arakawa, Y.; Shibata, N.; Shibayama, K.; Kurokawa, H.; Yagi, T.; Fujiwara, H.; Goto, M. Convenient test for screening metallo-beta-lactamase-producing gram-negative bacteria by using thiol compounds. *J Clin Microbiol*, **2000**, *38*, 40-43.
[168] Payne, D. J.; Hueso-Rodriguez, J. A.; Boyd, H.; Concha, N. O.; Janson, C. A.; Gilpin, M.; Bateson, J. H.; Cheever, C.; Niconovich, N. L.; Pearson, S.; Rittenhouse, S.; Tew, D.; Diez, E.; Perez, P.; De La Fuente, J.; Rees, M.; Rivera-Sagredo, A. Identification of a series of tricyclic natural products as potent broad-spectrum inhibitors of metallo-beta-lactamases. *Antimicrob Agents Chemother*, **2002**, *46*, 1880-1886.
[169] Walter, M. W.; Felici, A.; Galleni, M.; Soto, R. P.; Adington, R. M.; Baldwin, J. E.; Frere, J. M.; Gololobov, M.; Schofield, C. J. Trifluoromethyl alcohol and ketone inhibitors of metallo-beta-lactamases. *Bioorg Med Chem Lett*, **1996**, *6*, 2455-2458.
[170] Siemann, S.; Evanoff, D. P.; Marrone, L.; Clarke, A. J.; Viswanatha, T.; Dmitrienko, G. I. N-arylsulfonyl hydrazones as inhibitors of IMP-1 metallo-beta-lactamase. *Antimicrob Agents Chemother*, **2002**, *46*, 2450-2457.
[171] Toney, J. H.; Hammond, G. G.; Fitzgerald, P. M.; Sharma, N.; Balkovec, J. M.; Rouen, G. P.; Olson, S. H.; Hammond, M. L.; Greenlee, M. L.; Gao, Y. D. Succinic acids as potent inhibitors of plasmid-borne IMP-1 metallo-beta-lactamase. *J Biol Chem*, **2001**, *276*, 31913-31918.
[172] Greenlee, M. L.; Laub, J. B.; Balkovec, J. M.; Hammond, M. L.; Hammond, G. G.; Pompliano, D. L.; Epstein-Toney, J. H. Synthesis and SAR of thioester and thiol inhibitors of IMP-1 metallo-beta-lactamase. *Bioorg Med Chem Lett*, **1999**, *9*, 2549-2554.

[173] Nagano, R.; Adachi, Y.; Imamura, H.; Yamada, K.; Hashizume, T.; Morishima, H. Carbapenem derivatives as potential inhibitors of various beta-lactamases, including class B metallo-beta-lactamases. *Antimicrob Agents Chemother*, **1999**, *43*, 2497-2503.

[174] Nagano, R.; Adachi, Y.; Hashizume, T.; Morishima, H. *In vitro* antibacterial activity and mechanism of action of J-111,225, a novel 1beta-methylcarbapenem, against transferable IMP-1 metallo-beta-lactamase producers. *J Antimicrob Chemother*, **2000**, *45*, 271-276.

[175] Quiroga, M. I.; Franceschini, N.; Rossolini, G. M.; Gutkind, G.; Bonfiglio, G.; Franchino, L.; Amicosante, G. Interaction of cefotetan and the metallo-beta-lactamases produced in Aeromonas spp. and *in vitro* activity. *Chemotherapy*, **2000**, *46*, 177-183.

[176] Tsang, W. Y.; Dhanda, A.; Schofield, C. J.; Frere, J. M.; Galleni, M.; Page, M. I. The inhibition of metallo-beta-lactamase by thioxo-cephalosporin derivatives. *Bioorg Med Chem Lett*, **2004**, *14*, 1737-1739.

Received: December 14, 2012 **Revised: March 26, 2013** **Accepted: April 08, 2013**