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Succinic Acids as Potent Inhibitors of Plasmid-borne IMP-1 Metallo-β-lactamase*

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IMP-1 metallo-β-lactamase (class B) is a plasmid-borne zinc metalloenzyme that efficiently hydrolyzes β-lactam antibiotics, including carbapenems, rendering them ineffective. Because IMP-1 has been found in several clinically important carbapenem-resistant pathogens, there is a need for inhibitors of this enzyme that could protect broad spectrum antibiotics such as imipenem from hydrolysis and thus extend their utility. We have identified a series of 2,3-(*S,S*)-disubstituted succinic acids that are potent inhibitors of IMP-1. Determination of high resolution crystal structures and molecular modeling of succinic acid inhibitor complexes with IMP-1 has allowed an understanding of the potency, stereochemistry, and structure-activity relationships of these inhibitors.

Carbapenems such as imipenem (Scheme 1) have proven useful for the treatment of a variety of Gram-negative and Gram-positive infections (1, 2). Carbapenems and other β-lactam antibiotics covalently modify penicillin-binding proteins (PBPs)[1] involved in the peptidoglycan biosynthetic pathway of cell wall assembly in bacteria (3, 4). Resistance to carbapenems can arise because of acquisition of low affinity PBPs (3) (*e.g.* PBP2a of *Staphylococcus aureus*), altered membrane permeability (5), and expression of class A, B, and D β-lactamases (6–10). Class B β-lactamases (metallo-β-lactamases or MBLs) can hydrolyze a wide variety of substrates of the β-lactam class, including carbapenems, penicillins, and cephalosporins, rendering them ineffective as antibiotics.

The *IMP-1* gene encoding an MBL has been identified on a plasmid and in Japan has transferred among clinical isolates such as *Pseudomonas aeruginosa* (11, 12), *Klebsiella pneumoniae*, *Serratia marcescens,* and other members of the Enterobacteriaceae (13, 14). In addition, carbapenem-resistant clinical isolates expressing MBLs related to IMP-1 have been identified recently in Singapore (15), Italy (16), and Hong Kong (10). Such reports of plasmid-borne imipenem resistance highlight the need for inhibitors of IMP-1 that can restore the activity of carbapenems in resistant bacteria.

Several classes of MBL inhibitors have been reported (for reviews, see Refs. 17 and 18)) including phenazines (19), trifluoromethyl alcohol and ketone derivatives of L- and D-alanine (20), thioesters (18, 21–23), thiols (24–28), biphenyl tetrazoles (29, 30), and amino acid-derived hydroxamates (31). Biphenyl tetrazoles have been shown to reverse imipenem resistance in a clinical isolate of *Bacteroides fragilis* (29), and thioesters have been shown to reverse resistance to the carbapenem L-742,728 in a laboratory strain of *Escherichia coli* expressing IMP-1 (32). A 1β-methylcarbapenem substituted at C-2 with a benzothienylthio moiety has been reported to be a potent IMP-1 inhibitor that can reverse resistance to imipenem in an IMP-1-producing strain of *Serratia marcescens* (33). Although the inhibitors described above have been reported to have good activity against a specific MBL, only certain thiols (*e.g.* SB 264218) exhibit broad spectrum inhibition of MBLs (18).

The x-ray crystal structures of MBLs from several sources have been determined, including enzymes from *Bacillus cereus* containing one zinc atom (34) or two zinc atoms (35, 36) in the active site, from *B. fragilis* as a binuclear zinc enzyme (37, 38) or as a binuclear cadmium or heterometallic zinc/mercury enzyme (39), and from *Stenotrophomonas maltophilia* (40). The structures of enzyme-inhibitor complexes have also been reported in the case of the *B. fragilis* MBL bound to MES (41) and to a biphenyl tetrazole (29) or, more recently, IMP-1 bound to a mercaptocarboxylate (28). Taken together, the structures of the MBLs reveal a heterogeneous family of enzymes having conserved residues at the active site, with considerable variation in the conformation of a β-strand positioned above the binuclear metal center. Such structural heterogeneity within the MBL family is consistent with the paucity of reported broad spectrum inhibitors. Nevertheless, the knowledge of protein structure for several members of the MBL family offers the opportunity for structure-aided design of inhibitors as well as a better understanding of structure-activity relationships.

We report here that 2,3-(*S,S*)-disubstituted succinic acids are potent inhibitors of the IMP-1 enzyme and describe the mode of binding using x-ray crystallography and molecular modeling. Recently, members of this class of inhibitors were reported to reverse imipenem resistance in clinical isolates of *P. aeruginosa* expressing IMP-1.[2] The structure-activity relationships for succinic acids as inhibitors of IMP-1 can be understood by

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The atomic coordinates and structure factors (codes 1JJE and 1JJT) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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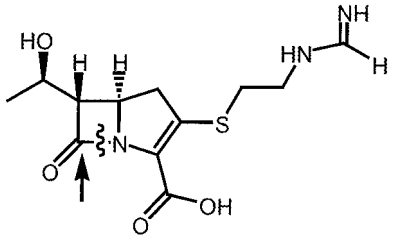
[1] The abbreviations used are: PBPs, penicillin-binding proteins; MBLs, metallo-β-lactamases; MOPS, 4-morpholinepropanesulfonic acid; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; MES, 4-morpholineethanesulfonic acid; CCD, charge-coupled device.

[2] J. Huber, K. Young, R. E. Painter, H. Rosen, and L. L. Silver, poster no. 1226 presented at the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Canada (September 17–20, 2000).



SCHEME 1. **Chemical structure of the IMP-1 substrate imipenem.** The *arrow* indicates the site of nucleophilic attack.

comparing the crystal structures of enzyme-inhibitor complexes and by molecular modeling of the inhibitors docked into the active site.

EXPERIMENTAL PROCEDURES

The IMP-1 metallo-β-lactamase lacking the N-terminal 18 hydrophobic amino acids was expressed and purified as described (32). Enzyme activity was assessed using the chromogenic substrate nitrocefin (Oxoid, Hampshire, UK). Substituted succinic acids were synthesized as described.[3] Succinic acid (butanedioic acid) (compound **5**) was obtained from Sigma. 2,2-Dimethyl succinic acid (compound **7**) and racemic 2-methyl-2-ethyl succinic acid (compound **8**) were obtained from Aldrich Chemical. All other chemicals were of reagent grade.

Kinetic Experiments—Hydrolysis of nitrocefin was monitored at 490 nm using a SPECTRAmax™ 250 plate reader (Molecular Devices, Sunnyvale, CA) in a final volume of 100 μl. The 50% inhibitory concentration (IC_{50}) for each compound was determined as described (32). Briefly, the IMP-1 enzyme (typically at a final concentration of 0.2 nM in 50 mM MOPS, pH 7, 2 mM CHAPS) was preincubated with compounds for 15 min at 37 °C. Initial velocities were measured after the addition of substrate at a concentration near K_m (60 μM).

Crystallography—Protein for crystallographic experiments was concentrated to ~10 mg/ml in a buffer containing 10 mM Tris(hydroxymethyl)aminomethane (Tris), pH 7.2, 2 mM dithiothreitol, and 0.1% β-octylglucoside. Crystals of IMP-1 in the presence of compound **1** were grown at 37 °C in hanging drop experiments by mixing equal volumes of protein solution with a well solution containing 25–32% polyethylene glycol momomethyl ether 550, 2 mM zinc acetate, and 100 mM MES, pH 6.7–7.1. The crystals were stable only if the crystallization experiments were maintained at 37 °C until crystal growth was complete (~1 week). The growth of crystals was irreproducible, and therefore a new construct of the protein, lacking 6 residues at the C terminus, which were found to be disordered in the structure of the complex with compound **1,** was cloned, expressed, and purified. Crystals of the complex with compound **11** were grown using this truncated construct (residues 1–222) under the same conditions described for the complex with compound **1**.

All diffraction data were measured at beamline 17-ID at the Advanced Photon Source, Argonne National Laboratory. Data were measured at ~100° K using crystals that were flash-frozen in a liquid nitrogen stream; the crystallization mother liquor was an adequate cryoprotectant. The data for the complex with compound **1** were measured using an ADSC Quantum-4 CCD detector mounted on a Bruker goniostat. The data for the complex with compound **11** were measured using a MAR165 CCD detector mounted on a Mar goniostat. All data processing calculations were performed with program X-gen (43); the data processing statistics are given in Table I.

The structure of the complex with compound **1** was phased using molecular replacement calculations preformed in the Merlot program package (44). The starting model was the structure of the IMP-1 metallo-β-lactamase determined previously in this laboratory in a different space group.[4] The results from both the rotation and translation function calculations were unambiguous.

The structure of the complex with compound **1** was initially refined in a number of cycles of iterative least-squares refinement and manual refitting of the model using programs Prolsq (45) and X-plor (46). At a later date, a data set at 1.30 Å resolution was obtained for a complex with a different succinic acid inhibitor, and that complex was refined using the program Shelxl (47). The protein model from that high resolution refinement was the starting point for the final refinements of the structures of both complexes reported here, again using the program Shelxl. All data with F greater than -3 sigma(F) were included in the calculations, and refinement was against F^2. The diffraction patterns for both complexes are extremely anisotropic; this was modeled in Shelxl using the HOPE overall anisotropic scaling procedure. The statistics for the refinement are given in Table I. The coordinates and structure factors for both complexes have been deposited with the Protein Data Bank (entry codes 1JJE and 1JJT).

Molecular Modeling—Compound **8** was docked manually into the enzyme active site using the x-ray structure of the complex between IMP-1 and compound **1**. One hundred conformations of compound **8** were then generated using the method described by Feuston *et al*. (48). This conformation set contains both enantiomers of compound **8**. The energy minimizations for the complexes of the IMP-1 enzyme and compound **8** conformers were carried out using two steps. First, the protein backbone was fixed, and only the inhibitor and enzyme side chains were minimized for 1000 steps. Next, the complexes were minimized by 2000 steps using harmonic atom constraints (k = 1.0 kcal/A^2) on the backbone atoms. The ABNR (adopted basis Newton Raphson) minimization algorithm and the Merck Mechanical Force Field (49) were used for the minimizations.

Because compound **2** is a very close analog of compound **1,** it was assumed that the binding modes to the IMP-1 active site were the same as that of compound **1**. The structures were docked into the active site overlapping with the structure of **1** as it is shown in the crystal structure, and the IMP-1-inhibitor complexes were energy-minimized using the same procedure as described for compound **8**.

For compounds **3** (*R,R*) and **4** (*R,S*) and the two stereoisomers of compound **2** (*S,S*), 100 conformations were generated, respectively, using the method described in Feuston *et al*. (48) and were docked into the IMP-1 active site. A detailed discussion of the docking of compounds **3** and **4** can be found under "Results."

For imipenem, 100 conformations were generated using the method described by Feuston *et al.* (48) and docked into the IMP-1 active site using the x-ray structure of the complex between IMP-1 and compound **1**. The conformers of imipenem were mapped onto compound **1** using the SQ procedure developed by Miller *et al*. (50). Two carbon atoms in the carboxylate groups of compound **1** were selected as essential points for the mapping. The complexes between imipenem and the IMP-1 enzyme were then energy-minimized using the same procedure as described for compound **8**.

RESULTS

Screening of the Merck chemical collection led to the discovery of the (2*S*,3*S*)-disubstituted succinic acid **1** as a potent inhibitor of IMP-1 metallo-β-lactamase (IC_{50} = 0.009 μM, see Table II). Both the relative and absolute stereochemistries of compound **1** (and analogs) have been determined critical for activity against IMP-1. This point is illustrated by comparing the activities of the isomeric 2,3-dibenzylsuccinic acids **2**, **3**, and **4**.[3] The (*S,S*)-isomer **2** is a potent inhibitor of IMP-1 (IC_{50} = 0.0027 μM), whereas the (*R,R*)-isomer **3** ($IC_{50} \geq$ 0.21 μM) and the (*R,S*)-isomer **4** (IC_{50} = 200 μM) are far less active. In addition to the (2*S*,3*S*) stereochemical requirement, two substituents of significant hydrophobicity on the succinic acid core (as in compounds **1** and **2**) have been found necessary for IMP-1 inhibition. Succinic acid itself (**5**) is essentially inactive (IC_{50} = 6.3 mM), whereas (2*S*)-benzylsuccinic acid (**6**) is only weakly active (IC_{50} = 490 μM). The 2,2-disubstituted succinic acids **7** (2,2-dimethyl) and **8** (2-ethyl-2-methyl) were found inactive against IMP-1. The addition of an *(R)*-methyl group at one of the benzylic positions of **2** (compound **9**), led to approximately a 5-fold loss of activity (IC_{50} = 0.013 μM), whereas the addition of an (*S*)-methyl group (compound **10**) resulted in a drastic loss of activity ($IC_{50} \geq$ 2.7 μM). The unsymmetrical compound **11,** which is a hybrid of the symmetrical compounds **1** and **2,** was found to have an IC_{50} of 0.0037 μM.

The binding modes of compounds **1** and **11** with IMP-1 were revealed by x-ray crystallographic analysis as illustrated in Figs. 1 and 2. The structure of the complex with compound **1,** shown in Fig. 2*A*, reveals that whereas the bicyclic substituent in R^1 (see Table II) is comfortably accommodated in the enzyme active site,

[3] M. L. Greenlee, G. P. Rouen, S. H. Olson, T. M. Sisk, J. M. Balkovec, M. L. Hammond, G. G. Hammond, J. H. Toney, P. M. D. Fitzgerald, and J. L. Huber, submitted for publication.

[4] P. M. D. Fitzgerald, unpublished results.

TABLE I
Crystallographic data collection and refinement of IMP-1 complexed to succinic acids

	Complex	
	1	**11**
Space group	$P2_1$	$P2_1$
Cell dimensions		
a (Å)	71.80	71.44
b (Å)	45.18	43.20
c (Å)	64.51	64.23
β (°)	100.32	100.93
Data processing		
Resolution	∞ − 1.80 (1.91–1.80)	∞ − 1.80 (1.91–1.80)
Unique reflections	37199 (5552)	35791 (5910)
Completeness	98.0 (89.0)	99.7 (100.0)
Redundancy	3.31 (2.32)	3.59 (3.70)
R-merge[a]	0.077 (.274)	0.075 (0.500)
Mean *Y*/sig(*Y*)[b]	19.77 (2.29)	25.77 (2.73)
Refinement		
Resolution range	10.0–1.80 (1.88–1.80)	10.0–1.80 (1.87–1.80)
Reflections	35190 (3786)	34043 (3653)
R-work (all data)	0.199 (0.351)	0.201 (0.365)
R-free (all data)	0.296	0.291
R-work (data > 4 σ)	0.175	0.178
R-free (data > 4 σ)	0.273	0.263
Rms deviations from ideal geometry		
Distances (Å)	0.006	0.006
Angles (Å)	0.023	0.023
Anti-bumping (Å)	0.015	0.007
Chiral volumes (zero) (Å^3)	0.031	0.035
Chiral volumes (non-zero) (Å^3)	0.039	0.038
Planes (Å)	0.324	0.337

[a] $R_{merge} = \Sigma_{hkl} \Sigma_i|I(hkl,i) - \langle I(hkl)\rangle|/\Sigma_{hkl} \Sigma_i|I(hkl,i)|$.
[b] Y = average of all observations of a given reflection.

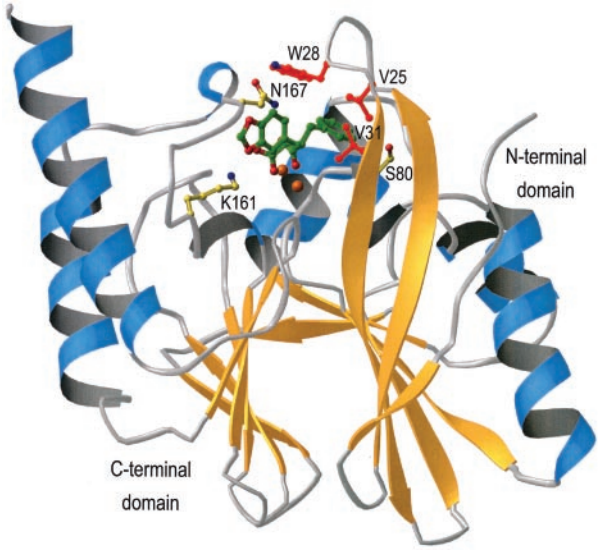


FIG. 1. **An overview of the structure of the complex between IMP-1 and compound 11.** There are two molecules in the crystallographic asymmetric unit, but the binding mode is roughly equivalent in both molecules, and therefore only one is illustrated. The zinc atoms are shown in *orange*, oxygen atoms in *red*, and nitrogen atoms in *blue*. Carbon atoms in the inhibitor are shown in *green*. Carbon atoms in the side chains of key residues in the active site (Ser-80, Lys-161, and Asn-167) are shown in *yellow*. Carbon atoms in the side chains of hydrophobic residues in the flap that interact with the inhibitor (Val-25, Trp-28, and Val-31) are shown in *red*.

the same substituent experiences strain in R^3 because of unfavorable steric interactions with the side chain of Ser-80. The complex with compound **11,** illustrated in Fig. 2*B*, shows that the smaller benzyl substituent at R^3 binds in an unstrained conformation.

Importantly, these structures also reveal the details of the interactions between the succinic acid group and the binuclear zinc cluster as illustrated in Fig. 2*C*. One oxygen of the right hand (as shown in Fig. 2*C*) carboxylate group interacts with both Zn-1 and Zn-2, whereas the second oxygen on the same carboxylate interacts with $N^{\delta 2}$ of Asn-167. One oxygen of the left-hand carboxylate interacts with Zn-2, and the second interacts with the main-chain nitrogen of Asn-167 as well as with N^{ϵ} of Lys-161. Thus, the succinic acid group is able to replace both the bridging and apical water positions that have been observed in unliganded structures of metallo-β-lactamases (37) as well as interact favorably with other key active site residues.

Stereoisomers **3** (*R,R*), **4** (*R,S*), and **2** (*S,S*) offer an opportunity to understand the structure-activity relationships of IMP-1 inhibition in greater detail. When compounds **3** and **4** were docked the into the IMP-1 active site as an initial trial, a low docking score was calculated, consistent with the observed weak enzyme inhibition. A further attempt at docking was made to determine whether the two (*R,R*)-benzyl groups could fit reasonably into the hydrophobic pocket. The two carboxylates in compound **3** adopt the same configuration as in compound **1** in order to interact effectively with both of the Zn^{2+} atoms and the side chain of Lys-161. This docking study (Fig. 3) revealed that both benzyl groups in compound **3** (shown in *red*) make unfavorable contacts with residues in the active site, one with Asn-167 and the other with Phe-51, in contrast to compound **2** (shown in *cyan*). Using this model, one would expect compound **4** (*R,S*) to have more potent inhibition against IMP-1 compared with compound **3,** because compound **4** contains only one benzyl group in an unfavorable position. However, the measured enzyme inhibition for compound **3** can be regarded as a lower limit, because the presence of 1% compound **2** in this sample could account for the observed result (Table II).

The x-ray crystal structure of IMP-1 complexed to compound **1** revealed that in addition to coordination to the two Zn^{2+} atoms, the remaining interactions are predominately hydrophobic, formed by residues Trp-28, Val-25, Val-31, Pro-32, Phe-51, Tyr-163, and Gly-166. Using molecular modeling, the aryl

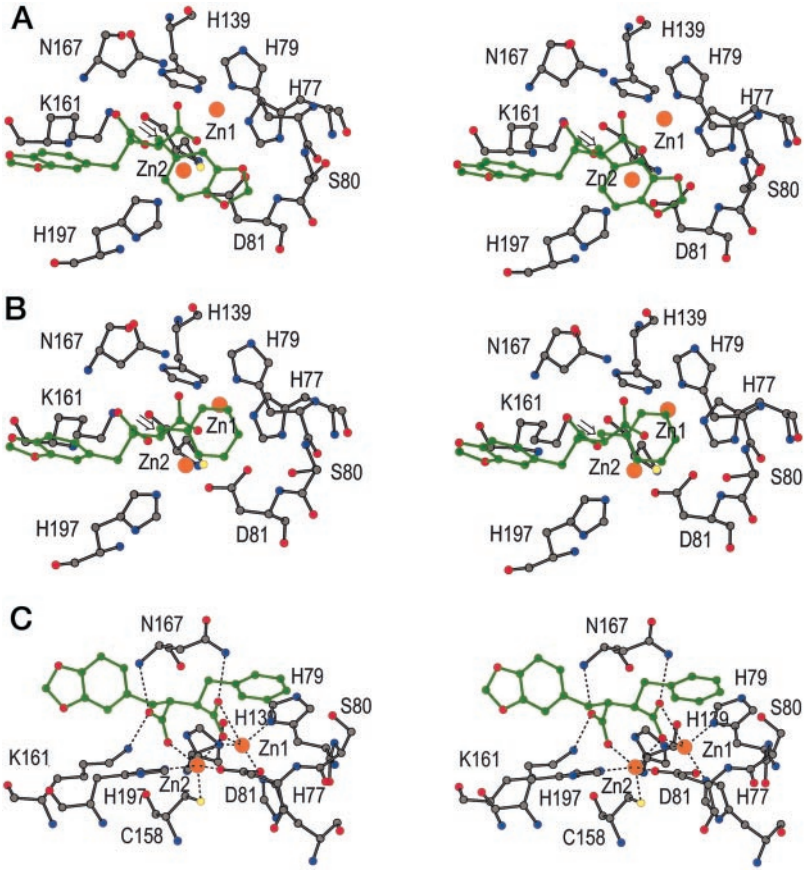


FIG. 2. **Binding details of the complexes between IMP-1 and compounds 1 and 11.** The zinc atoms are shown in *orange*, oxygen atoms in *red*, and nitrogen atoms in *blue*. Carbon atoms in the inhibitor are shown in *green*. *A*, in the complex with compound **1,** the conformation of the inhibitor is strained because of unfavorable steric interactions with Ser-80. The torsion angle about the bond, indicated with an *arrow*, is 144°. *B*, in the complex with compound **11,** the smaller phenyl group adopts an unstrained conformation; the torsion angle about the bond, indicated with an *arrow*, is −179°. *C*, details of the polar interactions between the inhibitor and the protein, as well as polar interactions between and the two zinc atoms and the protein, for the complex with compound **11**. Hydrogen bonds are indicated with *dashed lines*.

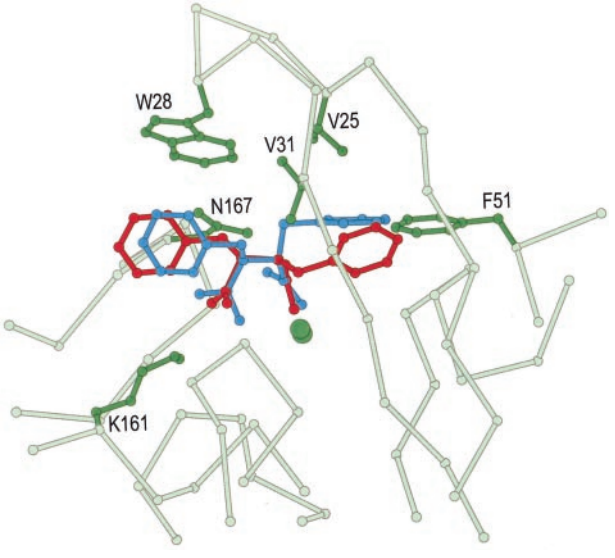


FIG. 3. **Binding modes of compounds 2 (shown in *cyan*) and 3 (shown in *red*) docked into the IMP-1 active site complexed with compound 1.**

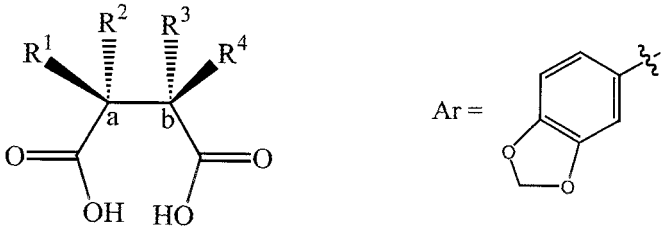
groups present in compounds **1** and **2** were found to fit into the hydrophobic pocket very well. In contrast, the methyl or ethyl group present in compound **8** was found to make significantly fewer favorable contacts within the enzyme active site (Fig. 4). The energy-minimized complexes of IMP-1 with compounds **2** and **8** were analyzed using CHARMM26 in the Merck Mechanical Force Field. Table III presents the interaction energies between IMP-1 and the inhibitors. Although the electrostatic interactions between IMP-1 and compounds **2** and **8** are similar, compound **2** is calculated to have 10 kcal/mol more favorable van der Waals interactions than compound **8,** consistent with the more than 3,700,000-fold binding difference between these two compounds (51). Interestingly, a binding energy difference of 10 kcal/mol is calculated to result in a selectivity of about 7.2 orders of magnitude or 15,000,000-fold.

DISCUSSION

2,3-Disubstituted succinic acids have been identified as potent inhibitors of the IMP-1 metallo-β-lactamase enzyme. The structure-activity relationship of these inhibitors indicates a preference for two hydrophobic groups substituted in a (2*S*,3*S*) configuration on the succinic acid core. The inhibition of IMP-1 exhibits a strong dependence on the 2,3-stereochemistry, with *S,S* > *R,R* > *R,S* in the case of 2,3-dibenzyl succinic acid. Succinic acid itself is a poor IMP-1 inhibitor with an IC_{50} of 6.3 mM, whereas the monosubstituted benzylsuccinic acid (**6**) is only weakly active (IC_{50} = 490 μM). The 2,2-disubstituted succinic acids **7** and **8** were inactive against IMP-1, whereas the (2*S*,3*S*)-disubstituted succinic acids **1**, **2**, and **11** were all potent inhibitors of IMP-1 with low nanomolar IC_{50} values.

When imipenem, a β-lactam substrate of IMP-1, is docked into the active site of the enzyme containing the succinic acid compound **1,** the model suggested that the β-lactam carboxylate group forms a salt bridge with the side chain of Lys-161 and also forms a hydrogen bond to the backbone NH of Asn-167 (Fig. 5). This model is consistent with that proposed by Concha *et al*. (37) in the case of the *B. fragilis* MBL. The [(iminomethyl)amino]-ethyl group of imipenem could be placed at several positions that were indistinguishable based upon their relative energies. A representative orientation is shown in Fig. 5. The carbonyl group of imipenem is about 2 Å from the water that bridges the two zinc atoms. As shown in Fig. 5, one of the carboxylates of the succinic acid inhibitor overlays with the carboxylate of imipenem. The other carboxylate group present in the succinic acid inhibitor is found to be about 1 Å below the β-lactam carbonyl, which is in the middle of the bridging water

TABLE II
Inhibition of IMP-1 metallo-β-lactamase by succinic acids



Compound	R^1	R^2	R^3	R^4	a,b-Configuration	IMP-1 IC_{50}
						μM
1	$ArCH_2$	H	$ArCH_2$	H	*S,S*	0.009
2[a]	$PhCH_2$	H	$PhCH_2$	H	*S,S*	0.0027
3[a]	H	$PhCH_2$	H	$PhCH_2$	*R,R*	≥0.21
4[b]	H	$PhCH_2$	$PhCH_2$	H	*R,S*	200
5	H	H	H	H		6300
6	H	H	$PhCH_2$	H	*S*	490
7	H	H	CH_3	CH_3		>10000
8	H	H	CH_3	CH_2CH_3	*Racemate*	>10000
9	$PhCH_2$	H	(*R*)-$PhCH(CH_3)$	H	*S,S*	0.013
10	$PhCH_2$	H	(*S*)-$PhCH(CH_3)$	H	*S,S*	≥2.7
11	$ArCH_2$	H	$PhCH_2$	H	*S,S*	0.0037

[a] D, L pair.
[b] Meso compound.

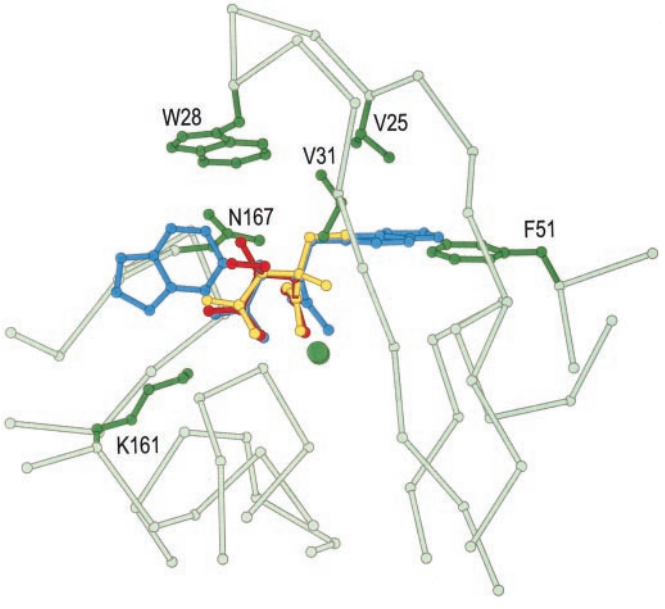


FIG. 4. **Binding modes of compound 8 docked into the active site of IMP-1 using the x-ray crystal structure of IMP-1 complexed with compound 1 (shown in *cyan*).** The two most probable conformations of the *S*-isomer of compound **8** are shown in *red* and in *yellow*.

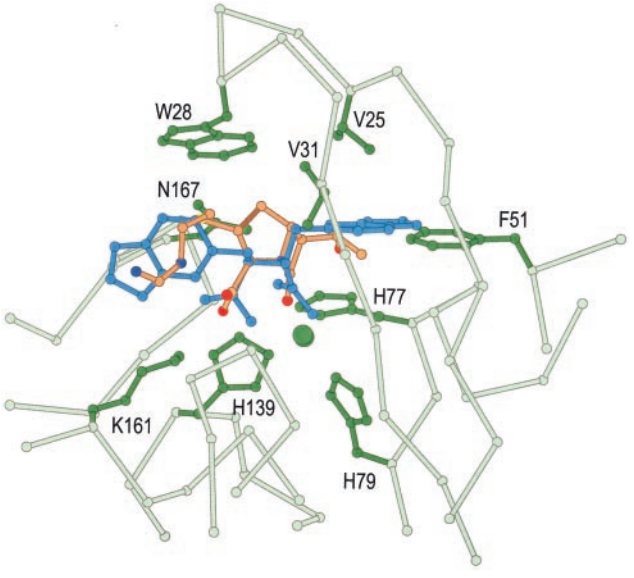


FIG. 5. **Imipenem docked into the active site of IMP-1 complexed with compound 1.** Compound **1** is shown in *cyan* only. The *colors* for imipenem, carbon, nitrogen, oxygen, and sulfur are *orange, blue, red,* and *yellow*, respectively.

TABLE III
Interaction energies calculated for IMP-1 bound to succinic acid inhibitors

Values are shown as kcal/mol.

Complex	van der Waals	Electrostatic	Total energy
IMP-1-**2**	9.66	−34.25	−24.59
IMP-1-**8**	20.96	−34.83	−13.87

between the two zinc atoms and the carbonyl of imipenem. The crystal structure of the IMP-1 complex with compound **1** revealed that this carboxylate replaces the bridging water and binds to both zinc atoms. The inhibitor binding to IMP-1 with low nM affinity effectively prevents any substrate from entering the enzyme active site considering the K_m value for substrate is in the micromolar range.

Gem-dimethyl succinic acid has been reported to be a potent inhibitor of carboxypeptidase (52, 53), a zinc-containing hydrolytic enzyme (54). Carboxypeptidases hydrolyze their substrates via a zinc-bound hydroxide (55). Similarly, the zinc-bound hydroxide is proposed to act as the attacking nucleophile in MBL-catalyzed hydrolysis (42, 56–57). Although substituted succinic acids can inhibit IMP-1 and carboxypeptidase A, the inhibitor profile is distinct for each enzyme. Inhibition of carboxypeptidases A and B strongly favors the *R* configuration of the substituents, in contrast to the preference for the *S* configuration for inhibition of IMP-1. In fact, crystals of carboxypeptidase A grown in the presence of the racemic mixture of compound **8** were found to contain only the *R*-form of the inhibitor (52).

2,3-(*S,S*)-Disubstituted succinic acids show promise in reversing imipenem resistance in clinical isolates of *P. aeruginosa*.[2] Knowledge of the binding modes of this new class of inhibitors to IMP-1 could reveal opportunities for inhibiting other MBLs and could extend their utility to other important clinical isolates.

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