X-ray Crystallography Deciphers the Activity of Broad-Spectrum Boronic Acid β -Lactamase Inhibitors

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Supporting Information

ABSTRACT: Recent decades have witnessed a dramatic increase of multidrug resistant (MDR) bacteria, compromising the efficacy of available antibiotics, and a continual decline in the discovery of novel antibacterials. We recently reported the first library of benzo[b]thiophen-2-ylboronic acid inhibitors sharing broad spectrum activity against β -lactamases (BLs). The ability of these compounds to inhibit structurally and mechanistically different types of β -lactamases has been here structurally investigated. An extensive X-ray crystallographic analysis of boronic acids (BAs) binding to proteins representative of serine BLs (SBLs) and metallo β -lactamases (MBLs) have been conducted to depict the role played by the boronic group in driving molecular recognition, especially in the interaction with MBLs. Our derivatives are the first case of noncyclic boronic acids active against MBLs and represent a productive route toward potent broad-spectrum inhibitors.

KEYWORDS: Broad-spectrum inhibitors, noncyclic boronic acid, NDM-1 metallo β -lactamases, tetrahedral intermediates, bacterial resistance

Boronic acids mimic natural NDM-1 tetrahedral intermediate, disrupt the active nucleophile and contribute to Zn coordination

Worldwide bacterial resistance is mining the efficacy of available antibiotics and the continuous dissemination of pan resistant bacteria represents a real menace to public health.¹ Notably, among the several mechanism of resistance bacteria employ, the production of β -lactamases (BLs) is the prevalent one against β -lactams antibiotics and has rapidly led to BLs with extended spectrum of action, e.g., the extended spectrum BLs (ESBLs) and the carbapenemases BLs able to inactivate nearly all available β -lactams, including last resort carbapenems.^{2–5} BL molecular classification, based on the amino acid sequence, divides β -lactamases into class A, C, and D enzymes, which utilize serine for β -lactam hydrolysis (SBLs), and class B metalloenzymes (MBLs), which require divalent zinc ions for substrate hydrolysis.⁶ While inhibitors are available in therapy for SBLs, for MBLs no inhibitor has been at the present approved, menacing the efficacious treatment of bacterial infections.^{7–9} Therefore, daily infections caused by clinical strains coproducing ESBLs and carbapenemases are not susceptible to available antibiotics and require last line antibacterial agents, e.g., colistin.^{10,11} The actual situation, in which health care costs and treatment failures rates augment, stresses the importance of the development of novel BL inhibitors (BLIs),^{12–14} targeting all four classes with broad-spectrum activity.¹⁵ However, the design of a BLI active

against SBLs and MBLs despite its attractiveness is challenged by the targets structural and mechanistic peculiarities.

In all SBLs, with some differences from one class to the other, the hydrolysis of the β -lactam antibiotic involves a catalytic serine and proceeds via acylation and deacylation steps.⁹ In MBLs, instead, a hydroxide ion, stabilized by one or two Zn atoms, attacks the carbonyl carbon of the β -lactam ring, leading to its opening. However, all BL classes share, in their hydrolytic mechanism of action, the occurrence of tetrahedral intermediates (Figure 1).¹⁶ As a consequence, compounds mimicking these intermediates may represent valuable candidates to overcome active site peculiarities among BLs.^{17,18}

Among recently disclosed BLIs, novel non- β -lactam-like chemical entities are rare, as well as broad-spectrum BLIs. In this scenario, boronic acids (BAs) deserve a key role:^{15,19,20} from vaborbactam, active against SBLs but inactive against MBLs, to the lately developed cyclic derivatives, BAs

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Figure 1. Proposed binding modes of tetrahedral intermediates in the SBL and MBL catalyzed hydrolysis of β -lactam antibiotics.

demonstrated their potentiality in the design of pan-spectrum BLIs.^{20–23}

If cyclic BAs were recently identify as first reported dual inhibitors,²³ we recently disclosed a small library of benzo[b]-thiophen-2-ylboronic acids active as the first noncyclic BAs active against all four BLs classes and with biological activity vs clinical strains (Table 1, see Supporting Information).²⁴

Moreover, cpd 5 in the series was validated as low micromolar inhibitor toward GES-5, a class A carbapenemase rapidly disseminating worldwide (Table 1; S2 and S3).¹⁸

To depict the full panel of interactions between our derivatives and targeted BLs and to deepen our computational predictions,²⁴ the binary X-ray complexes of these molecules binding SBLs (GES-5) and MBLs (NDM-1) were solved and the mechanism of action responsible for their affinity was clarified.

High-resolution structures of the BAs 1, 3, 5, and 6 in complex with NDM-1 MBL have been determined by single-crystal X-ray diffraction (Tables 2; S2–S6) bringing the number of X-ray structures of NDM-1 binding to a non- β -

lactam so far deposited in the PDB to seven. The binary complex of compound 3 binding with GES-5, the first X-ray structure of a GES-type carbapenemase binding a *de novo* inhibitor, has been determined as well. Along with a previous disclosed complex with AmpC (PDB 2I72),²⁵ they elucidate the structural determinants for BAs inhibitory activity vs SBLs and MBLs.

Two molecules per asymmetric units are present in our NDM-1 experimental models, as observed in other NDM-1 structures.^{26,27} Indeed, the two protein chains are present as unique elements in the crystal a.s.u., and the main features of the active site as well as compound binding orientations are conserved along complexes, except for cpd 3. The residues defining the active site undergo minor conformational changes, involving the intrinsically flexible loops L3 and L10.

In the NDM-1 active site, the inhibitors' boronic acid moiety is involved in the metal ions coordination. Indeed, boron is present in its hydrated tetrahedral coordination, displacing the conserved hydroxide nucleophile and allocating the two oxygens in a bridging position between the two zinc atoms. Most likely the same hydroxide nucleophile present in the NDM-1 apoprotein reacts with boronic acid and generates the tetrahedral intermediate observed in the four structures presented here. Trigonal boron(III) compounds, in fact, behave as Lewis acids and thus react with nucleophiles generating covalent, tetrahedral adducts.

The boron–oxygen are directly involved in the coordination of the two zinc cations, with distances ranging between 1.9 and 2.9 Å, *de facto* masking the reactive center of NDM-1.

Zn1 and Zn2 positions are only marginally shifted, maintaining a distance of about 4.25 Å between them. Side chain positions of key residues involved in the metal atoms coordination are all conserved: His120, His122, His189,

Table 1. Noncyclic Boronic Acid Derivatives as Broad-Spectrum β -Lactamases Inhibitors

Cpd	Structure	BLs panel K_i (μ M) ^[a]				
		GES-5	KPC-2 ^[b]	NDM-1 ^[b]	AmpC ^[b]	OXA-24 ^[b]
1		8,9	2,8	5.9 [§]	0,07	67
2		24	<i>n.t.</i>	<i>n.t.</i>	0.04 [§]	<i>n.t.</i>
3		15 [§]	4,4	5.5 [§]	0,05	96
4		17	3,2	3,2	1,3	27
5		0,16	4,9	3 [§]	0,42	16
6		5,7	1,4	11 [§]	1,4	64

^aEstimated K_i as per competitive inhibitor. Experiments were conducted as described in Materials and Methods (see Supporting Information). BAs, synthesized as pinacol esters, were assayed without previous deprotection since they spontaneously hydrolyzed to the free BA under assay conditions. All the experiments were performed in triplicate, and errors never exceeded 5%. ^b K_i data from Santucci et al.²⁴ [§]Derivative was crystallized with the target protein and disclosed here for the first time. [¶]Previously disclosed binary complex.²⁵

Table 2. NDM-1 Binary Complexes with Broad-Spectrum Noncyclic Boronic Acid Derivatives^a

^aThe residues lining the binding pockets are labeled according to the protein color: Zn atoms are represented in pink, water in cyano, cpd 1 in yellow, 3 in red, 5 in blue, and 6 in green. Omit map (phenix.composite_omit_map) is shown at 3.5 σ contour level.

His250, Cys208, and Asp124. Moreover, the histidine side chains establish polar contacts with the boronic acid moiety.

The benzo[b]thiophene skeleton, shared by all the compounds, lies inside the active site with same orientation, roughly perpendicular to the two Zinc ions axis. Solely cpd 5 is slightly shifted, most likely driven by the interactions established by the carboxylic group in position 7: this substituent is oriented toward the His122-Gln123 stretch and participates in a network of hydrogen bonds and polar contacts involving the above-mentioned residues and three water molecules.

BAs 1 and 5, with substituents in positions 5 and 7, respectively, place the benzo[b]thiophene aromatic core with same orientation, with the thiophene sulfur atom pointing toward His122, while cpd 6, carrying a bulkier substituent in position 7, places the sulfur atom, thus the substituent, in the opposite direction. Cpd 3 reveals a peculiar behavior as well, adopting both the alternative conformations, one in each active site of the two protein chains present in the asymmetric unit.

The two major loops, L3 and L10, defining the entrance of the active site, experience significant shift upon compound binding (Figure 2; S3). In all solved complexes, L10 moves

Figure 2. Superposition of four determined NDM-1 complexes highlighting loop-3 flexibility upon ligand binding. Cpd 1 is represented in yellow, 3 in red, 5 in blue, and 6 in green.

toward the active site, closer to the molecules lining in the catalytic pocket. The Asn220 side chain points toward the inhibitors, on top of the planar benzo[b]thiophene core, with the amide group nearly perpendicular to the latter. The same behavior has been observed in other NDM-1 structures, i.e., in the complex with meropenem.²⁷

Furthermore, in all the complexes, Asn220 main chain amide and Lys211 side chain form a water-bridged hydrogen bond with the boronic oxygen.

On the opposite side of the benzo[b]thiophene plane, a hydrophobic patch establishes direct interactions with the inhibitors and contributes to their stabilization. Such surface is mainly defined by Leu65, Met67, Val73, and Trp93, the latter with its side chain indole perpendicular to the heterocyclic core of the inhibitors. Loop L3 (from Ser63 to Ala74) is known to be involved in substrate recognition and undergoes major rearrangements upon substrate binding as documented by the structural studies of hydrolyzed substrates complexes.²⁷

In the structures of complexes with cpds 1, 3, 5, and 6, L3 establishes hydrophobic contacts with all the inhibitors but experience different orientations and peculiar interactions in

the turn segment, according to the differences in substituents nature and position (Figure 2; S3). In the complexes of compounds where the substituents are oriented toward L3 (cpd 1) or the solvent bulk (cpd 6), the loop between Asp66 and Gly71 is not clearly defined in the electron density maps and thus unstructured in our models.

On the contrary, in the case of cpd 3 and 5 pointing their heterocycle substituents to the opposite side, toward Gln123, the loop is fully defined. In particular, for cpd 5 (Figure 2; S3), the loop appears quite open and similar to apoprotein structures such as SZGU.²⁷

In the case of cpd 3 (Figure 2) L3 shows the closest conformation, with Met67 and Phe70 protruding to the internal side, toward the ligand. As mentioned, in the second chain present in the asymmetric unit, cpd 3 binds in the active site in a flipped orientation, similar to cpd 1. As a result of the substituent steric hindrance, L3 results undefined for cpds 1 and 6 complexes.

The binary complex of cpd 3 with the emergent carbapenemase GES-5⁴ has been solved as well: the binding mode of cpd 3 resembles that of already disclosed boronic acid derivatives binding KPC-2 (S2, S3).²⁸ The inhibitor forms covalent bonds, via its boron atom, with the O γ atom of Ser64. Both boronate oxygens interact extensively as in other complexes with class A BLs.^{19,29} The boronic acid O2 oxygen establishes multiple hydrogen bonds with the catalytic base Glu161, the backbone NH of catalytic Ser64, and the side chain of Ser165, whereas the boronic acid O1 oxygen hydrogen bonds to the backbone CO and NH groups of Thr232 and the backbone NH of Ser64. Cpd 3 adopts a deacylation transition-state analogue conformation with an inverted boron configuration previously described.^{19,29} Therefore, one boron oxygen is located in the oxyanion hole, while the other displaces the deacylation water normally positioned between Glu161 and Ser165. The benzo[b]thiophene ring is involved in cation- π and π - π interactions with Asn127 and Trp99, respectively, and creates hydrophobic interactions with the side chains of Glu98, Trp99, and Pro162. Interestingly, Cys63, a residue forming a disulfide bridge with Cys233, characteristic of all class A carbapenemases, is highly flexible, and it is represented in three different conformations. The electron density maps suggest that cpd 3 assumes two conformations, related by a rotation of 180° about the boron-benzo[b]thiophene axis, resulting in two roughly coplanar binding modes (S2). As a consequence, the major differences concern sulfur atom positioning, either oriented toward Ser165 or toward the carboxylic acid pocket (Ser125, Thr230, and Thr232), and the C5 amino-methyl moiety. In the two conformations, the latter is oriented in two positions, both toward the opening of the binding site, exposed to the solvent. In the orientation where the amino-methyl group points toward Pro162, it rather causes the displacement of Glu98 side chain. Interestingly, a molecule of dimethyl sulfoxide is trapped in a side pocket known to bind the carboxylic moiety of β -lactams and defined by Ser125, Thr230, and Thr232, well. In the apoprotein structure of GES-5, the cavity is occupied by the sulfonic group of HEPES buffer, while in this case, the solvent is bound and interacts with cpd 3.

Notably, for AmpC, a class C SBL, the close analog cpd 2 has been already described in its binding conformation via X-ray crystallography: the compound interacts covalently with the catalytic serine and establishes several interactions with the catalytic residues (PDB code 2I72;²⁵ S7).

Our X-ray crystallographic results evidence that, in NDM-1 MBL, BAs participate in bidentate coordination of the Zn(II) ions with both boron-bound oxygen atoms (Figures 3 and 4; S3).

Figure 3. Superposition of NDM-1 X-ray binary complexes binding meropenem (in green) and cpd 5 (in blue).

Figure 4. NDM-1 active site close-up. Compound 5 is represented in blue. Upon binding, boron-bound oxygen atoms participate in bidentate coordination of the Zn(II) ions.

The metals coordination adopts a distorted trigonal bipyramidal geometry, with the zinc ions slightly shifted out of the trigonal plane and one of the axial pyramidal ligands, represented by a boron oxygen, rather displaced (2.6 Å for Zn2 and 2.9 Å for Zn1) if compared to the ideal distances covered by β -lactam ligands (1.9–2.1 Å). BA binding geometry most closely resembles that predicted for the tetrahedral oxyanion formed during β -lactam hydrolysis (Figure 1).²³

The BA mechanism of action has been largely disclosed for SBLs and involves the covalent and reversible binding to the catalytic serine;^{19,29,30} for MBLs NDM-1, our evidence shows that these derivatives maintain the correct Zn coordination as per natural β -lactam substrates and explains their ability to overcome active site architecture peculiarities along BLs classes (S7). They represent valuable leads for the development of broad-spectrum inhibitors.

CONCLUSIONS

The X-ray crystallographic analysis conducted over a small library of BAs highlighted boron's unique capacity to interact with SBLs and MBLs, thus targeting all four BL classes.

Interactions with SBLs typically feature boron in an anionic tetrahedral form covalently bound to the protein, while in MBL a tetrahedral covalent adduct is formed between boron and the water coordinated by Zn ions.

Considering the remarkable differences between SBLs and MBLs, in terms of structure and mechanism of action, compounds mimicking the tetrahedral intermediates represent good candidates for the development of broad-spectrum BLI.

With recent findings on synthetic accessibility for the development of BAs/boron containing derivatives and the possibility to modulate the Lewis acidity of boron, thus enhancing activity, absorption, and distribution, the development of BAs as broad-spectrum BLIs could be successful.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.8b00607.

Materials and methods; NMR Data; Cpd 3 in complex with GES-5; BA binding interactions; reversibility of inhibition; crystallization methodologies and conditions; data collection and refinement statistics; Cpd 2 in AmpC active site (PDF)

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Notes

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ABBREVIATIONS

BA, boronic acid; BL, β -lactamase; BLI, β -lactamase inhibitor; cpd, compound; DMSO, dimethyl sulfoxide; ESBL, extended spectrum β -lactamase; GES-5, Guyana extended spectrum-5; KPC-2, *Klebsiella pneumoniae* carbapenemase-2; SBL, serine-based β -lactamase; MBL, metallo β -lactamase; NDM-1, New Delhi metallo β -lactamase-1; PDB, Protein Data Bank

REFERENCES

- (1) O'Neill, J. Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations. December 2014.
- (2) Bush, K. Carbapenemases: Partners in Crime. *J. Glob. Antimicrob. Resist.* **2013**, 1 (1), 7–16.
- (3) Papp-Wallace, K. M.; Endimiani, A.; Taracila, M. A.; Bonomo, R. A. Carbapenems: Past, Present, and Future. *Antimicrob. Agents Chemother.* **2011**, 55 (11), 4943–4960.
- (4) Tondi, D.; Cross, S.; Venturelli, A.; Costi, M. P.; Cruciani, G.; Spyridakis, F. Decoding the Structural Basis for Carbapenem Hydrolysis by Class A β -Lactamases: Fishing for a Pharmacophore. *Curr. Drug Targets* **2016**, 17 (9), 983–1005.

- (5) Farina, D.; Spyraakis, F.; Venturelli, A.; Cross, S.; Tondi, D.; Costi, M. P. The Inhibition of Extended Spectrum β -Lactamases: Hits and Leads. *Curr. Med. Chem.* **2014**, *21*, 1405–1434.
- (6) Bush, K. The ABCD of β -lactamase nomenclature. *J. Infect. Chemother.* **2013**, *19* (4), 549–559.
- (7) Linciano, P.; Cendron, L.; Gianquinto, E.; Spyraakis, F.; Tondi, D. Ten Years with NDM-1 Metallo β -Lactamase: From Structural Insights to Inhibitor Design. *ACS Infect. Dis.* **2019**, *5* (1), 9–34.
- (8) Bush, K.; Page, M. G. P. What We May Expect from Novel Antibacterial Agents in the Pipeline with Respect to Resistance and Pharmacodynamic Principles. *J. Pharmacokinet. Pharmacodyn.* **2017**, *44* (2), 113–132.
- (9) King, D. T.; Sobhanifar, S.; Strynadka, N. C. J. One Ring to Rule Them All: Current Trends in Combating Bacterial Resistance to the β -Lactams: Structure-Based Approaches to Overcome β -Lactam Resistance. *Protein Sci.* **2016**, *25* (4), 787–803.
- (10) Bush, K. A Resurgence of β -Lactamase Inhibitor Combinations Effective against Multidrug-Resistant Gram-Negative Pathogens. *Int. J. Antimicrob. Agents* **2015**, *46* (5), 483–493.
- (11) Shaikh, S.; Fatima, J.; Shakil, S.; Rizvi, S. M. D.; Kamal, M. A. Antibiotic Resistance and Extended Spectrum Beta-Lactamases: Types, Epidemiology and Treatment. *Saudi J. Biol. Sci.* **2015**, *22* (1), 90–101.
- (12) Spyraakis, F.; Celenza, G.; Marcoccia, F.; Santucci, M.; Cross, S.; Bellio, P.; Cendron, L.; Perilli, M.; Tondi, D. Structure-Based Virtual Screening for the Discovery of Novel Inhibitors of New Delhi Metallo- β -Lactamase-1. *ACS Med. Chem. Lett.* **2018**, *9* (1), 45–50.
- (13) Genovese, F.; Lazzari, S.; Venturi, E.; Costantino, L.; Blazquez, J.; Ibacache-Quiroga, C.; Costi, M. P.; Tondi, D. Design, Synthesis and Biological Evaluation of Non-Covalent AmpC β -Lactamases Inhibitors. *Med. Chem. Res.* **2017**, *26* (5), 975–986.
- (14) Klein, R.; Linciano, P.; Celenza, G.; Bellio, P.; Papaioannou, S.; Blazquez, J.; Cendron, L.; Brenk, R.; Tondi, D. In Silico Identification and Experimental Validation of Hits Active against KPC-2 β -Lactamase. *PLoS One* **2018**, *13*, e0203241.
- (15) Lomovskaya, O.; Sun, D.; Rubio-Aparicio, D.; Nelson, K.; Tsivkovski, R.; Griffith, D. C.; Dudley, M. N. Vaborbactam: Spectrum of Beta-Lactamase Inhibition and Impact of Resistance Mechanisms on Activity in Enterobacteriaceae. *Antimicrob. Agents Chemother.* **2017**, DOI: 10.1128/AAC.01443-17.
- (16) Meini, M.-R.; Llarrull, L. I.; Vila, A. J. Overcoming Differences: The Catalytic Mechanism of Metallo- β -Lactamases. *FEBS Lett.* **2015**, *589* (22), 3419–3432.
- (17) Docquier, J.-D.; Mangani, S. An Update on β -Lactamase Inhibitor Discovery and Development. *Drug Resist. Updates* **2018**, *36*, 13–29.
- (18) Spyraakis, F.; Bellio, P.; Quotadamo, A.; Linciano, P.; Benedetti, P.; D'Arrigo, G.; Baroni, M.; Cendron, L.; Celenza, G.; Tondi, D. First virtual screening and experimental validation of inhibitors targeting GES-5. *J. Comput.-Aided Mol. Des.* **2019**, *33*, 295.
- (19) Tondi, D.; Venturelli, A.; Bonnet, R.; Pozzi, C.; Shoichet, B. K.; Costi, M. P. Targeting Class A and C Serine β -Lactamases with a Broad-Spectrum Boronic Acid Derivative. *J. Med. Chem.* **2014**, *57* (12), 5449–5458.
- (20) Hecker, S. J.; Reddy, K. R.; Totrov, M.; Hirst, G. C.; Lomovskaya, O.; Griffith, D. C.; King, P.; Tsivkovski, R.; Sun, D.; Sabet, M.; et al. Discovery of a Cyclic Boronic Acid β -Lactamase Inhibitor (RPX7009) with Utility vs Class A Serine Carbapenemases. *J. Med. Chem.* **2015**, *58* (9), 3682–3692.
- (21) Zhou, M.; Yang, Q.; Lomovskaya, O.; Sun, D.; Kudinha, T.; Xu, Z.; Zhang, G.; Chen, X.; Xu, Y. In Vitro Activity of Meropenem Combined with Vaborbactam against KPC-Producing Enterobacteriaceae in China. *J. Antimicrob. Chemother.* **2018**, *73* (10), 2789–2796.
- (22) Castanheira, M.; Rhomberg, P. R.; Flamm, R. K.; Jones, R. N. Effect of the β -Lactamase Inhibitor Vaborbactam Combined with Meropenem against Serine Carbapenemase-Producing Enterobacteriaceae. *Antimicrob. Agents Chemother.* **2016**, *60* (9), 5454–5458.
- (23) Brem, J.; Cain, R.; Cahill, S.; McDonough, M. A.; Clifton, I. J.; Jiménez-Castellanos, J.-C.; Avison, M. B.; Spencer, J.; Fishwick, C. W. G.; Schofield, C. J. Structural Basis of Metallo- β -Lactamase, Serine- β -Lactamase and Penicillin-Binding Protein Inhibition by Cyclic Boronates. *Nat. Commun.* **2016**, *7*, 12406.
- (24) Santucci, M.; Spyraakis, F.; Cross, S.; Quotadamo, A.; Farina, D.; Tondi, D.; De Luca, F.; Docquier, J.-D.; Prieto, A. I.; Ibacache, C. Computational and Biological Profile of Boronic Acids for the Detection of Bacterial Serine- and Metallo- β -Lactamases. *Sci. Rep.* **2017**, *7* (1), 17716.
- (25) Venturelli, A.; Tondi, D.; Cancian, L.; Morandi, F.; Cannazza, G.; Segatore, B.; Prati, F.; Amicosante, G.; Shoichet, B. K.; Costi, M. P. Optimizing Cell Permeation of an Antibiotic Resistance Inhibitor for Improved Efficacy. *J. Med. Chem.* **2007**, *50* (23), 5644–5654.
- (26) Kim, Y.; Tesar, C.; Mire, J.; Jedrzejczak, R.; Binkowski, A.; Babnigg, G.; Sacchetti, J.; Joachimiak, A. Structure of Apo- and Monometalated Forms of NDM-1—A Highly Potent Carbapenem-Hydrolyzing Metallo- β -Lactamase. *PLoS One* **2011**, *6* (9), No. e24621.
- (27) Zhang, H.; Ma, G.; Zhu, Y.; Zeng, L.; Ahmad, A.; Wang, C.; Pang, B.; Fang, H.; Zhao, L.; Hao, Q. Active-Site Conformational Fluctuations Promote the Enzymatic Activity of NDM-1. *Antimicrob. Agents Chemother.* **2018**, DOI: 10.1128/AAC.01579-18.
- (28) Celenza, G.; Vicario, M.; Bellio, P.; Linciano, P.; Perilli, M.; Oliver, A.; Blazquez, J.; Cendron, L.; Tondi, D. Phenylboronic Acid Derivatives as Validated Leads Active in Clinical Strains Over-expressing KPC-2: A Step against Bacterial Resistance. *ChemMedChem* **2018**, *13* (7), 713–724.
- (29) Tondi, D.; Calò, S.; Shoichet, B. K.; Costi, M. P. Structural Study of Phenyl Boronic Acid Derivatives as AmpC Beta-Lactamase Inhibitors. *Bioorg. Med. Chem. Lett.* **2010**, *20* (11), 3416–3419.
- (30) Ke, W.; Sampson, J. M.; Ori, C.; Prati, F.; Drawz, S. M.; Bethel, C. R.; Bonomo, R. A.; van den Akker, F. Novel Insights into the Mode of Inhibition of Class A SHV-1 Beta-Lactamases Revealed by Boronic Acid Transition State Inhibitors. *Antimicrob. Agents Chemother.* **2011**, *55* (1), 174–183.