Aminocarboxylic acids related to aspergillomarasmine A (AMA) and ethylenediamine-\(N,N'\)-disuccinic acid (EDDS) are strong zinc-binders and inhibitors of the metallo-beta-lactamase NDM-1†

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Antibiotic resistance is a global public health concern with an increasing economic burden.1,2 Among Gram-negative pathogens, \(\beta\)-lactam resistance due to the production of \(\beta\)-lactamases is a major cause of antibiotic resistance.3 Based on their mechanism of \(\beta\)-lactam hydrolysis, \(\beta\)-lactamases can be classified as serine- or metallo-\(\beta\)-lactamases (SBLs and MBLs respectively). While SBLs hydrolyze \(\beta\)-lactams via an active site serine nucleophile, MBLs do so via a water molecule coordinated with active site zinc ion(s).4 Although there are clinically used SBL inhibitors available to counteract the infections caused by SBL-producing bacteria,5 there are currently no approved MBL inhibitors available.

Recent screening efforts led to the identification of aspergillomarasmine A (AMA, 1a, Table 1) as a potent inhibitor of the clinically relevant NDM- and VIM-type MBLs.6 This finding was followed by reports describing the chemical synthesis of AMA and its structural analogues.7–11 Among them, synthetic routes using either a key N-nosyl protected aziridine intermediate8 or a cyclic sulfamidate9 furnished AMA in relatively few steps and the highest reported yields (overall yields of 28% and 19% respectively).

Recently, we reported that ethylenediamine-\(N,N'\)-disuccinic acid (EDDS) lyase naturally catalyzes a reversible two-step sequential addition of ethylenediamine (2) to two molecules of fumaric acid (3), giving \((S)\)-\((2\text{-aminoethyl})\text{aspartic acid (AEAA, 4)}\) as an intermediate and \((S,S)\)-EDDS (5) as the final product (Table 1A).12 EDDS lyase was subsequently found to have broad substrate promiscuity,13–15 accepting a wide range of amino acids with terminal amino groups (6a–k) for regio- and stereoselective addition to fumarate, thus providing a straightforward biocatalytic method for the asymmetric synthesis of AMA (1a), AMB (1b), and related aminocarboxylic acids (1c–k, Table 1B).13 To further explore the substrate scope of EDDS lyase, as well as to prepare a small library of EDDS derivatives as potential NDM-1 inhibitors,16 we here describe the EDDS-lyase catalyzed reaction of fumaric acid with various diamines containing different aliphatic linkers between the two amino functional groups (7a–i) (Table 2).

Interestingly, diamine substrates with two to four atoms between the two amino groups (7a–g) were well accepted as substrates by EDDS lyase, giving good conversions (47–83%) and yielding the corresponding aminocarboxylic acid products (8a–g) in 21–60% isolated yield (Table 2, entries 1–7). Hence, EDDS lyase has a broad diamine scope, allowing the two-step sequential addition of appropriate diamines to fumaric acid, providing a powerful synthetic tool for the preparation of valuable aminocarboxylic acids. Conversely, the elongated diamines with five atoms between the two amino groups (7h–i) were not accepted as substrates by EDDS lyase (Table 2, entries 8 and 9).

The ability of the AMA and EDDS analogues to inhibit NDM-1 was evaluated using a fluorescence-based assay previously described by Schofield and coworkers.17 This assay makes use of a cephalosporin substrate known as FC5 which upon
hydrolysis releases 7-hydroxycoumarin. The well characterized NDM-1 inhibitors AMA, EDTA, and dipicolinic acid (DPA) were used as positive controls. In general, most of the AMA and EDDS analogues tested showed potent activity against NDM-1 with IC$_{50}$ values ranging from 1.3 m$\mu$M to 18.3 m$\mu$M (Table 3).

Compared with its analogues 8a–g, EDDS (5) proved to possess the highest activity (IC$_{50}$ = 2.2 M). Modifications to the central aliphatic spacer in length or steric bulk (or both) were generally tolerated. However, elongation of the linker to four methylene units (8g) led to the complete loss of activity. The inhibitory activity of the naturally occurring AMB (1b) was also promising (IC$_{50}$ = 2.63 $\mu$M). Insertion of a methylene group as in compounds 1c and 1d maintained the activity leading to equipotent new AMB analogues.

Toxin A (1e) is believed to be the biosynthetic precursor of the related fungal aminocarboxylic acid AMA and AMB. We found low IC$_{50}$ values for toxin A (1e) and its diastereomer 1f (IC$_{50}$ = 2.33 $\mu$M and 2.89 $\mu$M respectively). Replacing the dianaminopropionic acid moiety with the much simpler glycine unit as in 1j led to a slight reduction of potency. Notably, elongation of the aliphatic spacers in both 1c and 1j to generate compounds 1g–i and 1k, resulted in further or complete loss of NDM-1 inhibitory activity.

The majority of MBL inhibitors reported to date owe their activity to an ability to bind zinc. In general, MBL inhibitors either coordinate with zinc ions within the MBL active site or, if they are strong enough chelators, actively strip zinc from the MBL active site rendering the enzyme inactive. We have previously shown that the zinc-binding capacity of MBL inhibitors can be conveniently quantified using isothermal titration calorimetry (ITC). To this end, we next measured the zinc-binding affinity of the aminocarboxylic acids analogues listed in Table 3. These studies were conducted by titrating a zinc-containing solution into the test compound with the heat of binding monitored using a microcalorimeter (see supplementary information for more detail). The relevant thermodynamic parameters thus obtained ($K_d$ and $\Delta H$) are presented in supplemental Table S1.

For compounds 1c–f, 1j, and 8e strong zinc binding was established with $K_d$ values in the nM range. Notably, in the case of compounds 1b–d, 8a, 8b, 8d, 8e, and 8f, the zinc binding interactions were found to be so strong ($K_d < 100$ nM)
Table 3  Activity of AMA and EDDS analogues against NDM-1 and an E. coli strain producing the same enzyme

| Compound | IC_{50} [µM] | FICI |
|----------|-------------|------|
| 1a (AMA) | 0.94 ± 0.11 | 0.063 |
| 1b (AMB) | 2.63 ± 0.10 | 0.063 |
| 1c       | 1.35 ± 0.12 | 0.156 |
| 1d       | 1.37 ± 0.04 | 0.094 |
| 1e       | 2.33 ± 0.18 | >0.281 |
| 1f       | 2.89 ± 0.24 | >0.281 |
| 1g       | 18.34 ± 3.67 | >0.281 |
| 1h       | >400        | >0.281 |
| 1i       | >400        | >0.281 |
| 1j       | 7.87 ± 0.29 | >0.281 |
| 1k       | >400        | >0.281 |
| 5 (EDDS 3Na) | 2.21 ± 0.39 | 0.047 |
| 8a       | 4.33 ± 0.11 | 0.094 |
| 8b       | 9.65 ± 0.16 | 0.281 |
| 8c       | 3.11 ± 0.19 | >0.281 |
| 8d       | 2.85 ± 0.10 | >0.281 |
| 8e       | 3.50 ± 0.16 | >0.281 |
| 8f       | 2.85 ± 0.04 | 0.156 |
| 8g       | >400        | >0.281 |
| EDTA 2Na | 1.25 ± 0.06 | 0.047 |
| DPA      | 4.94 ± 0.22 | 0.094 |

FICI, fractional inhibitory concentration index. FICI < 0.5 indicates synergy (see main text for formula used to calculate FICI). The test microorganism was E. coli RC0089, an NDM-1 positive patient isolate with an MIC for meropenem of 32 µg mL^{-1}.

Conflicts of interest

There are no conflicts to declare.

Notes and references

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Interestingly, neither toxin A or its analogues (1e–k) demonstrated potent synergistic activity suggesting they may not be able to effectively access the enzyme target in the microorganism.

In conclusion, we here describe the application of a robust chemoenzymatic synthesis route in the preparation of a series of novel aminocarboxylic acids. A number of these compounds were found to be potent inhibitors of NDM-1, with inhibitory activities well correlated to zinc binding ability. In addition, a number of the most active compounds demonstrated promising synergistic activity against an NDM-1 producing E. coli when combined with meropenem. In the search for new agents to combat antibiotic resistance, chemoenzymatic methodologies such as those here described have the potential to provide access to novel inhibitors of metallo-β-lactamases of clinical relevance.

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