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Captopril analogues as metallo-β-lactamase inhibitors

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IMP-1 inhibitor

Captopril

Antibiotic resistance

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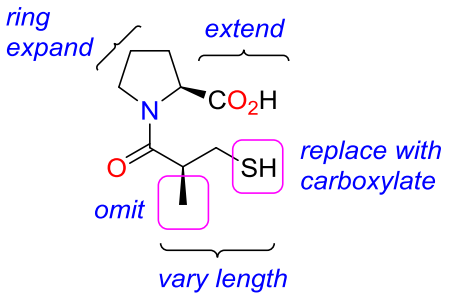
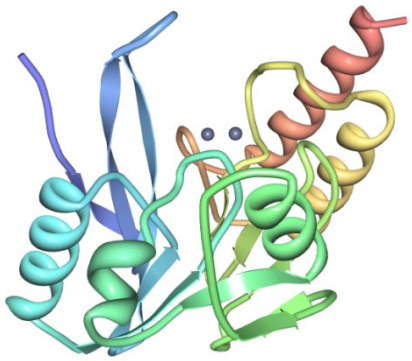
Graphical Abstract





Abstract

A number of captopril analogues were synthesised and tested as inhibitors of the metallo-β-lactamase IMP-1. Structure-activity studies showed that the methyl group was unimportant for activity, and that the potencies of these inhibitors could be best improved by shortening the length of the mercaptoalkanoyl side-chain. Replacing the thiol group with a carboxylic acid led to complete loss of activity, and extending the length of the carboxylate group led to decreased potency. Good activity could be maintained by substituting the proline ring with pipecolic acid.

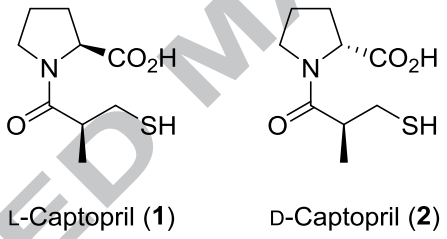
Due to their efficacy and safety, the β-lactam antibiotics, drugs that compromise the integrity of the bacterial cell wall, are the most widely prescribed drugs for the treatment of bacterial infections. Since the introduction of penicillin in the 1940s, several other classes of β-lactam antibiotics, such as carbapenems and cephalosporins, as well as many semi-synthetic members of these families, have been introduced into the clinic.[1]

Bacteria can overcome the effects of β-lactam antibiotics in a number of ways. In many cases resistant bacteria express lactamases, enzymes which hydrolyse the β-lactam ring of these drugs, thus rendering them ineffective.[2] Four classes of lactamase are known. Classes A, C and D are serine-β-lactamases (SBLs), so-called because they possess a nucleophilic serine residue in their active site which is responsible for the hydrolysis of the β-lactam ring of these compounds. The antibiotic resistance of bacteria that express SBLs may be overcome by co-administering β-lactam antibiotics with an SBL inhibitor, such as clavulanic acid. An example of this strategy is the widely prescribed drug Augmentin, a combination of the penicillin amoxicillin and clavulanic acid.[3]

The Class B lactamases are metallo-β-lactamases (MBLs) of which there are three subclasses, B1, B2 and B3, based on overall homology and active site geometry, and we have recently tentatively identified a fourth class, B4.[4] These enzymes require either one zinc ion (subclass B2) or two zinc ions (subclasses B1, B3 and B4) for activity.[5] MBLs have no structural or mechanistic relationship to the SBLs. The importance of the MBLs has increased markedly in recent years for a number of reasons: (1) they are capable of hydrolysing members of most β-lactam antibiotic classes; (2) pathogenic bacteria are increasingly expressing MBLs, including the notorious NDM-1 enzyme, which is capable of deactivating carbapenems; (3) the genes which code for MBLs can spread between bacteria via horizontal gene transfer, and (4) there are no clinically useful inhibitors of MBLs. Thus, the search for new MBL inhibitors and their development into drugs is both important and urgent.[5c]

Several classes of compounds have been reported as MBL inhibitors, including compounds which can coordinate to the zinc ions in the active site of the enzyme.[6] Thus, carboxylic acids[7] and thiol-

containing molecules[8] are particularly well-represented in the literature. An intriguing example of a good competitive MBL inhibitor is the anti-hypertensive drug L-captopril (**1**) (so-called because it is derived from L-proline), introduced as an inhibitor of angiotensin-converting enzyme,[9] but which also inhibits MBLs in subclasses B1-B3[7b, 10] (it has not yet been tested against the B4 subclass). We have determined L-captopril's K_i value against the IMP-1 enzyme (a B1 subclass MBL) to be 12.5 μM;[11] this compares well with values reported by other groups.[10a, 12] Since L-captopril is an established drug with good safety and bioavailability and contains the thiol and carboxylate functional groups often present in MBL inhibitors, it was of interest to examine how modifying its structure affected its ability to inhibit MBLs. This paper describes how inhibitory activity against the MBL IMP-1 varies with changes in the structure of captopril. A related strategy of testing simplified acyclic structures based on captopril as inhibitors of NDM-1 has been reported by Li *et al*.[8g]



L-Captopril (**1**) D-Captopril (**2**)

Figure 1.

Several crystal structures of L-captopril (**1**) and its stereoisomer D-captopril (**2**) in the active sites of MBLs have been solved, yet there still remains uncertainty regarding how these inhibitors bind in the active site in solution. While most studies have determined that the thiol group of captopril binds to the zinc ions in the active site,[12a, 13] other X-ray structures of the MBL-captopril complex show either no direct contacts between captopril and the metals (*e.g.*, with FEZ-1, a B3 MBL),[14] or show the carboxylate group of captopril, rather than the thiolate group, ligating a single metal ion (*e.g.*, with CphA, a B2 MBL).[10b] In the case of the complex between L-captopril and the MBL IMP-1, binding of the inhibitor to the metal ions in the active site is via the thiolate group which displaces a bridging water molecule, and binding is stabilised by an ionic interaction of the carboxylate group of captopril with a positively charged proximal Lys224 residue. An additional weak H-bonding interaction

between the captopril carboxylate and the backbone NH of Asn233 and a hydrophobic interaction between Trp64 and the pyrrolidine ring are also observed (Figure 2).[12a] In an attempt to improve upon these stabilising interactions, and thus obtain stronger binding inhibitors, we modified the structure of captopril by (1) varying the length of the pendent thiol chain, while removing the methyl group; (2) extending the distance between the thiol group and the carboxylate group by incorporating a (carboxylmethoxy)methyl group in place of the carboxylate group; (3) replacing the thiol functional group with a carboxylate group, and (4) expanding the pyrrolidine ring by one atom to a piperidine.

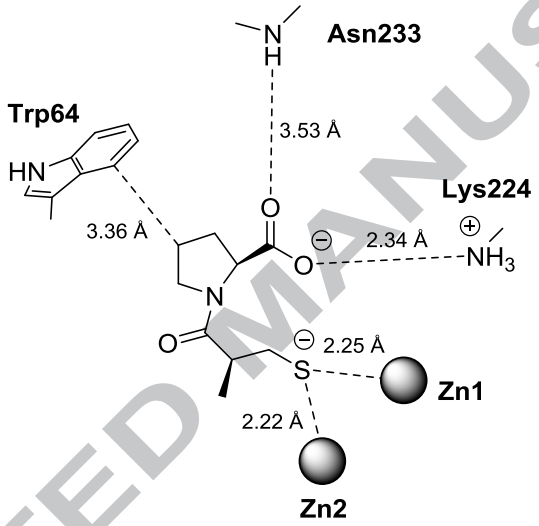
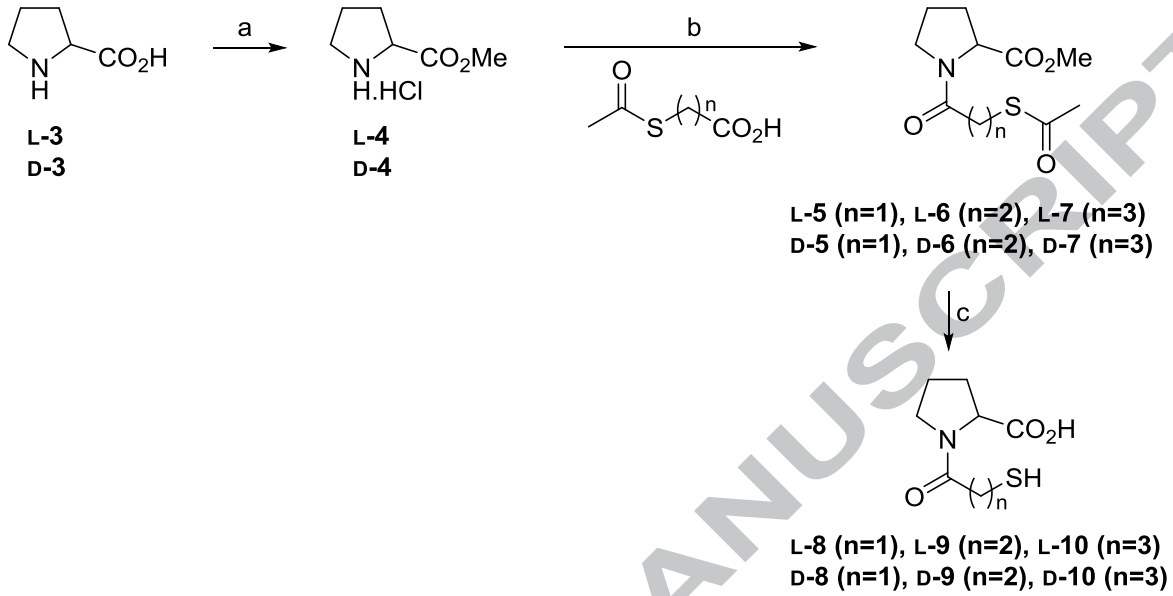


Figure 2. Binding interactions of L-captopril (**1**) in the active site of IMP-1.[12a]

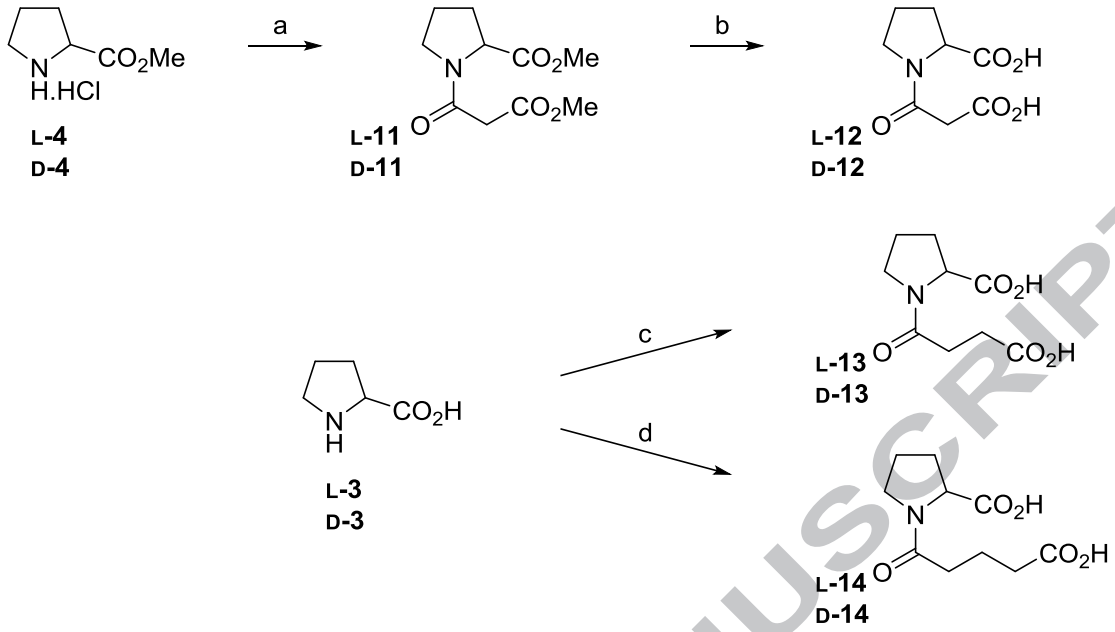
Scheme 1 shows the syntheses of mercaptoalkanoyl derivatives of D- and L-proline. Formation of the methyl ester hydrochlorides **L-4** and **D-4** could be achieved readily from proline (**3**) in quantitative yield using thionyl chloride in methanol, either overnight at room temperature or refluxing for 3 hours.[15] Coupling of **L-4** and **D-4** with either acetylthioacetic acid, 3-(acetylthio)propanoic acid or 4-(acetylthio)butanoic acid using *N,N,N′,N′*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) and *N,N*-diisopropylethylamine (DIPEA) in THF gave the corresponding amides **5-7**, generally in quantitative yields. Attempted acid hydrolysis of these compounds proved unsuccessful, resulting either in recovered starting material or selective hydrolysis of the thioester groups. However, base hydrolysis of **5-7** using sodium hydroxide in methanol

successfully cleaved both methyl ester and thioester groups of these compounds, giving the carboxylate-thiols **8-10** in excellent yields.



Scheme 1. Synthesis of the mercaptoalkanoyl derivatives of L- and D-proline. Reagents and conditions: (a) $SOCl_2$, MeOH, rt (**L-4**, 100%) or Δ (**D-4**, 100%); (b) HBTU, DIPEA, THF, rt (**L-5**, 65%; **L-6**, 100%; **L-7**,100%; **D-5**, 100%; **D-6**, 100%; **D-7**,100%); (c) 2 M NaOH, MeOH, rt (**L-8**, 100%; **L-9**, 99%; **L-10**, 100%; **D-8**, 89%; **D-9**, 84%; **D-10**, 89%).

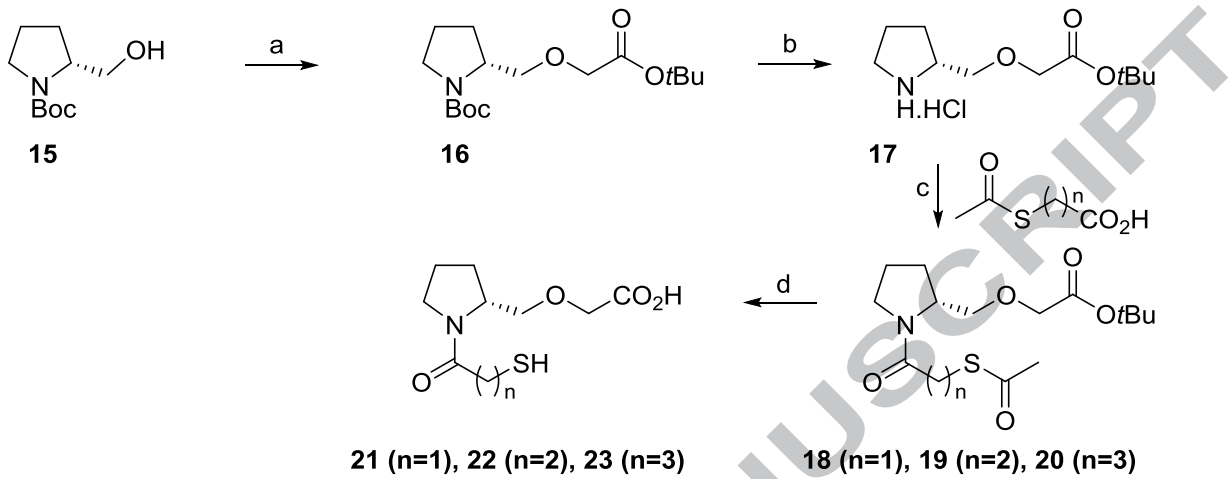
The syntheses of the carboxyalkanoyl derivatives of D- and L-proline are shown in Scheme 2. Coupling of **L-4** and **D-4** with methyl potassium malonate using HBTU and DIPEA gave the diesters **L-11** and **D-11** in quantitative yield. Subsequent base-mediated hydrolysis of both methyl ester groups of **11** using sodium hydroxide in methanol solution then afforded diacids **L-12** and **D-12**, in 72% and 60% yields, respectively. The higher homologues **13** and **14** were prepared more directly but in moderate yields by reacting D- or L-proline (**3**) with triethylamine and either succinic anhydride or glutaric anhydride, respectively.[16]



Scheme 2. Synthesis of the carboxyalkanoyl derivatives of L- and D-proline. Reagents and conditions: (a) $KO_2CCH_2CO_2Me$, HBTU, DIPEA, THF, (**L-11**, 100%; **D-11**, 100%); (b) 2 M NaOH, MeOH, rt (**L-12**, 72%; **D-12**, 60%); (c) succinic anhydride, Et_3N, MeCN, 0 °C (**L-13**, 46%; **D-13**, 51%); (d) glutaric anhydride, Et_3N, MeCN, 0 °C (**L-14**, 48%; **D-14**, 43%).

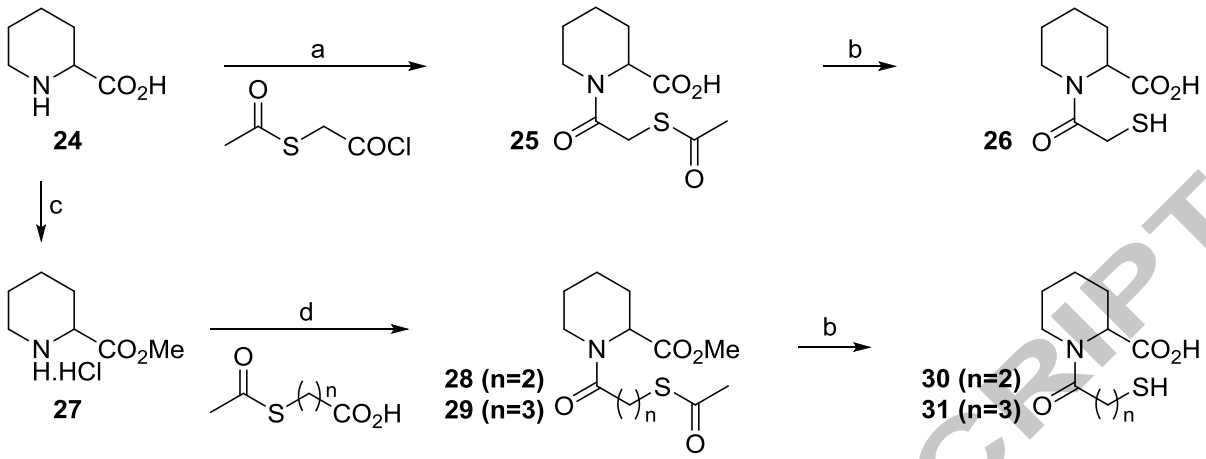
The preparation of the captopril analogues with the carboxylic group extended from the pyrrolidine ring is shown in Scheme 3. The synthesis starts from commercially available Boc-protected D-prolinol (**15**). Attempts to *O*-alkylate **15** with ethyl bromoacetate under phase-transfer conditions led only to the recovery of the prolinol starting material. However, the use of *tert*-butyl bromoacetate in its place gave under the same conditions a high yield of the alkylated product **16**.[17] It seems that ethyl bromoacetate (but not *tert*-butyl bromoacetate) hydrolyses under the reactions conditions to the less electrophilic bromoacetic acid. Selective removal of the Boc group of **16** in the presence of the *tert*-butyl ester was accomplished using anhydrous HCl in dioxane,[18] to give the hydrochloride salt **17** almost quantitatively. Coupling of **17** with either acetylthioacetic acid, 3-(acetylthio)propanoic acid or 4-(acetylthio)butanoic acid using the same protocol outined in Scheme 1 then gave compounds **18-20**,

albeit in poor yields. Finally, deprotection of the *tert*-butyl ester and the thioacetate groups of these compounds was achieved using 6 M HCl, which gave target inhibitors **21-23** in excellent yields.



Scheme 3. Synthesis of derivatives of D-prolinol. Reagents and conditions: (a) $BrCH_2CO_2tBu$, NaOH, PhMe, TBAHS, rt, 89%; (b) 4 M HCl/dioxane, rt, 97%; (c) HBTU, DIPEA, THF, rt (**18**, 16%; **19**, 36%; **20**, 24%); (d) 6 M HCl, rt (**21**, 100%; **22**, 100%; **23**, 76%).

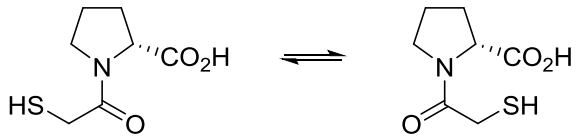
The pipecolic acid-based captopril analogues **26** and **30-31** were prepared by two slightly different routes, as outlined in Scheme 4. Reaction of racemic pipecolic acid **24** with (acetylthio)acetyl chloride gave the amide (**25**) but in poor yield. Subsequent base-mediated hydrolysis of **25** with sodium hydroxide in methanol then gave target **26**. The two other targets **30** and **31** were prepared more efficiently by coupling pipecolic acid methyl ester hydrochloride (**27**)[19] with either 3-(acetylthio)propanoic acid or 4-(acetylthio)butanoic acid to give **28** and **29**, respectively. Hydrolysis of the methyl ester and thioester groups then yielded compounds **30** and **31**, respectively, in almost quantitative yields.



Scheme 4. Synthesis of derivatives of pipecolic acid. Reagents and conditions: (a) DIPEA, THF, rt, 25%; (b) 2 M NaOH, MeOH, rt (**26**, 73%; **30**, 97%; **31**, 100%); (c) $SOCl_2$, MeOH, Δ, 100%; (d) HBTU, DIPEA, THF, rt (**28**, 100%; **29**, 99%).

Inhibitory activities of the captopril analogues against the metallo-β-lactamase IMP-1 are shown in Table 1. All of the inhibitors showed purely competitive inhibition.

Compounds **8-10** in both the L- and D-series all have comparable K_i values and were all slightly more potent inhibitors than L-captopril (**1**). This suggests that these inhibitors bind in the active site of IMP-1 in similar ways, but that the length of the pendent mercaptoalkyl chain is not crucial for tight binding. Presumably these inhibitors can adopt binding conformations that allow strong interactions between the metal ions and the thiolate group, while maintaining an effective salt bridge between the inhibitor's carboxylate and the proximal Lys224 near the active site. Molecular modelling of these compounds indicated that this was generally the case. An example is the *cis*-rotamer of the potent inhibitor **D-8** docked into the active site of IMP-1. The thiolate group coordinates to both zinc(II) ions and the carboxylate moiety forms ionic interactions with both Lys224 and one of the metal ions (Figure 3). In all cases modelling of inhibitors docked into the active site of IMP-1 was performed on both rotamers of each compound; generally the *cis*-rotamers appear to bind more strongly than the *trans*-rotamers (see Supplementary data for more details).



D-8 (*trans*-rotamer) **D-8** (*cis*-rotamer)

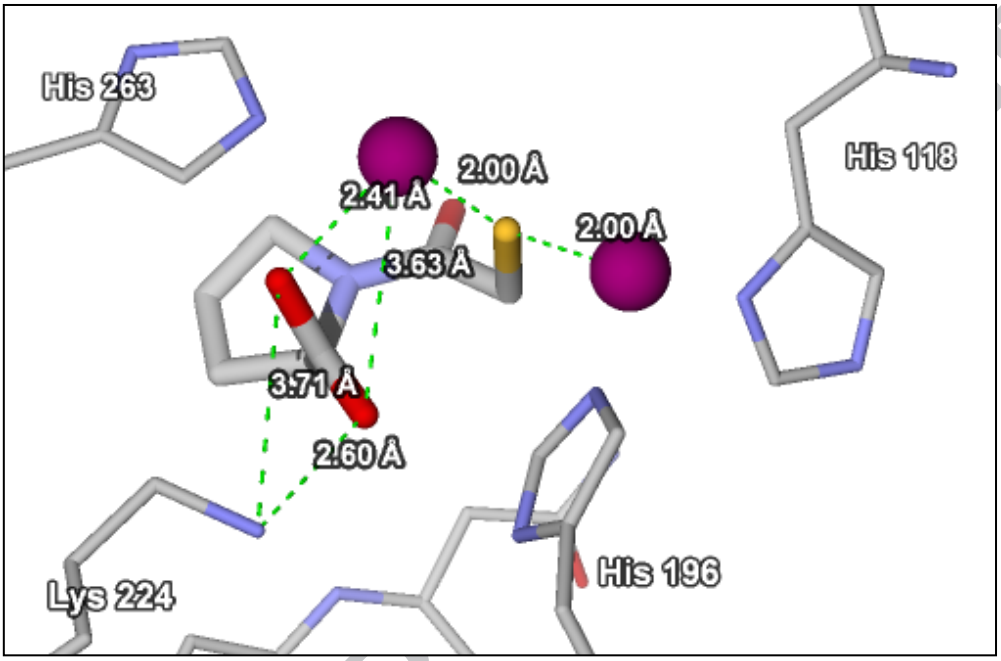


Figure 3. Docking of the *cis*-rotamer of compound **D-8** into IMP-1 active site. Colours: Blue – nitrogen; red – oxygen; grey – carbon; yellow – sulfur; magenta – Zn ions.

That both the L- and D-series of compound **8-10** show comparable inhibitory potency against IMP-1 is consistent with recent studies by Schofield's group who examined the inhibition constants of all four possible stereoisomers of captopril with various MBLs; with IMP-1 all isomers had similar potencies. This was rationalised as being due to similar binding modes of all stereoisomers in the active site and was supported by crystal structures of the same molecules bound in the active site of IMP-1.[12a]

Both the L- and D-series of compounds **12-14** surprisingly were devoid of inhibitory activity against IMP-1. This was unexpected because many potent inhibitors of MBLs are dicarboxylic acids, and X-ray structures of compounds from this class bound to IMP-1 show that one carboxylate typically binds directly to both metal ions, while the other binds to one metal ion while maintaining the salt bridge with Lys224.[7e] Modelling of compounds **12-14** in the active site of IMP-1 suggested that they could

adopt a similar mode of binding allowing ionic interactions between the carboxylate groups and both the metal ions and Lys224 of IMP-1 (data not shown). The reasons why compounds **12-14** are poor inhibitors of IMP-1 are unclear but, as Fast and Sutton have pointed out, the most potent reported dicarboxylic acid MBL inhibitors are based on succinic acid backbones, or have rigid templates holding the two carboxylate groups close in space (e.g., phthalates and maleates).[20]

The captopril analogues with the chain-lengthened carboxylate group (**21-23**) all display similar and weak inhibitory activity against IMP-1. Modelling again showed that the thiolate groups of these inhibitors coordinate to both zinc(II) ions, and that the carboxylate groups interacts ionically with both Lys224 and the metal ions (data not shown). It may be that the increased number of rotatable bonds in these molecules leads to decreased binding affinity for the active site of IMP-1.

The ring-expanded piperidine compounds (**26**, **30** and **31**) all possess low micromolar inhibitory activity against IMP-1, comparable in potency to those inhibitors bearing a pyrrolidine ring (**8-10**). This suggests that expanding the size of the ring has minimal impact on how these inhibitors bind to IMP-1. This is supported by the crystal structures of L- and D-captopril in the active site of IMP-1, which both suggest weak hydrophobic interactions of the pyrrolidine ring with Trp64, and scope for extending this region of the inhibitor towards this residue.[12a] Molecular modelling of the L-enantiomer of inhibitor **31** suggests that this is reasonable. Strong zinc(II)—thiolate bonds are predicted, as well as ionic interactions between the carboxylate of **L-31** and Lys224. A hydrogen bond between the carboxylate of **L-31** and the backbone NH of Asn233 is also predicted, similar to the binding interactions observed in the crystal structure of IMP-1 complexed with L-captopril (**1**) (Figure 2).

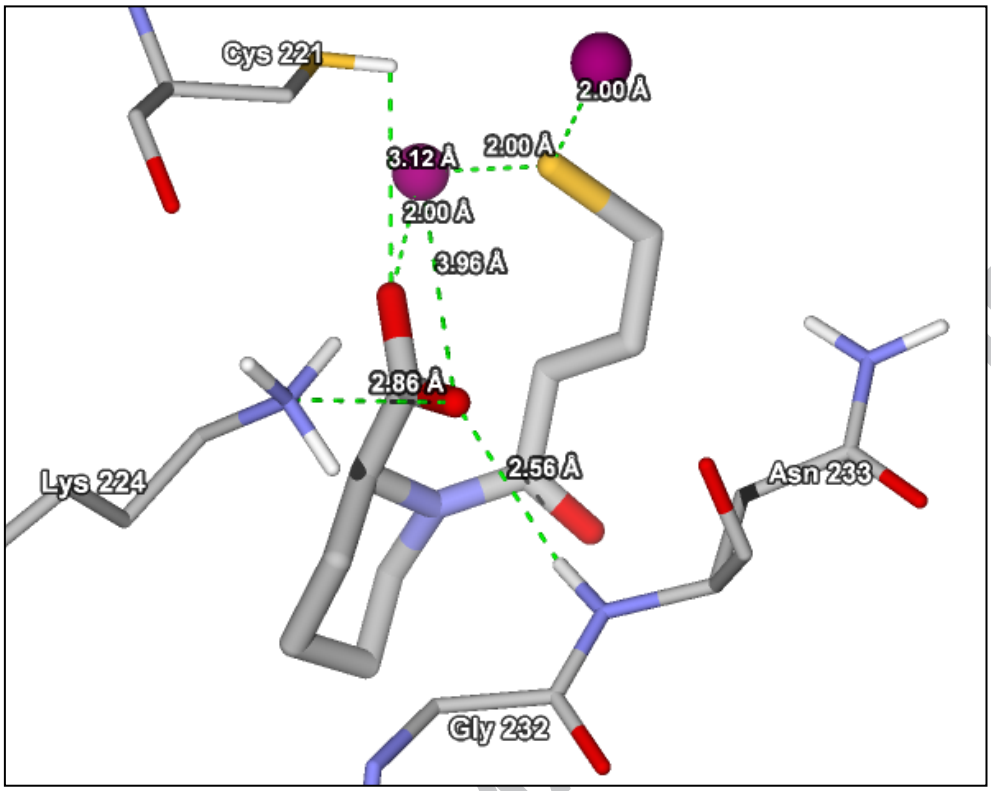
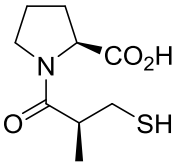
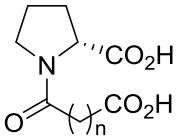
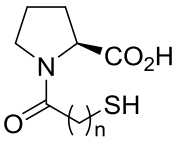
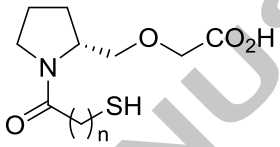
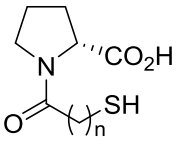
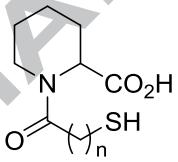
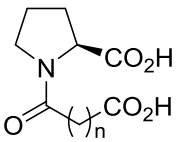
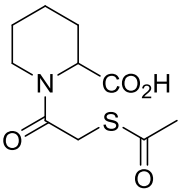


Figure 4. Docking of compound **L-31** into the active site of IMP-1. Colours: Blue – nitrogen; red – oxygen; grey – carbon; yellow – sulfur; magenta – Zn ions.

The synthetic intermediates described here were tested for their inhibitory activity against IMP-1. While most of these proved to be in inactive (data not shown) compound **25** exhibits a K_i value of 24 μM, making it only slightly less potent than compounds **8-10**, **26**, **30**, **31** or captopril. Other thioesters have been noted as potent IMP-1 inhibitors and it has been shown that these thioesters are readily hydrolysed *in situ* by IMP-1 to thiols, the actual inhibitory agents of this enzyme.[8c] Such a process may be operational here, although the direct inhibition of IMP-1 thioester by **25** cannot be ruled out.

Table 1. IMP-1 inhibitory activity of captopril analogues

Compound		K_{ic} (μM)	Compound		K_{ic} (μM)
	L-Captopril **1**	12.5 ± 2.4[a]		**D-12**, n=1	-
	**L-8**, n=1	2.2 ± 0.6		**D-13**, n=2	-
	L-9, n=2	9.9 ± 4.2		**D-14**, n=3	-
	L-10, n=3	7.2 ± 4.1		**21**, n=1	290 ± 146
	**D-8**, n=1	2.3 ± 1.5		**22**, n=2	390 ± 178
	D-9, n=2	2.9 ± 0.9		**23**, n=3	363 ± 129
	D-10, n=3	5.8 ± 4.0		**26**, n=1	3.4 ± 1.3
	L-12, n=1	-[b]		**30**, n=2	10 ± 4.0
	**L-13**, n=2	-		**31**, n=3	7.1 ± 1.7
	L-14, n=3	-		**25**	24 ± 3.4

[a] Value from Vella *et al.*[11]

[b] Indicates no significant inhibitory activity.

The structures of the MBL inhibitors L- and D-captopril (**1** and **2**) have been systematically varied to examine the effects of these structural changes on inhibitory activity against IMP-1. Deletion of the methyl group from either L- or D-captopril (compound **9**) led to a slight increase in potency, indicating that its presence is not needed for activity against IMP-1. Lengthening or shortening the pendent mercaptoalkyl chain by one methylene unit (compounds **8** and **10**) also gave inhibitors slightly more potent than captopril, indicating that a particular length of the thioalkanoyl chain is not essential for

activity. Replacement of the thiol group with a carboxylate led to complete loss of activity, in both the L- and D-series, suggesting that thiol group is essential for maintaining activity in captopril analogues (compounds **12**-**14**). Extending the carboxylate group out from the pyrrolidine ring gave compounds only very weakly active. The racemic piperidine-based inhibitors (**26**, **30** and **31**) showed good activity, with all of them being at least as potent as captopril.

Acknowledgements

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Supplementary data

Experimental procedures and characterisation of compounds; methodologies for enzyme inhibitory assays; ^{1}H NMR, ^{13}C NMR and HRMS spectra of compounds **L-8**, **L-9**, **L-10** and **30**; kinetics plots for compounds **D-8** and **31**; molecular modelling docking scores of inhibitor-IMP-1 complexes.

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