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STRUCTURE–ACTIVITY RELATIONSHIPS OF BIPHENYL TETRAZOLES AS METALLO-β-LACTAMASE INHIBITORS†

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Abstract: Resistance to carbapenem antibiotics in Gram-negative bacteria is due, in part, to expression of a wide spectrum metallo-β-lactamase, which renders the drug inactive. Biphenyl tetrazoles containing 3-*n*-butyl-1-phenylpyrazole-5-carboxylates or the corresponding 5-ethyl esters were found to inhibit metallo-β-lactamases as well as renal dehydropeptidase I to a lesser extent.

Introduction: Carbapenem antibiotics such as imipenem are useful for the treatment of a variety of Gram-negative and Gram-positive infections.[1] However, antibiotic resistance has emerged in part due to class B metallo-β-lactamases (MBLs), which hydrolyze β-lactams, cephalosporins and carbapenems rendering them ineffective.[2] One approach to this important medical problem is to combine the antibiotic with an enzyme inhibitor as was done with Augmentin™, which is comprised of the β-lactam antibiotic amoxicillin and clavulanic acid, a "suicide" inhibitor of the class A serine β-lactamases.[3] In a search for agents that could prevent the hydrolysis of carbapenems, biphenyl tetrazoles (BPTs)[4a] were found to be inhibitors of an MBL[4b] cloned from an imipenem-resistant clinical isolate of *Bacteroides fragilis* (*B. fragilis*). Some BPTs have been reported to be potent nonpeptide antagonists of angiotensin II and have been used for the treatment of hypertensive disorders.[5] The present paper describes emerging structure–activity relationships (SAR) of the BPT class as candidates for reversing antibiotic resistance in *B. fragilis* as well as in *Pseudomonas aeruginosa* (*P. aeru.*) mediated by the plasmid-borne IMP-1 enzyme. Recent reports of MBL inhibitors distinct from BPTs have appeared including thiol esters,[6a,b,g] thiols,[6c,d] trifluoromethyl alcohol and ketone derivatives of L- and D-alanine[6e] and amino acid-derived hydroxamates.[6f] However, only thiols have been shown to inhibit the IMP-1 enzyme present in *Serratia marcescens*.[6c]

Purified recombinant *B. fragilis*[4b] and IMP-1[6g] MBL was prepared as described. Activity was assessed using the chromogenic substrate nitrocefin at K_m levels in a 96-well microtiter plate as described.[4b] Renal

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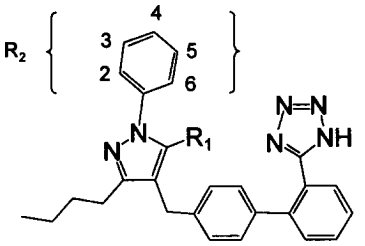
dehydropeptidase-I (DHP-I) was measured as described.[4a] K_i^{app} values are the apparent inhibition constant representing the average of two or more independent experiments. Preparation of the compounds described in this report has been described previously.[7] In brief, *C*-alkylation of ethyl 2-(methoxyimino)-4-oxooctanoate by 4'-(bromomethyl)-2-biphenylcarbonitrile was followed by regiospecific condensation with an arylhydrazine hydrochloride to give the 1-arylpyrazole-5-carboxylate. The tetrazole was generated from the nitrile by heating with trimethyltin azide, and saponification of the ester furnished the carboxylic acid.

Biological Activity: A series of BPTs containing 3-*n*-butyl-1-phenylpyrazole-5-carboxylates was explored as summarized in Table 1. The parent compound **1** bearing an unsubstituted phenyl group was found to be a weak *B. fragilis* MBL inhibitor with an IC_{50} value of ~ 200 ± 8 μM. A more detailed study revealed competitive inhibition with a K_i value of 90 ± 30 μM (correlation coefficient $r^2 = 0.9688$) although the data could also be fit to a non-competitive model with a K_i value of 150 ± 35 μM ($r^2 = 0.9662$). The parent compound **1** was also found to be a weak inhibitor of DHP-I, a metalloenzyme which is capable of hydrolyzing imipenem. Compound **2** containing one chlorine at the 2 position on the phenyl ring was much more potent with an IC_{50} value of ~ 30 ± 10 μM against the *B. fragilis* enzyme but was ineffective against both IMP-1 and DHP-I. Kinetics of inhibition were consistent with a competitive inhibitor with a K_i value of 15 ± 4 μM. Thus, a chlorine substituent on this phenyl ring plays an important role in inhibition of the *B. fragilis* enzyme contributing to a factor of at least a sixfold increase in binding affinity.

Various substitutions on the phenyl ring (R_2) were explored in order to determine whether the position of the chlorine was important for activity against the *B. fragilis* enzyme and whether other groups could replace the halogen. Single chlorines at the 3 or 4 position or a bulky phenyl group at the 2 position of the phenyl group (compounds **3**, **4**, and **13**, respectively) exhibited similar activity to that of the parent compound **1**. Addition of a nitro group at the 2 position led to a modest increase in activity (compound **12**). Substitution of a methyl group at the 2 position led to an increase in activity of two- to threefold (compound **10**). Interestingly, trifluoromethyl can substitute for the chlorine at the 2 position with similar enzyme inhibition (compound **11**). Two chlorines at positions 2/3, 2/4 or 2/5 (compounds **5**, **6**, and **7**, respectively) or three chlorines at the 2/4/6 positions (compound **9**) exhibited activities of three- to sixfold greater than that of the parent compound. In contrast, substitution of chlorine simultaneously at positions 2 and 6 led to only a modest increase in activity (compound **8**). Taken together, these data indicate that the presence of a single chlorine at the 2 position is a predominant factor in recognition by the *B. fragilis* enzyme and that similar binding can be achieved by substitution with a trifluoromethyl but not a methyl group. The SAR found for *B. fragilis* MBL do not seem to apply to DHP-I or to IMP-1, since these compounds are generally poor inhibitors. However, the biphenyl compound **13** was found to be a modest inhibitor of IMP-1.

Table 1. Enzyme Inhibition by Biphenyl Tetrazoles



			IC_{50} (μM)		K_i^{app} (μM)
Compound	**R_1**	**R_2**	***B. fragilis* MBL**	**IMP-1 MBL**	**DHP-I**
1	CO_2H	Ph	200 ± 8	>200	590 ± 100
2	CO_2H	Ph (2-Cl)	30 ± 10	>200	350 ± 100
3	CO_2H	Ph (3-Cl)	190 ± 20	>200	425 ± 60
4	CO_2H	Ph (4-Cl)	230 ± 4	>200	430 ± 70
5	CO_2H	Ph (2,3-Cl_2)	37 ± 3	>200	310 ± 20
6	CO_2H	Ph (2,4-Cl_2)	80 ± 40	>200	400 ± 100
7	CO_2H	Ph (2,5-Cl_2)	50 ± 20	>200	380 ± 130
8	CO_2H	Ph (2,6-Cl_2)	100 ± 40	>200	500 ± 50
9	CO_2H	Ph (2,4,6-Cl_3)	33 ± 2	>200	800 ± 300
10	CO_2H	Ph (2-CH_3)	80 ± 20	>200	470 ± 80
11	CO_2H	Ph (2-CF_3)	40 ± 20	>200	380 ± 130
12	CO_2H	Ph (2-NO_2)	120 ± 30	>200	640 ± 140
13	CO_2H	Ph (2-Ph)	180 ± 20	65 ± 10	420 ± 70
14	CO_2Et	Ph	100 ± 20	>200	250 ± 50
15	CO_2Et	Ph (2-Cl)	170 ± 20	200 ± 20	250 ± 50
16	CO_2Et	Ph (3-Cl)	170 ± 60	76 ± 10	450 ± 50
17	CO_2Et	Ph (4-Cl)	290 ± 60	70 ± 25	380 ± 120
18	CO_2Et	Ph (2,3-Cl_2)	100 ± 20	175 ± 20	170 ± 10
19	CO_2Et	Ph (2,4-Cl_2)	100 ± 40	66 ± 10	480 ± 20
20	CO_2Et	Ph (2,5-Cl_2)	100 ± 10	60 ± 30	80 ± 25
21	CO_2Et	Ph (2,4,6-Cl_3)	80 ± 30	>200	450 ± 80
22	CO_2Et	Ph (2-CH_3)	70 ± 30	>200	--
23	CO_2Et	Ph (2-CF_3)	22 ± 10	>200	--
24	CO_2Et	Ph (2-NO_2)	18 ± 2	>200	--
25	CO_2Et	Ph (2-Ph)	>200	150 ± 10	--

The series described above was expanded to include ethyl esters of the 5-carboxylic acids. By comparing equivalent compounds containing ethoxycarbonyl vs. carboxylic acid, compound **14** showed ~twofold increased enzyme inhibition. Compounds bearing a chlorine at the 2 position (compound **15**), two chlorines at positions 2/3 or 2/5 (compounds **18** and **20**, respectively), or three chlorines at positions 2,4 and 6 (compound **21**) led to a loss of activity. In contrast, compounds bearing a CH_3 or CF_3 group at the 2 position showed similar activity as their carboxylic acid counterparts. The ethoxycarbonyl series bearing a chlorine at position 3 (compound **16**) or position 4 (compound **17**) or two chlorines at positions 2/4 (compound **19**) exhibited the same inhibition as the corresponding compounds containing 5-carboxylic acid. In contrast, the presence of a nitro group at position 2 (compound **24**) in this series led to a sixfold increase in activity. Interestingly, of the compounds described in the present manuscript, compound **15** exhibits approximately equipotent inhibition, albeit modest, against the MBLs from *B. fragilis* and from *P. aeru.* as well as DHP-I. These observations indicate that the presence of carboxylic acid is important for enzyme recognition in conjunction with 2-chlorophenyl but not with chlorine present at other positions or with CH_3 or CF_3 substitutitions on the phenyl group. However, a nitro group at position 2 in this series exerts a greater influence on enzyme inhibition than does the presence of carboxylic acid at R_1.

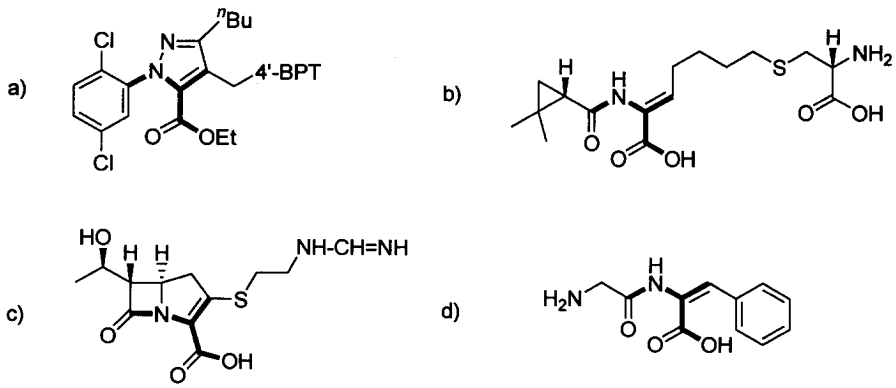


Figure 1

Since imipenem is co-administered with the DHP-I inhibitor cilastatin to prevent renal metabolism of the antibiotic,[1] a potential utility of the compounds described above would be to inhibit DHP-I as well as multiple MBLs. Although these compounds showed only a modest level of inhibition against DHP-I (e.g., compound **20**), the fact that such a small number of compounds from a much larger set of BPTs[4] showed any inhibition invited a closer inspection of the 4'-substituents on the BPT. Figure 1 illustrates a simple comparison

of compound **20** (a) with cilastatin (b), imipenem (c) and glycyldehydrophenylalanine (d), a substrate for DHP-I. Compounds constituting only the 4'-substituent without the BPT were not available. However, compounds were identified in the Merck chemical collection by simple similarity searches based on compound **20**. Since such compounds were found to be inactive in the enzyme assays (data not shown), the BPT portion of the molecule may contain some element required for enzyme binding. This suggests that there is a reasonable prospect for identifying a compound not only capable of broad spectrum inhibition of different enzymes, but one in which the SAR of the binding modes overlaps substantially. This would result in a compound lower in molecular weight than a simple chimera, a pharmacologically desirable property in the early stages of drug discovery.

In conclusion, BPT derivatives are emerging as inhibitors of MBLs from *B. fragilis* and from *P. aeru.* (i.e., plasmid-borne IMP-1). This report describes different SAR between the *B. fragilis* and IMP-1 enzymes, consistent with structural heterogeneity amongst the MBL family. A challenge for the pharmaceutical industry will be to identify potent, balanced inhibitors against MBLs found in clinically important bacteria. Such agents could be used to reverse resistance to carbapenem antibiotics analogous to that achieved with Augmentin™ with the reversal of β-lactam resistance.

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