SAR Leading to the Discovery of ANT3310, a Novel DBO β-lactamase Inhibitor which Potentiates Meropenem Activity Against Carbapenemase-Resistant *Acinetobacter baumannii* (CRAB) and Enterobacteriaceae (CRE)

Simon LEIRIS, Clarisse LOZANO, Justine BOUSQUET, Jerome CASTANDET, Agustina LLANOS, Laethitia ALIBAUD, Nicolas SPRYNSKI, Magdalena ZALACAIN, Martin EVERETT, David DAVIES

Antabio SAS, 31670 Labège, France

ABSTRACT

Background: There is an urgent need to develop improved therapies for the treatment of hospital infections caused by carbapenem-resistant Gram-negative pathogens. Of particular concern is the spread of class D OXA-type carbapenemases among carbapenem-resistant *Acinetobacter baumannii* (CRAB) and Enterobacteriaceae (CRE). We here describe the rational design and optimization of a novel diazabicyclooctanone (DBO) β-lactamase inhibitor, ANT3310, with broad-spectrum activity against class A, C and D serine β-lactamases (SBLs), including KPC- and OXA-type carbapenemases.

Materials/methods: Compound synthesis was carried out by GVKBio (India). MIC determinations on clinical isolates were performed using the CLSI broth micro-dilution method with fixed concentrations of SBL inhibitors. Enzyme inhibition assays were performed using purified SBL enzymes; the hydrolysis of nitrocefin was followed using a Perkin Elmer Envision plate reader and IC_{50} values determined using Dotmatics analytical software. Molecular modelling was performed with Flare, part of the Cresset™ suite of packages.

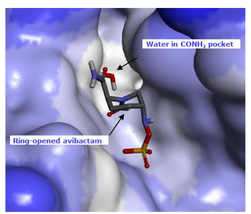
Results: A novel series of DBO analogues bearing halogen-containing substituents was prepared. Lead optimisation was guided by enzymatic inhibition of representative SBLs, potentiation of meropenem against carbapenemase-producing clinical strains (including *A. baumannii, Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae*) expressing a variety of SBLs (including AmpC, ESBL, KPC and OXA variants) and modelling of the covalent products formed by reaction of the active serine of OXA-48 and the inhibitor compound. Exploration of the SAR led to insights into both enzyme inhibition and efficient entry into the periplasm. Smaller, electron-withdrawing substituents, restricted to one carbon combined with various halogens, led to improved meropenem potentiation. Taking the small size of the substituent to the limit, a simple fluorine substituent led to ANT3310, which showed broad-spectrum inhibition of relevant SBLs (IC_{50} values between 1 – 175 nM), excellent potentiation of meropenem against clinical isolates (MICs between 0.06 – 1 μg/mL) and promising drug-like properties.

Conclusions: Rational-design and optimization led to the identification of a novel DBO compound, ANT3310. This compound shows broad-spectrum SBL inhibition and restores the activity of meropenem against CRAB and CRE pathogens and is a candidate for preclinical development.

INTRODUCTION

Multi-drug resistant (MDR) Gram-neg bacteria are an increasingly serious healthcare threat[1]. The main resistance mechanism for β-lactam antibiotics is the production of β-lactamase enzymes. Co-dosing of β-lactams with a β-lactamase inhibitor (BLI) is a clinically successful strategy to combat resistance but established inhibitors (such as clavulanic acid) have become less effective due to bacterial production of extended spectrum β-lactamases (ESBLs). The diazobicyclooctane (DBO) BLI avibactam[2] has entered clinical practice, with other analogues following behind (eg relebactam, zidebactam). However current DBOs are not ideal as (i) they do not have BLI activity against OXA-producing *A. baumannii* and (ii) their activity often partly relies on weak intrinsic antibacterial activity via PBP-2 inhibition, introducing a vulnerability to resistance selection.

We explored replacement of the primary carboxamide of avibactam with small, electron-withdrawing substituents, as we reasoned that the larger secondary carboxamides of other DBOs were contributing to the weak intrinsic antibacterial activity. We also gave consideration to the X-ray complex of OXA-48 covalently bound to avibactam which has a small pocket in the active site containing two water molecules and the carboxamide group. We hypothesized that a slightly larger substituent might be accommodated, possibly by displacement of the water molecules, thus more completely filling the pocket and increasing the interaction with the enzyme. The med chem campaign began by exploring the SAR of a carbon substituent substituted by electron-withdrawing halogen atoms.



X-ray of Avibactam covalently bound to OXA-48 via active site serine (PDB 4S2K)

METHODS

Chemistry: The synthesis of all new compounds was carried out by GVKBio (India) and will be reported elsewhere

Enzymology: Enzyme inhibition assays were performed using purified SBL enzymes (such as TEM-1; AmpC; CTX-M15; KPC-2; OXA-48) in 10mM HEPES buffer pH 7.5 in 96-well microtiter plates. Nitrocefin (100μM for TEM-1, AmpC, KPC-2, OXA-48; and 50μM for CTX-M15) was used as substrate. Hydrolysis was followed (after an initial 10min incubation at 30° C) at 482 nm for 12 minutes every 30 seconds using a Perkin Elmer Envision UV fluorescence plate reader. Hydrolysis rate data in presence of a range of inhibitors was analysed and IC_{50} determined for each compound using Dotmatics database software.

***In vitro* antimicrobial susceptibility testing:** Imipenem MIC values of SBL-producing clinical isolates were determined by the CLSI broth microdilution method (10) using Ca-adjusted Mueller-Hinton broth with an inoculum size of 5 x 10^4 CFU/well. Results were recorded after 18 h of incubation at 35-37 °C.

Molecular modelling: This was performed with Flare software (Cresset™). X-ray structures of avibactam in OXA-48 (PDB 4S2K) were modified with regard to the avibactam substituent and minimized (with and without water molecules). Potential substituents were ranked on the basis of movement of the DBO core and key amino acid residues compared to the starting position – larger movements were indicative of a poor fit.

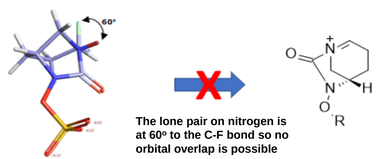
RESULTS

Table of Enzyme Inhibition and Meropenem Potentiation

Cpd	R	Volume R (Å3)	IC50s (μM) of compound for serine-β-lactamases					MICs (μg/mL) of meropenem in presence of compound (at 4 μg/mL)				
			AmpC	CTX-M15	TEM-1	OXA-48	KPC-2	*Enterobacter cloacae* **KPC-2** + TEM-1	*Klebsiella pneumoniae* **OXA-48** + TEM + CTX-M14 +	*Klebsiella pneumoniae* **KPC-3** + SHV-11 + TEM-1	*Klebsiella pneumoniae* **OXA-181** + SHV-11	*Acinetobacter baumannii* **OXA-23**
No cpd	-	-	-	-	-	-	-	8	16	128	16	32
1 avibactam	$CONH_2$	35.2	0.008	0.001	0.005	0.252	0.008	0.03	0.5	0.25	1	16
2	H	5.1	>3.0	1.200	0.511	1.337	>3.0	2	4	64	8	32
3	CH_2F	26.9	0.304	0.119	0.492	0.154	0.051	0.125	1	2	1	32
4	CH_2Cl	36.0	0.026	0.084	0.135	0.443	0.028	0.06	8	2	16	32
5	CHF_2	31.9	0.035	0.017	0.208	0.064	0.007	0.06	2	1	1	16
6	CF_3	36.9	0.004	0.010	0.810	0.019	0.006	0.06	2	2	2	16
7	SCF_3	55.7	0.002	0.010	0.026	0.099	0.002	0.03	8	4	8	32
8	SO_2CF_3	72.8	<0.001	0.002	0.001	0.015	0.015	0.06	8	8	16	32
9	Cl	19.1	0.004	0.008	0.001	0.269	0.031	0.06	4	64	4	32
10 ANT3310	**F**	**10.0**	**0.010**	**0.002**	**0.001**	**0.175**	**0.019**	**0.06**	**0.25**	**1**	**1**	**2**

This series was baselined with the hydrogen substituent **(2)**, which showed significant loss of both enzymic activity and meropenem potentiation compared to avibactam **(1)**. We explored several 1-carbon substituents bearing halogen atoms and found we could achieve good enzyme inhibition and acceptable meropenem potentiation with a wide range of electronic properties, from the strongly electron-withdrawing CF_3 **(6)** to the weakly electron-withdrawing CH_2Cl substituent **(4)**. Interestingly this potent enzyme inhibition was maintained with the large electron-withdrawing SCF_3 group **(7)** and the even larger and much more powerfully electron-withdrawing substituent SO_2CF_3 **(8)**. However with these latter two analogues there was a drop off in potentiation. We hypothesized that this was due to increased size (the volume of the $CONH_2$, SCF_3 and SO_2CF_3 substituents are 35.2, 55.7 and 72.8 Å, respectively[3]). Drugs like meropenem and BLIs enter the periplasm through water-filled porins, such as OmpF in *E.coli,* which have a narrow "constriction point" that presents a size barrier to entry, although an alternative hypothesis to the reduction in accumulation is that the efflux pumps are better at binding to DBOs with larger substituents.

We resolved to investigate smaller electron-withdrawing substituents such as halogens. *A priori* this functionality (N-C-Halogen) might be expected to be unstable but a consideration of the whole molecule shows that decomposition via iminium species due to loss of halide ion is not feasible from molecular orbital considerations. Orbital overlap in the iminium species is not possible due to ring strain (the iminium species would contravene Bredt's rule[5] that you cannot have a double bond at a bridgehead) so chemical stability is achieved through stereoelectronic control[6].

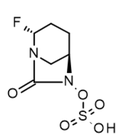


The lone pair on nitrogen is at 60º to the C-F bond so no orbital overlap is possible

Gratifyingly, both chloro **(9)** and fluoro **(10)** analogues were stable compounds with excellent enzyme inhibition and meropenem potentiation, supporting the "small is beautiful" hypothesis. Furthermore, fluoro analogue **(10)**, ANT3310, is clearly differentiated from avibactam, with additional activity in restoring the antibacterial activity of meropenem against an OXA-producing *A. baumannii* strain, which represents a significant advance from a clinical perspective. ANT3310 has been prepared in large-scale for further evaluation.

CONCLUSIONS

- A brief medicinal chemistry campaign exploring alternative substituents to the primary carboxamide of avibactam led to the discovery of ANT3310, a unique pan-active DBO bearing the fluoro substituent.
- Initially larger electron-withdrawing groups were investigated in an effort to completely fill the small pocket around the carboxamide by displacing the water molecules, but the SAR directed us towards smaller substituents until eventually arriving at a simple fluorine atom.
- ANT3310 is clearly differentiated from other DBOs by virtue of its activity in combination with meropenem against OXA-producing *A. baumannii* strains.



ANT3310

REFERENCES

1. O'Neill, J. (2016) Review on Antimicrobial Resistance, Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations
2. Lagace-Wiens, P., *et al* (2014) Ceftazidime-avibactam, a Review, *Core Evidence*, 9, 13-25 http://dx.doi.org/10.2147/CE.S406
3. Substituent volumes were calculated using Marvin (Chemaxon)
4. Richter, M.F., et al, (2017) Predictive Compound Accumulation Rules Yield a Broad-Spectrum Antibiotic, *Nature*, 545, 299 doi: 10.1038/nature22308
5. Bredt, J., et al (1902) Uber Isomere Dehydrocamphersauren, Lauronolsauren und Bihydrolauro-Lactone, *Ber. Dtsch. Chem. Ges*. 35, 1286 doi:10.1002/cber.19020350215
6. Kirby, A.J., (1992), "Stereoelectronic Effects", Oxford University Press, ISBN 9780198558934

Contact info: martin.Everett@antabio.com