WCK 5107 (Zidebactam) and WCK 5153: Bicyclo-acyl Hydrazide PBP2 Inhibitors Showing Potent β-lactam Enhancer Activity Against *Acinetobacter baumannii*, Including Multidrug-resistant (MDR) Carbapenemase-producing Clinical Strains

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INTRODUCTION & PURPOSE

- *Acinetobacter baumannii* (AB) is one of the most challenging Gram-negative pathogens, especially in intensive care units. It often causes bloodstream, respiratory tract, and wound infections that are associated with higher rates of morbidity and mortality in critically ill patients. Furthermore, 60% of AB infections are multidrug resistant (MDR), leaving few safe options for therapy. In the past, carbapenems were considered the treatment of choice against severe AB infections. However, the unceasing rise of carbapenemase-producing isolates has led to a concerning scenario, relying only on colistin that shows high toxicity and frequent emergence of resistance[1, 2]. The suboptimal performance of tigecycline in managing AB infections highlights the need for an alternative agent with bactericidal action[3].
- Therapeutic situation is further complicated due to lack of novel antimicrobial agents in the drug development pipeline for these infections. In particular, the inability of β-lactamase inhibitors such as clavulanic acid, tazobactam (TAZ), avibactam (AVI) and relebactam (REL) in overcoming Class D OXA carbapenemase resistance associated with AB, is likely to keep this therapeutic segment void of effective therapies for foreseeable future.
- WCK 5107 (Zidebactam, ZID) and WCK 5153 are new Bicyclo-acyl Hydrazides (BCH) β-lactam enhancers (BLEs) discovered at the Wockhardt Research Centre (India) which are Gram-negative Penicillin Binding Protein 2 (PBP2) binders helping in overcoming diverse carbapenem impacting resistance mechanisms such as metallo-β-lactamases (MBL) and OXA carbapenemases.
- The objectives of this work were to determine the PBP affinity, OXA-23 inhibition profiles and antimicrobial activity of ZID and WCK 5153, alone and in combination with β-lactams, against MDR AB.

METHODS

Susceptibility testing

- The MICs of ZID, WCK 5153 and reference β-lactams (BLs) - cefepime (FEP), sulbactam (SUL) and mecillinam (MEC) were determined by standard CLSI broth microdilution method for the Class D Oxacillinase (OXA-23) producing AB MDR ST2 international high-risk clone. For combination MICs, BLEs were employed at fixed concentration of 8 µg/mL .

Enzyme inhibition assays

- Acinetobacter derived OXA-23 inhibition activity (IC_{50}) of BLEs – ZID, WCK 5153 and BLIs - clavulanic acid, AVI and REL was assessed by using nitrocefin as substrate. For nitrocefin assay, enzyme-BLI preparation was pre-incubated for 20 min at 37˚C. The reaction mixture was further incubated with nitrocefin for 5 min to measure the residual enzyme activity. Spectrophotometric readings were measured at 482 nm and IC_{50} values were determined using GraphPad Prism software.

PBP binding assay

- The PBP binding affinities for BLEs - ZID, WCK 5153 and reference drugs - FEP, SUL and MEC were determined by using membrane isolations from a reference strain - *A. baumannii* ATCC 19606. Briefly, membrane preparations were obtained by the protocol previously described[4]. PBP containing solution was incubated (30 min, 37ºC) in the presence of increasing concentrations (0.0156-2 mg/L) of ZID, WCK 5153 or reference drugs - FEP, SUL and MEC and labelled with BOCILLIN FL (25 µM). The reaction mixtures were denatured and PBPs separated by SDS PAGE. Labelled PBPs were visualised and IC_{50}s determined (Typhoon™ FLA 9500 biomolecular imager and ImageQuant™ TL- GE Healthcare Bio-Sciences AB Björkgatan 30 751 84 Uppsala). All experiments were performed in triplicate.

Kill kinetics

- Kill kinetics were initiated by inoculating 100 µL of mid log phase AB OXA-23 ST2 culture in plates with 100 µL of MHB (initial inoculum ~10^6 CFU/mL) containing a final concentration of 8 µg/mL of each BLEs (ZID, WCK 5153) alone and in combination with FEP (at 8 and 16 µg/mL) or SUL (at 4 µg/mL), 16 µg/mL of standalone FEP and SUL. Imipenem (IPM)- 16 µg/mL was used as comparator. Cultures were then enumerated by plating serial dilutions at 1, 2, 4, 6 and 8h. All experiments were performed in triplicate.

RESULTS

- While the MICs of standalone FEP and SUL were 32-64 and 16 µg/mL respectively, addition of ZID or WCK 5153 (8 µg/mL) significantly reduced the MICs of FEP by 2-4 times and SUL by 8 times (**Table 1**). Higher MIC of IPM indicates optimal expression of OXA-23 β-lactamase.

Table 1. Antimicrobial susceptibility of AB OXA-23, ST2

Antibiotic	MIC (µg/mL)
FEP	32-64
FEP + ZID (8 µg/mL)	16
FEP + WCK 5153 (8 µg/mL)	16
SUL	16
SUL + ZID (8 µg/mL)	2
SUL + WCK 5153 (8 µg/mL)	2
IPM	32
TGC	1
ZID	>1024
WCK 5153	>1024

- OXA-23 inhibition studies showed that neither BLEs nor BLIs were potent inhibitors of this carbapenemase as reported previously for AVI and REL[5,6,7,8] (**Table 2**).

Table 2. Enzyme inhibition of ZID, WCK 5153 and comparators

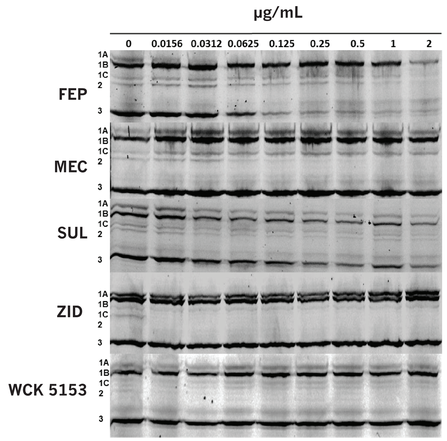
β-lactamase	IC_{50} (µM) Mean±SD				
	ZID	WCK 5153	AVI	REL	Clavulanic acid
OXA-23	2.19±1.06	2.61±0.49	11.13±1.03	5.98±1.08	>10

- The WCK enhancers-ZID and WCK 5153 showed potent PBP2 affinity with an IC_{50} value of 0.01 µg/mL which was 7-times higher than MEC - a known PBP2 binding agent. However, MEC demonstrated relatively higher affinity towards PBP2 as compared to FEP and SUL. First ever studied, FEP and SUL showed multiple AB PBP binding profile with high affinity to PBP3 (IC_{50}: 0.08 and 0.64 µg/mL, respectively) besides PBP1a and/or PBP1b inhibition, respectively (**Table 3** and **Figure 1**).

Table 3. PBPs IC_{50} of ZID, WCK 5153, and reference drugs

	IC_{50} A. baumannii ATCC 19606 (mg/L)				
PBP	FEP	MEC	SUL	ZID	WCK 5153
1A	0.05±0.02	>2	>2	>2	>2
1B	1.32±0.27	>2	0.9±0.5	>2	>2
1C	0.84±0.10	>2	>2	>2	>2
2	0.97±0.34	0.07±0.04	>2	0.01±0.003	0.01±0.002
3	0.08±0.03	>2	0.64±0.21	>2	>2

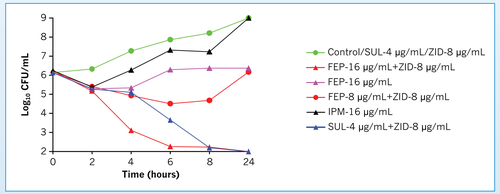
Figure 1. PBP competition assay for ZID, WCK 5153 and reference drugs



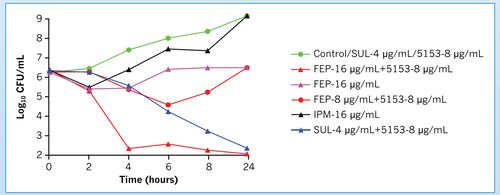
- As shown in **Figure 2**, FEP at 8 µg/mL (0.5× MIC) in combination with 8 µg/mL of ZID or WCK 5153, provided 1.1–1.5 log_{10} kill till 8h. Interestingly, higher concentration of FEP (16 µg/mL, 1× MIC) in combination with BLEs provided sustained cidality of >3 log_{10} till 24h. SUL at 4 µg/mL (0.25× MIC) in combination with 8 µg/mL of either ZID or WCK 5153 brought about ≥3 log_{10} kill within 4h which persisted till 24h. Standalone ZID and WCK 5153 did not elicit AB bactericidal effect possibly owing to their single PBP binding feature. Thus BLEs, despite being poor inhibitors of OXA-23, induced rapid killing of MDR AB when combined with labile PBP3 binding BLs. This enunciates the principle of β-lactam enhancers which trigger cidality through complementary PBP binding.

Figure 2. Time-kill curves of MDR AB ST2

2a) FEP and SUL in combination with ZID



2b) FEP and SUL in combination with WCK 5153



CONCLUSIONS

- **This is the first ever study to demonstrate high affinity Acinetobacter PBP2 binding property of BCH enhancers – WCK 5107 and WCK 5153. The PBP2 binding affinity observed for WCK enhancers was higher than MEC - a known PBP2 binding agent.**
- **BLE-BL combinations involving labile PBP3 binding BLs displayed potent cidality against MDR Acinetobacter indicating the role of complementary PBP binding in obviating the need for β-lactamase inhibition.**
- **This study for the first time, also established PBP binding profile for older agents such as FEP, SUL and MEC for Acinetobacter- a pathogen of growing importance.**
- **Unlike restoration of pharmacodynamics (PD) effect reported for BL-BLI combinations, WCK enhancers imparted rapid killing feature to FEP, resulting in a PD effect similar to carbapenems.**

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DISCLOSURES

The study was supported by Wockhardt Research Centre, Aurangabad, India.

Poster presented at the 26th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), 9th –12th April, 2016, Amsterdam.