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Evaluation of the SecA Inhibitors as Novel Anti-Microbial Agents

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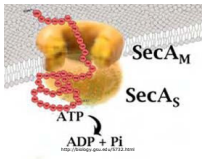
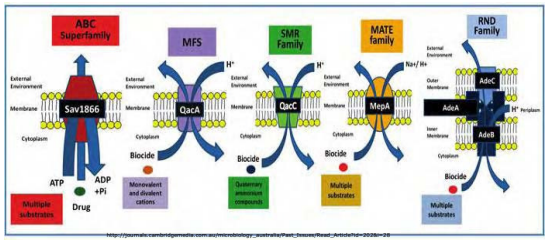
Abstract

Due to the misuse of conventional antibiotics and natural selection of the infectious bacterial population, drug resistance has emerged. Thus, there is an increasing need for novel, more effective antibiotic compounds that are successful in treating bacterial infections currently resistant to available therapies. SecA is an indispensable ATPase of the protein translocation machinery present in all bacteria. It is responsible for the secretion of many essential proteins, some toxins and virulence factors, and essential for bacterial survival. SecA has no counterpart in mammalian cells, thus providing an ideal target for developing antimicrobial agents. SCA-13 (HO) is a pyrimidine analog and was derived from virtual screening, exerts the ability to inhibit SecA translocation ATPase activity with an IC_{50} of 75 μM. Moreover, HO showed promising bacteriostatic activities against a vacomycin resistance strain of *S. aureus* Mu50 and *B. anthracis* Sterne. No significant difference in antimicrobial activity of HO was observed among efflux pump Strains of *S. aureus*, suggesting that compound HO is not a substrate of NorA or MepA efflux pumps. Resistant mutants of *E. coli* NR698 selected from HO will be characterized to gain a better understanding the mechanisms of resistance and subsequently find the drug target.





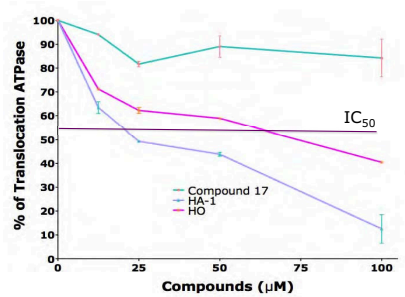
One of the methods of resistance is for the compound/ treatment to be pumped out of the cell via an efflux pump. We examined the effect of changes in the MepA and NorA efflux pumps on the HO compound. MepA and NorA efflux pumps are illustrated above. NorA is a member of the SMR family of efflux pumps.

Methods

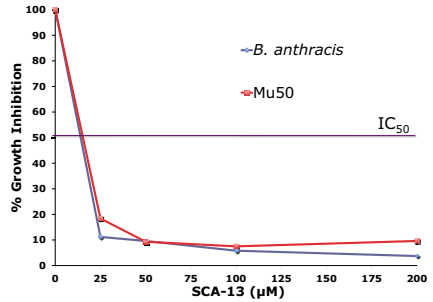
EMS Mutagenesis: *E. coli* strain NR698 was grown to mid-log phase (O.D. 600=0.5) and treated with 0.5% or 1% EMS for 1 h at 37ºC. Cells were washed with 5% sodium thiosulfate to inactivate the EMS. 10^4 to 10^6 cells were transferred to fresh LB agar plates containing various concentrations of compound HO and incubated at 37ºC until colonies formed. The resistant colonies were further confirmed by numerous passages on selection plates and untreated NR698 cells were used as a control.
Inhibition Assay: Efflux strains of *S. aureus* were grown on LB plates and inoculated in 2 mL of LB. Different strains included K1758 (NorA-), K2361 (NorA++), K2908 (MepA-), WT (83254), and K2068 (MepA++). The LB medium for the appropriate sample was treated with the corresponding antibiotic. The treatment for K2361 (NorA++) was Cm (2 μl/2 mL) and the Ery chemical treatment (8 μl/2 mL) was for the K1758 (NorA-) and K2908 (MepA-). The OD was measured and the mid-log samples were diluted to 0.05. The 5 different strains were then tested using different concentrations of antibiotic treatments. They were left in the incubator for 14 hrs at 37° C at 100 rpm.
SecA translocation ATPase assay: Translocation ATPase assay was carried out at 40ºC for 30 mins using Malachite Green colorimetric method. Urea-treated SecA-depleted BA-13 membranes and preprotein pOmpA were used in the reaction.
DARTS for purified protein binding: Ten- μg SecA protein was incubated at 37ºC for 10 mins after the addition of the proper inhibitor. The inhibitors include 0 mM, 2 mM, and 10 mM of HO, HA1, and NR698-17. This was followed by digestion with 2 μl trypsin on ice for 10 mins. Adding sample buffer and boiling for 20 mins stopped the digestion. SDS-PAGE (12%) was used to investigate the trypsin digestion pattern.

Results

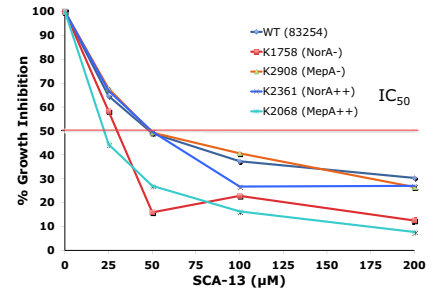
Inhibitory effect of HO on SecA Translocation ATPase



HO has an IC_{50} of approximately 15 μM



HO by-passes the effect of efflux pumps



The compound HO was tested in samples with the overexpression (++) and deletion (-) of MepA and NorA efflux pumps as shown by the IC_{50} values.

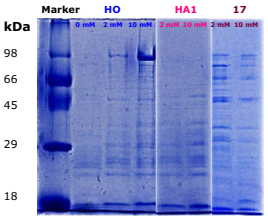
IC_{50} values of *S. aureus,* and *B. anthracis*

Strain	IC_{50} (μM)
WT (83254)	46
K2068 (MepA++)	22
K2908 (MepA-)	47
K2361 (NorA++)	47
K1758 (NorA-)	27
S. *aureus* Mu50	15
B. *anthracis*	12.5

The compound HO was tested in samples with the overexpression (++) and deletion (-) of MepA and NorA efflux pumps.

Trypsin digest altered conformation of SecA with binding of inhibitor

As HO increases, a new band is seen in the tryptic digest, indicating that the drug alters the SecA conformation. This could indicate that SecA is the target for HO.



Resistant mutants of HO

			HO (μM)			
Mutants	200	100	50	25	12.5	6.25
NR698	+	+	+	+	+	+/-
NR698-1	+/-	-	-	-	-	-
NR698-3	+	+/-	+/-	+/-	+/-	+/-
NR698-5	+	+	+/-	+/-	+/-	+/-
NR698-6	+	+/-	-	+/-	+/-	+/-
NR698-8	+	+/-	+/-	+/-	+/-	+/-
NR698-9	+/-	+/-	+/-	+/-	+/-	+/-
NR698-12	+	+	+	+	+	+/-
NR698-13	+	+	+/-	+/-	-	-
NR698-14	+	+/-	+/-	+/-	-	-
NR698-15	+	+	+	+	-	-

Conclusions

HO is a potent inhibitor for SecA that seems to be unaffected by an efflux pump. This particular compound, when bound to SecA alters the trypsin digest which indicates that HO changes the confirmation of SecA.

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