Analysis of the resistance profile of benzoxaborole AN11736 analogues in animal trypanosomes

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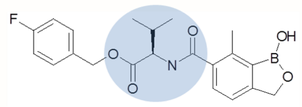
Introduction

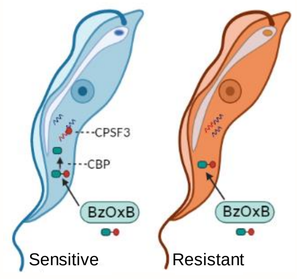
Benzoxaboroles (BXBs) are a class of compounds with broad antimicrobial activity. Some benzoxaboroles are under evaluation for the treatment of animal African trypanosomosis (or nagana), a wasting livestock disease caused by infection with *Trypanosoma* parasites.

As part of their pre-clinical development, we evaluated the mode of resistance to a class of valyl-ester benzoxaboroles in bloodstream animal trypanosomes, by analysing the genome of *in vitro*-selected resistant parasites.



Structure of AN11736 (above), with the valyl-ester linker, substrate of CBP cleavage, indicated by the blue circle.

Scheme depicting the mode of resistance to AN11736 in trypanosomes (right).

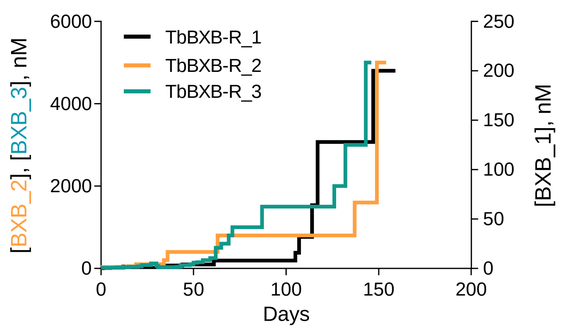


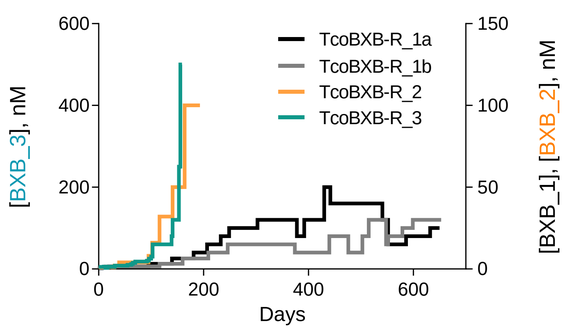
We previously showed that resistance to AN11736, an initial lead identified by GALVmed, arises by loss of serine carboxypeptidases (CBPs) activity [1]. In WT parasites, instead, these enzymes cleave the parental compound at its valyl-ester linker, releasing a carboxylate derivative that accumulates at high levels.

Here we describe the resistance and cross-resistance profiles of a series of three benzoxaboroles analogues of AN11736. Our results confirm previous findings but also hint to other potential routes for resistance development to benzoxaboroles.

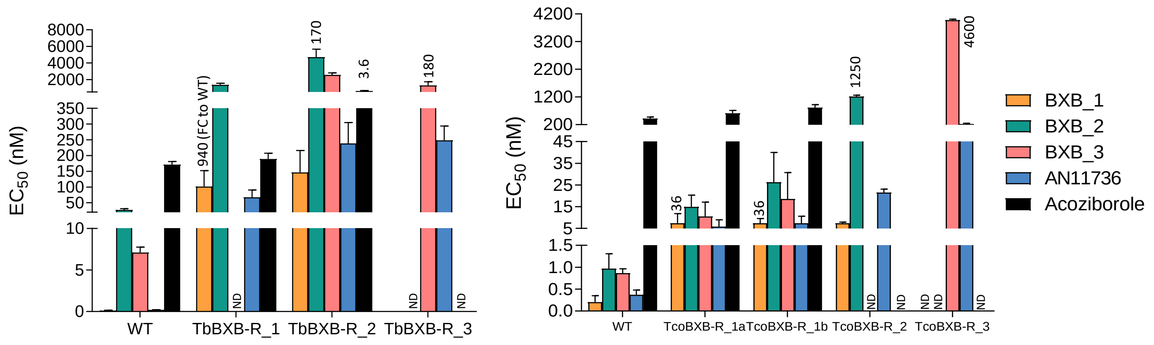
Resistance selection and resistance profile

In vitro selection of *T. brucei* (Tb) Lister 427 (left) and *T. congolense* (Tco) IL3000 (right) lines resistant to the three BXBs. High levels of resistance were obtained for most lines after ~5 months of culture. Resistance to BXB_1 was difficult to select in *T. congolense* (>21 months required for 36 fold resistance to WT).

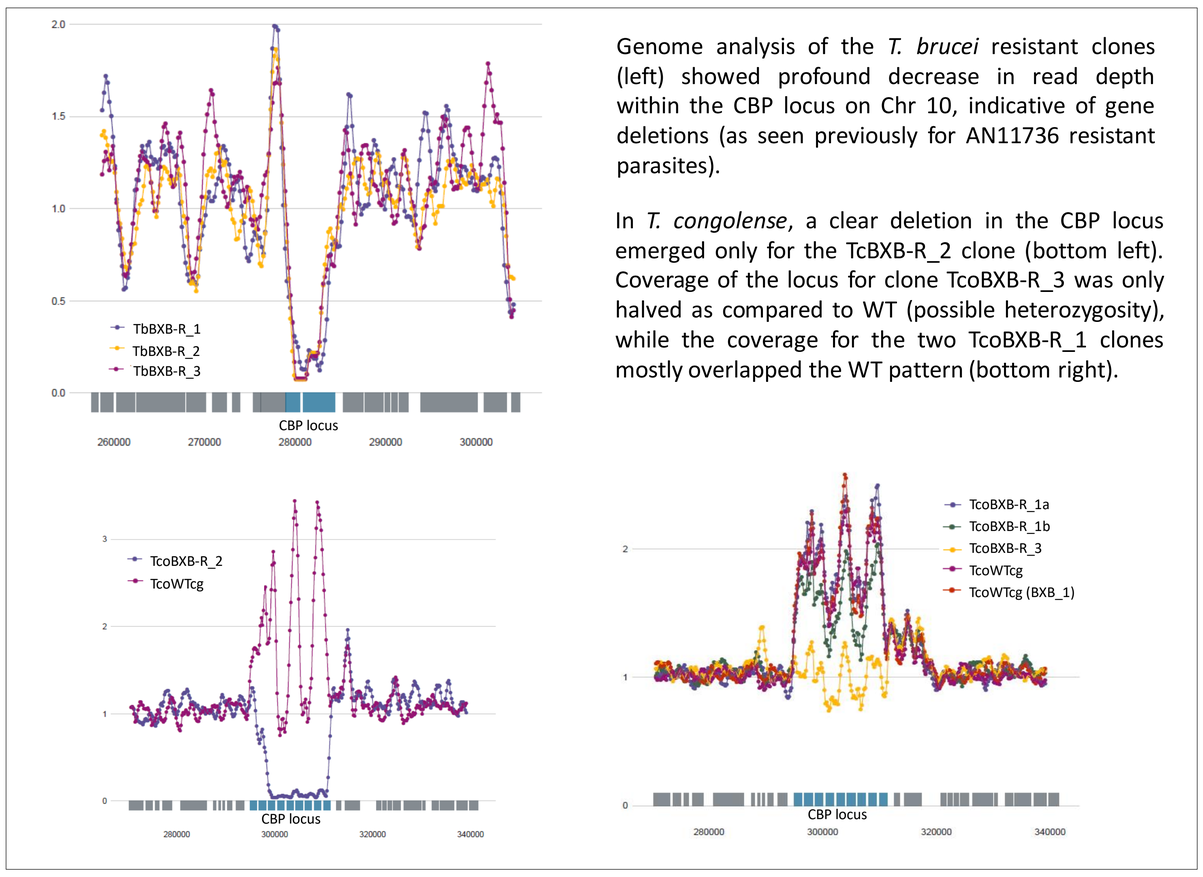




Sensitivity of *T. brucei* (left) and *T. congolense* (right) resistant clones to the selecting BXB and their cross-resistance to other BXBs. All clones were cross-resistant to the other BXBs, including AN11736. TbBXB-R_2 showed some cross-resistance (3.6 fold change – FC) to acoziborole [2], which lacks the valyl-ester linker.

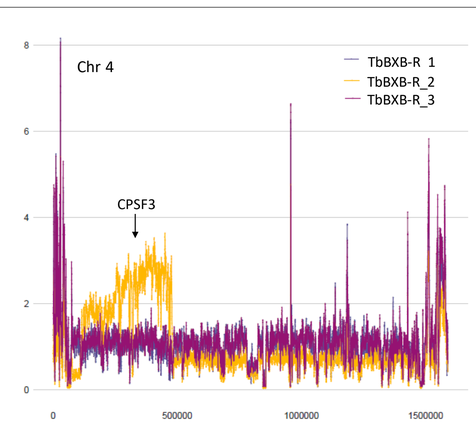


Whole genome analysis (CBPs and CPSF3)



Genome analysis of the *T. brucei* resistant clones (left) showed profound decrease in read depth within the CBP locus on Chr 10, indicative of gene deletions (as seen previously for AN11736 resistant parasites).

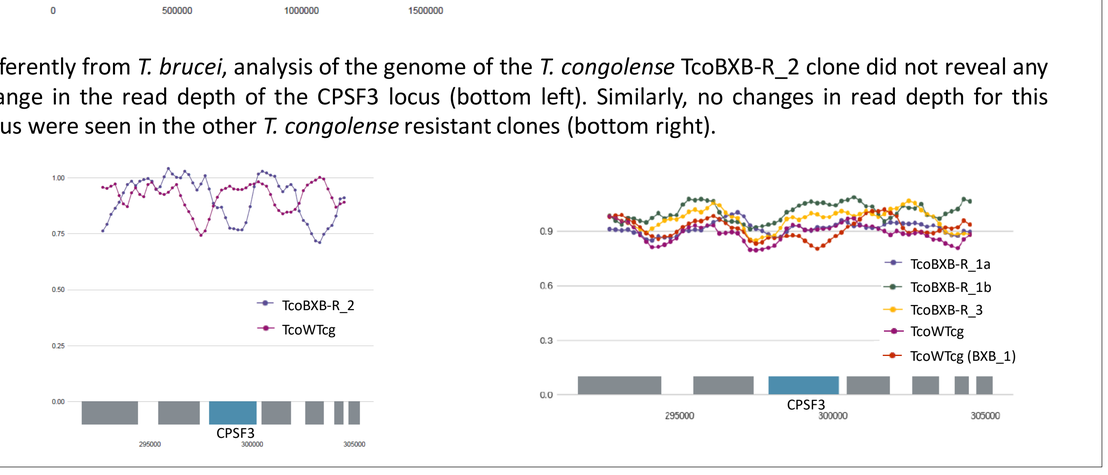
In *T. congolense*, a clear deletion in the CBP locus emerged only for the TcBXB-R_2 clone (bottom left). Coverage of the locus for clone TcoBXB-R_3 was only halved as compared to WT (possible heterozygosity), while the coverage for the two TcoBXB-R_1 clones mostly overlapped the WT pattern (bottom right).



CPSF3 (Cleavage and Polyadenylation Factor 3) has been identified as the molecular target of several benzoxaboroles including acoziborole and AN11736 [3].

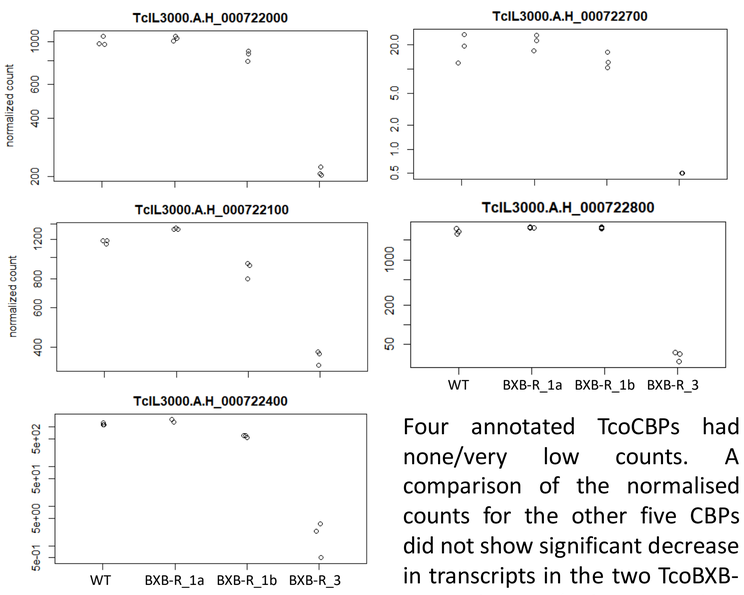
While we found no changes in the CPSF3 locus for resistant clones TbBXB-R_1 and TbBXB-R_3, we identified a 2-3 fold increase in read depth in the TbBXB-R_2 clone (left), indicative of a duplication of this locus (together with a big section of the surrounding Chr 4). This target amplification could explain the small degree of cross-resistance of these parasites to acoziborole seen by Alamar Blue.

Differently from *T. brucei*, analysis of the genome of the *T. congolense* TcoBXB-R_2 clone did not reveal any change in the read depth of the CPSF3 locus (bottom left). Similarly, no changes in read depth for this locus were seen in the other *T. congolense* resistant clones (bottom right).

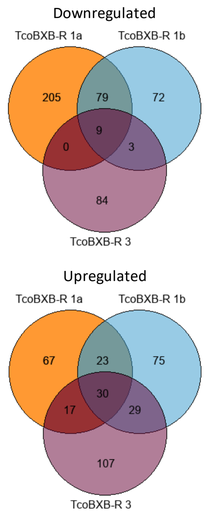


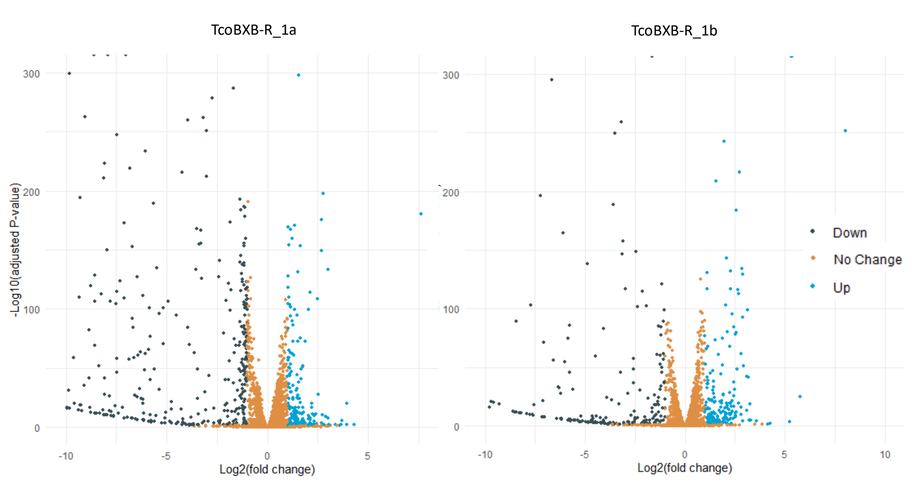
Transcriptomics of TcoBXB-R_1 lines

As TcoBXB-R_1 clones were the only parasites with no obvious deletion in the CBP locus, we checked their CBPs' expression levels to rule out downstream downregulation mechanisms. A PCA analysis (below) of the RNA-seq results showed a clear separation of the two independently selected clones TcoBXB-R_1a and TcoBXB-R_1b (below).



Four annotated TcoCBPs had none/very low counts. A comparison of the normalised counts for the other five CBPs did not show significant decrease in transcripts in the two TcoBXB-R_1 clones (only exception: 000722400 in 1b, 0.5 FC).





Analysis of the whole transcriptome revealed a series of genes upregulated and downregulated ($p < 0.05$, FC >2 or FC <0.5) in the TcoBXB-R_1 clones, mostly unique to each line.
TcoBXB-R_1a and 1b and TcoBXB-R_3 shared nine downregulated genes including four ATP-dependent DEAH-box RNA helicases. Most of the upregulated genes shared between the three clones were related to surface proteins.

Conclusions

- *T. brucei* resistance to the three new BXBs was linked to deletions within the CBP locus, as seen for AN11736. The amplification of BXBs target CPSF3, found in one of these clones, could also contribute to the resistance phenotype of these cells.
- Deletions within the CBP locus could also explain the resistance phenotype of two *T. congolense* clones. For one of them, the loss of CBP genes was shown to cause a significant decrease in CBP transcripts.
- *T. congolense* clones resistant to BXB_1 did not show any of the above genetic alterations and transcriptomics showed no decrease in CBP transcripts, hence other mechanisms must be responsible for resistance to BXB_1. Expression of several genes was altered in these cells as compared to WT and most of these genes were unique to the specific clones, indicative of different paths for resistance acquisition.
- Clone TcoBXB-R_1a was unable to establish infection *in vivo*, a phenotype likely due to the long culturing time required for selection of resistance to this BXB, as the parental line grown in parallel also struggled to infect mice.

References:

1. F Giordani F *et al,* PLoS Pathog 2020
2. P Steketee *et al*, PLoSNegl Dis 2021
3. R Wall *et al*, Proc Natl Acad Sci USA 2018

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