SUPPLEMENTARY METHODS AND FIGURES FOR : Identification of novel Bromodomain inhibitors of Trypanosoma cruzi Bromodomain Factor 2 (TcBDF2) using a fluorescence polarization-base high-throughput assay

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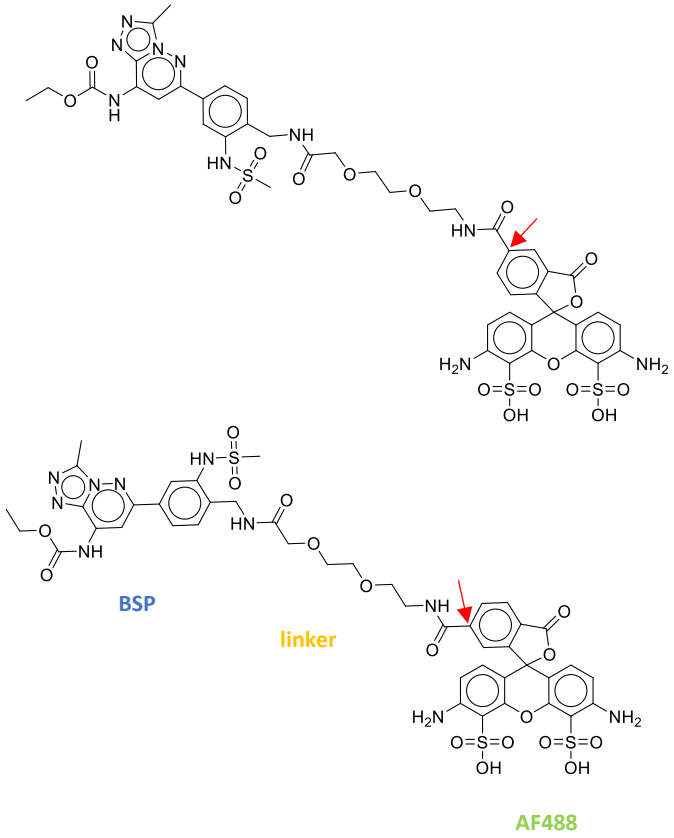


Figure S1: AlexaFluor-488 coupled to BSP. Mixture of regioisomers that differ at the atom on the benzene ring where the linker is bonded to the fluorophore (indicated with red arrows).

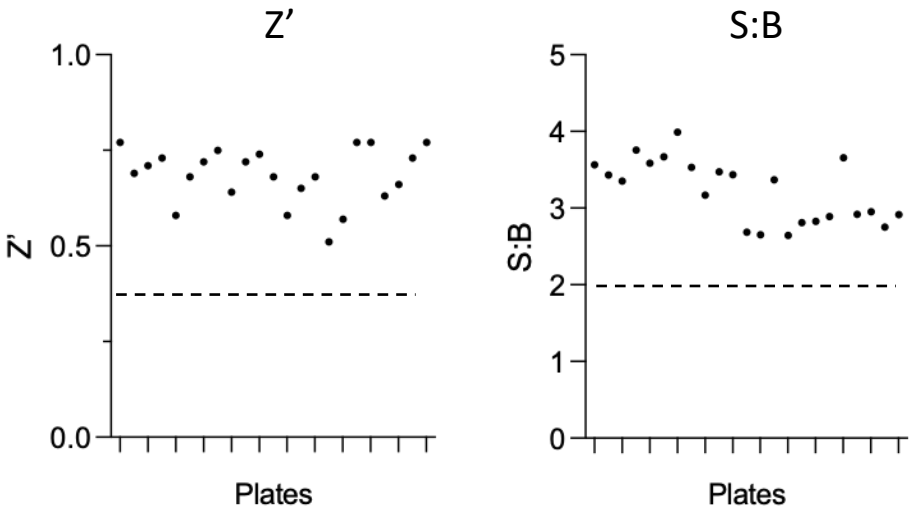
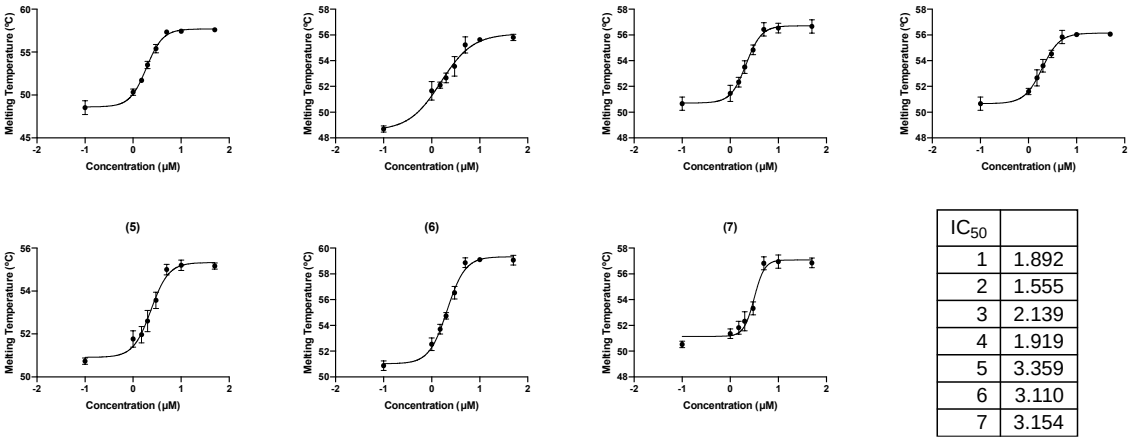


Figure S2: Quality control parameters used in this screening were applied to all plates. The Z' score (left panel) and the signal-to-noise ratio (S:B, right panel) were measured. The dotted line represents the threshold values: 0.4 for the Z' score and 2 for the S:B ratio.



IC_{50}	
1	1.892
2	1.555
3	2.139
4	1.919
5	3.359
6	3.110
7	3.154

Figure S3: *Kd* calculation using DSF (second round of experiments). Six concentrations of each compound were used (from 0.1 to 50 μM) and the melting temperatures of *Tc*BD2 with each concentration of compound obtained were used to calculate the *Kd*s. DMSO was used as a control sample. All thermal shift assays were performed at least in triplicate with reproducibility of parameters within ±10%. The change of melting temperature versus the log of the ligand concentration was plotted and fitted as described in the Methods section.

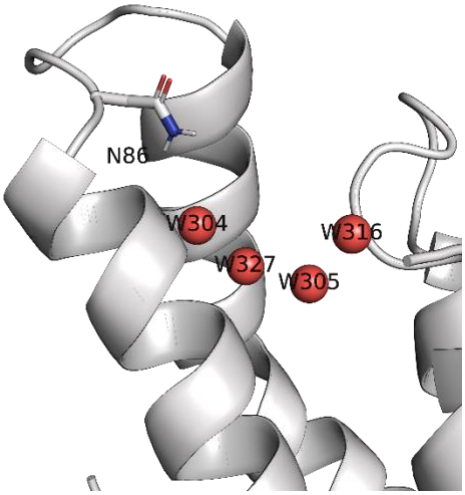


Figure S4: Structure of *Tc*BDF2 and the water network. Bromodomain structure with the conserved water molecules highlighted as red spheres. The four waters are referred to as W304, W305, W316, and W327.

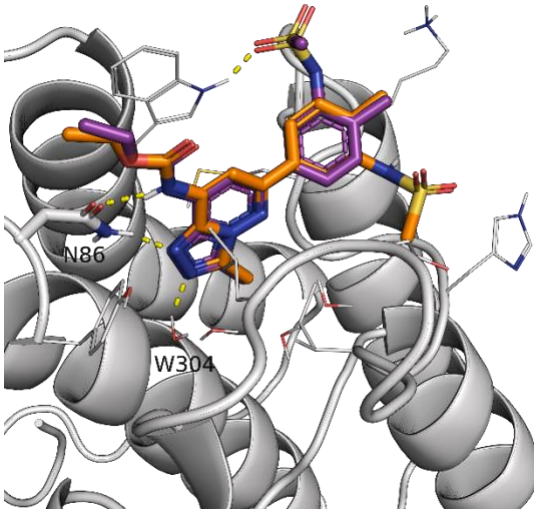


Figure S5: Binding mode validation of the docking protocol. Bromosporine (orange) was docked into the AcK pocket of TcBDF2 (PDBid 6NIM) obtaining an almost identical conformation as the X-ray ligand (purple) (core RMSD = 0.31 Å). Hydrogen bonds are shown in yellow dot lines.

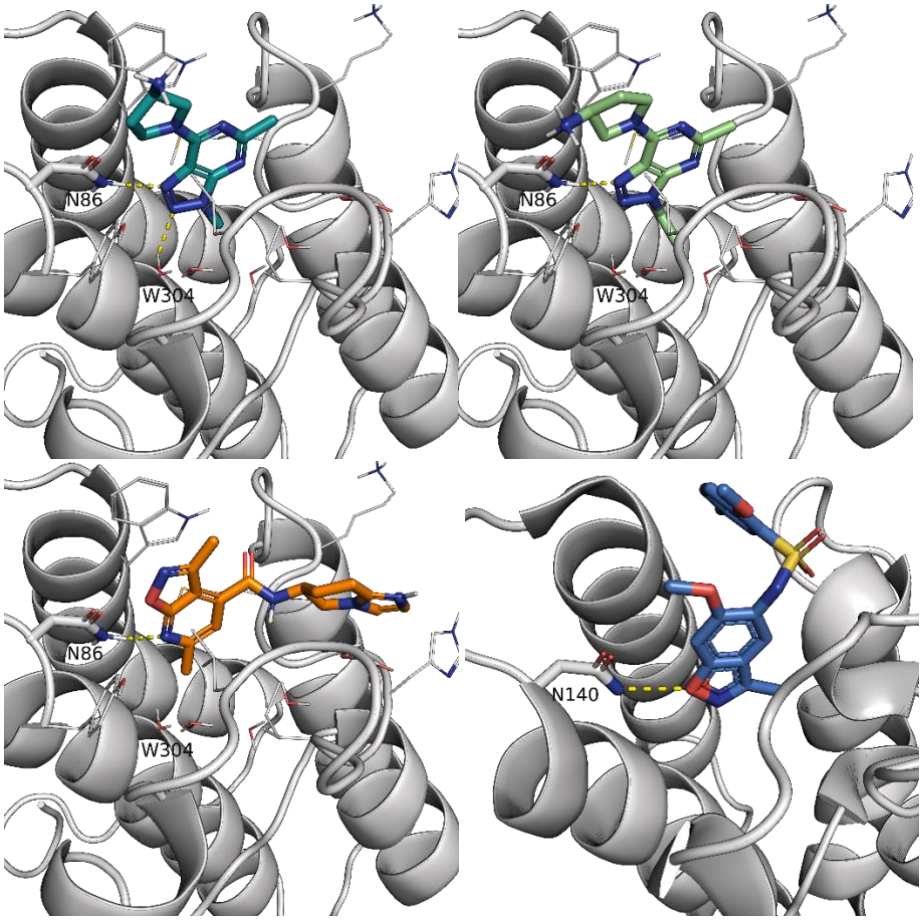


Figure S6: Binding mode prediction of compounds against *Tc*BDF2 and X-ray crystal structure of BRD4. Docked poses of compound 1 (dark cyan), 2 (green), 3 (orange), 3m (blue) and BRD4 (PDBid 5Y8C). Hydrogen bonds are shown in yellow dot lines.

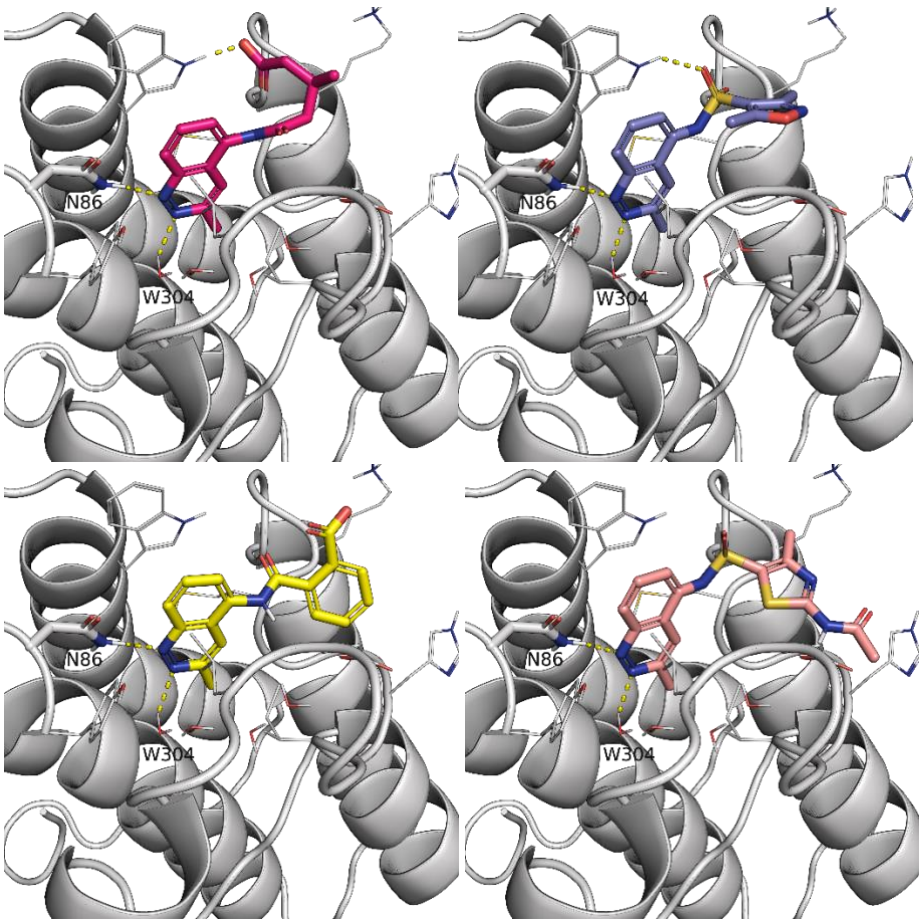


Figure S7: Binding mode prediction of compounds against *Tc*BDF2. Docked poses of compound 4 (dark pink), 5(purple), 6(yellow) and 7(pink). Hydrogen bonds are shown in yellow dot lines.