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Supporting information for article:

High-resolution crystal structure of *Trypanosoma brucei* pteridine reductase 1 in complex with an innovative tricyclic-based inhibitor

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S1. Synthetic procedure of 1

All commercially available chemicals and solvents were reagent grade and were used without further purification unless otherwise specified. Reactions were monitored by thin-layer chromatography on silica gel plates (60F-254, E. Merck) and visualized with UV light and alkaline $KMnO_4$ aqueous solution. NMR spectra were recorded on a Bruker 400 spectrometer with ^1H at 400.134 MHz and ^{13}C at 100.62 MHz. Proton chemical shift was referenced to the residual solvent peak. Chemical shifts are reported in parts per million (ppm, δ units). Coupling constants are reported in units of Hertz (Hz). Splitting patterns are designed as s, singlet; d, doublet; dd, double doublet; b, broad. Mass spectra were obtained on a 6520 Accurate-Mass Q-TOF LC/MS and 6310A Ion TrapLC-MS(n).

S1.1. 2,4-diamino-9H-pyrimido[4,5-b]indol-6-ol (1)

To a clear solution of 500 mg (1 eq., 4.07 mmol) pyrimidine-2,4,6-triamine in 10 mL of boiling glacial acetic acid, 527 mg (1.2 eq., 4.88 mmol) of 1,4-benzoquinone was added, and the mixture was refluxed for 3 h. The solvent was evaporated, and the residue purified over silica gel using chloroform/methanol 8:2 as eluent to give the desired product as a pale pink solid (366 mg, 42% yield). Mp [>300 °C with dec]. ^1H NMR (400 MHz, DMSO-d6) δ 5.79 (s, 2H), 6.43 (s, 2H), 6.63 (dd, J = 2.3, 8.4 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 7.35 (d, J = 2.4 Hz, 2H), 10.73 (s, 1H). ^{13}C NMR (101 MHz, acetone-d6) δ 105.43, 110.65, 110.85, 122.58, 130.19, 151.84, 158.64, 159.21, 161.78, 171.96. HRMS m/z [M+H]+ Calcd for $C_{10}H_9N_5O$: 216.0880, found: 216.0895.

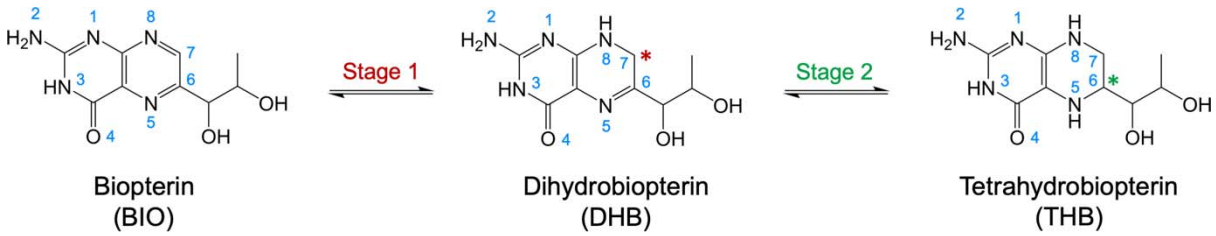

Figure S1 Scheme of the two-stage reduction catalyzed by *Tb*PTR1. In the first stage (Stage 1) biopterin (BIO) is reduced to dihydrobiopterin (7,8-dihydrobiopterin, DHB), that is further reduced to tetrahydrobiopterin (5,6,7,8-tetrahydrobiopterin, THB). In each reaction stage, the cofactor NADPH is oxidized to $NADP^+$. The carbons C7 of DHB and the C6 of THB, that accept the hydride from the cofactor, are highlighted by asterisks (*).

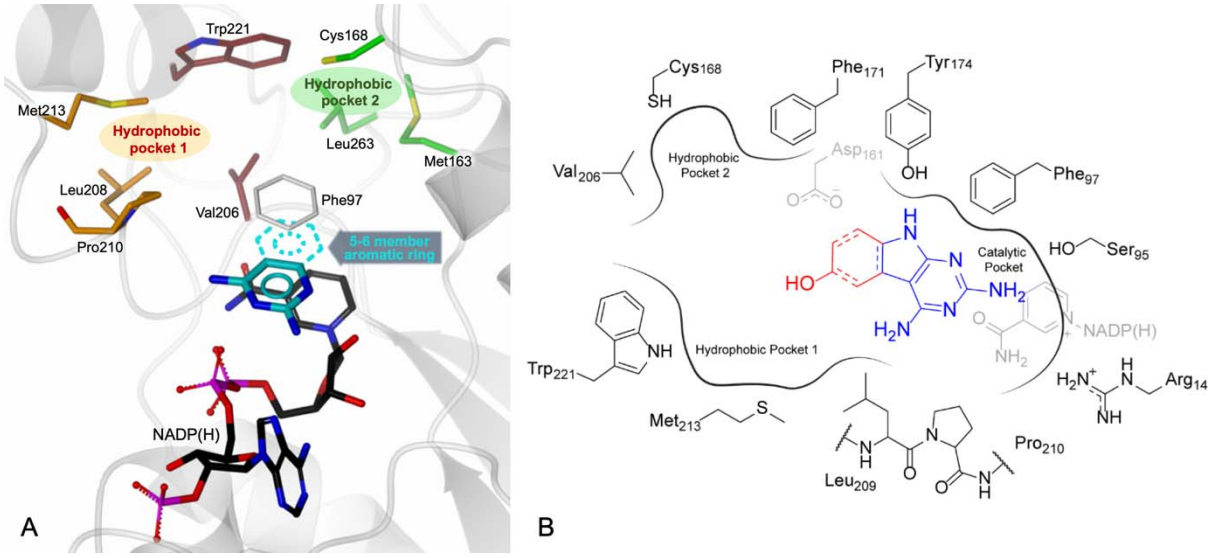

Figure S2 A) Active site view of *Tb*PTR1 (white cartoon and carbon atoms). Various bicyclic aromatic molecular systems (including either a five or a six-member aromatic ring, as second ring), mimicking the pterin of the natural substrates, have been explored to design *Tb*PTR1 inhibitors. Two peculiar hydrophobic pockets are present in the *Tb*PTR1 active site. The hydrophobic pocket 1 is defined by Val206, Leu208, Pro210, Met213, and Trp221, whereas pocket 2 is outlined by Met163, Cys168, Val206, Trp221, and Leu263. Carbon atoms are colored orange and green in the hydrophobic pocket 1 and 2, respectively, whereas those of the residues placed at the edge between the two pockets, namely Val206 and Trp221, are colored dark red. The cofactor NADP(H) is displayed in sticks, black carbon atoms. Oxygen atoms are colored red, nitrogen blue, and phosphorous magenta. B) 2D representation of the *Tb*PTR1 active site, highlighting the placement of the 2,4-diamino-pyrrolopyrimidine scaffold within the catalytic pocket and its potential expansion in a tricyclic derivative.