Supplementary Data

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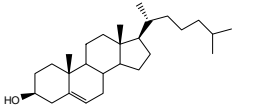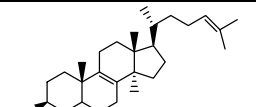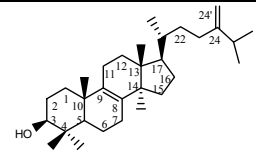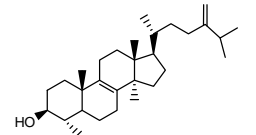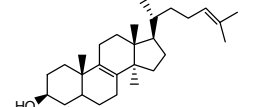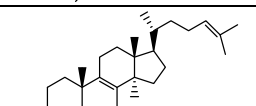
Supplementary Table 1. X-ray data collection and refinement statistics

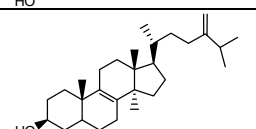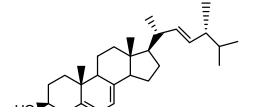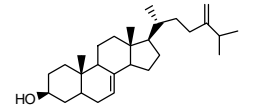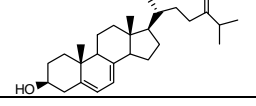|	*T. brucei* CYP51 in complex with VNI derivatives VFV and VNT	
Data collection	VFV [4G7G]	VNT [4G3J]
Wavelength, Å	0.979	0.979
Space group	P1	P1
Cell dimensions		
a, b, c, Å	59.9, 79.7, 117.9	60.0, 79.5, 116.4
α, β, γ, °	74.6, 81.2, 69.0	74.6, 79.3, 68.5
Molecules per asymmetric unit	4	4
Solvent content, %	50.7	49.7
Resolution (last shell), Å	30.0 2.05(2.09-2.05)	30.0 1.82(1.85-1.82)
R_{merge} (last shell)	0.046 (0.580)	0.048 (0.621)
I/σ (last shell)	31 (2.6)	28.3 (2.4)
Completeness (last shell), %	98.2 (97.1)	97.7 (96.2)
Redundancy (last shell)	4.6 (4.6)	4.6 (4.5)
Refinement		
Resolution, Å	50.0-2.05	29.9-1.83
R-factor	0.163	0.169
R-free	0.226	0.220
Reflections used	114765	157414
Test set size, %	5.0	5.0
Rms deviations from ideal geometry		
Bond lengths, Å	0.008	0.007
Bond angles, °	1.98	0.92
Ramachandran plot		
Residues in favorable regions (%)	97.9	97.4
Residues in allowed regions (%)	100	99.9
Outliers (%)	0	0.1
Model		
Total number of atoms	15068	15243
Residues per chain (average B-factor, Å²)		
protein	448 / 448 / 448 / 448 (44.9)	448 / 448 /448 /448 (36.4)
heme	1 / 1 / 1 / 1 (30.2)	1 / 1 / 1 / 1 (24.7)
inhibitor	1 / 1 / 1 / 1 (39.1)	1 / 1 / 1 / 1 (37.5)
Water	504 (44.1)	705 (41.0)
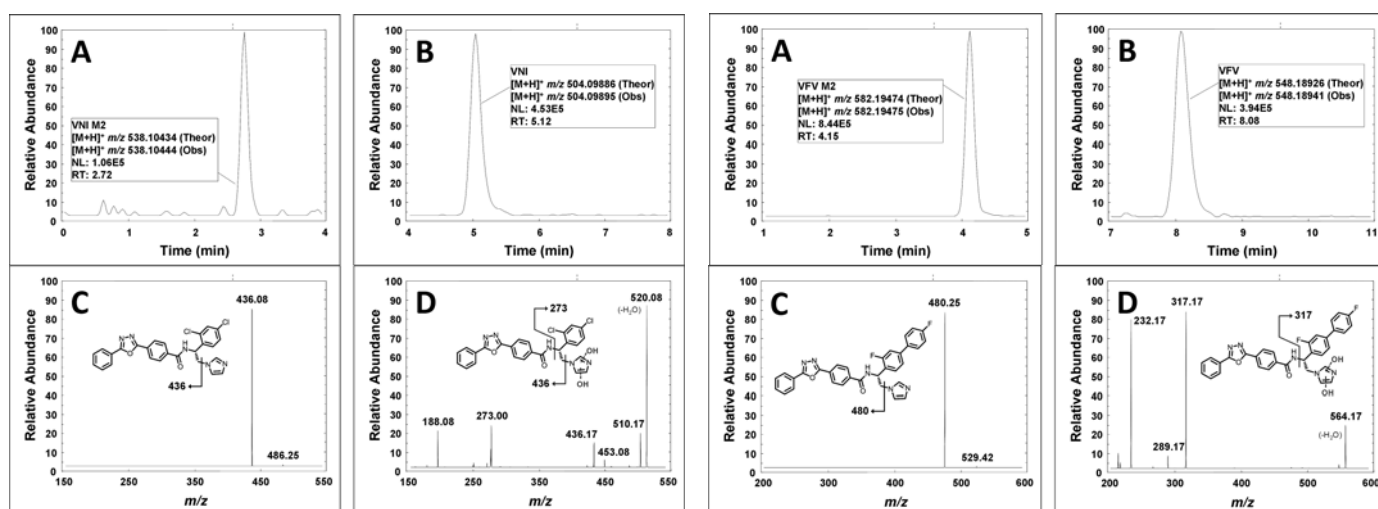
Supplementary Figure 1. Exact mass (A, B) and fragmentation patterns (C, D) of VNI (left panel), VFV (right panel) and their [M+34] metabolites extracted from mouse plasma. Mass-spectrometric experiments were performed using an *Acquity UPLC* system (Waters, Milford MA) and an *LTQ Orbitrap XL* mass spectrometer equipped with a standard electrospray ionization source (Thermo, San Jose CA). The *Acquity* system was equipped with a binary solvent manager, refrigerated sample manager, thermostated column heater, and a photodiode array UV detector. VFV, VNI, and related metabolites were chromatographically resolved on a Waters Symmetry reverse phase column (C18, 4.6 × 60 mm, 3.5μm) using isocratic elution conditions at a flow rate of 1.0 mL/min. Mobile phase consisted of 10 mM ammonium acetate in H_2O/CH_3CN (50:50). The eluent stream was split post-column to allow simultaneous UV and MS detection (70:30, UV detector /ESI source). The injection volume for all samples was 10 μl. The mass spectrometer was operated in positive ionization mode; the electrospray source parameters were as follows: N_2 sheath gas, 30; N_2 aux gas, 5; spray voltage, 4.0 kV; capillary temp, 300 °C; tube lens voltage, 60 V at *m/z* 500; capillary voltage, 21 V; skimmer offset, 0. Full scan spectra of 100-800 *m/z* were acquired in FTMS mode at a resolving power of 30,000 FWHM with the following AGC parameters: IT target, $3e^4$; FT target, $1e^6$; 1 microscan, maximum inject time, 50 ms. Data acquisition and analysis were carried out using *Xcalibur* v. 2.0.7 and *LTQ Orbitrap* v. 2.5.5 software (Thermo).

Supplementary Table 2. Sterol composition of *Leismania amazonensis*, non-treated and treated with VNI

Sterols (IUPAC nomenclature)	Structure	Rf TLC	HPLC RT/α_c	UV absorbance max	Molecular weight	Percentage of total sterol content (fg/cell)	
						Control (134)	1 μM VNI (130)
Cholesterol (cholesta-5-en-3β-ol) **(exogenous)**		0.36	26.6/1.0	204	386	3 (4)	6 (8)
Lanosterol (lanosta-8,24-dien-3β-ol)		0.57	25.6/0.96	204	426	<1	<1
Eburicol (24-methylenelanosta-8-en-3β-ol)		0.55	29.9/1.12	204	440	-	2 (3)
Obtusifoliol (4α,14α-dimethyl-5α-ergosta-8,24(24')-dien-3β-ol		0.49	23.8/0.89	204	426	-	6 (8)
C4-Norlanosterol (4α,14α-dimethylcholesta-8,24-dien-3β-ol)		0.49	20.8/0.78	204	412	4 (5)	8 (11)
14α-Methylzymosterol (14α-methylcholesta-8,24-dien-3β-ol)		0.33	17.2/0.65	204	398	-	13 (17)
14α-Methylfecosterol (14α-methyl-5α-ergosta-8,24(24')-dien-3β-ol)		0.32	19.7/0.74	204	412	-	18 (24)
Ergosterol (ergosta-5,7,22-trien-3β-ol)		0.31	19.4/0.71	282	396	12 (16)	5 (7)
Episterol (ergosta-7,24(24')-dien-3β-ol)		0.31	19.4/0.71	204	398	6 (8)	-
5-Dehydroepisterol (ergosta-5,7,24(24')-trien-3β-ol)		0.31	16.4/0.61	282	396	75(100)	39 (51)

About 300 mg of the cell pellet was saponified with 10 % KOH in 98% aqueous methanol at reflux temperature for 1 hour. The neutral lipids obtained by dilution with water and extraction with hexane were subjected to silica

gel TLC plates (0.25 μm, Whatman, Germany) for 30 min in hexane:ethyl acetate (8:2) as developing solvent and monitored in an iodine chamber. Individual sterols were separated by HPLC (Waters) using a reverse-phase Nova Pak C_{18} column (particle size 4 μM, 3.9 x 150 mm) and a solution of 23% acetonitrile, 72% methanol and 5% water as the mobile phase at a flow rate 0.8 ml/min for 35 min. The injection volume was 100μl and the UV detector wavelengths were set at 204 nm and 282 nm. The peaks were collected and the sterols were quantified and identified by combination of UV-spectrophotometry and mass-spectrometry as described in [16].