

Table 1 Effects of CYP5122A1 expression on the sterol profiles of *L. donovani* promastigotes at the log and stationary phases. The sterol levels in genetic mutants were normalized to the corresponding sterol levels in Ld1S WT. The values represent the mean ± 1/2 range (n = 2).

		Stationary phase			Log phase		
Sterols		Ld22A1+/- vs. Ld1S WT	Ld22A1+/- +pXNG4-22A1 vs. Ld1S WT	Ld22A1- +pXNG4-22A1 vs. Ld1S WT	Ld22A1+/- vs. Ld1S WT	Ld22A1+/- +pXNG4-22A1 vs. Ld1S WT	Ld22A1- +pXNG4-22A1 vs. Ld1S WT
4- and/or 14- methylated sterols	Lanosterol	9.3 ± 2.1**	0.20 ± 0.03	0.38 ± 0.06	23 ± 5***	0.35 ± 0.09	0.39 ± 0.16
	4-Hydroxylanosterol	3.3 ± 0.2**	0.28 ± 0.07	0.23 ± 0.00	14 ± 1***	0.21 ± 0.03	0.12 ± 0.04
	4,14-Dimethylzymosterol	2.0 ± 0.3*	0.47 ± 0.06	0.24 ± 0.01	3.3 ± 0.8**	0.20 ± 0.02*	0.20 ± 0.07*
	14-Methylzymosterol	0.81 ± 0.04	0.64 ± 0.05	0.74 ± 0.03	0.73 ± 0.01	0.52 ± 0.00	0.59 ± 0.20
	14-Methylfecosterol	ND	Accumulated***	ND	ND	Accumulated*	ND
	4-Methylzymosterol	1.6 ± 0.1	2.3 ± 0.0*	3.8 ± 0.1**	1.1 ± 0.0	1.7 ± 0.2	1.0 ± 0.3
	FF-MAS	ND	ND	ND	ND	ND	ND
	T-MAS	ND	ND	ND	ND	ND	ND
4,14- demethylated sterols	Zymosterol	1.2 ± 0.2	1.7 ± 0.1	0.89 ± 0.07	1.2 ± 0.0	1.6 ± 0.1*	0.30 ± 0.11*
	Fecosterol	1.6 ± 0.1	2.3 ± 0.0*	3.8 ± 0.1**	1.1 ± 0.0	1.7 ± 0.2	1.0 ± 0.3
	Episterol	1.6 ± 0.1	2.3 ± 0.0*	3.8 ± 0.1**	1.1 ± 0.0	1.7 ± 0.2	1.0 ± 0.3
	Cholesta-7,24-dienol	1.2 ± 0.2	1.7 ± 0.1	0.89 ± 0.07	1.2 ± 0.0	1.6 ± 0.1*	0.30 ± 0.11*
5-dehydro sterols	7-Dehydrodesmosterol	0.75 ± 0.14	0.47 ± 0.07*	0.14 ± 0.01**	0.66 ± 0.10	0.48 ± 0.02*	0.04 ± 0.02**
	5-Dehydroepisterol	0.99 ± 0.18	0.83 ± 0.10	0.84 ± 0.07	0.82 ± 0.07	0.67 ± 0.03	0.30 ± 0.13*
	Ergosta-5,7-dienol	1.2 ± 0.2	1.3 ± 0.2	2.0 ± 0.1*	0.79 ± 0.08	0.78 ± 0.07	0.52 ± 0.23
	Ergostateraenol	1.0 ± 0.2	0.64 ± 0.10	1.0 ± 0.1	1.1 ± 0.1	0.96 ± 0.15	0.30 ± 0.10*
	Ergosterol	1.0 ± 0.2	1.2 ± 0.1	1.8 ± 0.1*	0.79 ± 0.14	1.0 ± 0.1	0.53 ± 0.16
Cholesterol		1.2 ± 0.2	1.2 ± 0.0	1.5 ± 0.0	0.9 ± 0.1	0.9 ± 0.1	0.52 ± 0.17*

^a Episterol, fecosterol and 4-methylzymosterol could not be chromatographically separated.
^b Zymosterol and Cholesta-7,24-dienol could not be chromatographically separated.
ND: not detected. Accumulated: This sterol was detected in the genetic mutants but not in Ld1S WT.
* P<0.05; ** P<0.01; *** P<0.001. Statistical analysis was performed by ordinary one-way ANOVA, comparing the mean of each genetic mutant to the mean of WT.

Color coding:
fold≥32
32>fold≥8
8>fold≥2
2>fold≥0.5
0.5>fold≥1/8
1/8>fold≥1/32
fold<1/32

Table 2 In vitro antileishmanial activity and CYP inhibition of antifungal azoles and non azole compounds

Compound	EC ₅₀ or IC ₅₀ (μM)			SI ^f
	EC ₅₀ (<i>L. donovani</i>) ^a	IC ₅₀ (<i>LdCYP51</i>) ^e	IC ₅₀ (<i>LdCYP5122A1</i>) ^e	
Imidazole				
Bifonazole	12 ± 1	0.057 ± 0.008	0.33 ± 0.07	5.7
Butoconazole	1.9 ± 0.4	0.073 ± 0.008	0.32 ± 0.07	4.4
Clotrimazole	5.0 ± 0.1	0.068 ± 0.004	0.26 ± 0.08	3.8
Econazole	9.4 ± 0.3	0.046 ± 0.004	0.47 ± 0.13	10
Fenticonazole	5.6 ± 0.3	0.081 ± 0.002	0.65 ± 0.08	8.0
Isoconazole	6.2 ± 0.5	0.049 ± 0.003	0.43 ± 0.04	8.8
Ketoconazole	22 ± 6	0.048 ± 0.007	0.42 ± 0.05	8.7
Miconazole	7.6 ± 0.7	0.057 ± 0.003	1.1 ± 0.2	19
Oxiconazole	3.8 ± 0.4	0.032 ± 0.002	0.62 ± 0.02	20
Sertaconazole	7.1 ± 0.5	0.064 ± 0.007	1.1 ± 0.2	17
Sulconazole	7.4 ± 0.6	0.064 ± 0.003	0.38 ± 0.06	5.9
Tioconazole	9.5 ± 0.6	0.054 ± 0.008	1.1 ± 0.2	20
Triazole				
Efinaconazole	16 ± 1	0.068 ± 0.002	6.2 ± 1.7	92
Fluconazole	>100 ^b	0.96 ± 0.05	>100	>105
Isavuconazole	12 ± 0	0.043 ± 0.007	2.6 ± 0.7	60
Itraconazole	6.0 ± 1.1	0.049 ± 0.009	2.1 ± 0.4	43
Posaconazole	2.8 ^b	0.059 ± 0.003	1.5 ± 0.3	25
Ravuconazole	13 ± 2	0.049 ± 0.008	12 ± 4	248
Terconazole	13 ± 1	0.083 ± 0.009	12 ± 3	140
Voriconazole	>100	0.085 ± 0.017	>100	>1176
Non azole compounds				
Miltefosine	2.7 ^c	12 ± 1	43 ± 1	3.5
Pentamidine	15 ± 1	No inhibition at 100 μM	No inhibition at 100 μM	Not applicable
Paromomycin	>50 ^c	No inhibition at 100 μM	No inhibition at 100 μM	Not applicable
Amphotericin B	0.14 ± 0.01	<25% inhibition at 10 μM	<25% inhibition at 10 μM	Not applicable
DB766	0.036 ^d	<30% inhibition at 30 μM	1.0 ± 0.1	<0.035

^aMean ± standard deviation (n ≥ 3)^bPublished data from Feng et al. (ref. 16)^cIC₅₀ vs. intracellular amastigotes from Wang et al. (ref. 49)^dIC₅₀ vs. intracellular amastigotes from Wang et al. (ref. 49), see also Figure 10^eMean ± standard deviation (n = 3)^fSI (selectivity index) = IC₅₀ against *LdCYP5122A1*/IC₅₀ against *LdCYP51*

Table 3 Effects of different CYP inhibitor treatments on the sterol profile of LV82 promastigotes. The sterol levels in inhibitor-treated parasites were normalized to the corresponding sterol levels in DMSO-treated parasites. The values represent mean $\pm$ standard deviation. Each ratio is an average of three individual experiments conducted on separate days. Each experiment had biological triplicates.

Sterol group	Sterol name	20 μ M clotrimazole vs. DMSO	50 μ M voriconazole vs. DMSO
4- and/or 14-methylated sterols	Lanosterol	2.7 $\pm$ 0.9**	1.6 $\pm$ 0.2
	4CH ₂ OH-LS	2.3 $\pm$ 0.5	0.83 $\pm$ 0.09
	4,14-Dimethylzymosterol	43 $\pm$ 18***	17 $\pm$ 2**
	14-Methylzymosterol	2.3 $\pm$ 0.4**	8.0 $\pm$ 1.0**
	14-Methylfecosterol	Accumulated ^{c,***}	Accumulated ^{c,***}
	4-Methylzymosterol ^a	0.025 $\pm$ 0.013***	0.18 $\pm$ 0.06**
	FF-MAS	Not detected	Not detected
4,14-demethylated sterols	T-MAS	Not detected	Not detected
	Zymosterol ^b	0.018 $\pm$ 0.006**	0.067 $\pm$ 0.034**
	Fecosterol ^a	0.025 $\pm$ 0.013***	0.18 $\pm$ 0.06**
	Episterol ^a	0.025 $\pm$ 0.013***	0.18 $\pm$ 0.06**
	Cholesta-7,24-dienol ^b	0.018 $\pm$ 0.006**	0.062 $\pm$ 0.030**
5-dehydro sterols	7-Dehydroadosterol	0 ^{d,**}	0 ^{d,**}
	5-Dehydroepisterol	0.20 $\pm$ 0.04**	0.078 $\pm$ 0.043***
	Ergosta-5,7-dienol	0.79 $\pm$ 0.26	0.39 $\pm$ 0.11**
	Ergostatetraenol	0 ^{d,**}	0.086 $\pm$ 0.027**
	Ergosterol	0.26 $\pm$ 0.07**	0.30 $\pm$ 0.08***
Exogenous	Cholesterol	1.1 $\pm$ 0.2	0.88 $\pm$ 0.07

^a Episterol, fecosterol and 4-methylzymosterol could not be chromatographically separated.

^b Zymosterol and Cholesta-7,24-dienol could not be chromatographically separated.

^c This sterol was detected in inhibitor-treated samples but not in vehicle-treated samples.

^d this sterol was detected in vehicle-treated samples but not in inhibitor-treated samples.

*, **, *** unpaired two-tailed t-test (* P<0.05; ** P<0.01; *** P<0.001)

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fold $\geq$ 32
32>fold $\geq$ 8
8>fold $\geq$ 2
2>fold $\geq$ 0.5
0.5>fold $\geq$ 1/8
1/8>fold $\geq$ 1/32
fold<1/32