



Table S1. Statistics of data collection and structural refinement for EtDHODH and HsDHODHferulenol complex structures

	EtDHODH	EtDHODH	HsDHODH		
	(ligand-free form)	(ferulenol complex)	(ferulenol complex)		
Data collection					
Space group	<i>I</i> 23	<i>P</i> 6 ₃	P3221		
Cell parameters					
<i>a, b, c</i> (Å)	248.8, 248.8, 248.8	133.0, 133.0, 215.0	90.2, 90.2, 123.3		
$\alpha \beta \gamma$ (°)	90,0, 90.0, 90.0	90,0, 90.0, 120.0	90,0, 90.0, 120.0		
X-ray source	SPring-8 BL44XU	SPring-8 BL44XU	KEK-PF BL17A		
Wavelength (Å)	0.90000	0.90000	0.98000		
Resolution (Å)	50-3.5 (3.56-3.5)	50-3.65 (3.71-3.65)	50-1.90 (1.93-1.90)		
Total No. of reflections	283,621	100,597	348,775		
No. of unique reflections	32,456	24,158	46,303		
Rmerge	0.132 (0.792)	0.075 (0.797)	0.081 (0.729)		
$R_{ m means}$	0.140 (0.849)	0.085 (0.908)	0.085 (0.783)		
I /σ(I)	5.6 (2.6)	7.9 (1.7)	9.5 (3.0)		
Completeness (%)	100.0 (100.0)	96.1 (96.7)	100.0 (100.0)		
Redundancy	9.3 (8.3)	4.3 (4.2)	7.5 (7.6)		
<u>Refinement</u>					
Resolution (Å)	20-3.5	20-3.65	20-1.90		
Rwork / Rfree	0.272 / 0.359	0.221 / 0.303	0.163 / 0.178		
Used reflections	29,340	21,056	43,740		
No of non-hydrogen atoms					
Protein	11,285	11,764	2,823		
Ligand	168	276	69		
Solvent	-	-	159		
Average <i>B</i> -factors ($Å^2$)					
Protein	48.0	62.4	29.6		
T. 1	34.1	50.7	31.1		
Ligand	(FMN, ORO)	(FMN, ORO, ferulenol)	(FMN, ORO, ferulenol)		
Solvent	-	-	36.1		
RMSD					
Bond length (Å)	0.013	0.009	0.009		
Bond angle (°)	1.796	1.522	1.539		
Ramachandran plot (%)					
Favored regions	78.8	79.9	93.4		
Allowed regions	20.0	19.1	6.6		
Outers	1.2	1.0	0		
PDB code	6AJ5	6AJE	6IDJ		

Values in parentheses are for the highest resolution shell.

Palament Image: State of the s	E.tenella									
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Makanana Endit Model Figure Sector	H.sapiens	· MHLFVRFALS	KGIVAPAS · SCAAR	GARCEALAFS	SCRWEARG	HASSACSIKGLI	CKT · QARASLERTARQ			G1192 •••
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Impair Impair<	H.sapiens 1				MA	WRHLKKRAQDAV	IILGGGGLLFASYLM.	ATGDERFYAEF	ILMPTLQ · · ·	- G L 48
Image Image <th< td=""><td>H.vulori 1 · · · ·</td><td></td><td></td><td></td><td></td><td></td><td>·····························</td><td>· · · · MLYSLV</td><td>KKYLFSLDAEI</td><td>AHE 20</td></th<>	H.vulori 1 · · · ·						·····························	· · · · MLYSLV	KKYLFSLDAEI	AHE 20
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Instrument Instrum	P.falciparum 179 IDGE	ICHDLFLLLGK	YNIL PYDT SNDSIY	ACTNIKHLDF	INPFGVAAGFDKN	IGVCIDSILKL <mark>G</mark>	SFIEIGTITPRGQTG	NAK PRIFRDVI	E S R S I I N S C G F	N NM281
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$ \begin{array}{c} Lam \\ Find \\ Fi$	S.aureus 20 L T I D	ALKTLQKFPVL	FPVVDKLFTYKNPT	LSQTIQGNTY	D N <mark>P</mark> I <mark>G L A A G F D K</mark> S	C E V P K A L E H L <mark>G</mark> I	FGALEL <mark>G</mark> GI <mark>T</mark> PKPQP <mark>G</mark>	N P Q <mark>P R M F R</mark> L L I	E D D A L I <mark>N</mark> R M <mark>G F</mark>	N N I 122
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Hygien 10	H.sapiens 246 M tuberculosis 223				VHR PAVLVKIAPD	LTSQDKEDIAS	V K E L G I D G L I V T N T T A V F L D L A G I V A T N T T	V S R P A G L Q G A I	LRSETG - GLSG	K P L 308 P P L 282
	H.pylori 210				· · · · P L F L <mark>K I A P D</mark>	LETDDMLEIVNS	A I EAGAN <mark>G</mark> I I AT NTT	IDKSLVFAP -	KEMG · · GLSG	К С <mark>L</mark> 265
LundJoinJ	S.aureus 209				DVTVPIYL <mark>K</mark> LTS <mark>D</mark> HKYVPIAVKIAPD	MDFDGLKPLLP	AITETFDGIILANTT	RQRDGLTSAN	VEEG GLSG	R P L 269
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E. tenella $P.$ falciparum $T.$ gondii $N.$ caninum $H.$ sapiens $M.$ tuberculosis $H.$ pylori $S.$ aureus $E.$ coli $E.$ tenella $P.$ falciparum $T.$ gondii $N.$ caninum $H.$ sapiens $M.$ tuberculosis $H.$ pylori $S.$ aureus $E.$ coli $N.$ caninum $I.$ caninum	P.falciparian 482 KDIS T.gondii 494 KHLS	TACVSDMYKLT	OGKLAIIAT GGVES	GLDALEKTEA GRDALDKIEA	GASVCQLYSCLVF GASLVELYSSMVY	IGPOVARRVKNI	ELNHLLYQRGYYNLKE. ELYHALNEKGYKDVAA.	AIGRKHSKS - ·	EKKLOAPKFD	569
Hargion 369 RDLSTOTTREMALTOGRVPH GVGVSVSGODALEKERAGAS LVGGVTGFTYGGVVGKVRE FALLKRGGFGGVTDATGADHR	N.caniman 487 KNLS	TACVSDMYKLT	QGK L V I I A T GGV E S	GRD <mark>A</mark> LDKIEA	GAS LVEL Y S SMIY	LGPQVARRVKNI	E LYCALNEKGYKDVAA.	AVGRKHRQTPI	EKKLQAPTFD	585
Hyper 26* TEKSRETEFEELAKAFFNKSVUSVSCI SDAKERY EN KNGAS LLOI ASHTYNE UN CON LKRETVKLOORDELSVEALGADEN	H.sapiens 309 R D L S M.tuberculosis 283 A O R A	TQTIREMYALT VOVLERLYDEV	QGRVPIIGVGGVSS GDRLALISVGGIET	GQD <mark>A</mark> LEKIRA ADDAWERITA	GASLVQLYTALTF GASLLOGYTGFLY	G C F R WAKDIHE	ELEALLKEQGFGGVTD.	AIGADHRR · AVGSARRROPS		395 372
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E. tenella P. falciparum T. gondii N. caninum H. sapiens M. tuberculosis H. pylori S. aureus E. coli E. tenella 41.56 52.17 52.42 45.52 36.06 32.43 33.25 35.70 P. falciparum 32.93 31.80 34.23 28.24 29.30 28.12 36.95 T. gondii 72.13 43.65 33.41 32.09 33.83 36.32 N. caninum 1 1 1 42.59 33.01 31.36 32.18 35.88 H. sapiens 1 </td <td>S.aureus 270 FERN</td> <td>LKLIKYAYQQT</td> <td>NGEFLIIGT GGVFS</td> <td>T E D <mark>A</mark> I KMMRH</td> <td>GASLIQIYSSLVI</td> <td>E G P G L T K K M N K G</td> <td>GIARY LKDHHFDNVSD</td> <td>IIGLDA</td> <td></td> <td>354</td>	S.aureus 270 FERN	LKLIKYAYQQT	NGEFLIIGT GGVFS	T E D <mark>A</mark> I KMMRH	GASLIQIYSSLVI	E G P G L T K K M N K G	GIARY LKDHHFDNVSD	IIGLDA		354
E. tenellaP. falciparumT. gondiiN. caninumH. sapiensM. tuberculosisH. pyloriS. aureusE. coliE. tenella41.56 52.17 52.42 45.52 36.06 32.43 33.25 35.70 P. falciparum32.93 31.80 34.23 28.24 29.30 28.12 36.95 T. gondii672.13 43.65 33.41 32.09 33.83 36.32 N. caninum66642.59 33.01 31.36 32.18 35.88 H. sapiens666640.63 36.14 35.77 41.62 M. tuberculosis66666 42.59 33.01 31.36 34.08 42.98 H. pylori6666666 34.55 41.23 S. aureus6666666 34.55 41.23 E. coli666666666	2.00 2.12 Q 2 K 2						•••			000
E. tenella 41.56 52.17 52.42 45.52 36.06 32.43 33.25 35.70 P. falciparum 32.93 31.80 34.23 28.24 29.30 28.12 36.95 T. gondii 72.13 43.65 33.41 32.09 33.83 36.32 N. caninum 42.59 33.01 31.36 32.18 35.88 H. sapiens 40.63 36.14 35.77 41.62 M. tuberculosis 40.63 36.14 35.77 41.62 S. aureus 40.63 36.14 35.55 41.23 S. aureus 40.63 40.63 36.14 35.77 41.62		E. tenella	P. falciparum	T. gondii	N. caninum	H. sapiens	M. tuberculosis	H. pylori	S. aureus	E. coli
P. falciparum 32.93 31.80 34.23 28.24 29.30 28.12 36.95 T. gondii 72.13 43.65 33.41 32.09 33.83 36.32 N. caninum 6 72.13 43.65 33.01 31.36 32.18 35.88 H. sapiens 6 6 42.59 33.01 31.36 32.18 35.88 M. tuberculosis 6 6 6 8 40.63 36.14 35.77 41.62 M. tuberculosis 6 6 6 8 42.98 34.08 42.98 S. aureus 6 6 6 6 34.55 41.23 S. aureus 6 6 6 6 6 6 6	E. tenella		41.56	52.17	52.42	45.52	36.06	32.43	33.25	35.70
T. gondii Image: Constraint of the second secon	P. falciparum			32.93	31.80	34.23	28.24	29.30	28.12	36.95
N. caninum Image: Marcol of the sapiens Marcol of the sapiens 33.01 31.36 32.18 35.88 H. sapiens 40.63 36.14 35.77 41.62 M. tuberculosis 38.87 34.08 42.98 H. pylori 34.55 41.23 S. aureus 5. aureus 5. aureus 5. aureus 5. aureus	T. gondii				72.13	43.65	33.41	32.09	33.83	36.32
H. sapiens Image: Constraint of the second seco	N. caninum					42.59	33.01	31.36	32.18	35.88
M. tuberculosis 38.87 34.08 42.98 H. pylori 34.55 41.23 S. aureus 6 6 6 36.18 E. coli 6 6 6 6 6	H. sapiens						40.63	36.14	35.77	41.62
H. pylori 34.55 41.23 S. aureus 36.18 E. coli	M. tuberculosis							38.87	34.08	42.98
S. aureus 36.18 E. coli 36.18	H. pylori								34.55	41.23
E. coli	S. aureus									36.18
	E. coli									

Figure S1. Top: sequence alignment of amino acid sequences of DHODH from *E. tenella* and other organisms. Residues were colored according to percentage identity by Jalview. Conserved residues involved in the binding of orotate and coumarin group of ferulenol, according to the crystal structure of *H. sapiens* DHODH (PDB: 6IDJ), are shown with "#" and "*" symbols, respectively. The long insertion (R217 to T252) found only in apicomplexan DHODHs is highlighted in red.

Bottom: Amino acid sequence identity. Abbreviations; *E. tenella*: *Eimeria tenella* (XP013227840), *P. falciparum*: *Plasmodium falciparum* (AAC37170), *T. gondii*: *Toxoplasma gondii* (AAM46067), *N. caninum*: *Neospora caninum* (XP003880770), *H. sapiens*: *Homo sapiens* (AAH65245), *M. tubercu*: *Mycobacterium tuberculosis* (KDA15626), *H. pylori*: *Helicobacter pylori* (ADZ49526), *S. aureus*: *Staphylococcus aureus* (SBB47760), *E. coli*; *Escherichia coli* (KIG45581). All the numbers in the parentheses are GenBank Accession Nos.



Figure S2. The purity and oligomeric state of EtDHODH in solution evaluated by SDS-PAGE and HrCNE, respectively. The enzyme eluted by 100 mM and 300 mM imidazole was loaded (5 μ g) onto SDS-PAGE (12%) and stained by CBB (left panel). The fraction eluted at 300 mM was used for subsequent studies. The oligomeric state of EtDHODH in solution was evaluated together with PfDHODH and HsDHODH by high-resolution clear native electrophoresis (HrCNE), followed by CBB and DHODH activity staining (right panel), which were performed essentially as previously reported (see reference 22 from the main text). The calculated molecular weight (MW) from the amino acid sequences are 46 kDa, 47 kDa and 40 kDa for EtDHODH, PfDHODH and HsDHODH, respectively. The buffer used for HrCNE contained dodecylmaltoside (DDM) which has a micelle size of 72 kDa and cause the shift in MW of the three enzymes to 110-130 kDa. Small variations in the size of the three enzymes are characteristic of a globular protein. Altogether, these results indicate that the three enzymes are active as monomer.



Figure S3. Differences in the sensitivity to ascofuranone derivatives between EtDHODH and HsDHODH. The inhibition of each compound against EtDHODH (dark gray) and HsDHODH (light gray) at 2.5 μ M are shown as the average. The error bars represent the SD (n = 4). The structure of the compounds selected for this figure (compounds **1-10**) is shown at the bottom.



HsDHODH-Ferulenol: cyan PfDHODH-Ferulenol (model): pink



EtDHODH-Ferulenol: green PfDHODH-Ferulenol (model): pink

Figure S4. Model structure of ferulenol bound to PfDHODH. Binding of ferulenol to PfDHODH is not favorable due to steric hindrance of isoprenyl chain with F188 (red circle). The F188 from PfDHODH is replaced by A59 and V26 in HsDHODH and EtDHODH, respectively. Structure, residues and ligands shown in cyan, pink and green are from HsDHODH, PfDHODH and EtDHODH, respectively.



PfDHODG-DSM265: green EtDHODH-DSM265 (model): pink

Figure S5. Superposed structure of DSM265 bound to PfDHODH and EtDHODH. Binding of DSM265 from PfDHODH (4RX0) was modelled into EtDHODH, which show several steric hindrance with the pentafluorosulfanyl moiety and side chain from V26, M78 and F75 in EtDHODH. Ligands and residues from PfDHODH and EtDHODH are shown in green and pink colors, respectively.