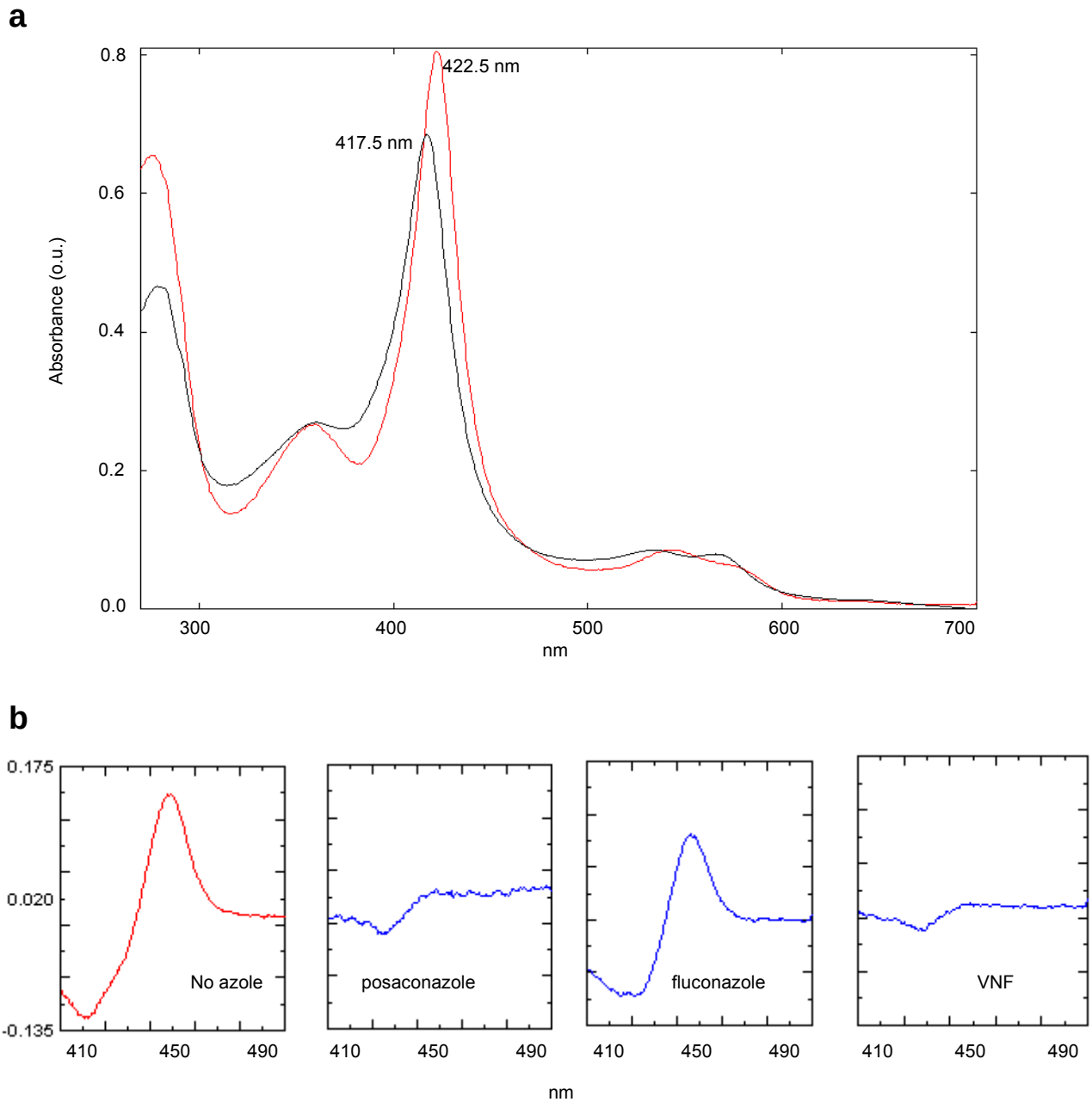
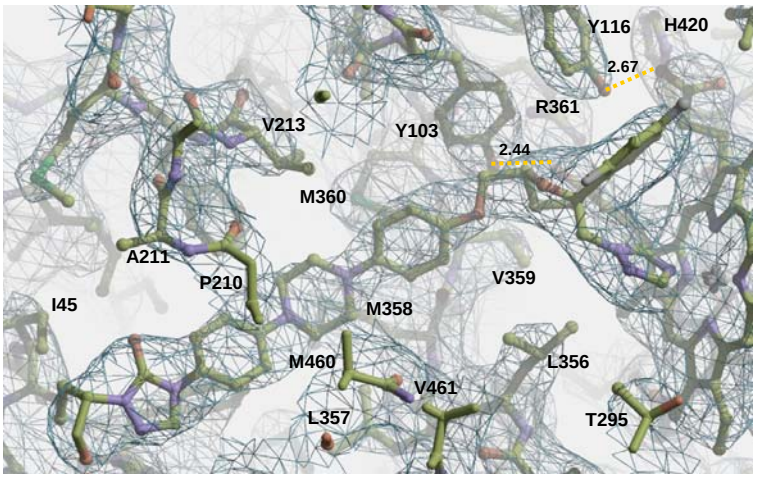
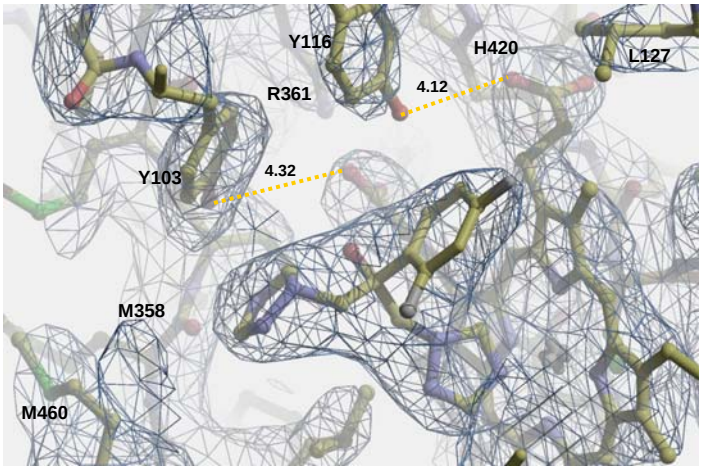


Supplemental Figure S1. **Spectral responses of TC14DM to azole inhibitors. a.** Absolute absorbance spectra of ligand-free and posaconazole bound ferric TC14DM (5.85 μM). The red shift in the Soret band maximum reflects coordination of the basic triazole nitrogen to the heme iron. **b.** Difference CO binding spectra used as an alternative approach to evaluate relative strength of the N-Fe coordination in the azole-TC14DM complexes (1.6 μM). The spectra reflect the ability of carbon monoxide, whose binding produces a red shift in the reduced Cys-coordinated hemoprotein Soret band maximum from 417 to ~450 nm (the origin of the cytochrome P450 name), to replace the nitrogen in the Fe-coordination sphere. The CO-spectra were taken after reduction of TC14DM with 100 mM $Na_2S_2O_4$ followed by bubbling CO-gas through the test cuvette. In the control (no azole) sample, the protein shows a clear 450 nm peak, in fluconazole-bound TC14DM the amount of spectrally detectable CO-complexes is decreased, while TC14DM bound to posaconazole or VNF does not produce any observable CO-difference spectra.

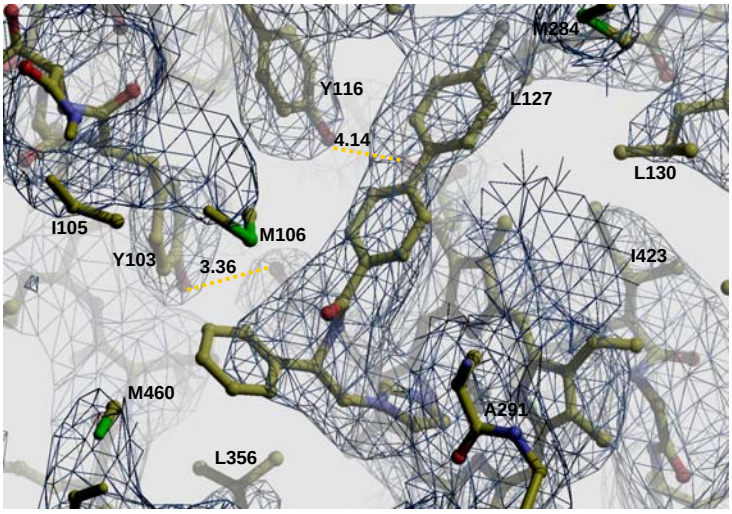
a



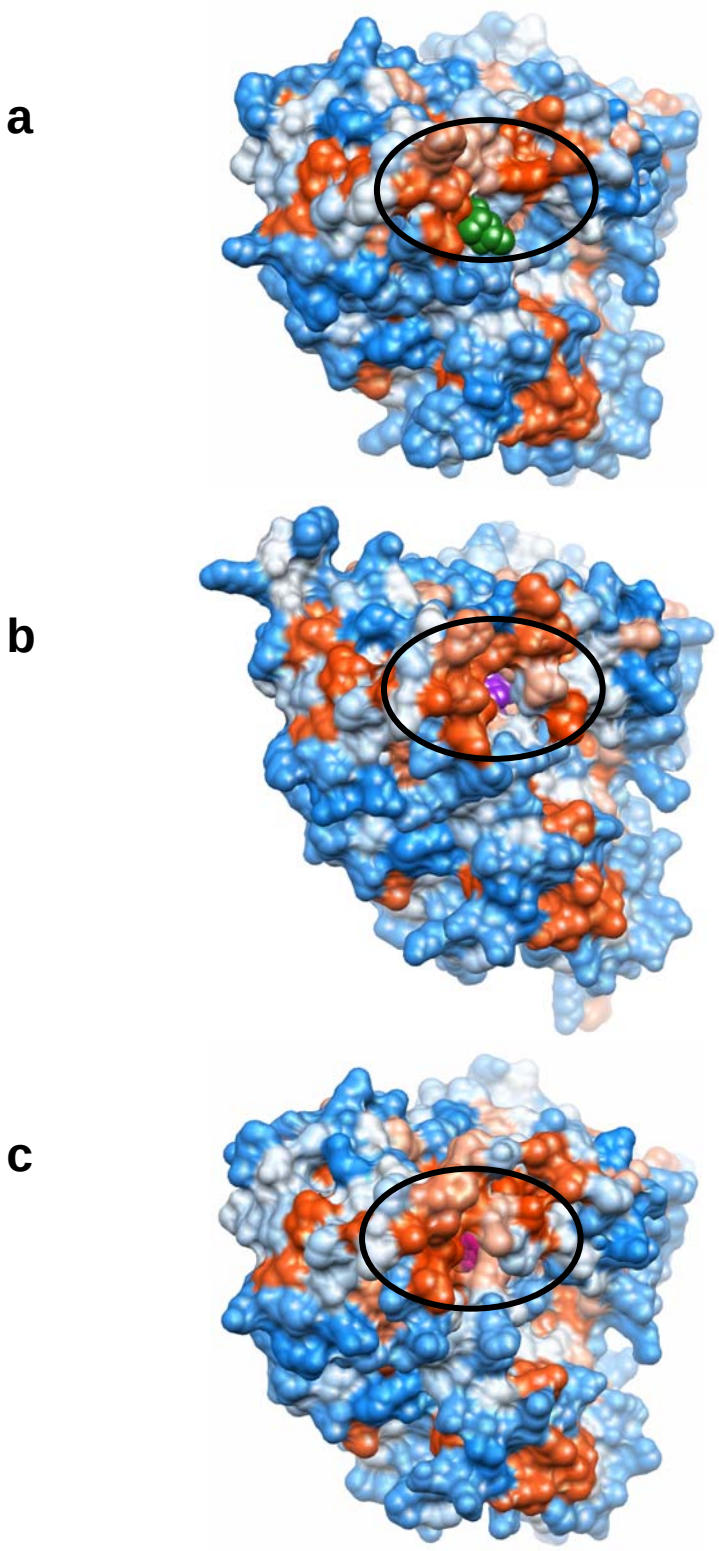
b



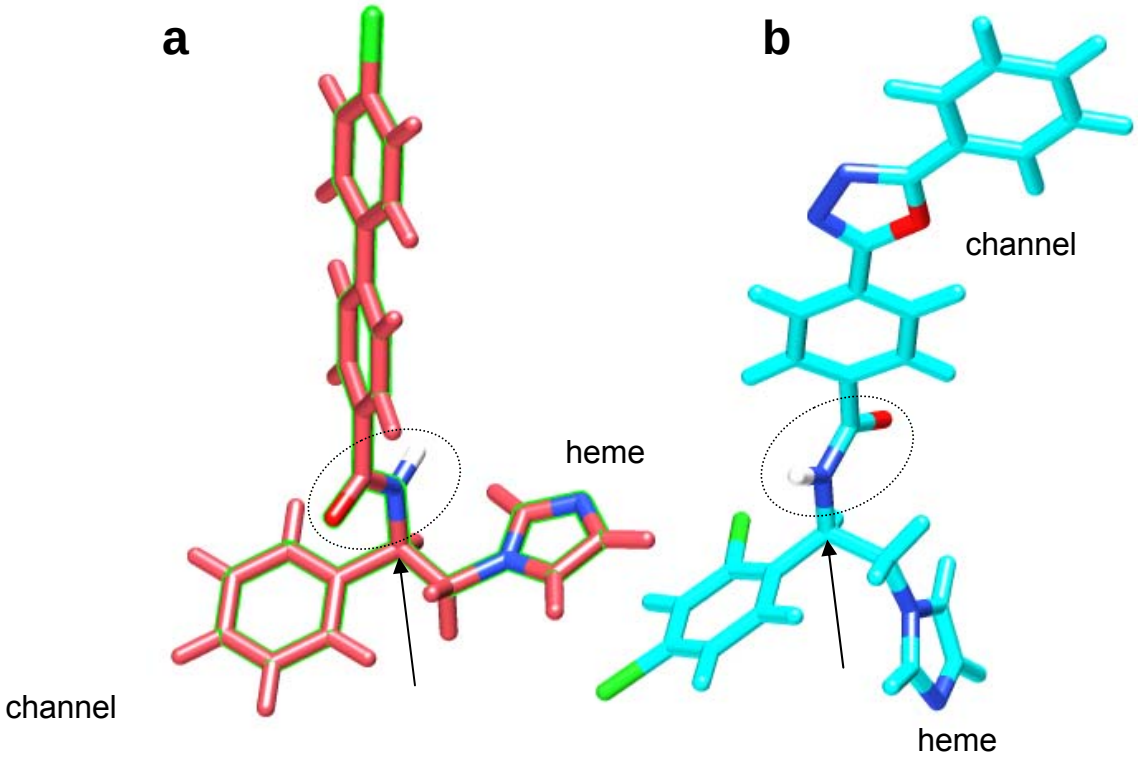
c



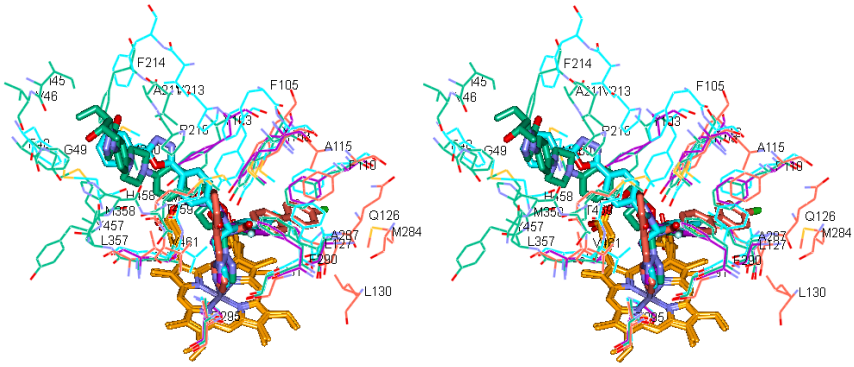
Supplemental Figure S2. 2Fo-Fc electron density map for posaconazole (a), fluconazole (b) and VNF (c) in the active site of TC14DM at 1σ. Selected distances (Å) are marked with dashed yellow lines



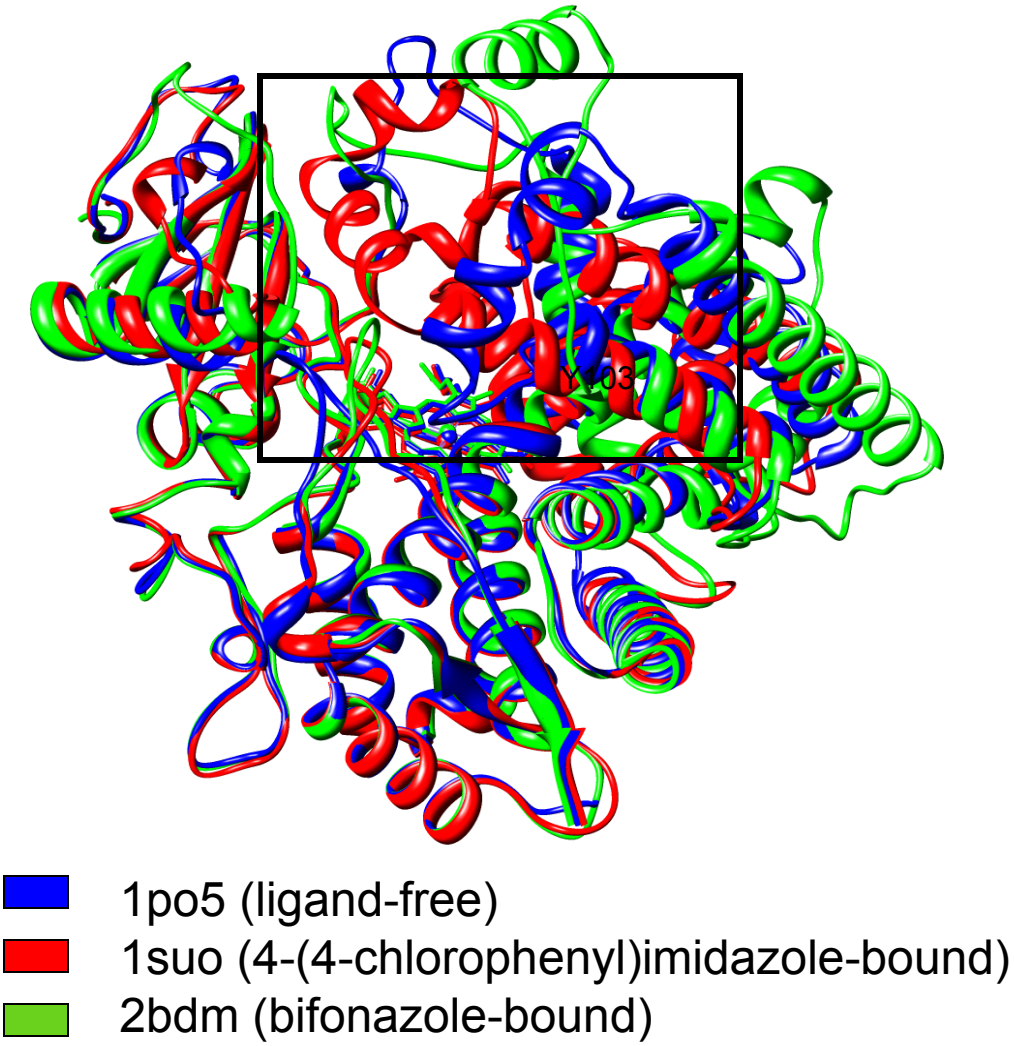
Supplemental Figure S3. **Hydrophobicity-colored surface representations of TC14DM complexed with posaconazole (a), fluconazole (b) and VNF (c).** Left-side view. Carbon atoms of the inhibitors are colored in green, violet and pink, respectively and presented as spheres. The entrance channel is circled. Highly hydrophobic area around the access channel entrance strongly supports that this part of the molecule, which is adjusted to the (truncated) N-terminal membrane anchor sequence *in vivo* must be immersed into the membrane.



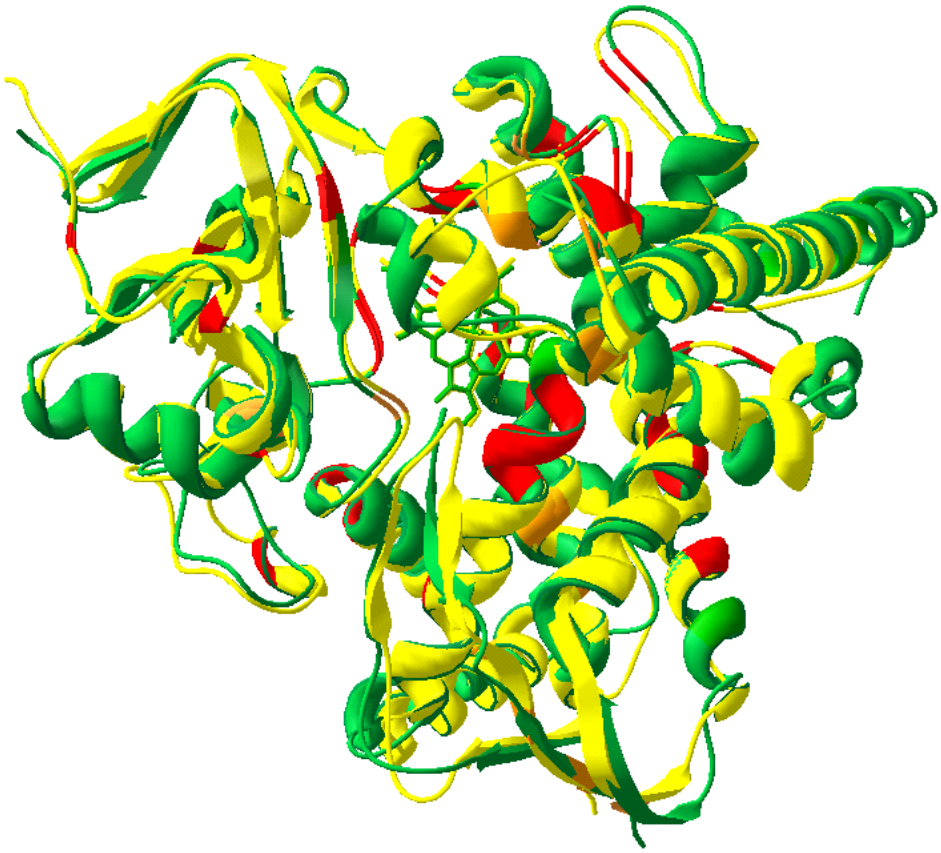
Supplemental Figure S4. Structures of VNF (a) and VNI (b) in complexes with 14DMs from TC and Tbb, respectively. Hydrogens were added in Chimera; Both compounds are in the R-configuration, chiral atoms are marked with arrows. Carboxamide fragment is circled.



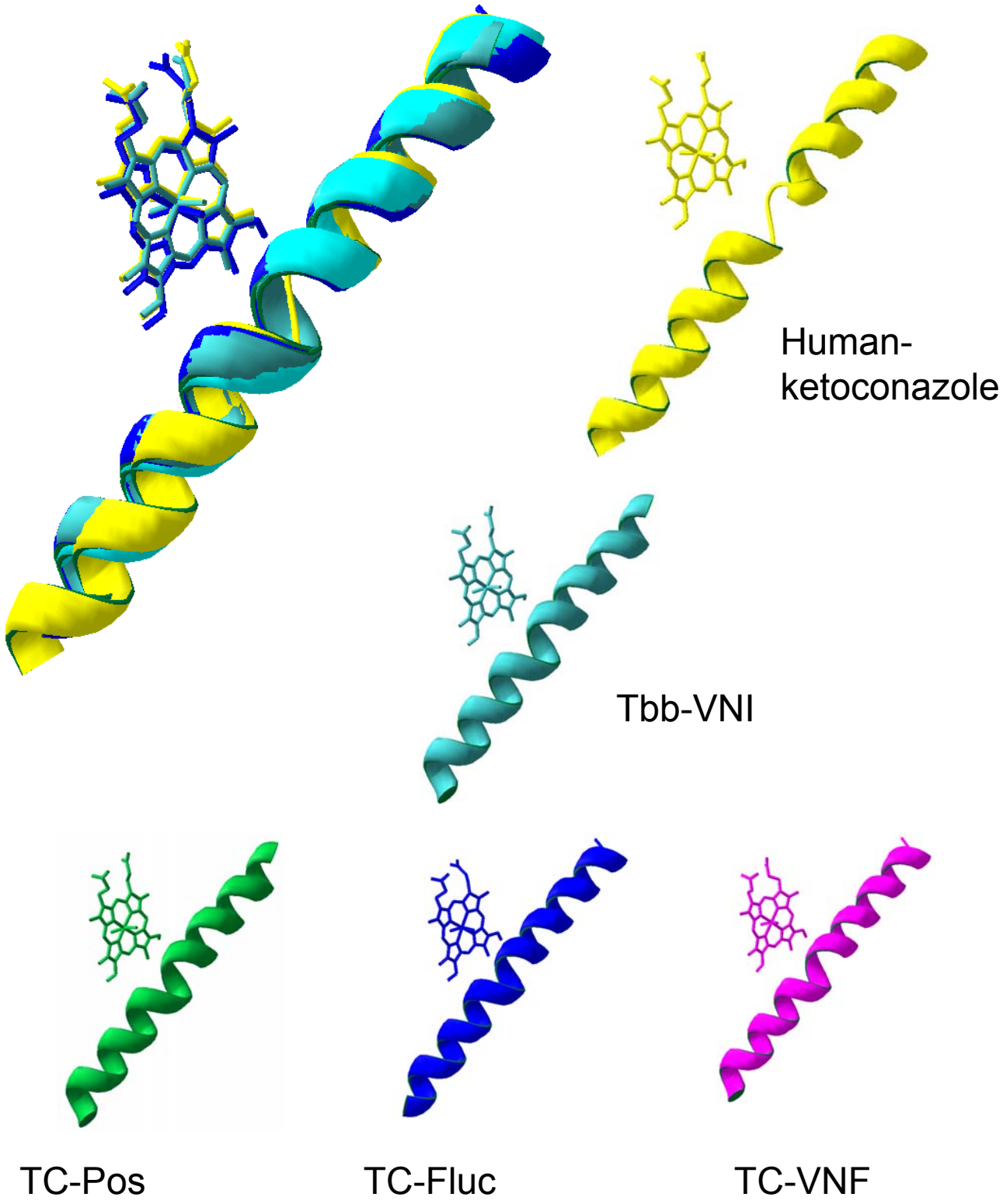
Supplemental Figure S5. Inhibitors bound in four superimposed 14DM structures. Residues located within Van der Waals contact distances are shown, the residue color correspond to the color of the inhibitor: green for posaconazole (TC), violet for fluconazole (TC), salmon for VNF (TC) and cyan for VNI (Tbb).



Supplemental Figure S6. Superimposition of three crystal structures of a drug-metabolizing P450 2B4, ligand-free and bound to two different inhibitors. The ligands are deleted for clarity. The active site pocket area is marked.



Supplemental Figure S7. **Superimposition of TC14DM (green) with the human 14DM (3ld3, most recent release, yellow).** Residues identical in all eukaryotic 14DM (sequences from~160 organisms known to date) are marked in red, highly conserved residues are orange.



Supplemental Figure S8. **The core helix (helix I) in the human 14DM has an interruption in the middle**. Lack of helical hydrogen bonding must make this area more flexible. In trypanosomal 14DMs the I-helix must be more rigid.

Supplemental Table S1. Data collection and refinement statistics

TC14DM-inhibitor complex	Posaconazole [3K1O]	Fluconazole [3KHM]	VNF [3KSW]
Data collection			
Wavelength, Å	0.9787	0.9793	1.0539
Space group	P3(1)21	P3(1)21	P3(1)21
Cell dimensions			
a, b, c, Å	63.12, 63.12; 229.30	62.59, 62.59, 222.25	66.49, 66.49, 234.28
α, β, γ, °	90.00, 90.00, 120.00	90.00, 90.00, 120.00	90.00, 90.00, 120.00
Number of molecules per asymmetric unit	1	1	1
Resolution, Å	30-2.89 (2.94-2.89)*	50-2.85 (2.95-2.85)	50-3.05 (3.10-3.05)
R_{merge}	0.067 (0.588)	0.060 (0.502)	0.098 (0.369)
I/σ(I)	25.7 (4.1)	24.8 (1.4)	13.1 (2.0)
Completeness (%)	99.5 (100)	96.0 (95.9)	91.7 (43.4)
Redundancy	8.5 (8.8)	3.6 (3.3)	7.6 (2.4)
Refinement			
Resolution, Å	28.7-2.89	28.8 – 2.85	28.6-3.05
Number of reflections	11910	11141	10505
$R_{work/}$ / R_{free}	0.228/ 0.276	0.227/0.280	0.245/0.292
No. atoms			
protein	3491	3517	3514
heme/inhibitor	94	65	72
water	10	0	0
Rms deviations			
Bond lengths, Å	0.014	0.003	0.003
Bond angles, °	1.697	0.365	0.620

*Values in parenthesis are for highest-resolution shell.

Supplemental Table S2. Inhibitor contacting residues in the 14DM structures

Posaconazole TC14DM	Fluconazole TC14DM	VNF TC14DM	VNI TB14DM
I45 (αA')			
V46 (αA')			
F48 (αA')			F48
G49 (αA')			
Y103 (αB')	Y103		Y103
			F105 (αB')
M106 (αB')	M106	M106	
F110 (αB')	F110	F110	F110
		A115 (B'C loop)	
Y116 (B'C loop)	Y116	Y116	Y116
		Q126 (αC)	
L127 (αC)	L127	L127	
		L130 (αC)	
P210 (αF'')			
A211 (αF'')			
V213 (αF'')			
F214 (αF'')			F214
		M284 (αI)	
A287 (αI)	A287	A287	A287
F290 (αI)	F290		F290
A291 (αI)	A291	A291	A291
T295 (αI)	T295	T295	T295
L356 (αKβ1-4 loop)	L356	L356	L356 (Kβ1-4 loop)
L357 (αKβ1-4 loop)			
M358 (αKβ1-4 loop)			M358
M360 (β1-4)			M360 (β1-4)
Y457 (β4 hairpin)			
H458 (β4 hairpin)			
T459 (β4 hairpin)			
M460 (β4 hairpin)		M460	M460
		V461	V461 (β4-hairpin)
25	10	14	15