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Supporting Information for:

Amiodarone has intrinsic anti-*Trypanosoma cruzi* activity and acts synergistically with posaconazole

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Lipid analysis:

For the analysis of the effects of drugs on epimastigote lipid composition, total lipids from control and drug-treated cells were extracted and fractionated into neutral and polar fractions by using silicic acid column chromatography and gas-liquid chromatography[1,2]. The neutral lipid fractions were analyzed by thin layer chromatography (on Merck 5721 silica gel plates, using heptane-isopropyl ether-glacial acetic acid [60:40:4] as the developing solvent), and by conventional gas-liquid chromatography (isothermal separation in a 4-m glass column packed with 3% OV-1 on Chromosorb 100/200 mesh, nitrogen carrier gas, at 24 mL min^{-1}; flame ionization detection on a Varian 3700 gas chromatograph). For quantitative analysis and structural assignments, the neutral lipids were then separated on a high resolution capillary column (25 m x 0.20 mm i.d., Ultra-2 column, 5% phenyl-methylsiloxane, 0.33 μm film thickness) using a Hewlett-Packard 6890-Plus gas chromatograph, equipped with a HP5973A mass sensitive detector. Lipids were injected in chloroform and the column kept a 50ºC for 1 min, then the temperature was increased to 270ºC at a rate of

25ºC·min^{-1} and finally to 300ºC, at a rate of 1ºC·min^{-1}. The carrier gas (He) flow was kept constant at 0.5 mL·min^{-1}. The injector temperature was 250ºC and the detector was kept at 280ºC. The effects of amiodarone on squalene synthase purified from *T. cruzi* epimastigotes were determined as described elsewhere[3,4].

Theoretical calculations

The pharmacophore features selected for model construction were: two aromatic ring features, an unspecified number of hydrogen bond acceptor features, an unspecified number of hydrophobic features, and one positive charge feature. The importance of both aromatic ring features is supported by the 1W6J x-ray structure[5], in which both of the aromatic rings present in the Ro48-8071 inhibitor (**3**, Chart 1) form aromatic-aromatic interactions with the protein. The carbonyl group in Ro48-8071 is hydrogen bonded to a water molecule that also interacts with the protein, and a water molecule is present in the lanosterol/OSC structure (PDB File 1W6K[5]) and has virtually identical coordinates. This, together with the fact that all of the training set molecules contain a hydrogen bond acceptor group that is geometrically similar to that in **3**, provides a likely structural basis for the hydrogen bond acceptor feature seen in the pharmacophore. Compound **16** (Chart 2), however, does not have two

aromatic ring features, so the number of complete misses allowed in the pharmacophore model was set to one (the default is zero), and the max omit features value for compound **16** was set to two (the default is zero).

For the docking investigations, we used the Autodock 3.05 program[6] to dock lanosterol, amiodarone, and Ro48-8071 to oxidosqualene cyclase using the reported crystallographic structures (PDB files 1W6J and 1W6K[5]). 1W6J and 1W6K were prepared for docking by using the Sybyl 7.0 program (Tripos, Inc., St. Louis, MO). Crystallographic ligands and water molecules were deleted from the PDB structures, with the exception of water molecules within 5 Å of Ro48-8071 or lanosterol (in 1W6J and 1W6K, respectively). For 1W6J, Glu42, Arg43, and Ala44 were missing atoms and therefore required repair. The Structure Preparation Tool in Sybyl 7.0 was used to assign Lovell rotamers to these residues, with rotamers being chosen to reduce steric clashes with the protein. The termini were truncated with neutral end groups, and essential hydrogen atoms were added to the model, with H-bonding orientations being assigned to the water molecules. His232 was taken to be protonated at the epsilon position, since this permitted hydrogen bonding with Tyr503 and a nearby water molecule (HOH31). Following the addition of hydrogen atoms, the protein was assigned Kollman united atom charges and water molecules were assigned Gasteiger charges. To correct the geometries of the non-crystallographic residues in 1W6J, Glu42, Arg43, and Ala44 were annealed by using the Minimize Subset command

in Sybyl 7.0. The “hot region” was set to only include the corrected residues, which were annealed by using the Powell algorithm, the Tripos force field, a dielectric constant of 80, and a convergence gradient of 0.05 kcal·mol^{-1}·Å^{-1}. For 1W6K, the preparation varied somewhat from that of 1W6J since residues 42 and 43 were completely missing from the structure. After adding these residues to the protein, the conformations of Glu42 and Arg43 were copied from the corrected 1W6J structure and then annealed as described above. 1W6K required further preparation still, since the hydrogen atom orientations in the water molecules did not provide the hydrogen bonding patterns observed in 1W6J. To address this, the water hydrogen atoms were minimized by using the settings described in the above annealing protocol.

Ligand preparation for lanosterol and **3** was initiated by extracting the ligands from the x-ray structures, while amiodarone was built in Sybyl 7.0. The geometries of all alkyl chains were set to be all-trans. For **3** and amiodarone, the nitrogen atoms were taken to be positively charged (ammonium) groups, and hydrogen atoms were added before the geometries were optimized. Minimization was performed by using the MMFF94 force field with MMFF94 charges, and a dielectric constant of 80 was used. The minimization algorithm employed was BFGS, with a gradient convergence criterion of 0.001 kcal·mol^{-1}·Å^{-1}. Following minimization, ligands were assigned Gasteiger atomic charges.

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