Electronic Supplementary Information (ESI)

Heme Protein Binding of Sulfonamide Compounds: A Correlation Study by Spectroscopic, Calorimetric and Computational Methods

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MATERIALS

High purity standard Myoglobin (95-100%), Sulfamethazine (4-amino-N-(4,6-dimethylpyrimidin-2-yl) benzenesulfonamide, 98%) and Sulfadiazine (SDZ, 4-amino-Npyrimidin-2-yl-benzenesulfonamide, 98%) were obtained from Sigma-Aldrich Corporation. Citrate phosphate (cp) buffer at pH 7.0 was prepared and used for all the experimental titrations maintaining the concentration of [Na^+] at 10 mM. The pH of the mentioned buffer was measured on a digital systronics high precision pH meter (model: MK-VI) having an accuracy of >0.01. All the chemicals and reagents used in the experiments were of analytical grade purchased from Sigma-Aldrich. Double distilled and deionised (millipore) water was used for the preparation of buffer during the experiment.

METHODS

UV-Vis Absorption Spectroscopy

UV-vis absorbance spectra of SMZ and SDZ binding with Mb were carried out on an Agilent, Cary 100 series UV-Vis spectrophotometer. The cuvettes used were of 3.5 ml standard quartz cells with 10 mm optical path length. Buffer solutions were used as the reference in the reference cell, which was then corrected as baseline to get the proper undisturbed absorbance value of the protein. After each fraction of the drug to the protein, the samples were properly mixed together for the interaction study.

Fluorescence Spectroscopy

Fluorescence quenching studies in steady state, temperature dependent, synchronous fluorescence spectra and FRET analysis were recorded on an Agilent Cary eclipse spectrofluorophotometer at (25 ± 0.5) °C. The fluorescence measurements employed 10 mm optical path length standard quartz cuvettes. The excitation wavelength of the protein for the steady state was kept at 295 nm, scanning the excitation maximum and emission spectra for the amino acid fluorophores. For the synchronous fluorescence spectra's, the measurement of tyrosine and tryptophan moiety in the absence and presence of the drugs (SMZ, SDZ) were set in the wavelength difference ($\Delta\lambda$) of 15 and 60 nm, respectively. All measurements were done keeping the excitation and emission slit at 5 nm.

Fluorescence Energy Resonance Transfer (FRET) analysis of Mb with the antibiotic drugs were determined taking the emission spectra of the protein maintaining the excitation wavelength at

295 nm and the absorbance spectra of the drugs. The concentrations of the samples were recorded at 8 μM for both the protein and the drugs.

Circular Dichroism Measurement

The changes in the conformation of the secondary and tertiary structures of the protein molecule, Mb on interaction with SMZ and SDZ was obtained on a Jasco J815 spectropolarimeter (Jasco International Co., Ltd.) enhanced with temperature controller PFD 425 L/15 and a Peltier cell holder. The concentration used for the protein molecule was maintained at 4.0 μM and performed at 25°C. The parameters used for CD measurements were set as; a scanning speed of 50 nm min^{-1}, a sensitivity of 100 milli degrees and a bandwidth of 1.0 nm. Number of scans implemented was Five and smoothened to rebuild the signal-to-noise ratio. The molar ellipticity values are expressed in terms of mean residue molar ellipticity [θ], in units of deg cm^2 $dmol^{-1}$.

FT-IR Spectrometer

The vibration FT-IR spectra of Mb on binding with SMZ and SDZ at 298 K were measured on an Agilent Cary 630 FTIR spectrometer in the range of 490–4000 cm^{-1}. The spectra were recorded in citrate phosphate buffer in the presence and absence of the drugs. The concentration was maintained in the form of 1:1 molar ratio keeping 10 μM constant for both the protein and the drugs respectively.

Differential Scanning Calorimetry

The stabilization or destabilization of the protein, Mb upon interaction with SMZ and SDZ drug was monitored on a MicroCal VP-differential scanning calorimeter (DSC) (MicroCal, Inc., Northampton, MA, USA). The excess heat capacity was measured as a function of temperature to determine the temperature dependent transitions in the protein. The sample and the reference cells were filled with degassed buffer solution, balanced and scanned from 333.15 K to 373.15 K at a scan rate of 50 K h^{-1}. The required buffer scans were repeated for about 10-12 times and a stable baseline was acquired. The DSC thermograms of excess heat capacity and temperature were further examined and plotted using origin 8.0 software.

Isothermal Titration Calorimetry

ITC experiments were performed using a MicroCal VP-ITC unit at 20°C. In order to avoid bubble formation in the calorimeter cell, the solutions were degassed on the MicroCal's Thermovac unit. The titration was done filling the syringe with 500 μM of the drug solution with

Mb. SMZ/SDZ solution of 10 µl aliquots was continuous injected into 50 µM of Mb solutions at a rotating speed of 416 rpm into the syringe. The heat of dilution data were further corrected and plotted as a function of molar ratio to determine the binding affinity (*K*), standard molar enthalpy of complex formation ($\Delta H°$) and the binding stoichiometry (*N*). The remaining thermodynamic parameters, molar Gibbs energy change ($\Delta G°$) and molar entropy contribution ($T\Delta S°$) where $\Delta S°$ is the calculated standard molar entropy were calculated using the following equations.

$$\Delta G° = -RT \ln K \quad \text{(s1)}$$

and

$$\Delta G° = \Delta H° - T\Delta S° \quad \text{(s2)}$$

where *R* is the universal gas constant (1.9872041 cal mol^{-1} K^{-1}) and *T* is the absolute temperature in Kelvin.

Zeta Potential

The study of charge distribution of the protein (Mb) with and without the drugs - SMZ/SDZ was analyzed on a Horiba Particle Zetasizer SZ-100 instrument. To determine the charge on the surface of particles, the sample was injected into the cell and the measurement of the particle electrophoretic mobility resulted in the calculated zeta potential. The measurements were repeated at least five times to get average size and value.

Computational Details

Density Functional Theory (DFT) Study – The electronic structures and transitions of antibiotic drugs (SMZ/SDZ) and geometry optimization were performed using DFT calculations. GAUSSIAN 09W program package was used for the calculations utilizing the hybrid method B3LYP function and keeping the basis sets as 6-31G (d, p). The electronic properties - highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energies were calculated using B3LYP method of the time-dependent DFT (TD-DFT).

Molecular Docking – Docking analyses for the interaction of myoglobin with antibiotic drugs, SMZ and SDZ were performed using Swissdock online server (http://www.swissdock.ch/docking) & Autodock 1.5.6 software. The structure of myoglobin was taken from RCSB Protein Data Bank having PDB ID: 1A6N. Receptor and ligands were prepared in UCSF Chimera and Open Babel software; followed by energy minimization of the ligands using Gaussian Version. SwissDock online server was used to obtain the blind docking

clusters for each ligand on the surface of the Mb receptor. Next, top scoring complexes obtained were prepared for Autodock docking protocol. The autodock software utilizes Lamarckian Genetic Algorithm (LGA). In order to prepare protein and the ligands for docking, water molecules were removed and polar hydrogen atoms, Gasteiger charges were added. The grid box was set at 110 Å, 110 Å and 110 Å along the X-, Y- and Z-axis with a grid spacing of 0.422 Å. The resultant minimum energy docked model was chosen for further analysis and viewed in PyMOL, Ligplot and chimera for better visualization.

FIGURES

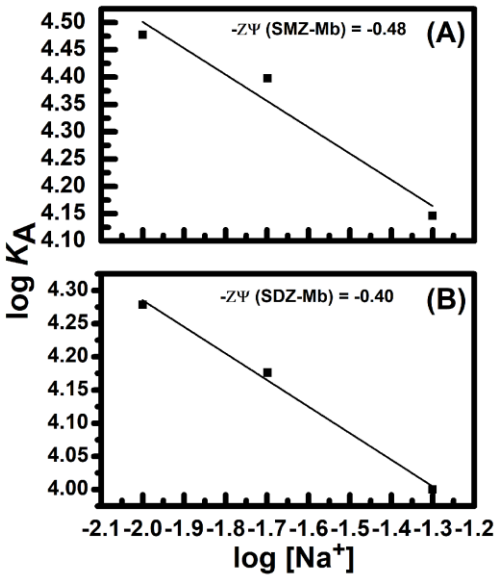

Figure S1. Plot of log K_a *versus* log [Na^+] of (A) SMZ-Mb complex and (B) SDZ-Mb complex.

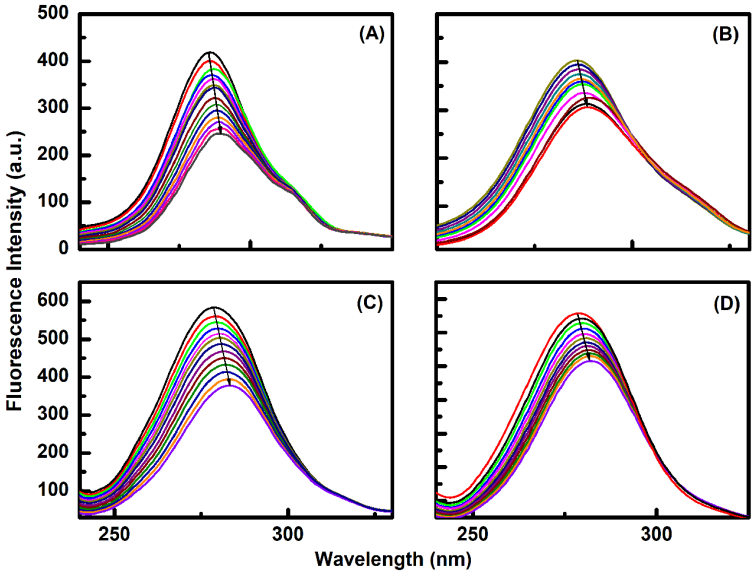

Figure S2. Synchronous fluorescence spectra of Mb in the presence of different concentrations of SMZ and SDZ. Panel (A) and (B) represents SMZ-Mb and SDZ-Mb at $\Delta\lambda$= 15, Panel (C) and (D) represents SMZ-Mb and SDZ-Mb at $\Delta\lambda$= 60.

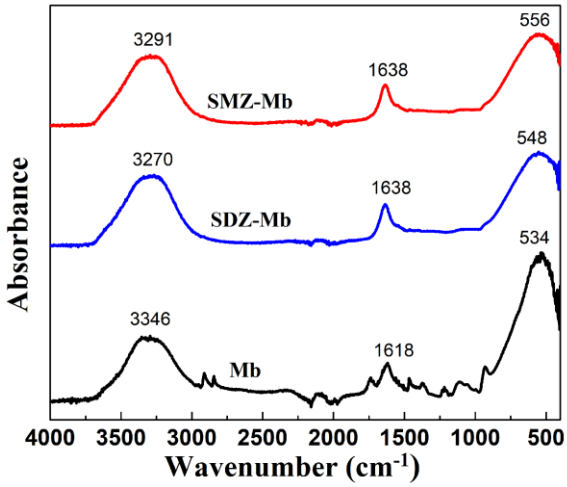

Figure S3. FT-IR spectra of Mb in presence and absence of the drug (SMZ/SDZ).

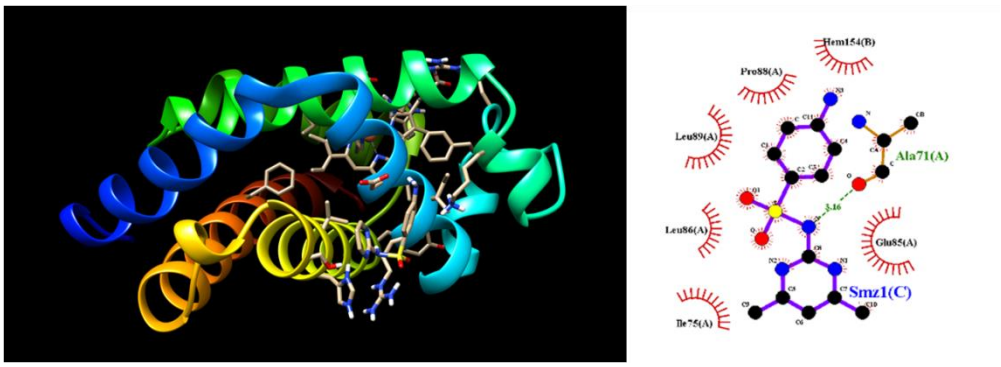

Figure S4. Docking position and interaction of Ist top scoring of SMZ with ligplot analysis.

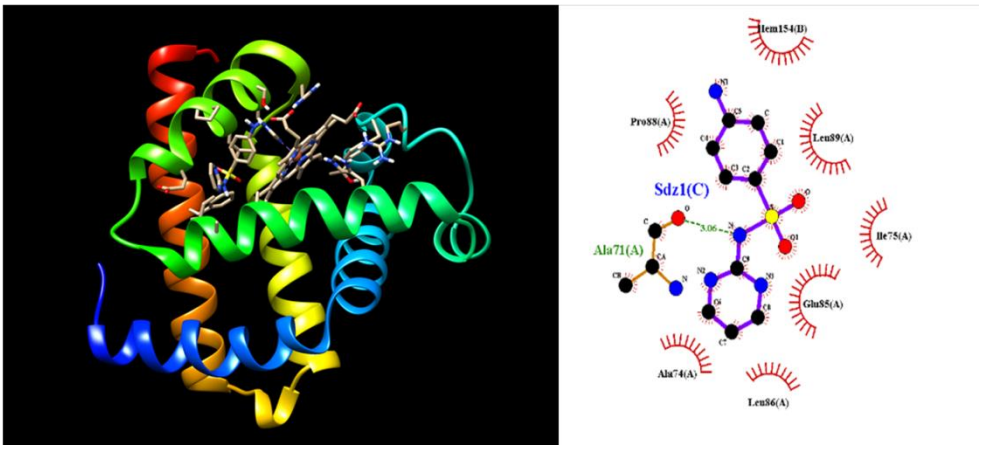

Figure S5. Docking position and interaction of Ist top scoring of SDZ with ligplot analysis.

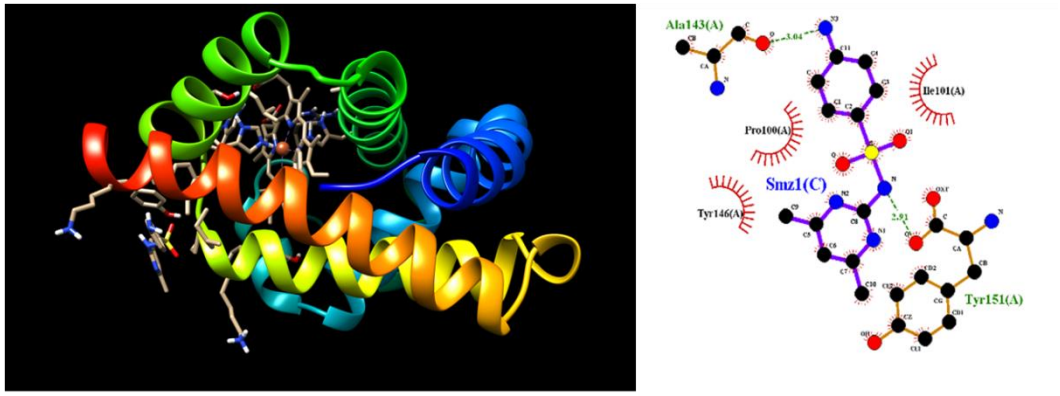

Figure S6. Docking position and interaction of 2nd top scoring of SMZ with ligplot analysis.

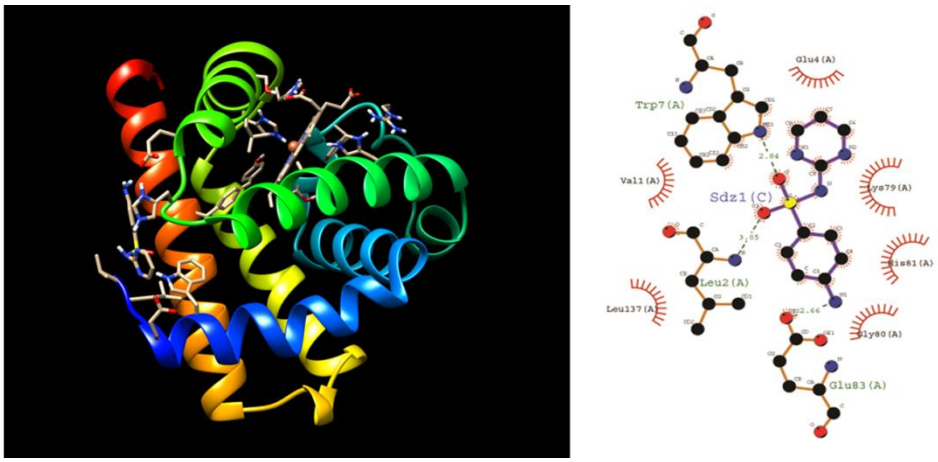

Figure S7. Docking position and interaction of 2nd top scoring of SDZ with ligplot analysis.

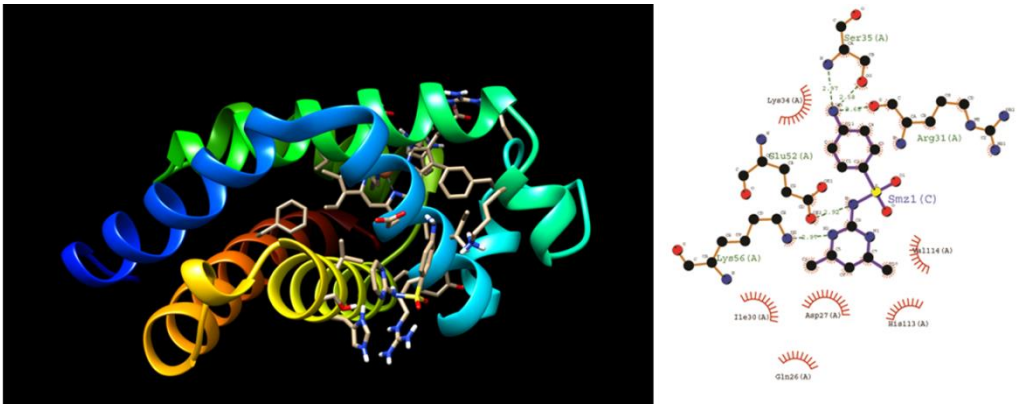

Figure S8. Docking position and interaction of 3rd top scoring of SMZ with ligplot analysis.

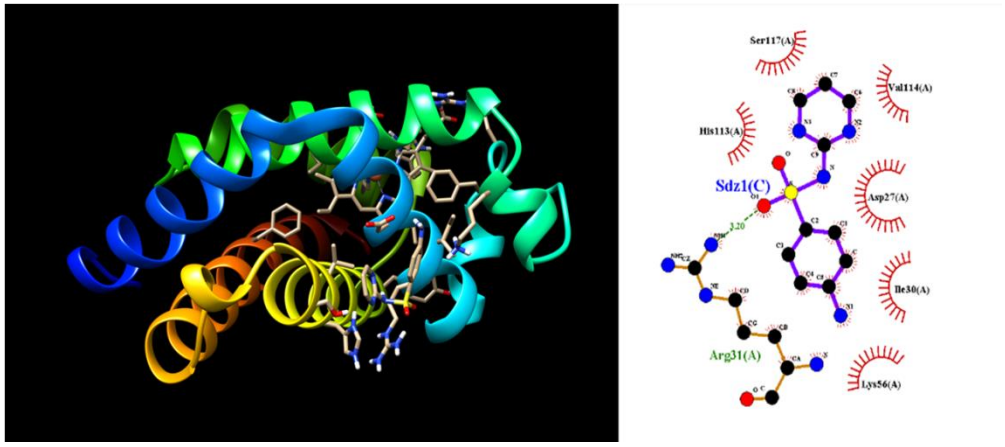

Figure S9. Docking position and interaction of 3rd top scoring of SDZ with liplot analysis.

Table S1. AUTODOCK RESULTS ON SMZ-MB AND SDZ-MB COMPLEXES

Complex	Top Sites	Estimated Free Energy of Binding (kcal/mol)	Estimated Inhibition Constant, Ki (uM)	Final Intermolecular Energy (kcal/mol)	Final Total Internal Energy (kcal/mol)	Torsional Free Energy (kcal/mol)	Unbound System's Energy (kcal/mol)
SMZ-Mb	1st	-7.44	3.53	-8.63	-1.39	+1.19	-1.39
	2nd	-7.48	3.32	-8.67	-0.66	+1.19	-0.66
	3rd	-7.42	3.62	-8.62	-0.75	+1.19	-0.75
SDZ-Mb	1st	-6.99	7.57	-8.18	-0.88	+1.19	-0.88
	2nd	-7.27	4.67	-8.47	-0.79	+1.19	-0.79
	3rd	-6.77	10.84	-7.97	-1.08	+1.19	-1.08