Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Bioactive Supra Decorated Thiazolidine-4-carboxylic acid derivative attenuates cellular oxidative stress by enhancing catalase activity.

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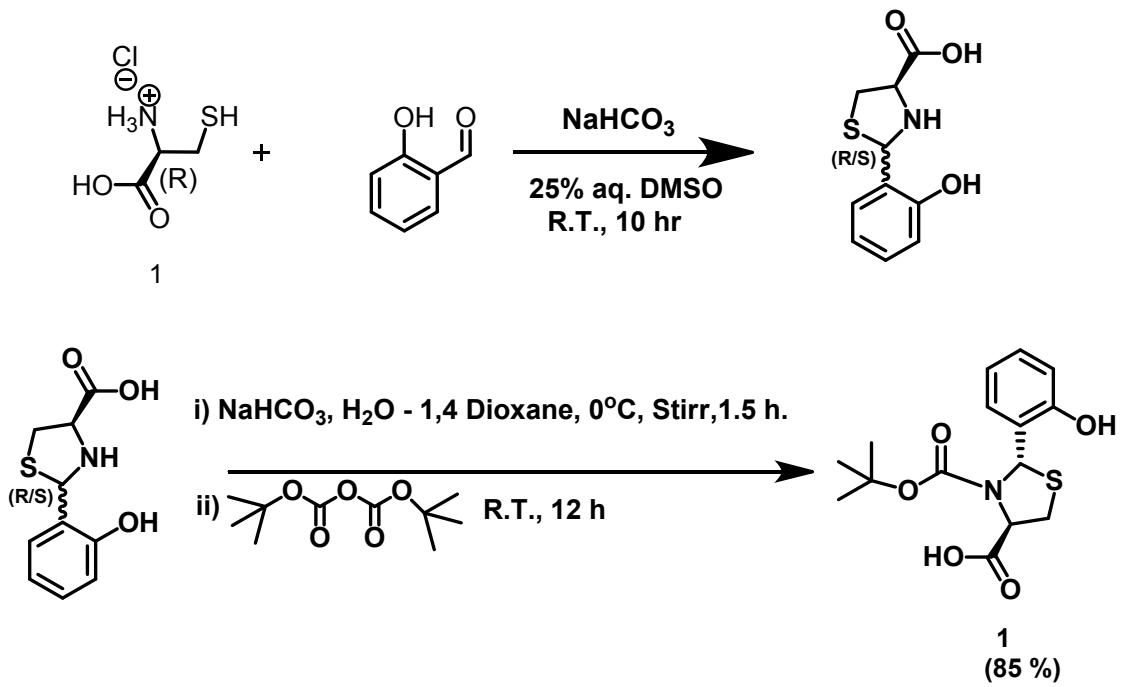
Supporting information

Synthesis of TZT {(2S,4R)-3-(tert-butoxycarbonyl)-2-(2-hydroxyphenyl) thiazolidine-4 carboxylic acid

The target synthesis of compounds **TZT** was carried out under ambient conditions. The melting points were measured in glass capillaries. The infrared spectrum (KBr disc) was obtained using a Perkin–Elmer Spectrum One FT-IR spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded over a Bruker 500 MHz spectrometer (with Me_4Si internal standard). The ESI-MS studies were carried out on Bruker maXis impact mass spectrometer. Solvents were purified according to standard procedures prior to use, commercially available starting materials were used as procured.

Synthetic procedure: The compound **(TZT)** was synthesized as per optimized procedure of our earlier work (1). At room L-Cysteine hydrochloride (0.175 g, 1.0 mmol) and $NaHCO_3$ (0.084 g, 1.0 mmol) were stirred in 40 ml of 25% aq. DMSO to a homogenous solution. Salicylaldehyde (0.122 g , 0.95mmol) was added to this clear solution and the resultant mixture was again stirred for 10h at room temperature. On completion of reaction as evidenced from TLC, reaction mixture was acidified with cold 2M HCl and extracted in ethyl acetate. The organic extract was washed with cold saturated $NaHCO_3$ and further extracted in DCM solvent. On evaporation of the organic layer a white compound was obtained which on purification over silica column was found to be a mixture of (2R/2S,4R)-2-(2-hydroxyphenyl) thiazolidine-4-carboxylic acid. In the second step compound a (0.225 g, 1.0 mmol) and $NaHCO_3$ (0.21 g, 2.5 mmol) were taken in a two neck round bottom flask(100 ml) and stirred in 25 ml 50% aqueous 1,4-dioxane at 0°C for 1.5 h. To this solution di-ter-butyloxycarbonyl (0.305 g, 1.4 mmol) was added in a drop wise manner, and the mixture was magnetically stirred at room temperature for 12 h. The reaction mixture was acidified by cold 2M HCl (up to pH 4.0) and extracted in ethyl acetate. The extract was washed with cold saturated $NaHCO_3$ and further extracted in DCM. The organic phase on solvent evaporation gave an oily solid which was purified over silica column using 40% ethyl acetate in hexane as eluent, yielding two diastereomer's. The major 85% was *2S,4R* diastereomer (TZT**)** and minor 15% as *2R,4R* diastereomer. The scheme 1 summarizes the two step synthesis of compound TZT**.** The TZT compound showed all major/ diagnostic spectroscopic responses {$C_{15}H_{19}N_1O_5S_1$} white solid (85%),: IR :3097 (ν O—H), 1627 (ν C=O), 831 (ν C-S), 1097, 1093, 761 (aromatic C-H bending) cm^{-1}, ^{1}H-NMR (DMSO-*d*6): *δ* 10.3 (s, 1H,broad, carboxylic acid proton), *δ* 7.83 (d, 1H, *J* = 8Hz, aromatic proton), *δ* 7.17 (t, 1H, *J* = 8Hz, aromatic proton), *δ* 6.83 (m, 2H,*J* = 8Hz, aromatic protons), *δ* 5.93 (s, 1H, H-2), *δ* 4.86 (q, 1H, *J* = 7.2Hz, H-4), *δ*, 3.43 (dd,1H, *J* = 7.2, 4.5Hz, H-5), *δ* 2.73 (dd, 1H, *J* = 7.2, 4.5Hz, H-5), *δ* 1.38 (s, 6H, $HCH_3C(CH_3)_2$), *δ* 1.25 (s, 3H, H $CH_3C(CH_3)_2$). ^{13}C NMR (DMSO-*d*6): 172.69 (C=O), 172.24 (C=O), 154.60, 130.29, 127.50, 120.47, 119.90, 115.13(C Ar), 83.35 (C $C(CH_3)_3$), 60.89 (C-2), 49.43 (C-4), (C-5, merged in DMSO-*d*6 signals), 28.10 (C $C(CH_3)_3$). MS: 326.00 (M+1).



Scheme 1: Synthesis of (2S,4R)-3-(tert-butoxycarbonyl)-2-(2-hydroxyphenyl) thiazolidine-4-carboxylic acid (TZT).

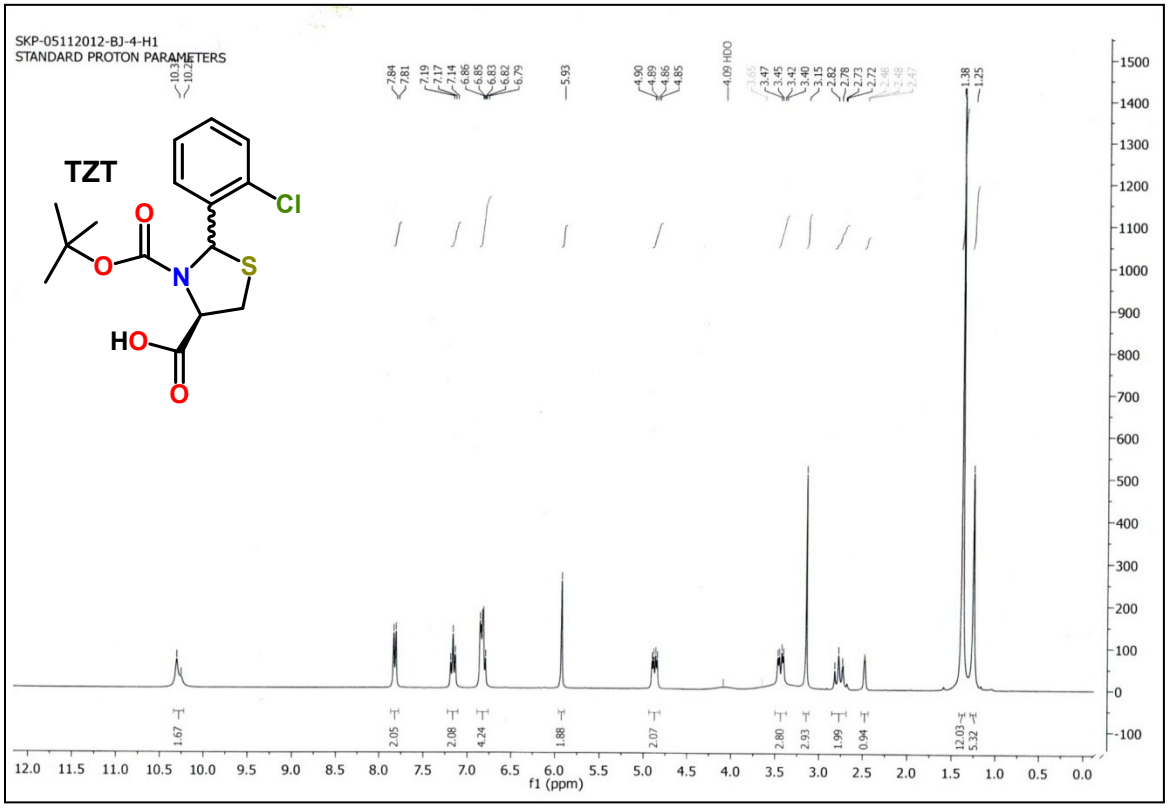


Figure S1: *^{1}H NMR of the TZT Compound*

^{13}C NMR of the TZT Compound

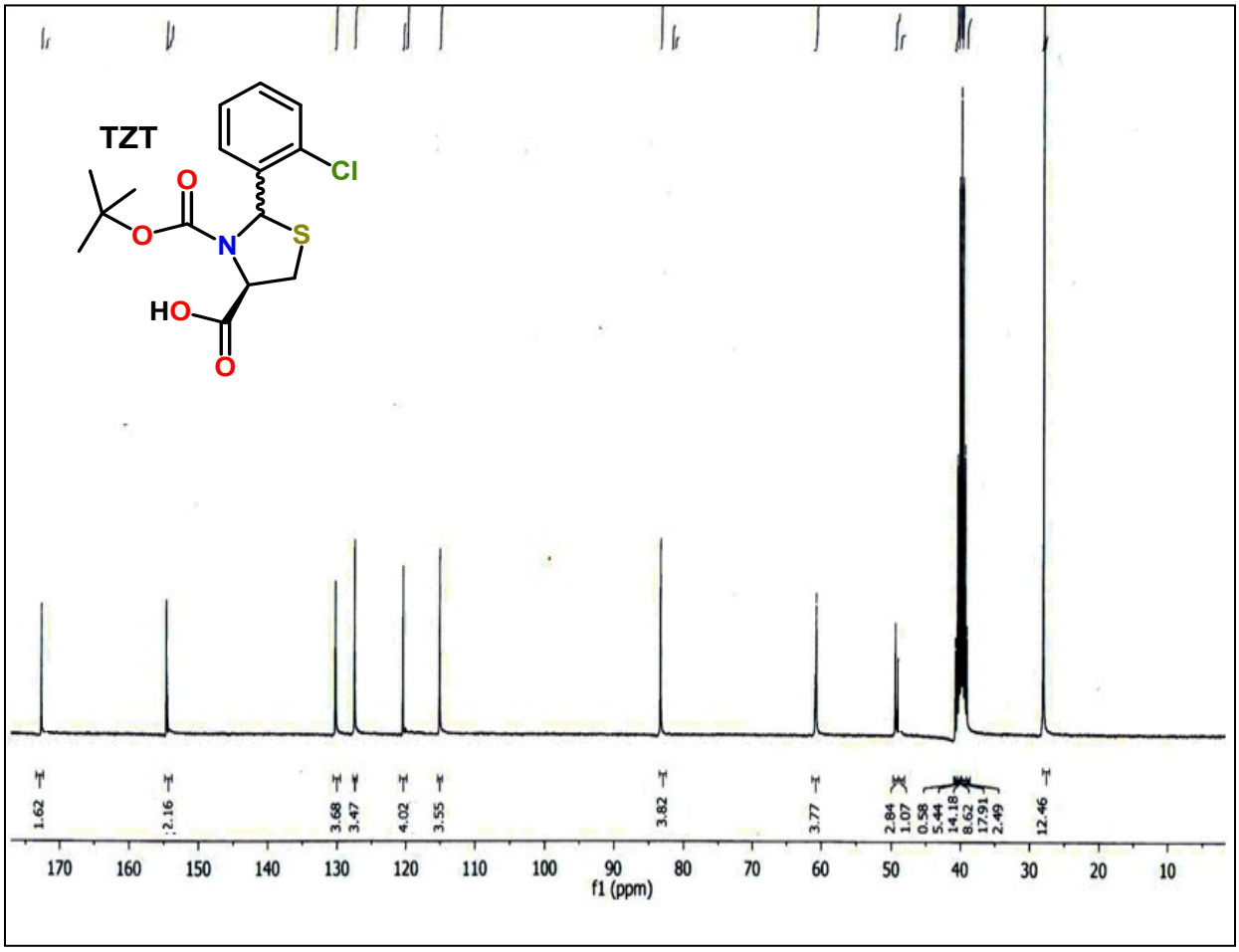


Figure S2: ***^{13}C NMR of the TZT Compound***

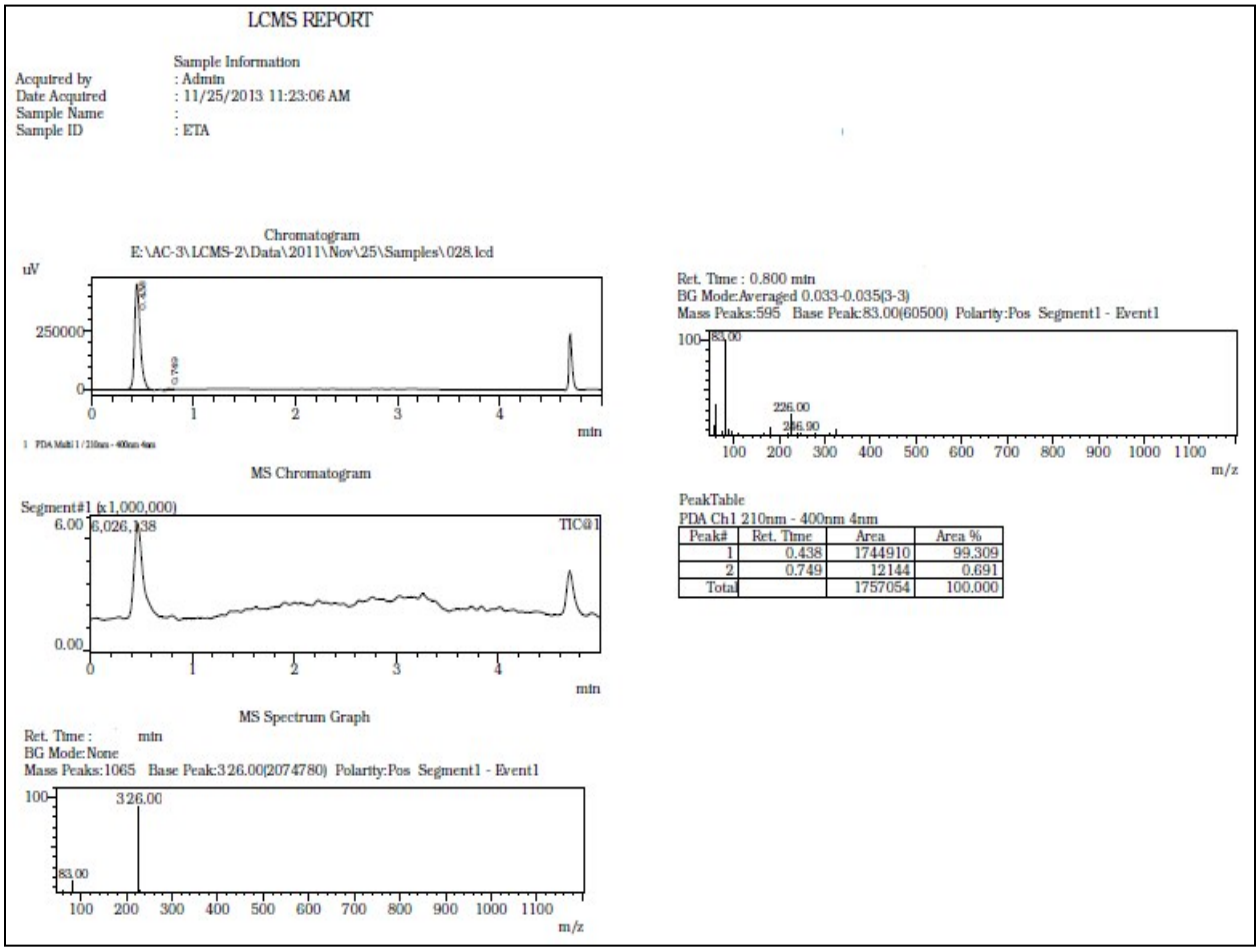


Figure S3: LCMS *of the TZT Compound*

Stern Volmer Plots of BSA- TZT and BLC- TZT interaction.

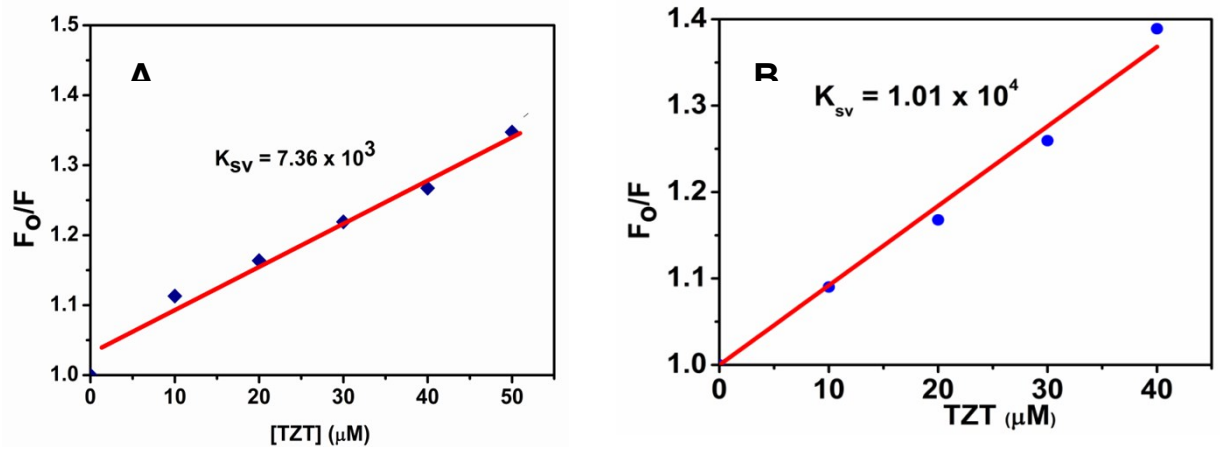


Figure S4. **The Stern Volmer plots for A: BSA –TZT and B: BLC-TZT quenching at 298 K**.
The calculated Stern Volmer quenching constantfor BLC-TZT quenching interaction was higher than that of TZT-BSA.

Equations used for calculations.

Michaelis–Menten model of enzyme kinetics

$$\frac{1}{V0} = \frac{Km}{Vmax} \times \frac{1}{[S]} + \frac{1}{Vmax}$$

…….. S1

Where K_m is the Michaelis constant, V_{max} the maximum velocity, V_0 is the initial velocity of the enzyme. [S] is substrate concentration.

Wolfee-Shimmer equation for quantifying binding propensity of compound TZT (absorption data):

$$\frac{A^0}{A - A^0} = \frac{\varepsilon_{TZT}}{\varepsilon_{TZT-Protein}} + \frac{\varepsilon_{TZT}}{\varepsilon_{TZT-Protein}\, x\, Kb} x \frac{1}{[TZT]}$$

…….S2

Where, A° and A are absorbance of pure TZTand TZT–protein complex respectively at 278 nm. $\boldsymbol{\varepsilon}_{TZT}$ and $\boldsymbol{\varepsilon}_{TZT\ -protein}$ are the molar extinction coefficient of TZT alone and TZT –protein complex, respectively.

Stern Volmer equation for Steady state fluorescence measurements

$$\frac{F^o}{F} = 1 + K_{SV}[Q] = 1 + k_q \tau^o [Q] \quad \text{.....S3}$$

Where F^o and F are the fluorescence intensities of proteins in the absence and presence of quencher, K_{SV} is the Stern Volmer constant, [Q] is the quencher concentration, kq is the quenching rate constant and τ_0 is the fluorescence lifetime in the absence of quencher which is 10^{-8} seconds.

Scatchard equation for the binding constant (Ka) and number of binding sites (n) of TZT binding

$$\log \frac{[F^0 - F]}{[F^0]} = \log K + n \log [Q] \quad \text{.....S4}$$

Where Fo and F are the fluorescence intensities of protein in absence and presence of TZT quencher [Q], K and n are the binding constant and the number of binding sites, respectively.

Van't Hoff's equation for determination of enthalpy change (ΔH) of TZT binding.

$$ln\left[\frac{K_2}{K_1}\right] = \left(\frac{1}{T_a} - \frac{1}{T_b}\right)\frac{\Delta H}{R}$$

.....S5

Where K_2 and K_1 are Stern Volmer constants at temperatures "a" and "b" respectively. ΔH is the enthalpy change of TZT binding with proteins (BSA and BLC) and "R" is molar as constant.

$$\Delta G = -nRTlnK = \Delta H - T\Delta S$$

.....S6

$$\Delta S_{total} = \Delta S_{conf} + \Delta S_{solv.} + \Delta S_{r/t}$$

.....S7

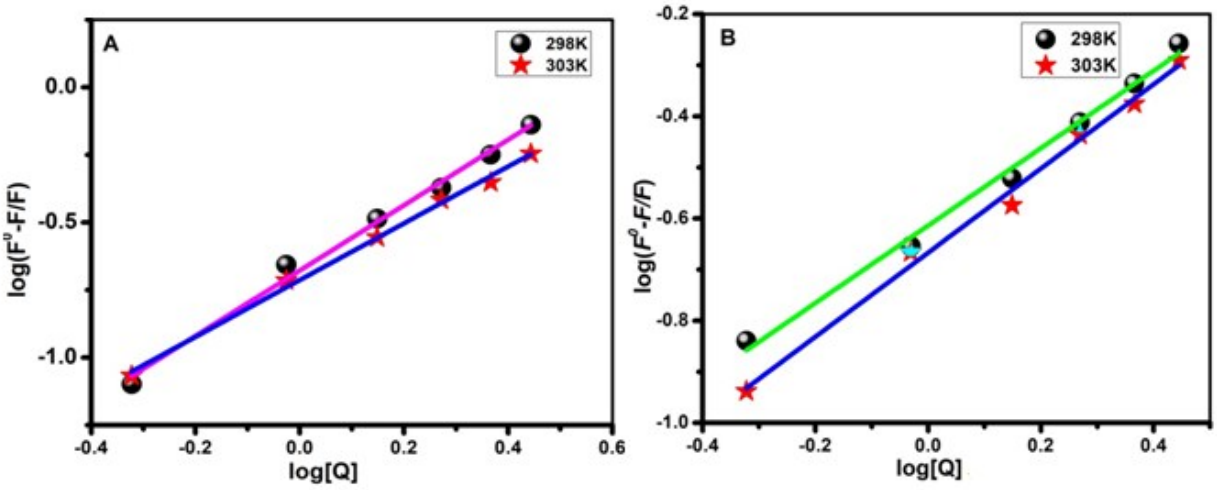


Figure S5: Scatchard plots for BSA-TZT (A) and BLC-TZT (B) used to calculate the binding numbers and binding constants of TZT with target proteins.

Molecular Docking investigation of TZT protein interaction:

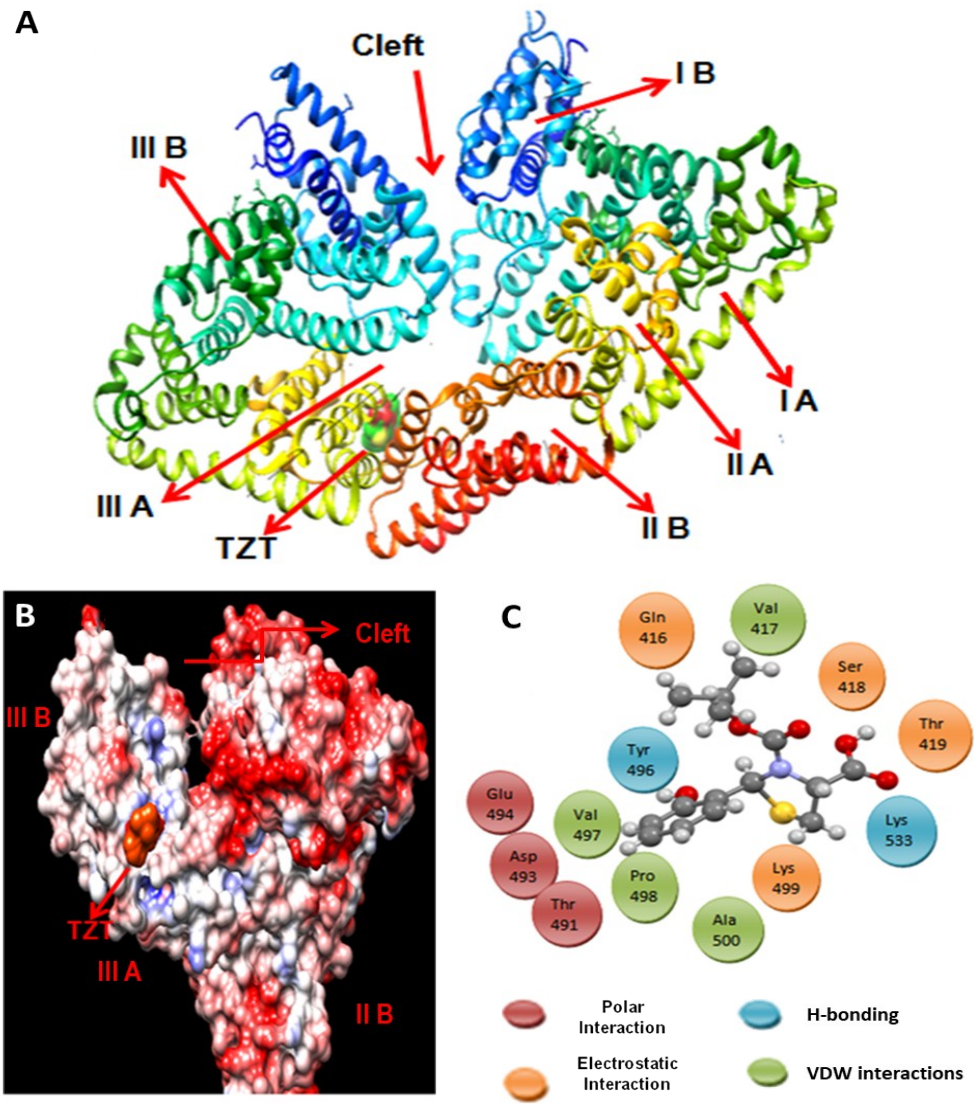


Figure S6: **Molecular docking of TZT interaction with BSA.** Ribbon representation of optimum docked binding site of TZT with BSA (A). Coulombic surface model of binding site (B). Schematic representation of TZT non-covalent interactions with amino acid residues from BSA in its binding pocket.

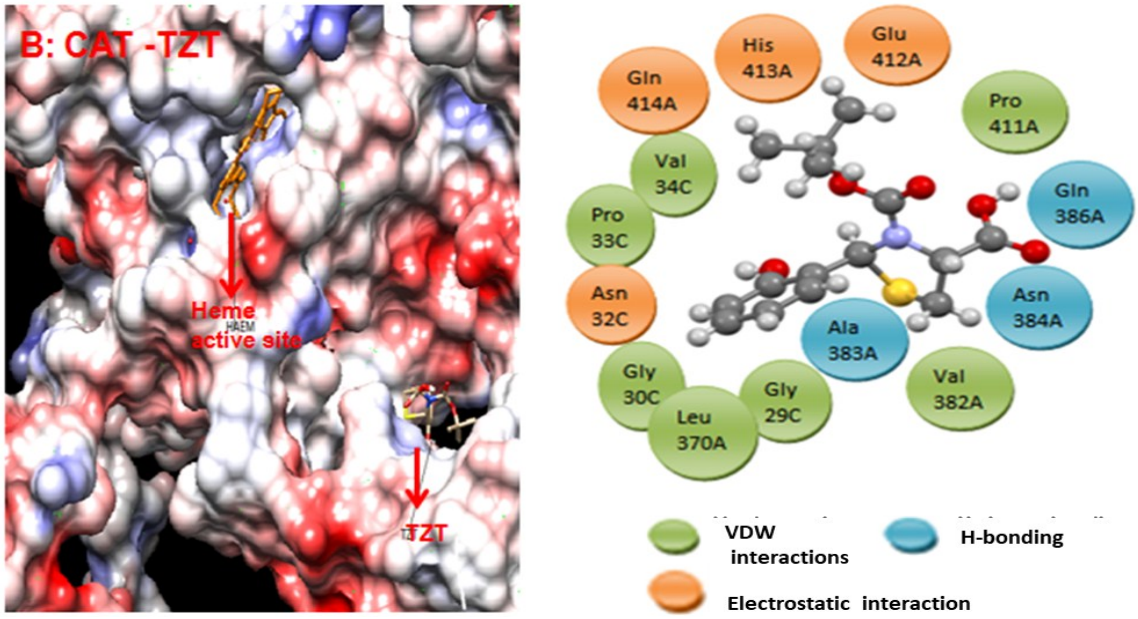


Figure S7: **Molecular docking of TZT interaction with BLC.** Coulombic surface model of binding site and schematic representation of TZT non-covalent interactions with amino acid residues from BLC in its binding pocket.

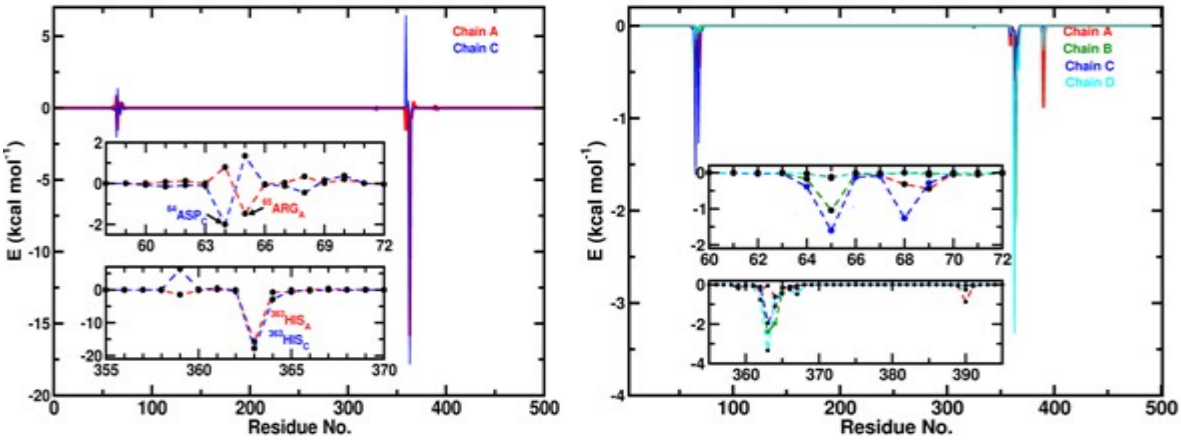


Figure S8: Residue-wise electrostatic interaction energy of chain A and chain C of BLC with TZT molecule (A). Residue-wise VDW interaction energy of all chains of BLC with TZT molecule (B).

Table S-1. Average electrostatic and VDW interaction energy between different chains of BLC and TZT. Standard deviation is shown in brackets.

Chain type	Electrostatic energy (Kcal.mol^{-1})	VDW energy (Kcal.mol^{-1})
Chain A	-18.48 (2.81)	-3.46 (1.87)
Chain B	-2.75 (2.32)	-6.32 (1.01)
Chain C	-15.26 (2.8)	-7.51 (2.1)
Chain D	-3.45 (1.97)	-6.54 (1.57)

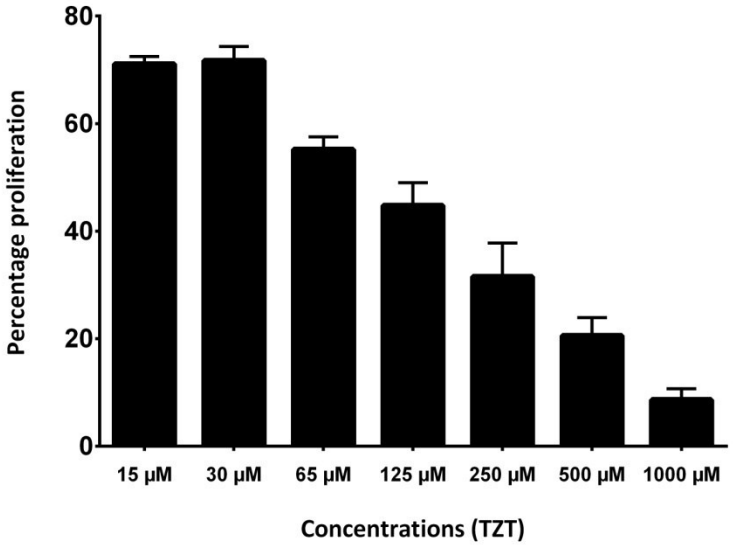


Figure S9 Effect of TZT on the proliferation of A549 cells. A549 cells were treated with various concentrations (15 µM –1000 µM) of TZT for 24 h. After 24 h, rate of cell proliferation was determined by MTT assay. Data represented as mean ± SD of results from three independent experiments.

References:

1.R. M. Jagtap, M. A. Rizvi, Y. B. Dangat and S. K. Pardeshi, Crystal structure, computational studies, and stereoselectivity in the synthesis of 2-aryl-thiazolidine-4-carboxylic acids via in situ imine intermediate, *Journal of Sulfur Chemistry*, 2016, **37**, 401–425.

2.Y. Bai , J. Du, X. Weng, Synthesis, characterization, optical properties and theoretical calculations of 6-fluoro coumarin.*Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2014, **126**, 14–20.

3 A. Solankee, S. Lad, G. Patel, S. Solankee, Synthesis and Antibacterial Activity of Chalcones, Aminopyrimidines and Pyrazolines. *Orient J Chem,* 2009, **1**, 25