Synthesis and molecular interaction of a novel diphenolic hidrazinyl-thiazole compound with strong antioxidant and antiradical activity with HSA

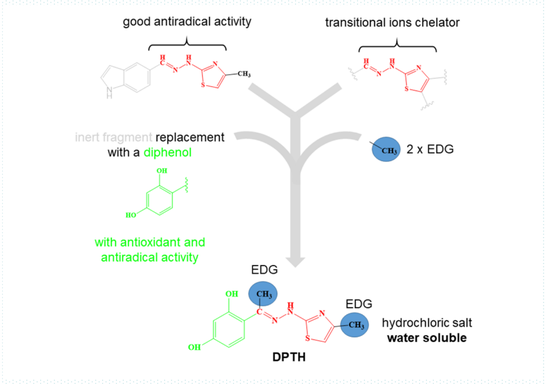
M Mic[1], A Pîrnău[1], C G Floare[1], G Marc[2], A H Franchini[2], O Oniga[2], L Vlase[3], M Bogdan[1]

[1]National Institute for Research and Development of Isotopic and Molecular Technologies, 67-103 Donat, 400293 Cluj-Napoca, Romania

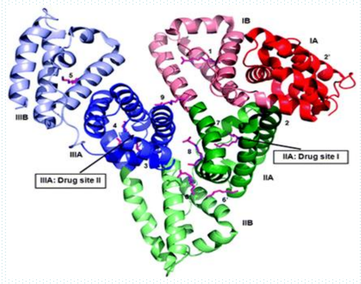
[2]Department of Pharmaceutical Chemistry "Iuliu Haţieganu" University of Medicine and Pharmacy, 41 Victor Babeş, 400012 Cluj-Napoca, Romania

[3]Department of Pharmaceutical Technology and Biopharmaceutics, "Iuliu Haţieganu" University of Medicine and Pharmacy, 41 Victor Babeş, 400012 Cluj-Napoca, Romania

Abstract. In this study we designed, synthesized and analyzed a water-soluble hybrid molecule presenting a good antioxidant and antiradical activity, namely Dihydroxy-Phenyl-Thylthiazol-Hydrazinium chloride (DPTH) which contain 2',4'-dihydroxyaceto¬phenone and the 2-hydrazinyl-4-methyl-thiazole linked through a Schiff base. This molecule presented a very good antiradical scavenging and antioxidant activity and half of the EDTA Fe(II) chelation activity in the in vitro evaluations. The main goal of this study is to quantitatively evaluate the interaction between DPTH and HSA to characterize the nature and forces underlying the formation of a molecular complex. To fulfil this goal, we analysed their interaction using ITC, NMR and molecular docking.

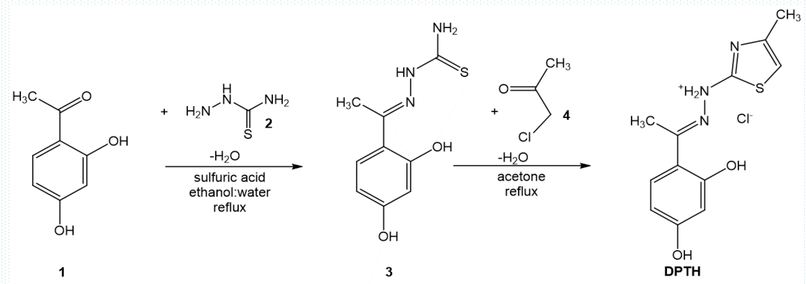


Conceptualization of designing of **DPTH**



Human serum albumin (HSA)

Host – guest vehicle



The synthetic route followed in order to obtain **DPTH**

Calorimetric study of the thermodynamics of DPTH binding

ITC measurements:

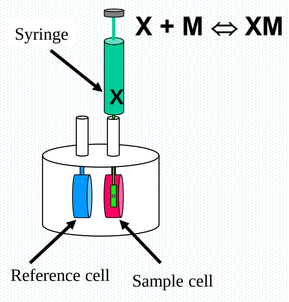
- NanoITC^{2G} nanocalorimeter
- AGP concentration 300μM
- IMT concentration 2mM
- Volum/injection: 10μL
- Injection: 300s

In a single ITC experiment you get…

- Affinity (K) – strength of binding
- Heat of binding (ΔH) and entropy (ΔS) – mechanism and driving force of interaction
- Stoichiometry (n) - Number of binding sites

The value of the free energy change (ΔG) was subsequently calculated with the following equation:

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



The equilibrium in guest–host reaction is described by the following hypothetical scheme:

$$H + nG \leftrightarrow HG_n \equiv C$$

$$K = \frac{[C]}{[H][G]^n}$$

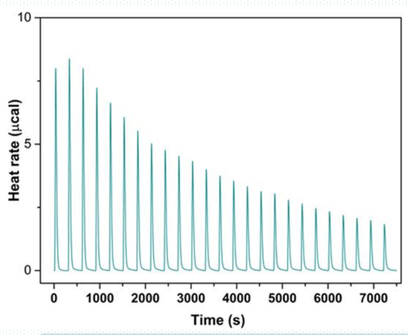
where [H], [G], and [C] stand for the concentrations of β-CD, 1MPTMPC and complex respectively.

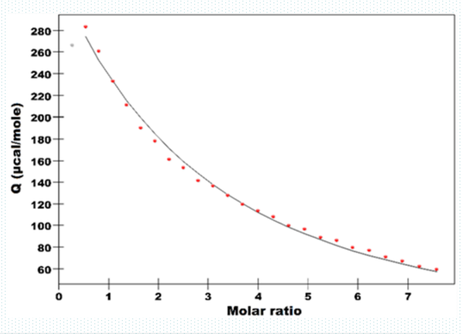
In an ITC experiment the reaction heat per mole of injected molecules after the ith injection is given by:

$$\frac{q_i}{vG_0} = \frac{\Delta H}{2}\left[1 + \frac{1 - r_i/n - (r_0 + r_i)/nKG_0}{\sqrt{(1 + r_i/n + (r_0 + r_i)/nKG_0)^2 - 4r_i/n}}\right]$$

where $r_0 = \frac{G_0}{H_0}$; $r_i = \frac{G_i}{H_i} = r_0\frac{iv}{V}$, H_0 and G_0 are the initial concentrations of β-CD and 1MPTMPC of respectively, H is the reaction enthalpy, V is the volume of reaction cell and v is the small volume injected at each titration step. The software supplied with the calorimeter was used to fit thermodynamic parameters K and ΔH to the heat profiles.

Calorimetric titration of HSA with DPTH





K_a = 4.31x10^2 M^{-1}

n = 1

ΔG = - 3.51 kcal/mol

ΔH = 21.12 kcal/mol

TΔS = 24.7 kcal/molK

Calorimetric titration of HSA with DPTH. Raw data obtained from 25 injections of 10μl aliquots of 10 mM DPTH into 400 μM HSA at 25 ºC . The incremental heat/mol of added ligand as a function of molar [DPTH]/[HSA] ratio of reaction partners. The solid line is obtained by fitting the experimental data by independent model.

^{1}H NMR spin-lattice selective relaxation

The selective spin lattice relaxation rates of DPTH was measured as a function of its concentration, keeping the HSA concentration constant at 0.1 mM. The dissociation constant, K_d for DPTH/HSA complex and the number of the binding sites, n, are evaluated by the application of Langmuir isotherm. The relaxation times were fitted by means of an exponential regression analysis of the longitudinal magnetization components via the standard equation:

$$A(t) = A(0)\{1 - 2\exp(-\tau/T_1)\}$$

$$\frac{R_{1obs} - R_{1\,free}}{R_{1b} - R_{1\,free}} = \frac{C_L + nC_p + K_d}{2C_L} - \sqrt{\left(\frac{C_L + nC_p + K_d}{2C_L}\right)^2 - \frac{nC_p}{C_L}}$$

$T_1 = 1/R_1$

$\Delta R = R_{1obs} - R_{1\,free} = y$

C_L = X = [DPTH] = variable

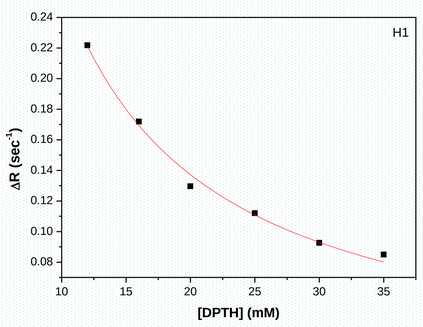
C_P = [HSA] = 0.1 mM

K_a = 9.65x10^2 M^{-1}

K_d = 1.035 mM

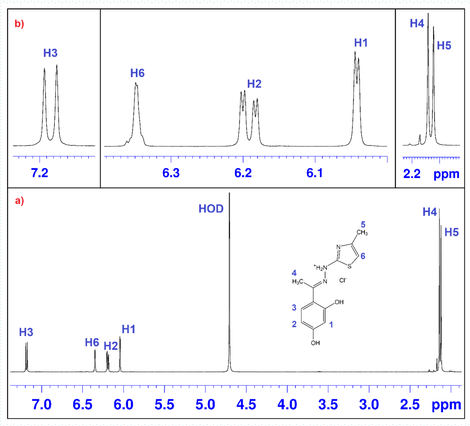
n = 1

R_{1bound} = 29.sec^{-1}

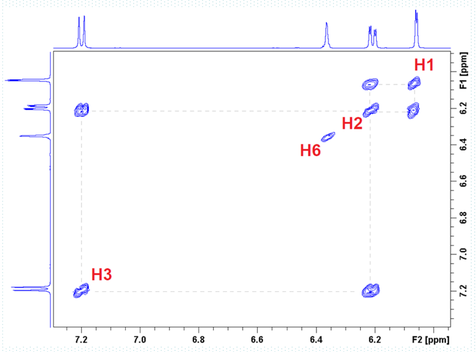


The binding affinity of the DPTH determined by NMR method is weaker than the previous method. This can be due to the fact that the NMR method is susceptible to interference from additional non-specific bindings.

1D and 2D NMR spectroscopy



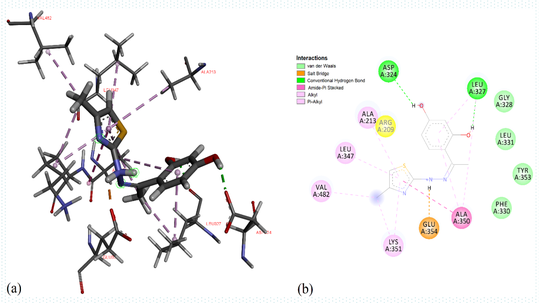
^{1}H NMR for DPTH in D_2O: a) total spectrum and proton numbering; b) inset - details zoom.



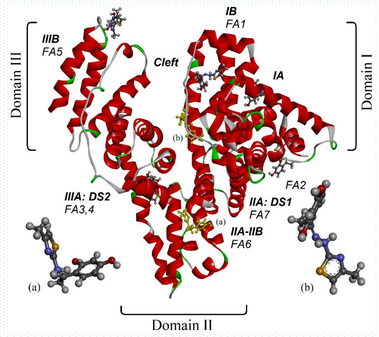
^{1}H-^{1}H COSY NMR spectrum of DPTH in D_2O, details zoom to aromatic zone.

Molecular docking

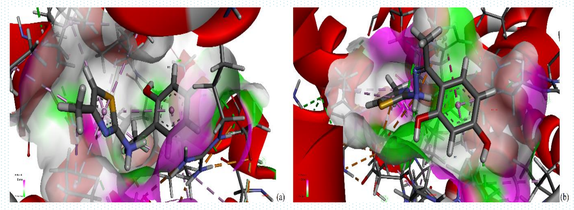
Computational molecular investigation aiming to obtain the optimal conformation of DPTH in interaction with human serum albumin (HSA) was performed using the Monte Carlo simulated annealing search implemented in Autodock v4.2. The initial 3-dimensional molecular structure of DPTH was optimized using M06-2X functional and 6-311++G(d,p) basis set using Gaussian 09 software and no imaginary frequencies were obtained. This structure is presented in Figure 3. The crystal structure of the albumin was downloaded from Protein Data Bank, PDB ID: 1AO6



Close interactions of DPTH in the FA6 binding pocket (a) and the 2D interaction map (b).



Binding conformations of DPTH to the albumin as resulted from molecular docking simulations. Conformations of the first (a) and second (b) highest binding energies are highlighted. Albumin structure is presented with detailed domains and subdomains (IA-IIIB) specification, and we mentioned also its seven fatty-acids binding sites (FA1-FA7) and Sudlow's drug sites (DS1, DS2).



DPTH in the FA6 (a) and FA1 (b) binding pockets of the albumin including the receptor hydrogen-bond surface map, with a view to the closer interactions of the H1 proton.

Acknowledgements.

This research was financially supported by the Core Program PN 19 35 02 01 and by "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania through PCD 1529/39/18.01.2019 .