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Investigation of the interaction between isopropyl derivative of thiosalicylic acid and human serum albumin

Chaired by Dr. Alfredo Berzal-Herranz and Prof. Dr. Maria Emília Sousa

pharmaceuticals MDPI

Nikola Nedeljković [1], Ratomir Jelić [1], Emina Mrkalić [2], Gordana Radić [1], Zoran Ratković [3], Andriana Bukonjić [1], and Miloš Nikolić [1,*]

[1] University of Kragujevac, Faculty of Medical Sciences, Department of Pharmacy, Svetozara Markovića 69, Kragujevac 34000, Serbia;
[2] University of Kragujevac, Institute for Information Technologies, Department of Science, Jovana Cvijića bb, Kragujevac 34000, Serbia;
[3] University of Kragujevac, Faculty of Science, Department of Chemistry, Radoja Domanovića 12, Kragujevac 34000, Serbia.

* Corresponding author: milos.nikolic@medf.kg.ac.rs

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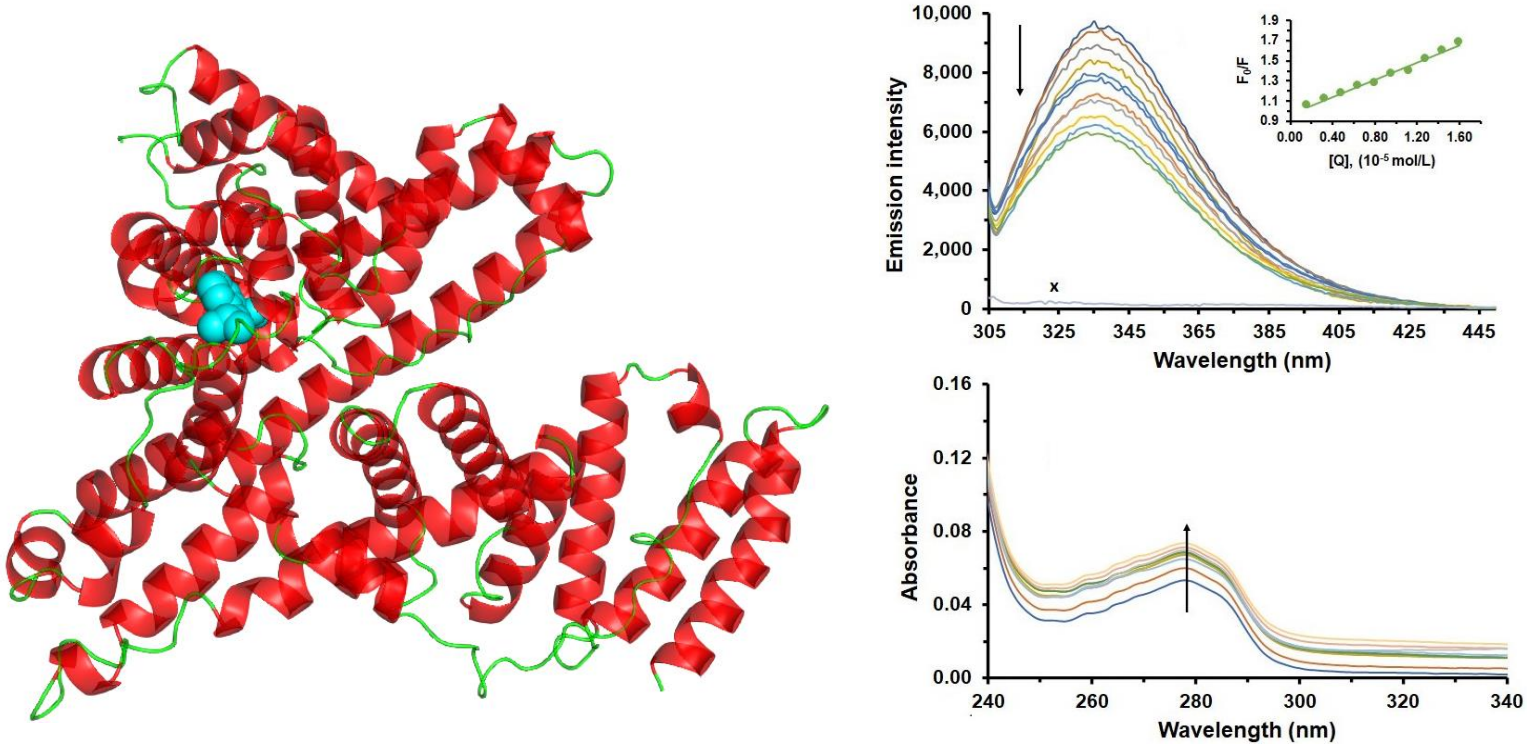
Graphical Abstract



Abstract:

According to research, thiosalicylic acid has anti-inflammatory, antioxidant, and analgesic effects. Additionally, some of its derivatives have shown significant antimicrobial and antitumor activity. *In vitro* studies have also shown that S-alkyl derivatives of thiosalicylic acid exhibit moderate and dose-dependent cytotoxic effects on human colon and lung carcinoma cells. In this research, we utilized various spectroscopic methods and molecular docking simulation to examine the binding interaction between human serum albumin (HSA) and potential biologically active isopropyl derivatives of thiosalicylic acid (ligand, L). To analyze the quenching mechanism, the association constants and number of binding sites were utilized based on the obtained results. The tested L and HSA had a static fluorescence quenching mechanism, while their binding processes were spontaneous. Additionally, UV-Vis absorption spectroscopy revealed that the binding of the tested L induced slight conformational changes in HSA.

Keywords: Human serum albumin; Thiosalicylic acid; Spectroscopic measurements; Docking simulations

Introduction

Thiosalicylic acid (TSAH2) is a fascinating heterodifunctional ligand. Combining both hard and soft donor atoms, it offers a wide array of bonding opportunities with various metal centers. Thiosalicylic acid and its derivatives find applications in several areas, including:

- Metal determination [1, 2],
- Modification of graphite paste electrodes [3],
- Use as photoinitiators for free radical polymerization [4],
- Incorporation into cosmetic products [5],
- Utilization in the treatment of inflammatory, allergic, and respiratory diseases [6],
- Potential as inhibitors of Ras-driven tumor growth [7]

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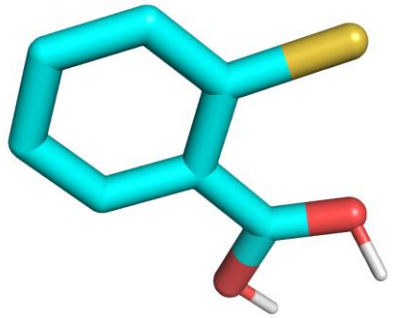

Figure 1. Crystal structure of thiosalicylic acid visualized in Pymol

Introduction

The antitumor effects of acetylsalicylic acid have been documented, with some of its efficacy attributed to the induction of apoptosis through the regulation of the PTEN (phosphatase and tensin homolog)/AKT (serine/threonine kinase Akt)/NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells)/survivin signaling pathway [8]. Additionally, extensive research has explored the capacity of thiosalicylic acid derivatives to form complexes with various metal ions, exhibiting diverse geometries [9,10]. Recently, it was shown that S-isopropyl derivative of thiosalicylic acid and copper(II) complex with this ligand moderately reduce the viability of murine lung cancer cells [11].

In accordance with previously established cytotoxic effect of isopropyl derivative of thiosalicylic acid (L), the interaction of investigated compound and HSA would be of great importance in the search for potential new strategy for targeted drug delivery to tumor tissues. The objective of present study is to examine the binding interaction between human serum albumin (HSA) and L using various spectroscopic methods and molecular docking simulations.

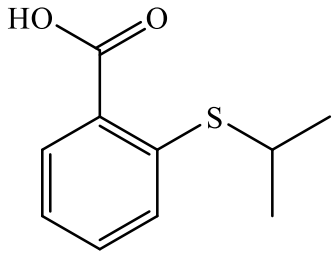

Figure 2. Chemical structure of investigated ligand

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Results and discussion

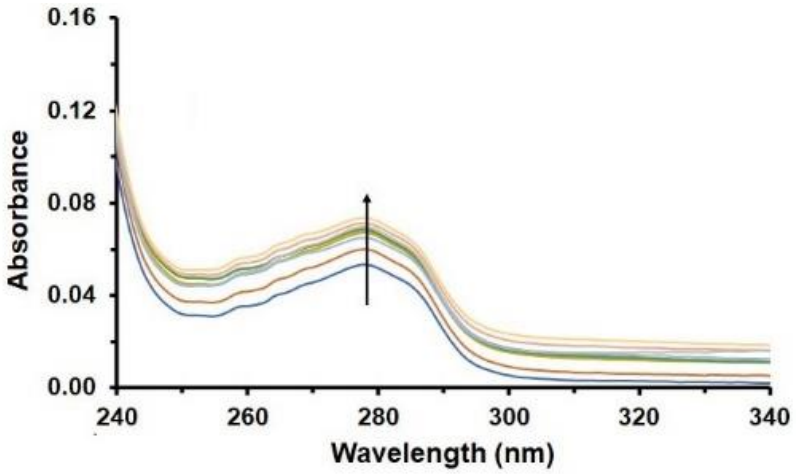

Figure 3. UV-Vis absorption spectra of HSA in the absence and presence of increasing amounts of ligand L. (T = 298 K, pH = 7.4). [HSA] = 1.6 µM; [ligand] = 0 -16 µM.

The UV-Vis absorption spectra of HSA were initially recorded in the absence of ligand L and subsequently upon their addition, as depicted in Figure 2. As the concentration of the ligand gradually increased, the UV absorption of HSA exhibited a corresponding rise, while retaining its original peak shape and position, as illustrated in Figure 2. Dynamic quenching exclusively impacts the excited states of fluorophores, causing no alterations in the absorption spectra, whereas static quenching leads to modifications in the absorption spectrum of the protein [12]. The acquired spectra provide evidence of the formation of a complex between albumin and the ligand L. Moreover, the presence of these ligand L had an impact on the polarity and hydrophobicity of the microenvironment surrounding tryptophan (Trp) and tyrosine (Tyr) residues [13]. These findings indicate that the interaction between the ligand (L) and HSA followed a static quenching process, as deduced from the emission spectra.

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Results and discussion

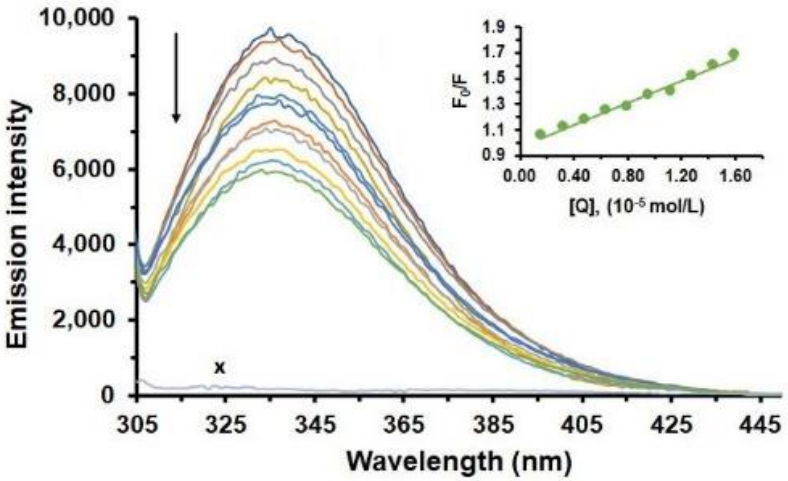

Figure 4. Emission spectra of HSA in the presence of various concentrations of ligand (L) (T = 296 K, pH = 7.4). [HSA] = 1.6 µM; [ligand] = 0 - 16 µM. x represents a 16 µM ligand only. The inset: plot of F_0/F vs. [ligand].

The constant K_{SV} can be calculated through linear regression by plotting F_0/F against [Q], where K_q ($K_q = K_{SV} / \tau 0$) represents the rate constant. The values of K_{SV} are obtained from the slopes of the fitted lines, and K_q is determined for the interaction of ligand (L) with HSA.

Results and discussion

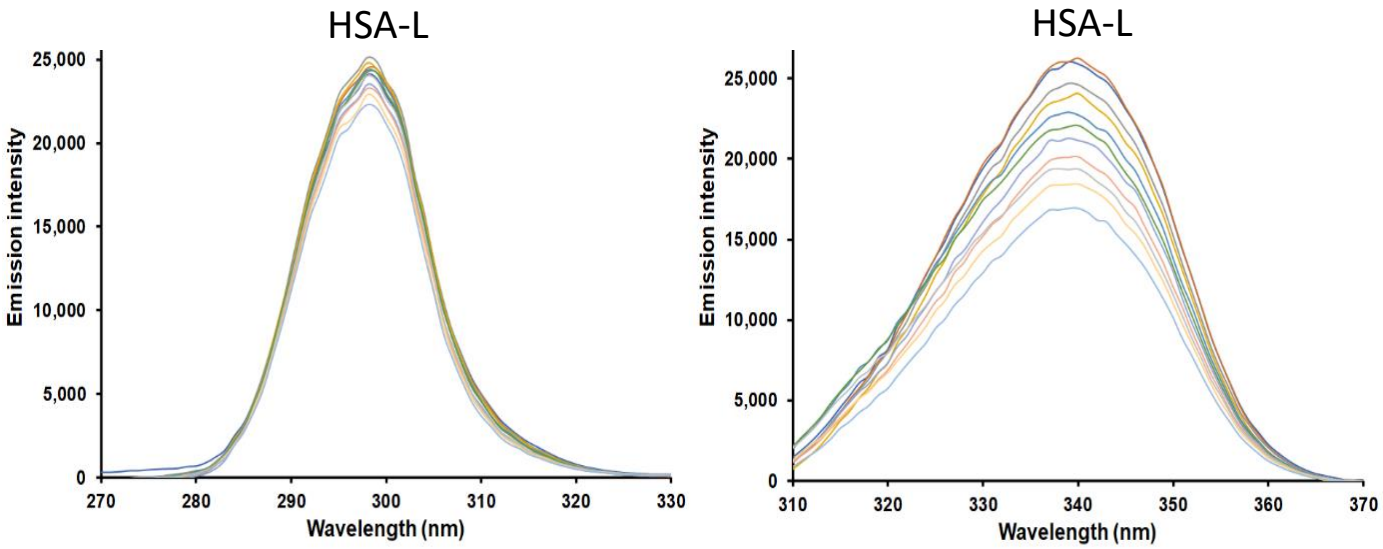

Figure 5. The effect of L on the synchronous fluorescence emission spectra of HSA (left: $\Delta\lambda$=15 nm and right: $\Delta\lambda$=60 nm) (T = 298 K, pH = 7.4). [HSA] = 1.6 µM, [L1] = 0-1.6 x 10^{-5} M.

The fluorescence intensity of HSA decreased with the increase of ligand (L) concentration when Δλ = 15 nm and Δλ = 60 nm. The fluorescence quenching was more intense at Δλ = 60 nm, confirming that L binding to HSA occurs near the tryptophan residue.

Results and discussion

Table 1

Stern-Volmer quenching constants (K_{SV}), quenching rate constants (K_q), binding constants (K_a), and number of binding sites (n) for the interaction of ligand (L) with HSA.

Ligand	K_{SV} (M^{-1})	K_q (M^{-1} s^{-1})	R^{2a}	K_a (M^{-1})	n	R^{2a}
L	4.18×10^4	4.18×10^{12}	0.9820	6.89×10^4	1.05	0.9946

[a]R is the correlation coefficient

As indicated in Table 1, the calculated value of n is approximately 1 for the examined ligand (L), suggesting the presence of a single binding site in HSA for this ligand. Based on the K_a values, it can be inferred that ligand L forms stable complex with HSA, with the K_a value of 6.89×10^4 M^{-1}. These findings demonstrate that the binding constants (K_a) for the HSA-ligand system are significantly greater than 10^4, indicating a strong binding affinity between ligand (L) and HSA [14]. Additionally, it can be concluded that the investigated ligands can be efficiently transported and stored in the body when bound to HSA [15].

References:

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Results and discussion

Molecular docking protocol:

- Energy optimization: Chem3D Ultra 7.0 [16]
- Preparation of L: AutoDockTools 1.5.6 [17]
- The crystal structure of the HSA (PDB ID: 1HA2) [18]
- Preparation of target molecule: Discovery Studio Visualizer [19]
- Semi-flexible docking procedure: AutoDock Vina [20]

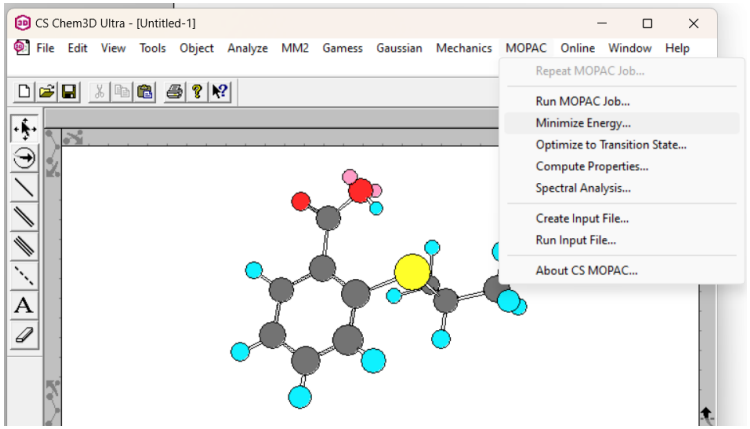

Figure 6. Energy optimization of L

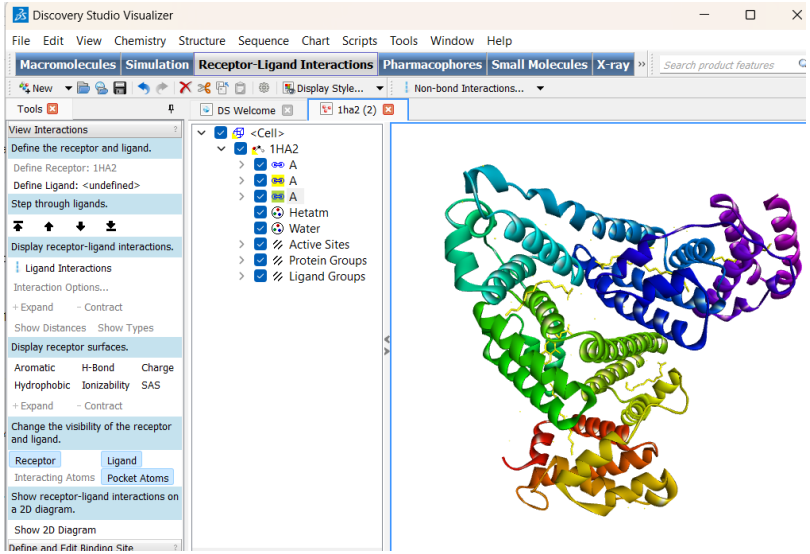

Figure 7. Preparation of target molecule

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Results and discussion

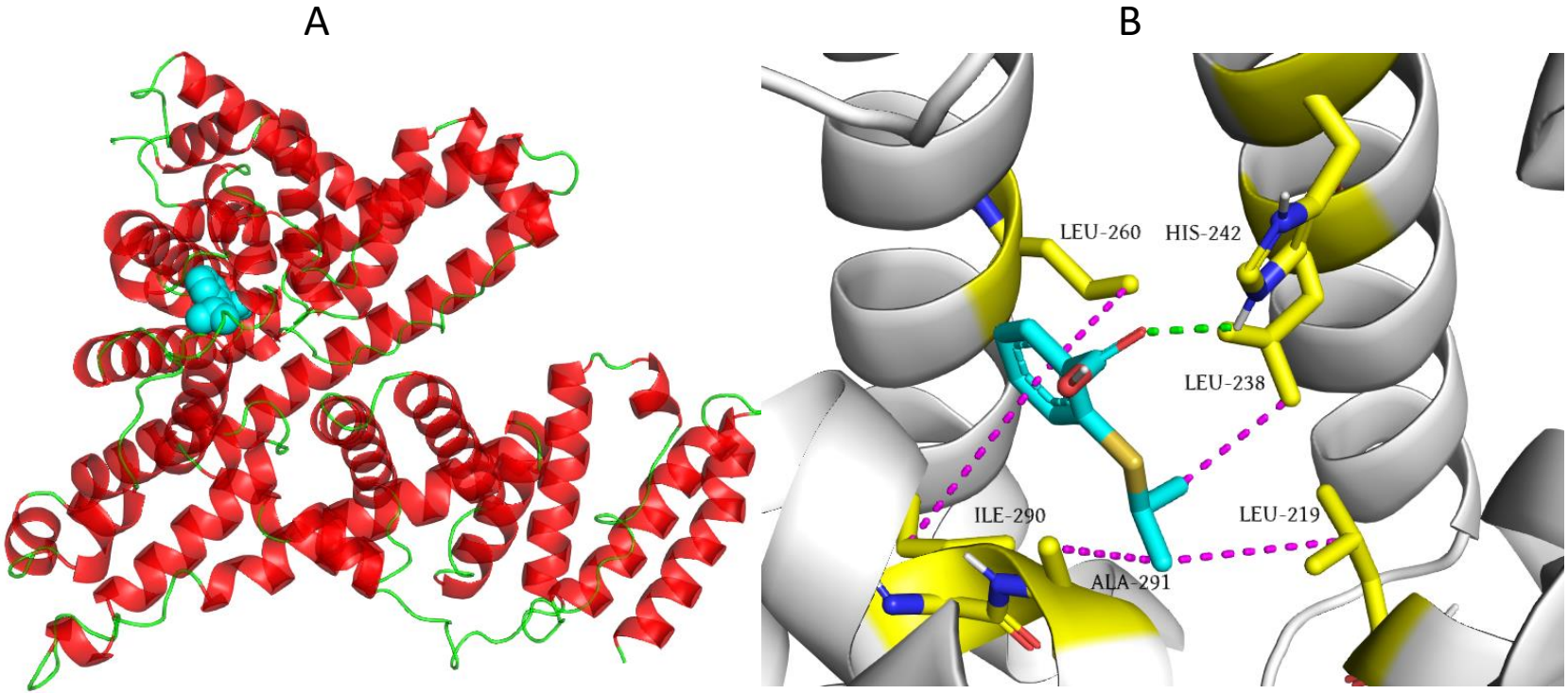

Figure 8. A) Schematic representation of L bound to the HSA. B) Molecular docking of L into the HSA. Conventional hydrogen bond is presented as green dashed line, while hydrophobic interactions are shown as magenta dashed lines.

Carbonyl oxygen atom of derivative L (hydrogen bond acceptor) forms a single hydrogen bond with imidazole ring of His242 (hydrogen bond donor), whereas aromatic ring and isopropyl side chain of L establish multiple hydrophobic interactions.

Conclusions

- ✓ To investigate the quenching mechanism, we employed the association constants and determined the number of binding sites.
- ✓ The interaction between the tested ligand (L) and HSA exhibited a static fluorescence quenching mechanism, characterized by spontaneous binding processes.
- ✓ UV-Vis absorption spectroscopy indicated that the binding of the tested ligands induced minor conformational changes in HSA.
- ✓ Carboxyl group of L is involved in the formation of a hydrogen bond with imidazole ring of His242, while aromatic ring and isopropyl side chain of L interact with carbon atoms of amino acid residues, establishing multiple hydrophobic contacts.

Acknowledgments

ИНСТИТУТ ЗА ИНФОРМАЦИОНЕ ТЕХНОЛОГИЈЕ КРАГУЈЕВАЦ
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