

Supplementary material for the article:

Vilipić, J. P.; Novaković, I. T.; Zlatović, M. V.; Vujčić, M. T.; Tufegdžić, S. J.; Sladić, D. M. Interactions of Cytotoxic Amino Acid Derivatives of Tert-Butylquinone with DNA and Lysozyme. *Journal of the Serbian Chemical Society* **2016**, *81* (12), 1345–1358.
<https://doi.org/10.2298/JSC160725101V>



SUPPLEMENTARY MATERIAL TO
**Interactions of cytotoxic amino acid derivatives of
tert-butylquinone with DNA and lysozyme**

JOVANA P. VILIPIC¹, IRENA T. NOVAKOVIC^{2**}, MARIO V. ZLATOVIC³,
MIROSLAVA T. VUJIC², SRĐAN J. TUFEGDŽIĆ² and DUŠAN M. SLADIĆ^{3*}

¹Innovation Center of the Faculty of Chemistry, University of Belgrade, Studentski trg 12–16, Belgrade, Serbia, ²Institute of Chemistry, Technology and Metallurgy, Center for Chemistry, University of Belgrade, Njegoševa 12, Belgrade, Serbia and ³Faculty of Chemistry, University of Belgrade, Studentski trg 12–16, Belgrade, Serbia

J. Serb. Chem. Soc. 81 (12) (2016) 1345–1358

SOME EXPERIMENTAL DETAILS

Mass spectrometry

For recording the mass spectra of lysozyme and its possible conjugates with quinones, the prepared samples were introduced into a mass spectrometer by direct injection. Ionisation was performed by HESI (spray voltage 4.5 kV, vaporizer temperature 50 °C, sheath gas flow rate 12.00 $\mu\text{L min}^{-1}$, auxiliary gas flow rate 3.00 $\mu\text{L min}^{-1}$, capillary voltage 42 V, capillary temperature 250 °C, tube lens 110 V). The analysis was performed in the positive mode in the 800–2200 m/z range. Data analysis was realised by Xcalibur software and ToxID Automated Screening Software.

UV-Visible spectroscopic methods

Reaction mixtures of final volume of 1 mL containing 40 mM bicarbonate solution (pH 8.4), different volumes of tested compounds (5, 10 and 20 μL of the stock solutions) and 10 μL of CT-DNA solution were incubated at 37 °C for 90 min with occasional vortexing. The absorbance titrations were performed at a fixed concentration of quinone derivatives (50 μM) with varying concentrations of double stranded CT-DNA (6, 8, 10, 12, 14 and 16 μL of CT-DNA solution). UV-Vis spectra were recorded on spectrometer operating from 200 to 800 nm in 1.0 cm quartz cells. Individual spectra of compounds of the same concentrations and CT-DNA were also recorded.

Fluorescence studies

The competitive interactions of quinones and fluorescent dyes, ethidium bromide (EB) and Hoechst 33258 (H), with CT-DNA were studied by monitoring the changes of fluorescence intensity upon binding of the tested compounds to complexes of CT-DNA with the dyes. The reaction mixtures containing 10 μL of CT-DNA in 1 mL of bicarbonate solution (40 mM, pH 8.4) and different concentrations of amino acid derivatives were incubated at 37 °C

* Corresponding authors. E-mail: (*)dsladic@chem.bg.ac.rs; (**)irenan@chem.bg.ac.rs



for 90 min with occasional vortexing. The probe solution (25 μM of EB or 28 μM of H at final concentrations) was added to each reaction mixture and the incubation was prolonged for 20 min. The change in the fluorescence intensity was measured by excitation at 500 nm in the range 520–700 nm for EB and by excitation at 350 nm in the range 400–550 nm for H. The EB–CT-DNA and H–CT-DNA solutions were used as control. The solutions of the free quinones did not fluoresce under the applied conditions.

DPPH radical scavenging activity test

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined by the method of Blois.¹ All compounds and commercially available free radical DPPH were dissolved in methanol. Into a 96-well microplate, 100 μL of DPPH solution (6.58×10^{-5} M) was loaded and 50 μL methanol solutions of the tested compounds diluted to ten different concentrations were added, or just pure methanol as the control. After incubation for 30 min at room temperature in the dark, the absorbance was measured at 517 nm. All the measurements were performed in triplicate and the scavenging activity of the tested derivatives was calculated as:

$$\text{Scavenging activity} = \frac{100(A_{\text{control}} - A_0 - A_{\text{sample}})}{A_{\text{control}}}$$

where A_{control} and A_{sample} refer to the absorbance of DPPH in control solution and sample, respectively, while A_0 refers to the absorbance of the quinone solutions, because of their intense colour.

The IC_{50} was defined as the antioxidant concentration necessary to decrease the amount of the initial DPPH radical by 50 % and was calculated from the plotted graph of scavenging activities against the concentrations of the tested compounds. Ascorbic acid was employed as the positive control (concentrations from 50 to 500 $\mu\text{g mL}^{-1}$).

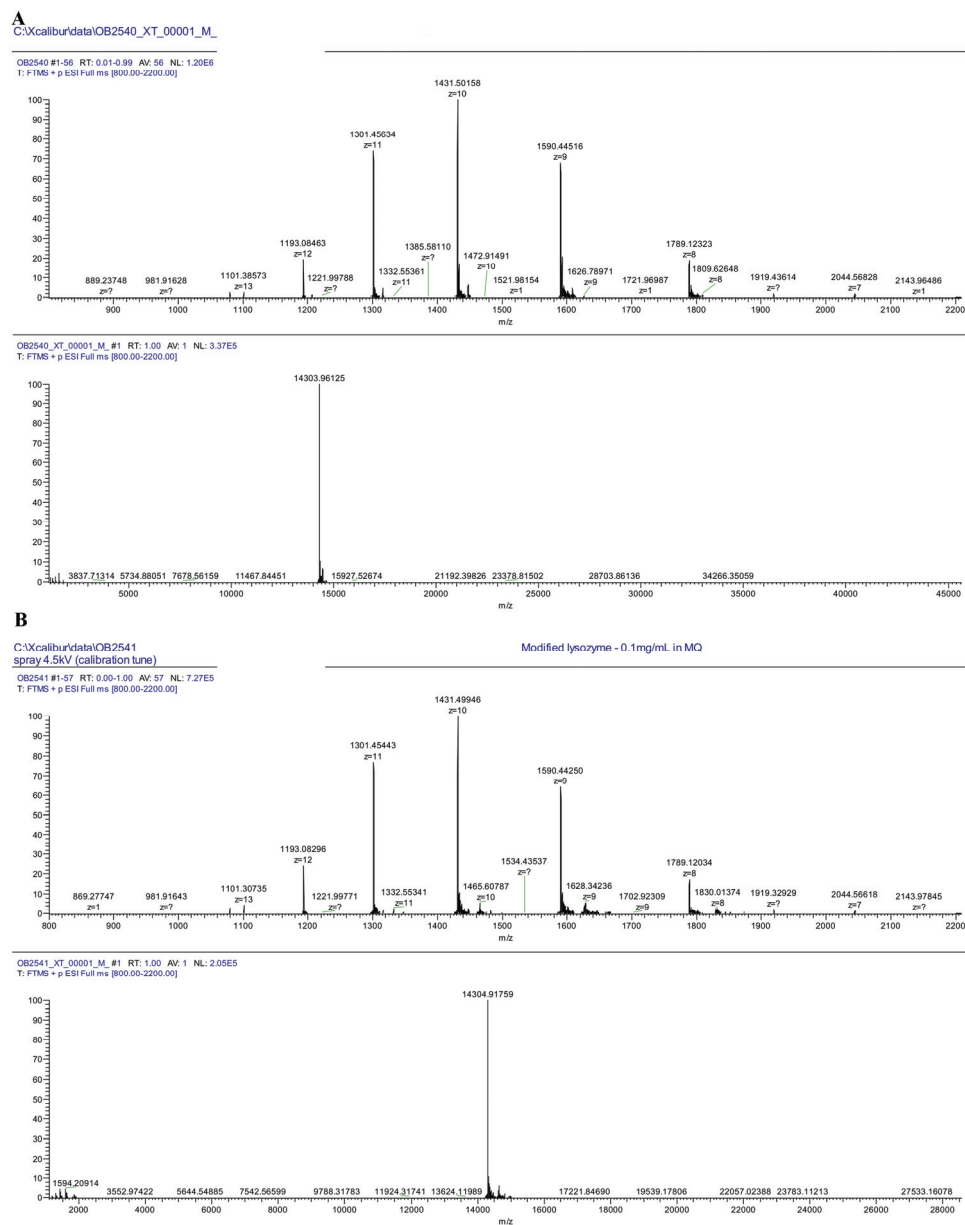


Fig. S-1. Mass spectra of unmodified lysozyme (A) and lysozyme after treatment with **1h** (B).

DETERMINATION OF BINDING CONSTANT BY ABSORPTION TITRATION

The absorbance at 259 nm was monitored for each concentration of DNA. The binding constant K_B was determined from:²

$$\frac{[DNA]}{\varepsilon_A - \varepsilon_F} = \frac{[DNA]}{\varepsilon_B - \varepsilon_F} + \frac{1}{K_B(\varepsilon_B - \varepsilon_F)}$$

where ε_A , ε_F , ε_B are the extinction coefficients of the derivative–CT-DNA complex, the free derivative and the bound derivative, respectively.

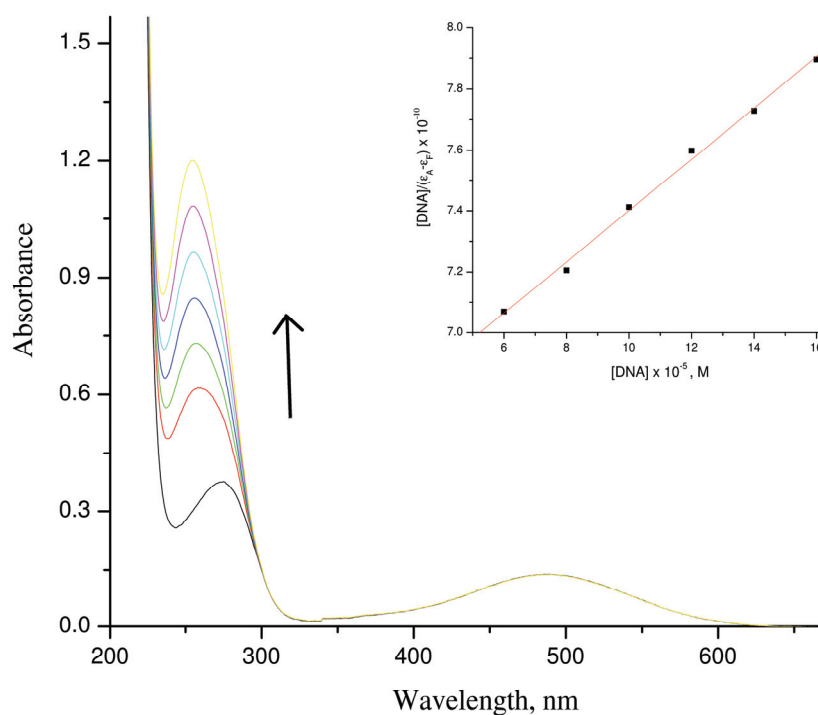


Fig. S-2. Determination of binding constant by absorption titration of **1h** with CT-DNA. Absorption spectra of **1h** (50 μ M) without (0 M) and with CT-DNA at different concentrations (60, 80, 100, 120, 140 and 160 μ M, from bottom to top).

TABLE S-I. The values of binding constants K_B (M^{-1}) of the derivative–CT-DNA complexes

Compound	1a	1b	1c	1d	1e	1f	1g	1h	1i
$K_B / 10^3$	1.1	1.1	1.8	1.5	2.1	0.6	1.5	1.3	1.2

REFERENCES

1. M. S. Blois, *Nature* **181** (1958) 1199
2. R. Vijayalakshmi, M. Kanthimathi, V. Subramanian, B. U. Nair, *Biochem. Biophys. Res. Commun.* **271** (2000) 731.