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DNA/BSA binding affinities and *in vivo* toxicity of dinuclear silver(I) complexes with phthalazine

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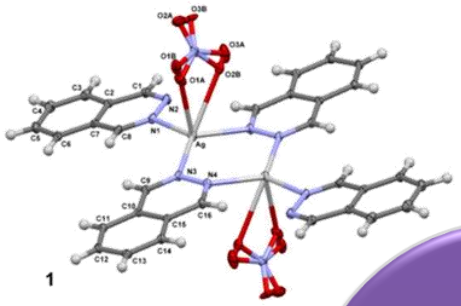
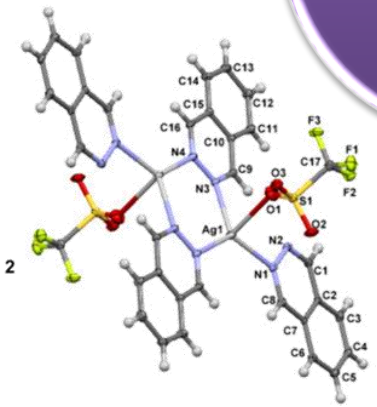


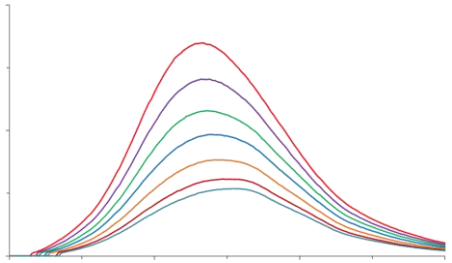
1



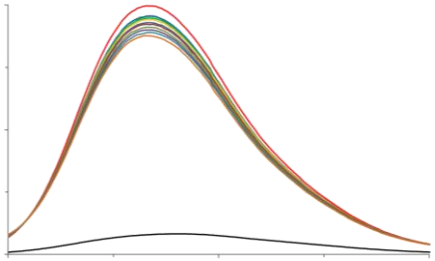
2

Silver(I) complexes

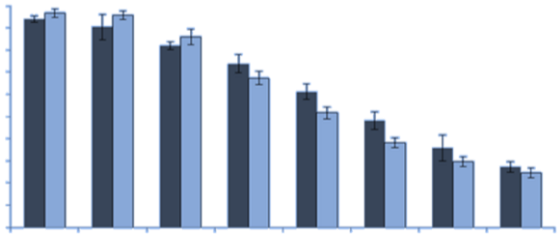
BSA



DNA



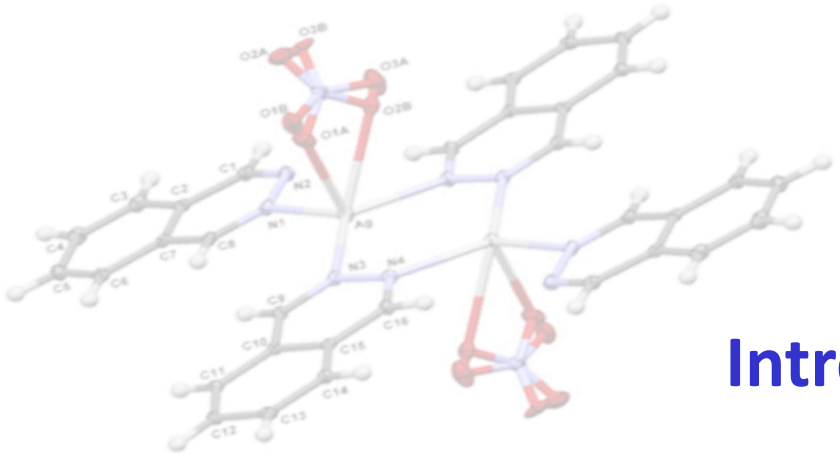
Caenorhabditis elegans

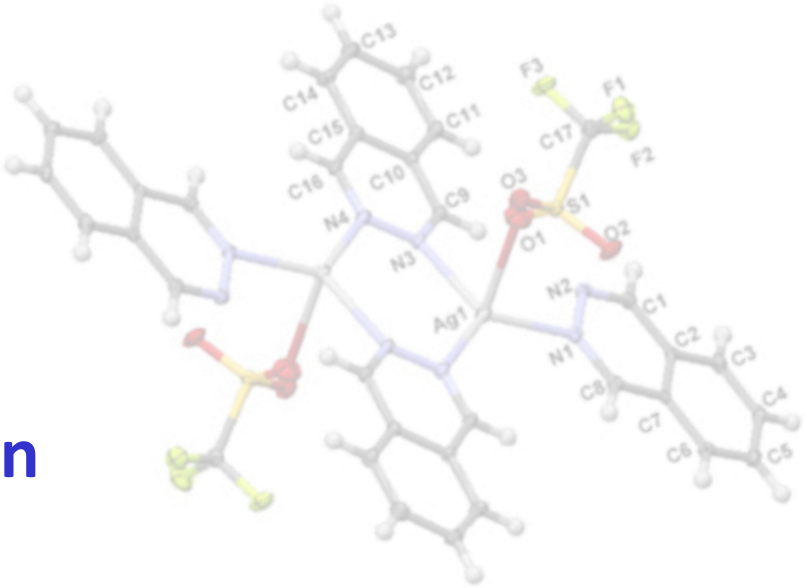


Abstract

Silver(I) complexes with aromatic nitrogen-containing heterocycles have shown an effective and wide-spectrum antimicrobial activity. The possible mechanism of their antimicrobial activity can be attributed to interactions of these complexes with biomolecules, including DNA and proteins. Herein, we investigated the interactions of two antimicrobial active dinuclear phthalazine-silver(I) complexes, $[\{Ag(NO_3)(phtz)\}_2(\mu\text{-}phtz)_2]$ (**Ag1**) and $[\{Ag(CF_3SO_3)(phtz)\}_2(\mu\text{-}phtz)_2]$ (**Ag2**) (phtz is phthalazine), with calf thymus DNA (ctDNA) and bovine serum albumin (BSA) to evaluate their binding affinities towards these biomolecules for possible insights on their mode of antimicrobial activity. The value of binding constants (K_A) of **Ag1** and **Ag2** to BSA is higher than that for DNA, indicating greater affinity of the complexes toward this model protein. The partition coefficient (logP) values for **Ag1** and **Ag2** are 0.0035 and -0.0063, respectively, what is in accordance with higher cellular uptake efficiency and better antibacterial activity of **Ag1** in respect to **Ag2**. In order to determine the therapeutic potential of **Ag1** and **Ag2** complexes, their toxicity *in vivo* against nematode, *Caenorhabditis elegans*, was investigated.

Keywords: Silver(I) complexes; Phthalazine; DNA/BSA interaction; Lipophilicity; *Caenorhabditis elegans*.



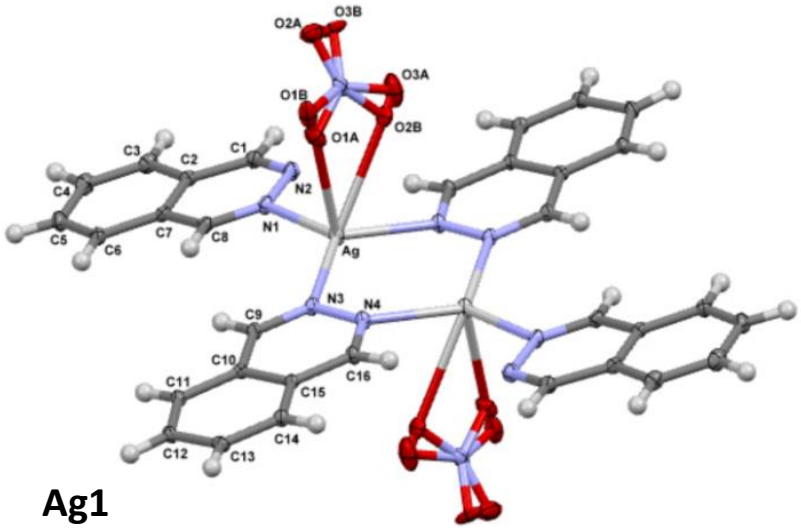


Introduction

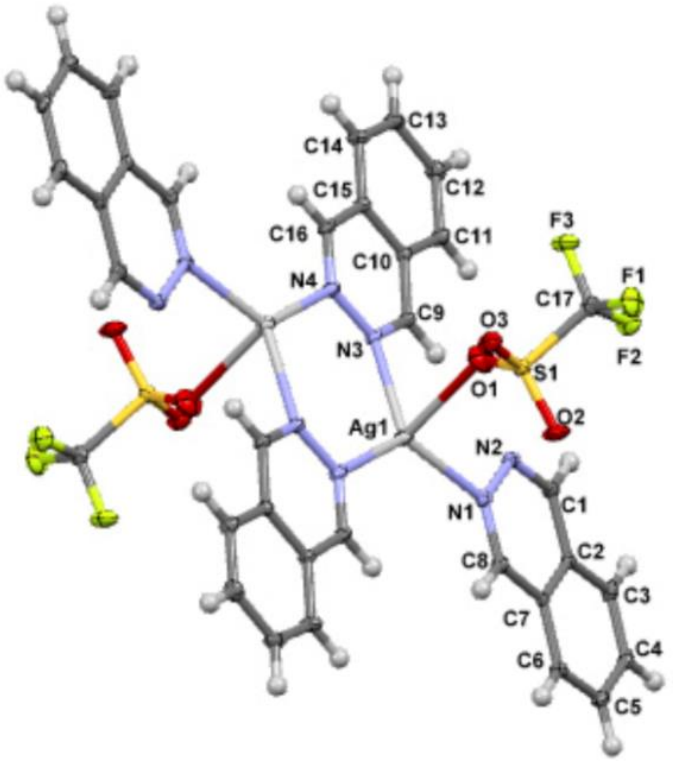
- ✓ Silver(I) complexes showed significant antimicrobial activity against the strains which are resistant to the currently used antimicrobial drugs, while their toxicity to the normal human cells was not pronounced
- ✓ One of the mechanism of antimicrobial activity of silver(I) complexes is their interactions with biological targets including DNA and proteins

Results and discussion

Molecular structures of Ag1 and Ag2 complexes



Ag1



Ag2

B.Đ. Glišić et al., J. Inorg. Biochem. 155 (2016) 115.

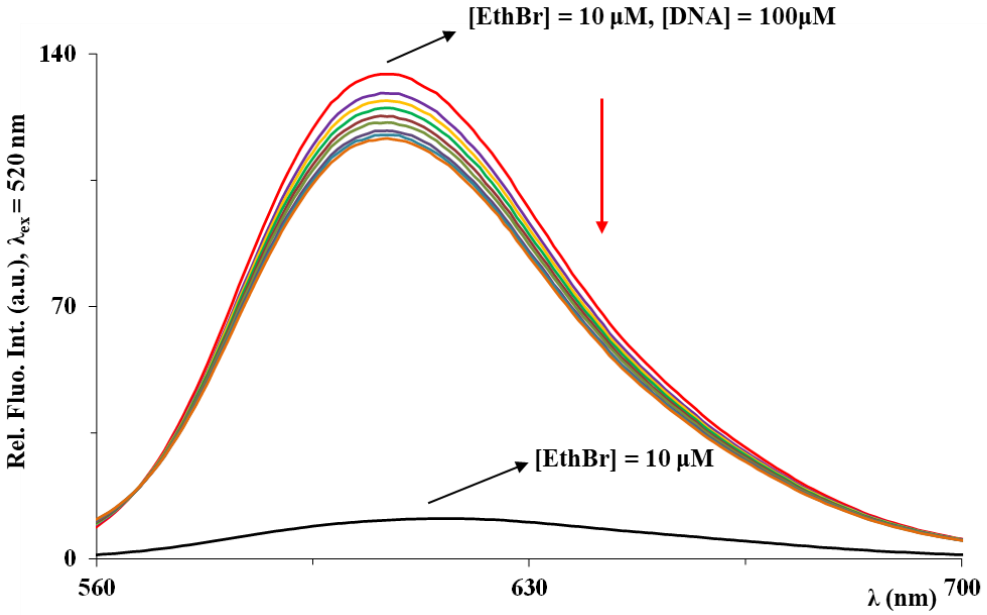
Interaction of Ag1 and Ag2 complexes with ct-DNA

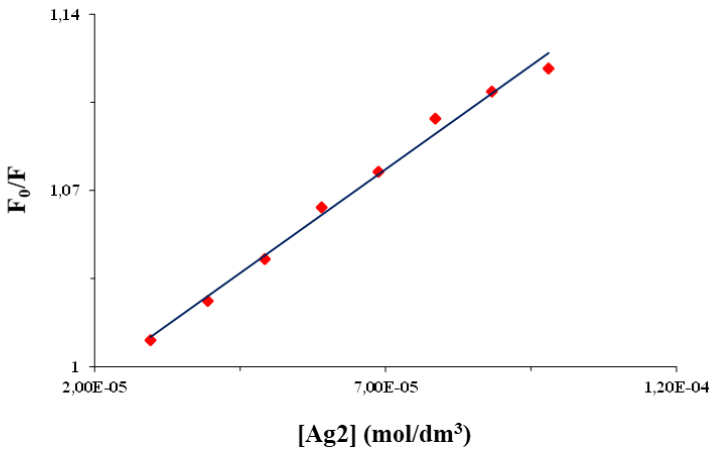
- Interaction between synthesized complexes and ct-DNA was studied using fluorescence spectroscopy and cyclic voltammetry
- The K_A values for both silver(I) complexes are similar, but much lower than that for EthBr itself

Complex	K_{SV} (M^{-1})	*Hypochromism (%)*	K_q $(M^{-1}s^{-1})$	K_A (M^{-1})	*n*
Ag1	$(8.50 \pm 0.19)\cdot10^2$	12.24	$8.51\cdot10^{10}$	$9.30\cdot10^2$	1.01
Ag2	$(1.71 \pm 0.01)\cdot10^3$	12.99	$1.71\cdot10^{11}$	$4.91\cdot10^3$	1.12

✓ The results of fluorescence spectroscopy suggest the electrostatic mode of binding of Ag1 and Ag2 complexes to ctDNA

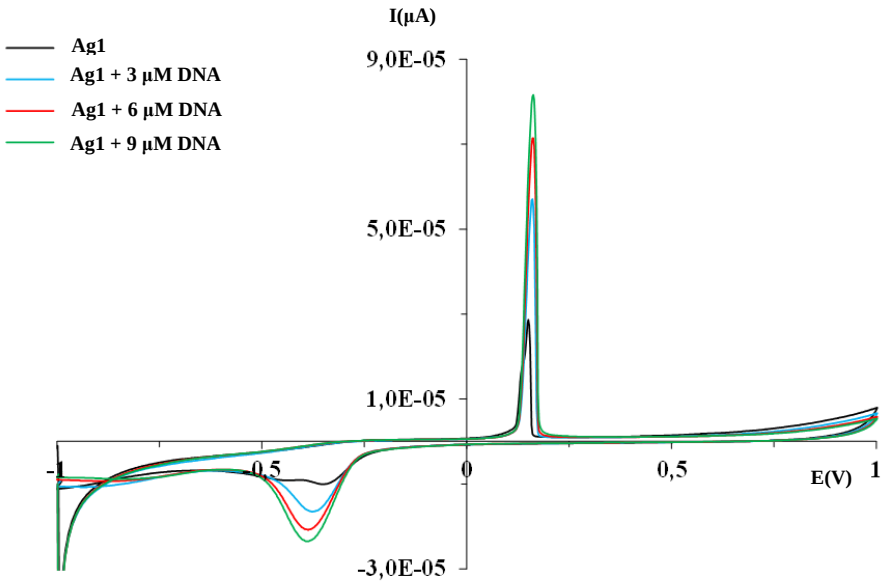
[Ag2] = 0 – 160 µM, Phosphate buffer saline (pH = 7.4)

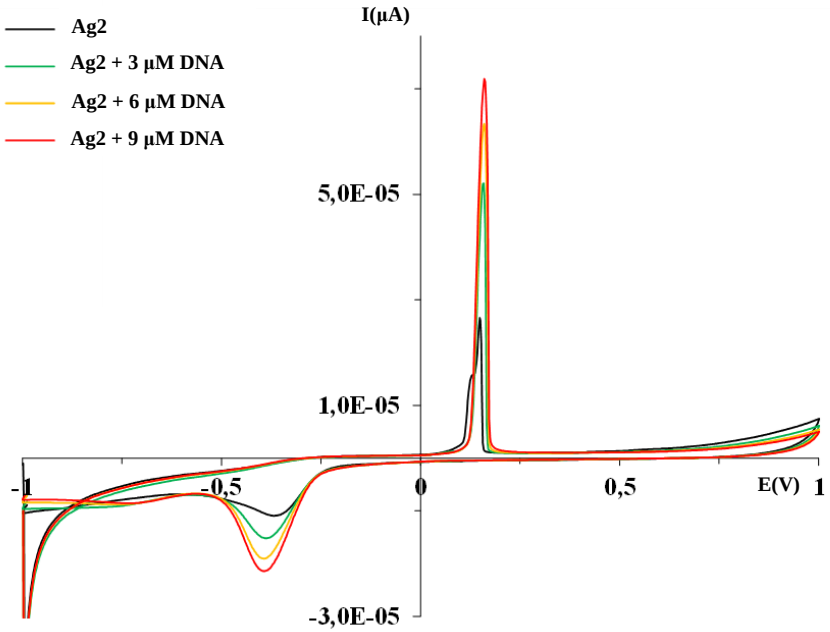




Fluorescence emission spectra of DNA-EthBr system in the presence of an increasing concentration of **Ag2** complex. Arrow shows the intensity changes upon increased amount of the complex. Inserted graph: Stern-Volmer plots of F_0/F *vs* [complex]

- ✓ Non-intercalative mode of binding was confirmed for Ag1 and Ag2 by cyclic voltammometry





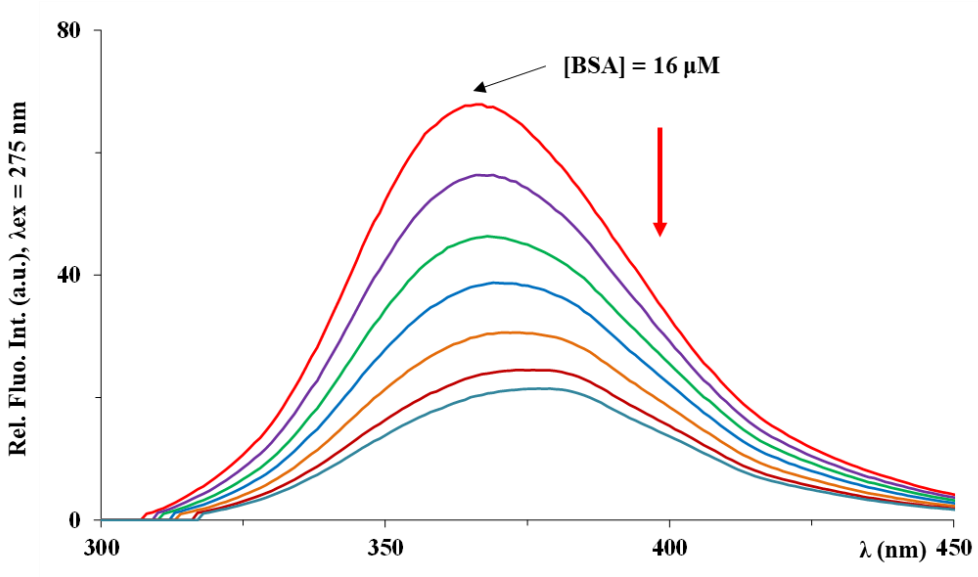
Interaction of Ag1 and Ag2 complexes with BSA

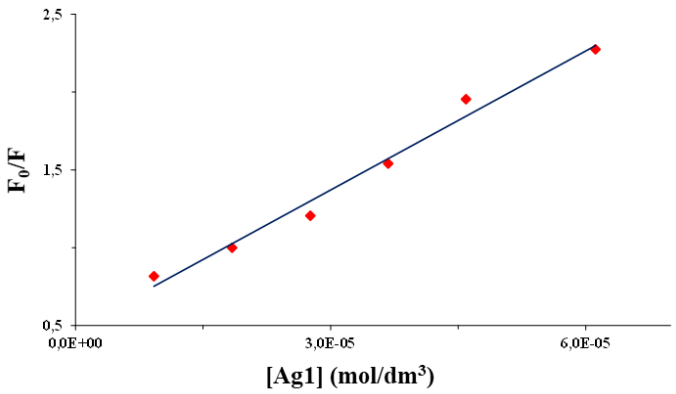
- The protein binding study of Ag1 and Ag2 was performed by tryptophan fluorescence quenching experiments using BSA in phosphate buffer solution (pH = 7.4)

- The addition of increasing amounts of the complexes to the BSA solution resulted in a remarkable quenching of BSA fluorescence, as a consequence of the complexes binding to BSA

Complex	K_{sv} (M^{-1})	*Hypochromism (%)*	K_q ($M^{-1}s^{-1}$)	K_A (M^{-1})	*n*
Ag1	$(6.23 \pm 0.19)\cdot10^4$	70.19	$6.23\cdot10^{12}$	$1.05\cdot10^6$	1.34
Ag2	$(2.99 \pm 0.15)\cdot10^4$	83.69	$3.00\cdot10^{12}$	$4.81\cdot10^5$	1.30

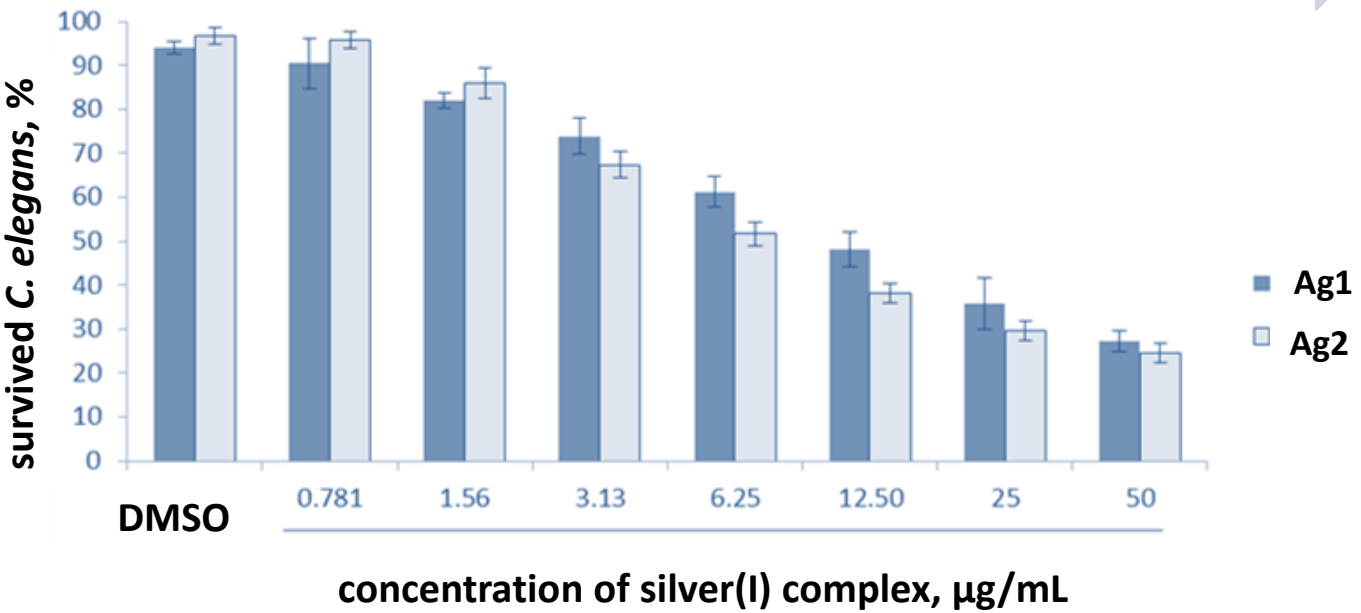
[Ag1] = 0 - 60 µM, Phosphate buffer saline (pH = 7.4)





Fluorescence emission spectra of BSA in the presence of an increasing concentration of **Ag1** complex alongside. Arrow shows the intensity changes upon increased amount of the complex. Inserted graph: Stern-Volmer plots of F_0/F *vs* [complex]

In vivo cytotoxicity of Ag1 and Ag2 C.elegans model



- IC_{50} values of **Ag1** and **Ag2** are 12.5 and 6.25 µg/mL, respectively

Conclusions

- [{Ag(NO_3)(phtz)}$_2$(μ-phtz)$_2$] (**Ag1**) and [{Ag(CF_3SO_3)(phtz)}$_2$(μ-phtz)$_2$] (**Ag2**) complexes interact with ctDNA through electrostatic mode
- Complexes **Ag1** and **Ag2** have greater affinity toward BSA with respect to DNA
- The partition coefficient values for **Ag1** are in accordance with higher cellular uptake efficiency and better antibacterial activity of **Ag1** in respect to **Ag2**
- The presently investigated complexes **Ag1** and **Ag2** are moderatly cytotoxic *in vivo* in *C. elegans* model

Acknowledgments

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МИНИСТАРСТВО ПРОСВЕТЕ,
НАУКЕ И ТЕХНОЛОШКОГ РАЗВОЈА