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Hesperetin's antigenotoxicity: alleviation of chemically induced mutations on somatic cells understood through molecular modeling

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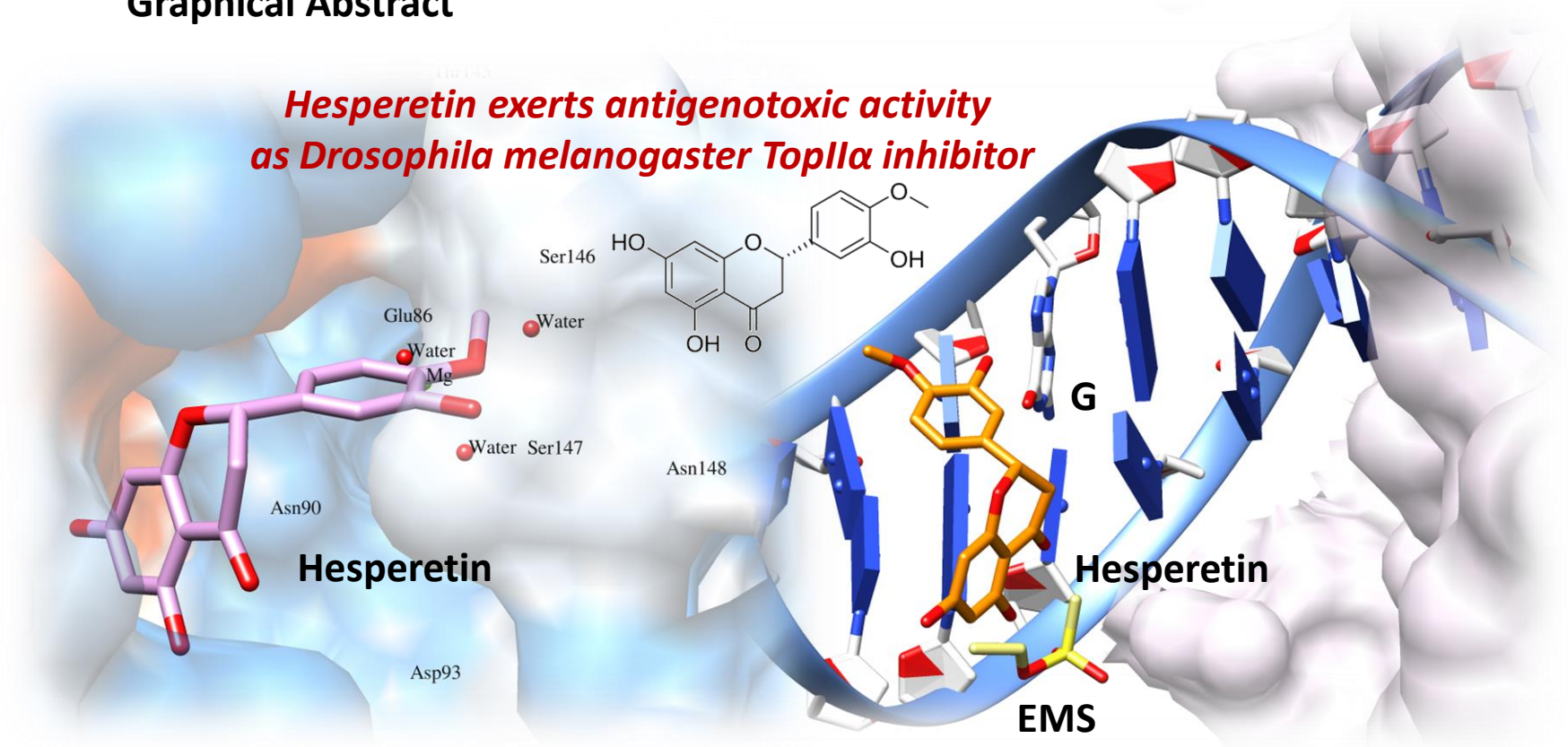
Graphical Abstract



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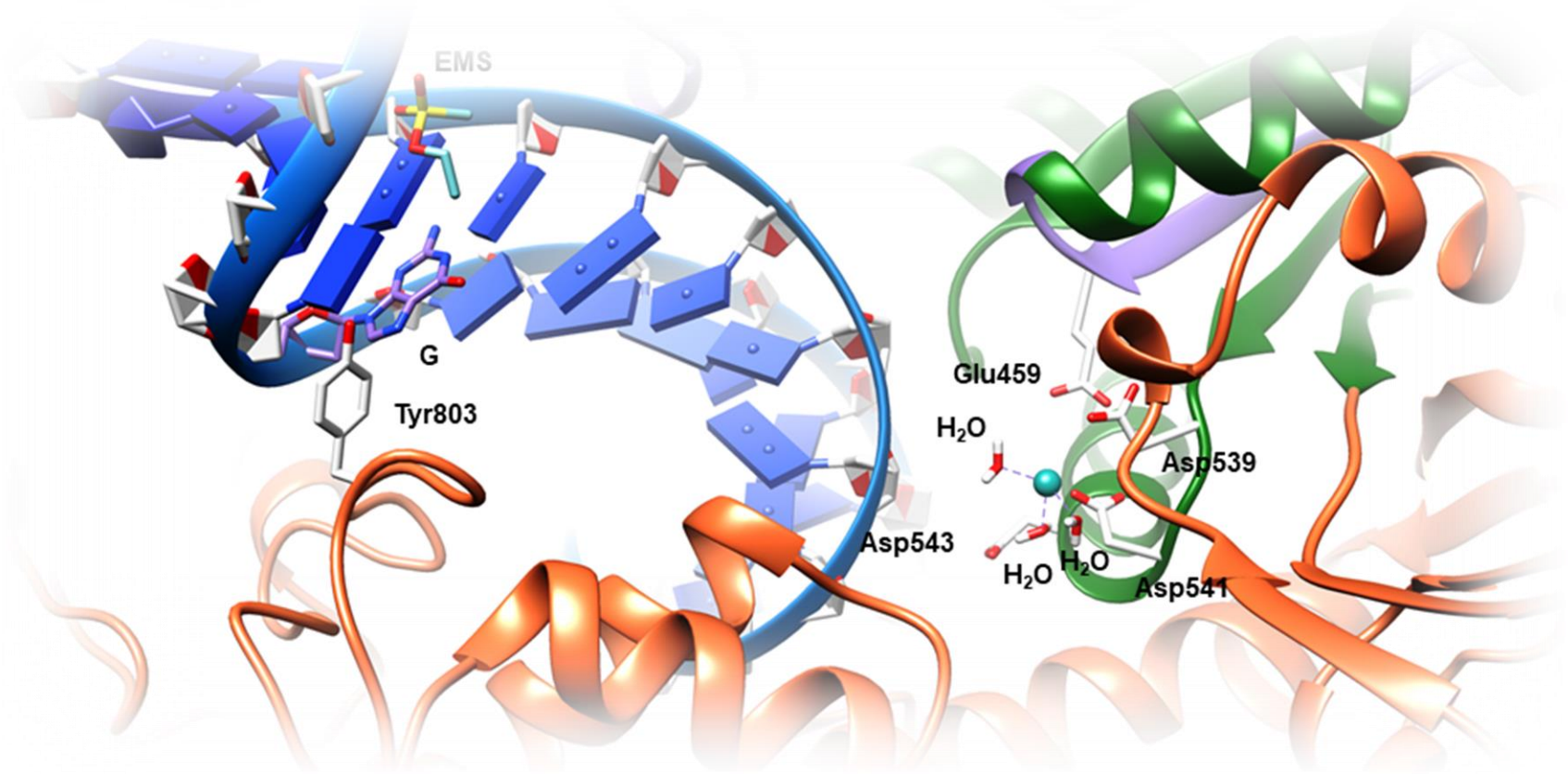
Abstract: Previously undefined genotoxic and antigenotoxic potentials of hesperetin were elaborated in *Drosophila melanogaster*, upon inducing the DNA damage with ethyl methanesulfonate (EMS), proven alkylating agent and mutagen, within somatic cells. Upon the EMS-mediated *in vivo* alkylation, the O^6-ethylguanine and O^4-ethylthymine lesions emerged leading to the aberrant G=T and T=G pairing. The *dm*TopIIα inhibition has been confirmed employing the electrophoresis on *Drosophila melanogaster* plasmid DNA (*dm*PDNA) relaxation level, enzymatic and fluorescence assaying on dmTopIIα's ATPase level and DNA-Binding and Cleavage Region, respectively, and molecular docking. Thus, as an antigenotoxic agent, hesperetin exerted dual pharmacology: within the *dm*TopIIα hesperetin acted as an ATPase uncompetitive inhibitor (as confirmed by spectrophotometric studies), denying the *dm*TopIIα energy for enzyme-catalized cleavage of double-strand containing the G=T and T=G pairings; within the *dm*TopIIα DNA-Binding and Cleavage Region hesperetin exerted no intercalating features (as verified by fluorescence quenching) but instead and blocked the EMS approach to either guanine and thymine, prevented the alkylation, and consequent dmTyr803-catalysed cleavage of normal double-strand (as certified by molecular docking). Conclusively, hesperetin could be used as a supplement for alkylating agent-based cancer therapy in terms of preventing the alkylation agent to cause unnatural lesions and aberrant pairing.

Keywords: hesperetin; genotoxic; antigenotoxicity; *Drosophila melanogaster*; pharmacological studies; molecular docking

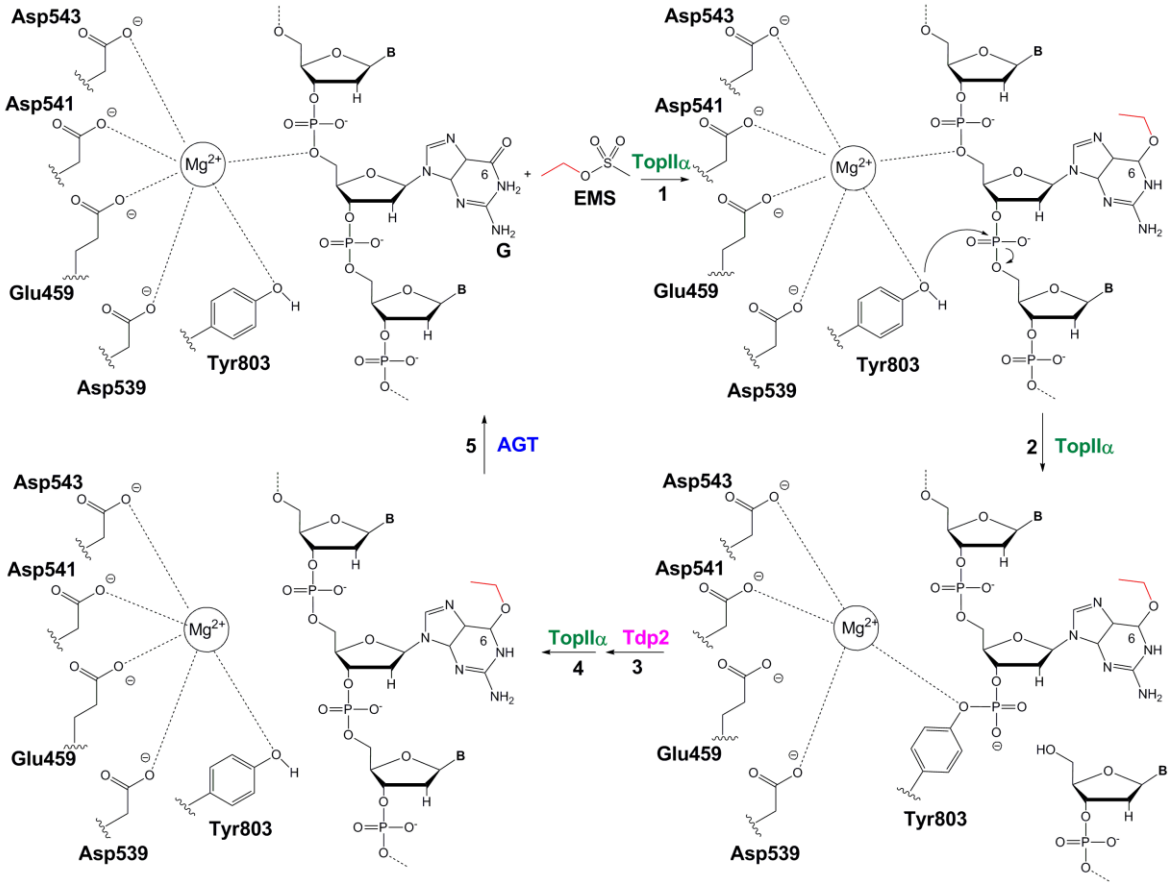
Introduction

The ethyl methanesulfonate (EMS), proven alkylating agent and mutagen, induces the DNA damage within somatic cells by means of *in vivo* alkylation. Targeting the guanine and thymine, the O^6-ethylguanine and O^4-ethylthymine lesions emerge, leading to the aberrant G=T and T=G pairing.



Introduction

The O^6-ethylguanine and O^4-ethylthymine lesions lead to the aberrant G=T and T=G pairing. Previously undefined genotoxic and antigenotoxic potentials of hesperetin were elaborated in *Drosophila melanogaster*, while preventing the EMS' action.



Results and discussion

Being applied in the equimolar concentration related to the alkylating agent, hesperetin significantly reduced the EMS-influenced DNA damage, as verified by the comet assay, implying that it has acted as a powerful *Drosophila melanogaster*'s Topoisomerase IIα *(dmTopIIα)* inhibitor.

Table 1. Genotoxic and Antigenotoxic Activities of Hesperetin Using the Comet Assay.

Treatments	Comet classes[a]					Total score[a]	%R[b]
	0	1	2	3	4		
NC[c]	82.7±0.26	17.3±0.43	0.00±0.00	0.00±0.00	0.00±0.00	17.3±0.4[†]	/
EMS[d]	13±0.2	23.2±0.41	17.6±0.34	17.3±0.25	28.9±0.31	225.9±1.04[*]	/
H[e]	82.3±0.34	12.6±0.71	5.1±0.9	0.00±0.00	0.00±0.00	22.8±0.82[†]	/
EMS + H[f]	60.2±0.61	22.9±0.57	11±1.32	4.8±0.8	1.1±0.24	63.7±0.34[*†]	77.8

[a]The values are mean ± S.D. from three independent experiments. [b]%R; percentage of reduction [c]Negative control. [d]Ethyl methanesulfonate, 1 mM. [e]Hesperetin, 1 mM. [f]EMS + hesperetin; ethyl methanesulfonate 1 mM + hesperetin 1 mM. [*]$p < 0.05$ when compared with the negative control group; [†]$p < 0.05$ when compared with the positive control group.

Results and discussion

The *dm*TopIIα inhibition has been confirmed employing the electrophoresis on *Drosophila melanogaster* plasmid DNA (*dm*PDNA) relaxation level

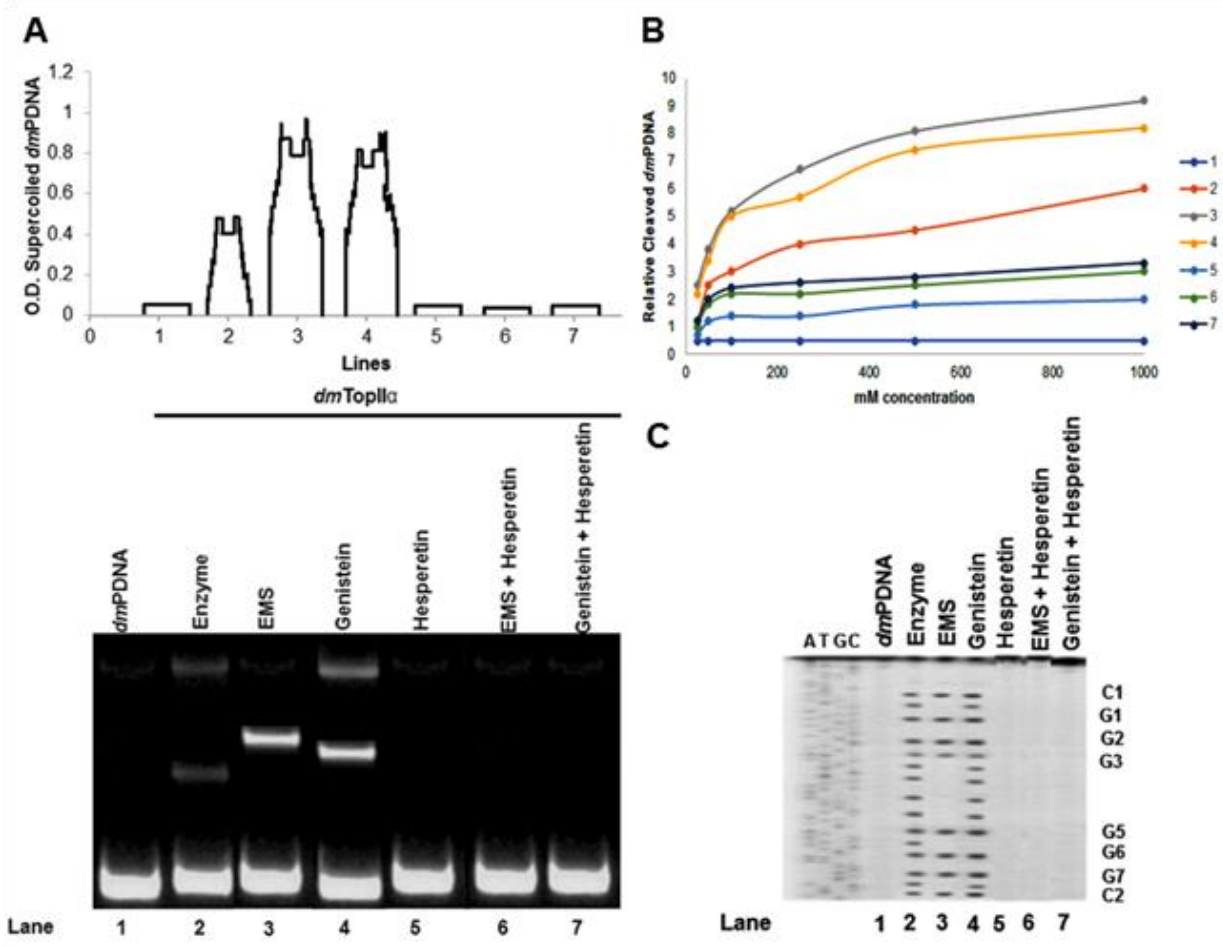


Figure 1. (A) The upper part: The optic densitometry and density of *dm*PDNA decatenation in the presence of no enzyme (line 1), *dm*TopIIα (line 2), *dm*TopIIα and EMS (line 3), *dm*TopIIα and genistein (line 4), *dm*TopIIα and hesperetin (line 5), *dm*TopIIα and EMS + hesperetin (line 6), and *dm*TopIIα and EMS + genistein (line 7). The lower part: The *dm*PDNA decatenation in the presence of no enzyme (line 1), *dm*TopIIα (line 2), *dm*TopIIα and EMS (line 3), *dm*TopIIα and genistein (line 4), *dm*TopIIα and hesperetin (line 5), *dm*TopIIα and EMS + hesperetin (line 6), and *dm*TopIIα and EMS + genistein (line 7) quantified by means of the ethidium bromide-stained agarise gel electrophoresis; (B) The double-stained *dm*PDNA breaks in the presence of enzyme (1), *dm*TopIIα (2), *dm*TopIIα and EMS (3), *dm*TopIIα and genistein (4), *dm*TopIIα and hesperetin (5), *dm*TopIIα and EMS + hesperetin (6), and *dm*TopIIα and EMS + genistein (7); (C) The audioradiograms of *dm*PDNA cleavage site utilization in the presence of no enzyme (line 1), *dm*TopIIα (line 2), *dm*TopIIα and EMS (line 3), *dm*TopIIα and genistein (line 4), *dm*TopIIα and hesperetin (line 5), *dm*TopIIα and EMS + hesperetin (line 6), and *dm*TopIIα and EMS + genistein (line 7).

Results and discussion

The *dm*TopIIα inhibition has been confirmed employing enzymatic assaying on dmTopIIα's ATPase level

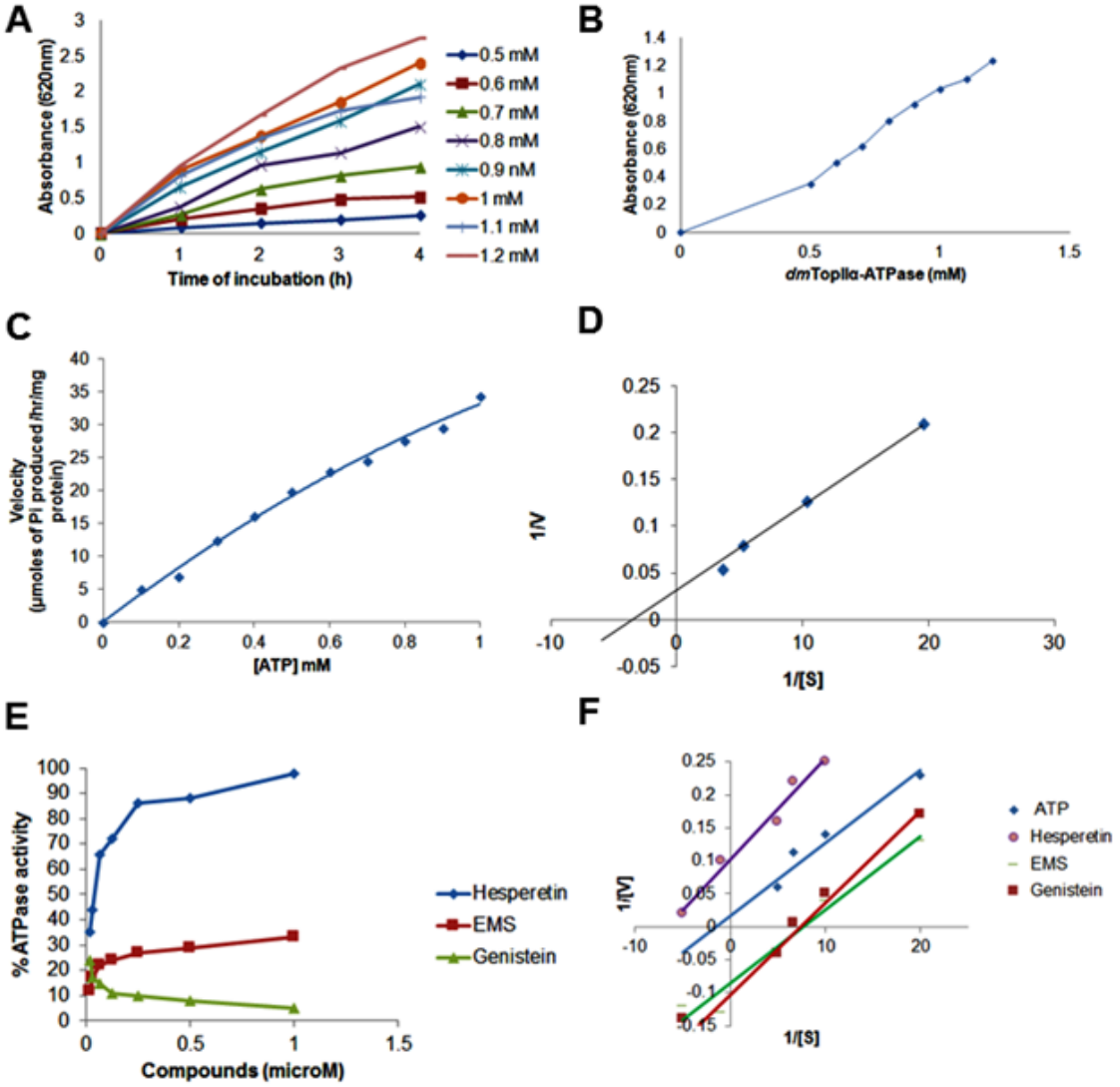


Figure 2. (A) The ATPase activity of dmTopIIα determined by the malachite green assay as a function of time and protein. (B) Data from (A) plotted as dmTopIIα-ATPase activity against protein for the 2 h time point. (C) Catalytic activity of the purified ATP-binding domain of dmTopIIα following Michaelis–Menten equation. (D) Double reciprocal Lineweaver–Burk plot (1/V) versus (1/S) is also shown. Dose-response curve for inhibition of dmTopIIα-ATPase domain activity by EMS and hesperetin (E). Double reciprocal plot of 1/[V] versus 1/[S] in presence of EMS and hesperetin (F).

Results and discussion

The *dm*TopIIα inhibition has been confirmed employing the fluorescence assaying on *dm*TopIIα's DNA-Binding and Cleavage Region level.

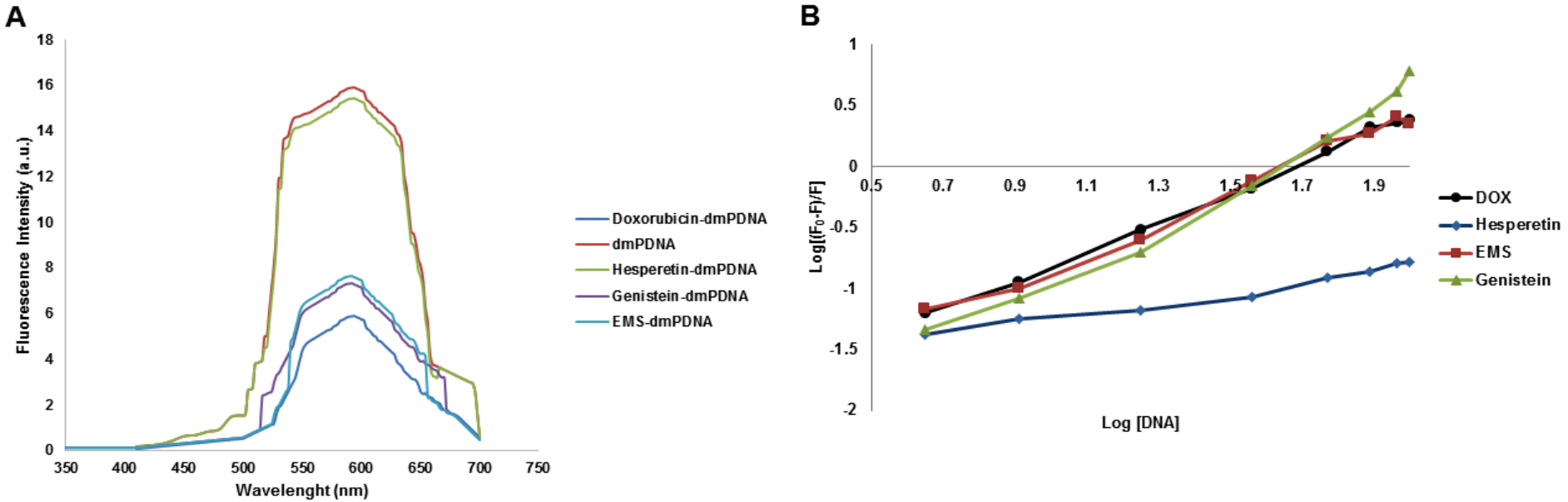
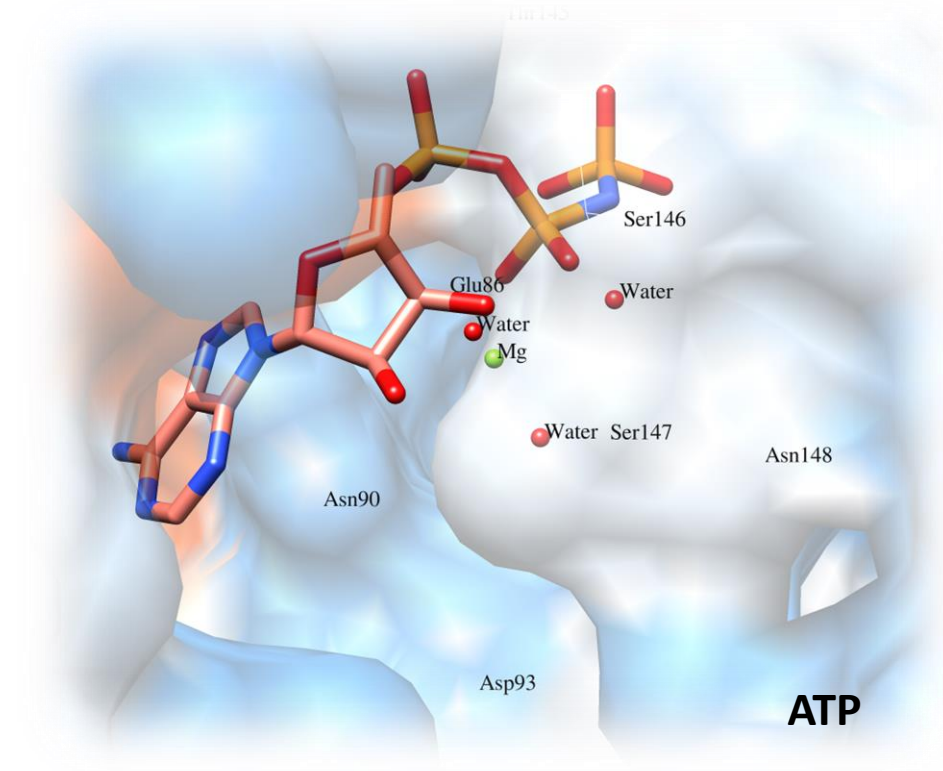


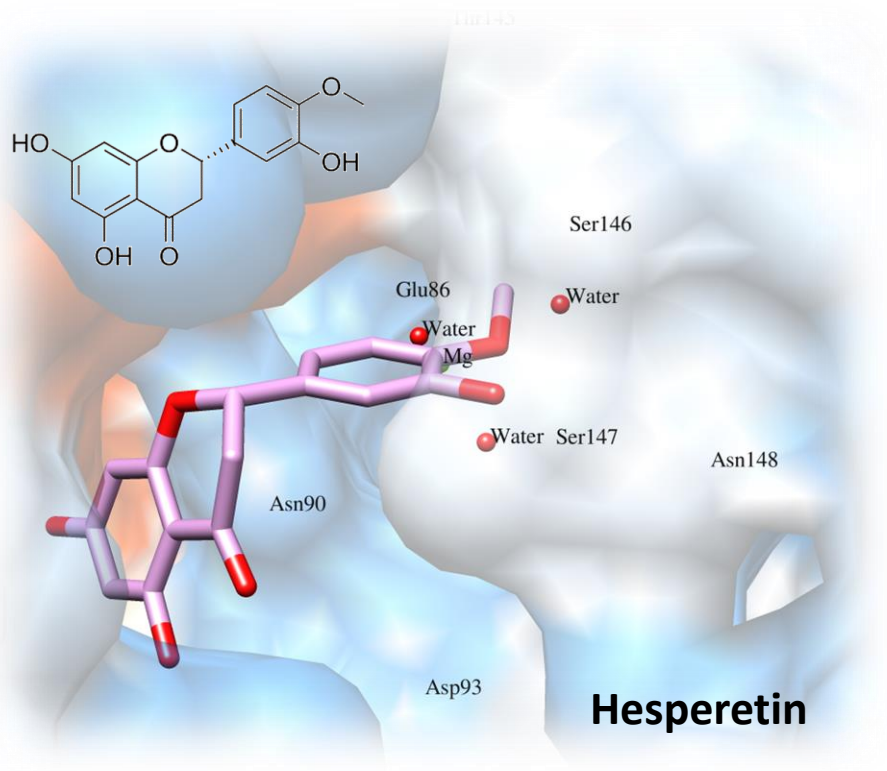
Figure 3. (A) Fluorescence emission spectra of either *dm*PDNA, *dm*PDNA-doxorubicin, *dm*PDNA-EMS, *dm*PDNA-genistein or *dm*PDNA-hesperetin systems in 10 mM Tris–HCl buffer pH 7.4 at 25°C. (B) The plot of $\log(F_0\text{-}F)/F$ as a function of log[DNA] for calculation of number of bound drug molecules (*n*) per *dm*PDNA.

Results and discussion

As an antigenotoxic agent, hesperetin exerted dual pharmacology: within the *dm*TopIIα hesperetin acted as an ATPase uncompetitive inhibitor (as confirmed by spectrophotometric studies), denying the *dm*TopIIα energy for enzyme-catalized cleavage of double-strand containing the G=T and T=G pairings (as certified by molecular docking).



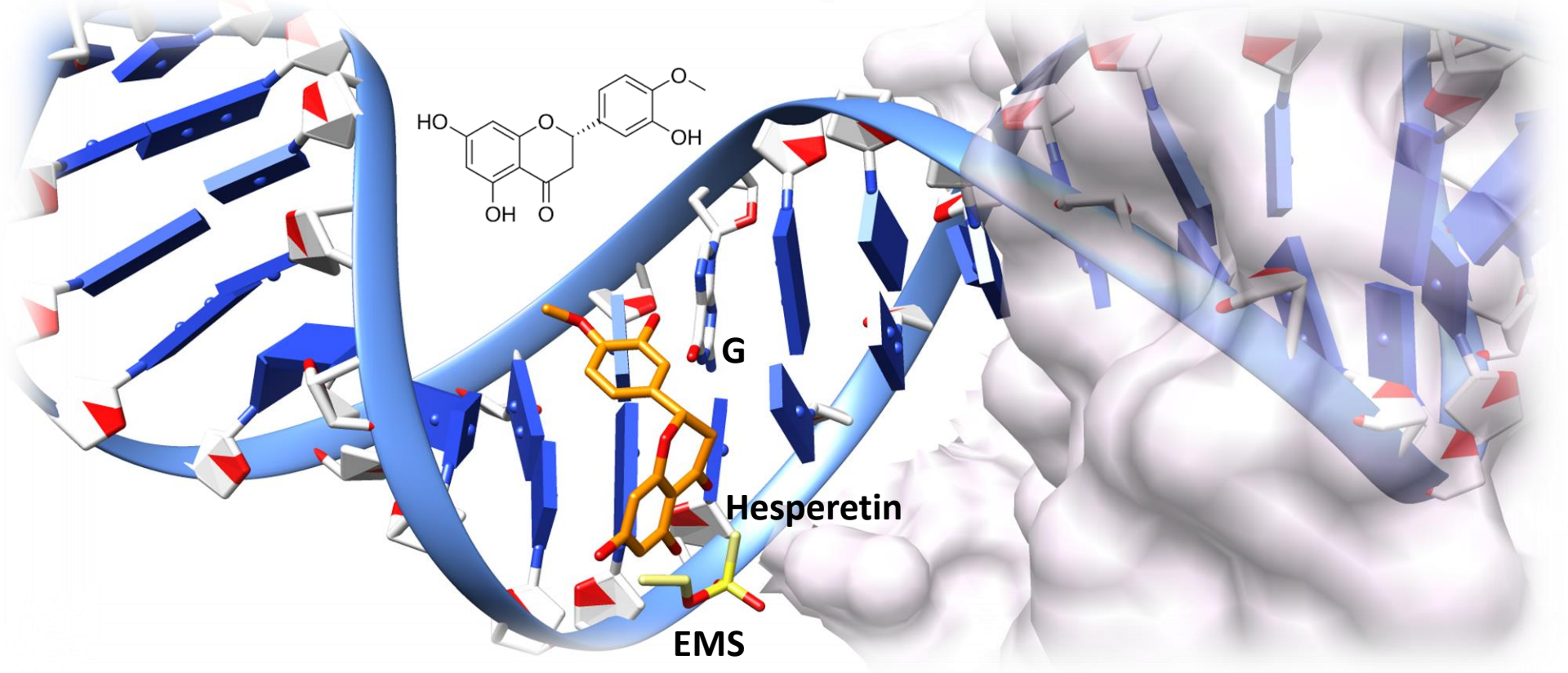
ATP



Hesperetin

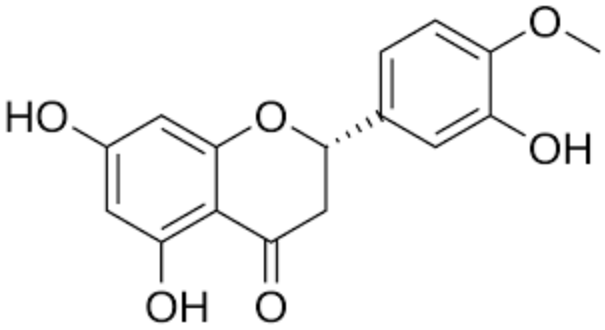
Results and discussion

As an antigenotoxic agent, hesperetin exerted dual pharmacology: within the *dm*TopIIα DNA-Binding and Cleavage Region hesperetin exerted no intercalating features (as verified by fluorescence quenching) but instead and blocked the EMS approach to either guanine and thymine, prevented the alkylation, and consequent *dmT*yr803-catalysed cleavage of normal double-strand (as certified by molecular docking).



Conclusions

Conclusively, hesperetin could be used as a supplement for alkylating agent-based cancer therapy in terms of preventing the alkylation agent to cause unnatural lesions and aberrant pairing.



Acknowledgments

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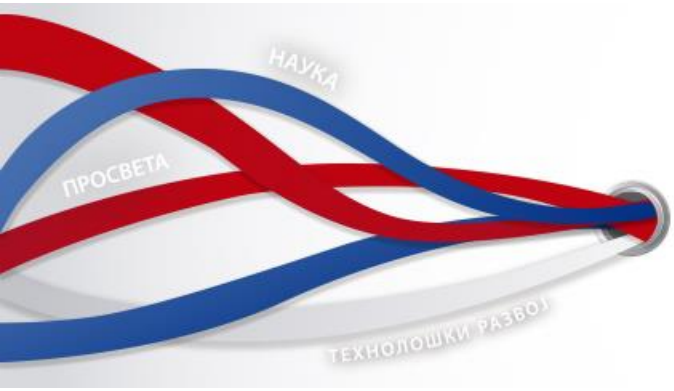

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