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# EXPLORING THE DNA INTERCALATING POTENTIAL OF GLYCOSYLATED TETRATHIAFULVENE (TTF) AND DITHIOLENE DERIVATIVES

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**abstract:** The DNA intercalating potential of the previously prepared water-soluble glycosylated tetrathiafulvene (TTF) and dithiolene derivatives was assessed through comparative docking analysis using doxorubicin as the reference molecule. Comparing the binding orientations and statistical evaluation of the ten lowest energy poses of each molecule with doxorubicin revealed that the glycosylated TTF derivatives are more likely to possess DNA intercalating activity since they posses lower energy values although adopted a different binding orientation. Results obtained will provide important preliminary insight on the possible biological applications of these glycoconjugates and may aid further development of these compounds for the intention of utilizing them as anti-tumor agents.

**key words:** tetrathiafulvalene; dithiolene; DNA intercalation; docking.

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## 1. Introduction

Tetrathiafulvalene (**TTF**) and its corresponding half-analogue, dithiolene are organo-sulfur compounds which have found widespread applications as molecular conductors due to its aromatic nature [1]. The π-accepting and π-donating properties of **TTF** have been extensively exploited to be used as sensors [2], electrodes [3] and redox agents [4]. However, its limited solubility in water has confined its applications mostly for these purposes and has restricted its potential utility in biological systems. In order to circumvent this restriction and to fully exploit the properties of **TTF** and its analogue, the derivatization of **TTF** [5] and dithiolene with glucose [6] have been previously conducted (Figure 1). These accounts have opened new frontiers for **TTF** chemistry since the resulting conjugates exhibit optimum solubility in water and can thus be evaluated for any biological activity they may possess.

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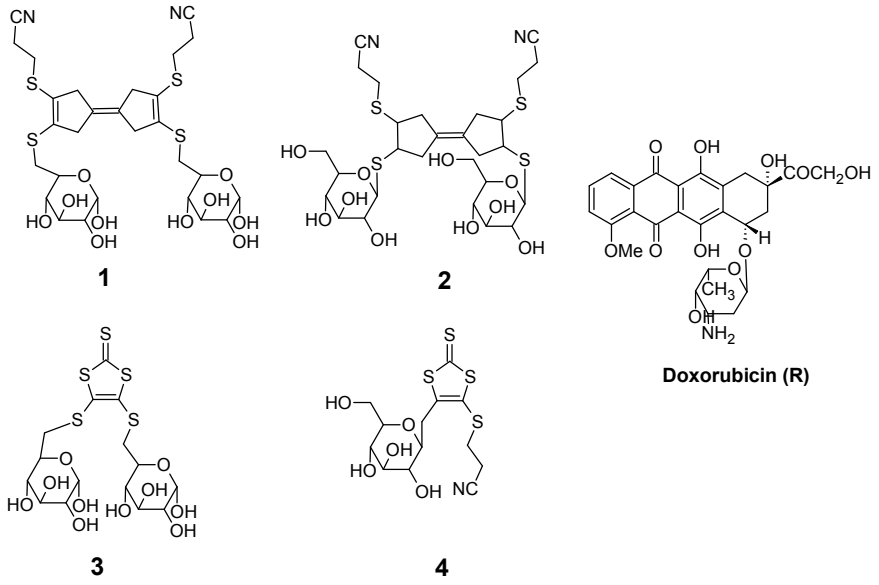



**Fig. 1** *Structures of the water-soluble TTF (1,2) and dithiolene (3,4) glycoconjugates and doxorubicin.*

The study presented herein aims to evaluate the **DNA** intercalating potential of the water-soluble **TTF** and dithiolene glycoconjugates by comparing its docking score with a known **DNA** intercalating agent, doxorubicin. The similarity of the planar structure of the TTF and dithiolene glycoconjugates with doxorubicin presents a rational motivation that the former class of molecules may exhibit **DNA** intercalating activity as well. Molecular docking has been extensively used in the drug design process wherein it can be used to screen viable compounds [7] as well as to study ligand-receptor interactions [8]. Results obtained will provide important preliminary insight on the possible biological applications of these glycoconjugates and may aid further development of these compounds for the intention of utilizing them as anti-tumor agents.

## 2. Methodology

### Geometry Optimization

The structures of the molecules to be docked were individually drawn using the molecular builder kit of ArgusLab 4.0.1 [9]. Each of the molecules was structurally refined using the Universal Force Field (UFF) through molecular mechanics calculations. The resulting structures were further optimized through geometry optimization calculations at the semi-empirical level of theory utilizing the AM1 hamiltonian. The restricted shell calculations were repeated until convergence was achieved for each. The geometry optimized molecules exhibited the expected aromatic and planar structure, which signifies the validity of the methodology.

## Molecular Docking

The geometry optimized molecules were then individually docked onto a **DNA** segment [10] retrieved from the Protein Data Bank using Hex 6.3 Docking software [11]. The docking parameters were set to include non-covalent, ligand-receptor interactions in order to obtain a comprehensive account of the different possible docking poses of the ligands onto the receptor. The results provided by Hex 6.3 are arbitrary energy values which serve as the score and determine which among the given solutions is the best fit. The best solution is judged as to which energy value possesses the greatest magnitude in the negative order. The image of the best solution for each docked model was analyzed, but the 10 lowest energy models were recorded as well and were subjected to statistical comparison.

## Statistical Comparison

The 10 lowest energy models for each docked molecule were subjected to statistical comparison using the *t test* utilizing the values obtained from the docked model of doxorubicin as the standard. Variance homogeneity was first established by conducting Levene's test. All statistical calculations were conducted with a significance level of 0.05 using Statistica 9 (Statsoft) [12] as the software.

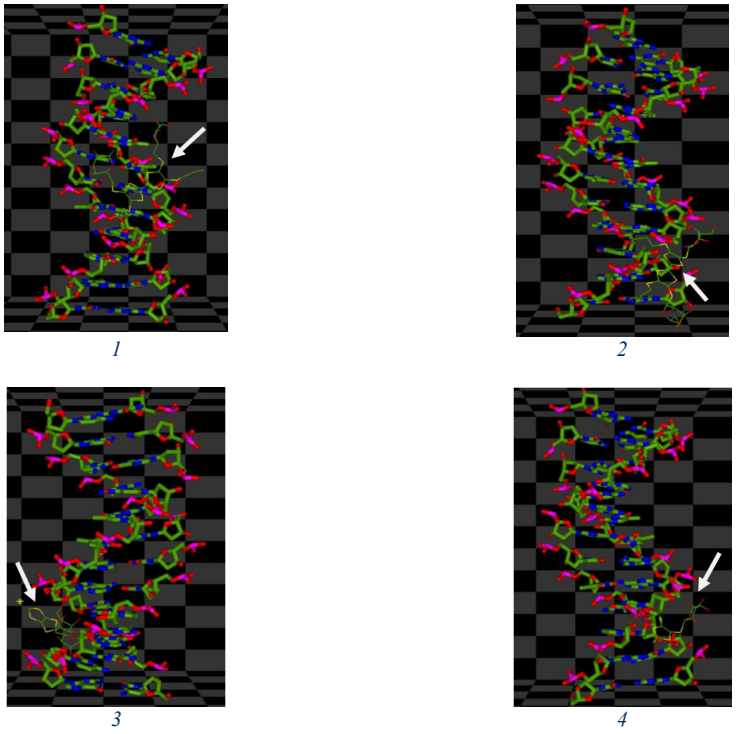
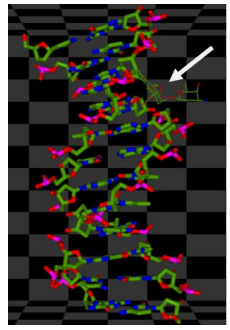



**Fig. 2** *Lowest energy models for the docked molecules, arrow shows the corresponding ligands*
(From the top, left to right: (1), (2), (3), (4), (R))



*R*

**Fig. 2** *continued.*

## 3. Results and Discussion

The geometry-optimized molecules were successfully docked onto the **DNA** segment which acts as the receptor for the docking analysis. The qualitative analysis of the docked models was based on the lowest energy poses for each molecule. Comparison of the binding orientation of the four molecules with the reference revealed that the dithiolene molecules (**3** & **4**) exhibited similarity with the reference molecule. Both the dithiolene derivatives and doxorubicin were found to orient themselves towards the minor groove of the **DNA** whereas the **TTF** derivatives (**1** & **2**) oriented themselves differently which was near the coil of the double helix.

Since assessing the binding orientation alone is insufficient to determine whether the **TTF** and dithiolene derivatives possess potential intercalating activity, the 10 lowest energy models for each docked molecule were statistically compared with the 10 lowest energy models of the reference molecule, doxorubicin.

**Table 1** Ten lowest energy-based docking scores for each of the docked molecules.

| Solution | 1 | 2 | 3 | 4 | R |
|---|---|---|---|---|---|
| 1 | –310.6 | –314.32 | –239.38 | –215.01 | –279.71 |
| 2 | –269.26 | –271.91 | –215.4 | –192.3 | –246.5 |
| 3 | –261.68 | –266.24 | –208.91 | –187.55 | –240.59 |
| 4 | –257.38 | –261.72 | –205.41 | –184.71 | –236.76 |
| 5 | –253.81 | –258.85 | –202.96 | –182.37 | –234.28 |
| 6 | –251.22 | –256.44 | –200.83 | –180.59 | –232.02 |
| 7 | –249.16 | –254.39 | –199.12 | –178.96 | –230.13 |
| 8 | –247.38 | –252.56 | –197.63 | –177.55 | –228.3 |
| 9 | –245.49 | –251.03 | –196.21 | –176.4 | –226.34 |
| 10 | –243.72 | –249.66 | –195.01 | –175.42 | –224.25 |
| Average | –258.97 | –263.712 | –206.086 | –185.086 | –237.888 |

The statistical comparison aims to acquire an extensive insight on the possible binding behaviour of the glycosylated derivatives onto the **DNA** since no experimental studies have been reported yet to verify that the lowest energy pose is the actual orientation in which the glycosylated derivatives adopt. The statistical comparison was commenced by first establishing variance homogeneity which is a pre-requisite prior for parametric tests can be conducted. A p-value that is greater than the significance level which is 0.05 is indicative that parametric tests such as the *t* test is a valid statistical analysis for the system of interest.

**Table 2** Summary of statistical analysis.

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| $P_{Levene}$ | 0.698 | 0.780 | 0.746 | 0.557 |
| *T* value | –2.610 | –3.263 | 4.810 | 8.350 |
| Conclusion | 1<R | 2<R | 3>R | 4>R |

Results of the Levene's test revealed that the average energy value of the individually docked molecules can be statistically compared with the reference molecule using the *t* test. Results of mean comparison using the *t* test indicated that the TTF derivatives possessed lower energy value, hence better fit, relative to the doxorubicin reference molecule which suggests that perhaps it can be an effective **DNA** intercalating agent. On the other hand, the dithiolene derivatives were determined to possess an energy value higher relative to doxorubicin. However, the percentage difference of the average energy values of **3** and **4** relative to the average energy value of the reference were only 14 % and 25%, respectively. It can thus be inferred that doxorubicin is a more potent **DNA** intercalating agent relative to the glycosylated dithiolene derivatives.

## 4. Conclusion

The results of both qualitative and quantitative analysis revealed that the glycosylated **TTF** derivatives exhibited more favourable attributes as a **DNA** intercalating agent relative to the doxorubicin reference molecule. Although the glycosylated **TTF** derivates adopted a different binding orientation with that of doxorubicin, it however possessed lower energy values as pointed out by the statistical comparison. The findings presented in this paper can serve as a starting point for further development of this class of compounds as **DNA** intercalating agents.

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