Design, Synthesis and Calf-Thymus DNA binding properties of highly functionalized Quinazolinones

Arunava Misra[§a], **Soumya Das**[§a] and Mohabul Alam Mondal[a]*

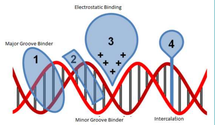
Department of Chemistry, Jadavpur University, Kolkata, 700032, INDIA

3rd Commonwealth Chemistry Posters
28-29 SEP 2022

Abstract: We have synthesized twelve new quinazolinone derivatives by a one-pot, intramolecular hydride transfer reaction starting from anthranilamide, glutaraldehyde, and aromatic amines. The developed method has advantages in terms of several green chemistry parameters such as atom economy, carbon efficiency, and mass efficiency E-factor. Kinetics of the DNA complex formation was measured by UV-Vis and fluorescence spectroscopy and it was found in the order of 10^4 M^{-1}. A thermal denaturation study revealed that the complex formation with the DNA and quinazoline resulted in enhanced stabilization of the DNA duplex. A model is constructed by docking study by using the AutoDoc Vina package to understand the bind interactions. All the experimental data are in agreement with the minor groove bind mode of interaction.

Introduction

- DNA, being a genetic material, has been identified as the primary target for developing therapeutics. Many molecules containing planar heterocyclic ring interacts with cellular DNA and such interactions are the basis to control different cellular functions including Genotoxicity and cytotoxicity.
- Drugs bind to DNA via several different mechanisms. The possible mode of interaction follows these models (**fig. 2**)
- However, there is growing interest that the small molecules consisting of heterocyclic scaffolds bind to cellular DNA in a reversible fashion and show significant potential to control tumor progression.
- There are several DNA-Binding drugs as anti-cancer and anti-tumor such as netropsin and distamysin which act as a minor-groove binding agent.[2]
- Quinazolinones exhibit diverse chemical activities and a wide range of biological properties. We hypothesized that the two different aromatic/heterocyclic moieties (**fig. 2**) within the molecules will show efficient binding potential with dsDNA and the spacer would assume a curved shape fit to the DNA it can even act as a double intercalator.[3]



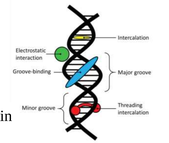


Figure 1. *Representation of DNA Bindings modes*

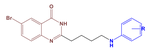


Figure 2. *Target molecule*

Methods

- A literature review was used to gather data and determine trends in anti-tumor drug discovery.
- Designing of novel and easy to synthesis DNA-Binding molecules.
- The synthesized molecules solution was made in DMSO and CT-DNA solution was made in Tris-HCl buffer. The experiments were conducted in 10mM Tris-HCl, 1mM EDTA (TE) buffer.
- UV-Vis and Fluorescence Spectrometry, CD spectrometry, Viscometry, Fluorescence Lifetime study, and Docking studies were performed to analyze the interaction with CT-DNA.

Synthesis

Quinazolinones are synthesized by the reaction with anthranilamide, glutaraldehyde, and amines in the presence of an acid catalyst. We have explored the method for synthesizing quinazolinones (6) having two different nitrogenous units attached via a 4-carbon spacer (**fig. 2**).

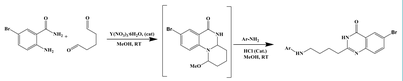


Figure 3. *Synthetic Scheme*

Synthetic route: The synthesis requires the coupling of anthranilamide at one end while the other end with a different amine (Ar-NH_2). Initially, we attempted a one-pot method under different reaction conditions. A stoichiometric mixture of the reactants (equal molar ratio) treated in methanol in presence of catalytic acid led to the formation of large numbers of unidentified products. However, we were able to isolate intermediate compound **5** from **1a** by using yttrium nitrate as a white solid in quantitative yield. Using compound **5**, we have synthesized a cross-coupled product where one end of the product is constructed from anthranilamide and the other from an aromatic amine. We have synthesized 12 (**6a-l**) different products as shown in **fig. 4**. The structure of the quinazolinone derivatives was confirmed by IR, NMR spectroscopy, and HRMS.[4]

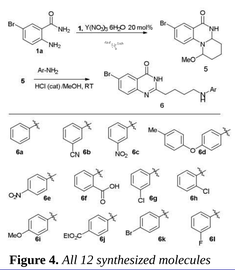


Figure 4. *All 12 synthesized molecules*

Green Chemistry Approach:

Our developed methodology has great advantages in terms of several green parameters, for example, atom economy of the synthesis of **6d** reached 93.70%; carbon efficiency of the reaction was 96%; reaction mass efficiency was observed up to 71.2%, and most importantly the E-factor was reduced to 0.39 (**Fig. 5**).

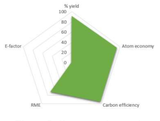


Figure 5. *Green matrix: a quick assessment of 6d synthesis.*

We used three (**6b, 6d & 6f**) of our synthesized molecules (**fig. 6**) to study the DNA binding.

We used several spectroscopic techniques to study the binding style.

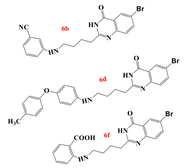


Figure 6. *Structures of analyzed molecules*

Results and Discussion

UV-VIS Study:

- These molecules with a constant conc. were titrated against an increasing conc. of CT-DNA to observe a change in the absorbance (**7. a**).
- Benesi-Hildebrand equation **Ao/(A-Ao) = εo/(εo-ε) + εo/{K_b(εo-ε)}[DNA]** was used to calculate K_b and the equation **ΔG = - RTlnK$_b$** was used to calculate Gibb's free energy change.
- The **Ao/(A-Ao)** value showed a linear relation to the **1/[DNA]** shown in **fig. 7(b)**.
- From the linear plot K_b was calculated and it was in the order of 10^4, which shows a strong binding interaction between CT-DNA and our synthesized molecules.
- The presence of isobestic point at 295nm is characteristic of more than one binding type.

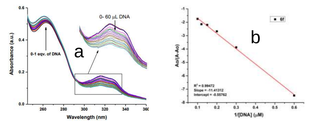


Figure 7. *UV-Vis Spectroscopy measurement of **6f** (a & b)*

Ligand	Abs. (nm)	Kb *10^4 (M^{-1})	ΔG kJ.mol^{-1}
6b	330	1.03	-22.85
6d	325	6.57	-27.48
6f	325	4.88	-26.75

Table 1. *Binding constant and free energy*

Fluorescence Study:

- These molecules were titrated against an increasing conc. of CT-DNA to observe a change in the fluorescence (**8. a**) when excited at 330 nm.
- We have calculated the binding constant (K_b) from the fluorescence titration by using the equation **Log[(F-Fo)/Fo] = K_b+ nLog[DNA]**.
- The **Log[(F-Fo)/Fo]** vs **Log[DNA]** value showed a linear relation, shown in **fig 8 (b)**.

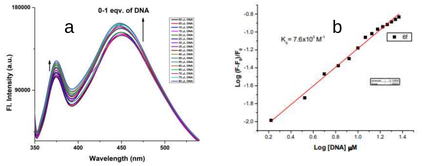


Figure 8. *FL Spectroscopy measurement of **6f** (a & b)*

Melting Point Study:

- The increased temperature of DNA solution weakens various binding forces, eventually separating the dsDNA into ssDNA (thermal denaturation of DNA), and the temperature at which half of the dsDNA structure is transformed into two individual ssDNA.
- This temperature could be determined by the UV-visible absorbance of DNA bases at 260 nm as a function of temperature ranging from 35° C to 95° C.
- The ligands (6b & 6f) made them stable by 6-7° C. Which shows the binding strength of the ligands, shown in **fig 9**.

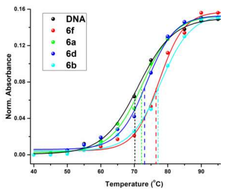


Figure 9. *Melting point study of 6a, 6b, 6d & 6f*

Viscosity Measurement:

- In the case of the intercalative mode of binding, the viscosity of the DNA solution increases; for the groove binding mode, the viscosity does not change significantly.
- A plot of (Ƞ/Ƞo)$^{1/3}$ versus **[6b, 6d & 6f]/[DNA]** was obtained to study any change in viscosity of CT-DNA solution in presence of 6b, 6d & 6f, shown in **figure 10**.
- It was observed that the variation of relative viscosity of the DNA-quinazolinones complexes is much less than that of EB dyes. This confirms that 6b, 6d, & 6f bind to DNA into the mixed / groove of the DNA helix.

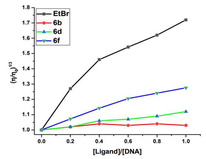


Figure 10. *Viscosity measurement of 6b, 6d , 6f &*

Competitive Binding Study:

- Competitive binding studies of unknown molecules with EB and Hoechst 33342 are extensively used to determine the mode of binding with CT-DNA. Small molecules with similar binding modes displace each other at low concentrations.
- EB 32 is a well-known intercalator display emission band at ~600 nm and shows enhancement when it intercalates into the dsDNA while Hoechst 33342 is a minor groove binder, that emits an enhanced fluorescence emission band at 450nm.
- Our molecules showed different behavior with both EB and Hoechst, we calculated Ksv using Stern-Volmer equation **F_0/F = 1 + Ksv[Q]**. where F_0 and F are the fluorescence intensity in the absence and presence of the molecule added [Q] respectively. Ksv is the Stern-Volmer quenching constant calculated from the slope of the [F_0/F] vs [Q] plot shown in fig. 11.
- A comparison study of Hoechst and EtBr study was done for all the molecules.

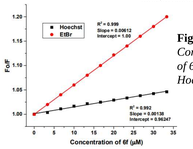


Figure 11. *Competitive binding of 6f with EB and Hoechst*

Sample	Ksv (Hoechst)	Ksv(EB)	Relative percentage of groove binding	Remarks
6b	28.41	1.38	95.3676	Mostly groove binding
6d	4.04	0.612	86.84437	Preferentially groove binding
6f	1.16	2.38	32.7684	Mix Binding, preferably intercalation

Table 2. *Competitive binding of 6b, 6d & 6f*

Circular Dichroism:

- CD spectra of the DNA in absence and presence of compound 6b, 6d, and 6f was measured.
- The characteristic negative band at 245nm and positive at around 275nm of B-DNA structure are observed in all spectra as shown in **fig. 12**.
- The CD spectrum of the DNA complex with the DNA itself suggests that the overall secondary structure of the complex has remained unaltered.
- However, the slight deviation in the ellipticity in the range 275nm and 245nm is due to the non-covalent interactions of the DNA helices with the quinazolinones via minor-groove binding.

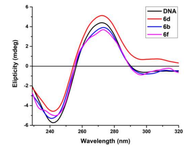
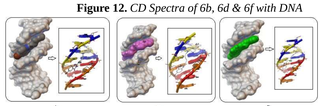


Figure 12. *CD Spectra of 6b, 6d & 6f with DNA*

Docking Studies:

Molecule docking poses of **6b** (black), **6d** (pink), and **6f** (green) in the minor groove of the DNA dodecamer are shown below. The inset diagram of each shows the different points of interactions and close contact with the quinazolinones and DNA backbone residues.



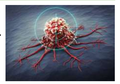
Scheme 12. *Docking Studies*

Conclusion

Using a highly medicinal useful moiety like quinazoline which could be synthesized with a one-pot reaction herein we reported 12 quinazolinone molecules having strong bonding with CT-DNA. We found a considerable substituent effect but they are binding through the minor grooves of the double helix structure of CT-DNA.

Ongoing / Future Work

- These synthesized molecules are being used to study for their anti-tumor and anti-cancer properties.
- Anti-microbial properties of these molecules will also be investigated.



References

1. Biebricher, A., Heller, I., Roijmans, R. *et al. Nat Commun* **6**, 7304 (**2015**)
2. Kopka *et al.* Proc. Nati. Acad. Sci. USA Vol. 82, pp. 1376-1380 (March **1985**)
3. Glazer, A. *et al. Nature* **359**, 859–861 (**1992**).
4. Mondal, M. A. *et al.* ChemistrySelect **2021**, 6, 11788
5. Hemant Sharma *et al. ACS Omega* **2019** *4* (3), 5829-5838

*mohabula.mondal@jadavpuruniversity.ac.in
§Equal Contribution