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Spectroscopic Investigation into Minor Groove Binders Designed to Selectively Target DNA Sequences

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jwalton13@gsu.edu

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SPECTROSCOPIC INVESTIGATION INTO MINOR GROOVE BINDERS DESIGNED TO
SELECTIVELY TARGET DNA SEQUENCES

by

JOSEPH WALTON

Under the Direction of W. David Wilson, PhD

ABSTRACT

Recently, there has been increasing focus toward the development of small molecules designed to
target a specific sequences of double stranded DNA for therapeutic purposes\(^1\). Minor groove
binding compounds have been shown to be capable of selectivity target GC sites in AT tract DNA\(^2\).
In this research, binding selectivity was investigated using absorption, fluorescence and circular
dichroic properties of selected DB minor groove binders in the presence of two unique DNA
sequences. Further insight was gained by comparing the electrostatic potential maps and optimized
structures of the compounds of interest. Using the results presented, potential selective minor
groove binders can be selected for further investigation and kinetic studies.

INDEX WORDS: DNA, Sequence recognition, Heterocyclic dications, Minor groove binding,
Fluorescence, Circular dichroism, Molecular modeling
SPECTROSCOPIC INVESTIGATION INTO MINOR GROOVE BINDERS DESIGNED TO
SELECTIVELY TARGET DNA SEQUENCES

by

JOSEPH WALTON

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of
Masters of Science
in the College of Arts and Sciences
Georgia State University
2015
SPECTROSCOPIC INVESTIGATION INTO MINOR GROOVE BINDERS DESIGNED TO SELECTIVELY TARGET DNA SEQUENCES

by

JOSEPH WALTON

Committee Chair: W. David Wilson

Committee: David Boykin

Ivaylo Ivanov

Electronic Version Approved:

Office of Graduate Studies
College of Arts and Sciences
Georgia State University
December 2015
Dedication

To my Family whose support has allowed me to follow my dreams no matter what
Acknowledgement

I would like to thank Kathryn T. Savitz who helped me run and calculate the Molar Extinction Coefficients.
# Table of Contents

Dedication.............................................................................................................................................. v

Acknowledgement.................................................................................................................................. vi

Table of Contents.................................................................................................................................. vii

List of Tables ........................................................................................................................................... ix

List of Figures .......................................................................................................................................... x

List of Equations ..................................................................................................................................... xiii

1. Introduction ......................................................................................................................................... 1
   1.1 Minor Groove Binding Compounds ................................................................................................. 1
   1.2 Molar Extinction Coefficients ......................................................................................................... 3
   1.3 Fluorescence Spectroscopy ............................................................................................................. 4
   1.4 Circular Dichroism .......................................................................................................................... 6
   1.5 Computational Chemistry ................................................................................................................ 9

3. Methods ............................................................................................................................................... 15
   3.1 Molecular Absorptivity .................................................................................................................. 15
   3.2 Fluorescence Spectroscopy ........................................................................................................... 15
   3.3 Circular Dichroism ........................................................................................................................ 17
   3.4 Molecular Modeling ....................................................................................................................... 17

4. Results ................................................................................................................................................. 18
   4.1 Molar Extinction Coefficients ......................................................................................................... 18
   4.2 Category 1 Compounds .................................................................................................................. 19
      4.2.1 Molecular Modeling ................................................................................................................. 19
      4.2.2 Fluorescence Titrations .......................................................................................................... 20
      4.2.3 Circular Dichroism .................................................................................................................. 28
   4.3 Category 2 Compounds .................................................................................................................. 36
      4.3.1 Molecular Modeling ................................................................................................................. 36
      4.3.2 Fluorescence Spectroscopy .................................................................................................... 37
      4.3.3 Circular Dichroism .................................................................................................................. 45
4.4 Category 3 Compounds .................................................................................................................. 53
  4.4.1 Molecular Modeling .................................................................................................................. 53
  4.4.2 Fluorescence Spectroscopy ...................................................................................................... 54
  4.4.3 Circular Dichroism ................................................................................................................... 62

5. Results .............................................................................................................................................. 70
  5.1 Structure Comparison ................................................................................................................. 70
  5.2 Category 1 Compounds ............................................................................................................... 72
  5.3 Category 2 Compounds ............................................................................................................... 74
  5.4 Category 3 Compounds ............................................................................................................... 75

6. Discussion ......................................................................................................................................... 77

7. Conclusion .......................................................................................................................................... 80

References ............................................................................................................................................ 82
List of Tables

Table 1 Fluorescence spectroscopy parameters for each DB compound .......................................................... 16

Table 2. Molar extinction coefficients ........................................................................................................ 18
List of Figures

Figure 1. (a) DNA binding (b) Major and minor grooves of DNA base pairs ........................................... 2
Figure 2. Structures of three DNA minor groove-binding compounds .......................................................... 3
Figure 3. Jabłoński diagram ......................................................................................................................... 5
Figure 4. Circularly polarized light ................................................................................................................ 8
Figure 5. (a) DNA sequences (b) Compounds synthesized by the Boykin group ........................................ 14
Figure 7. Spectral representation of a molecule excited at one wavelength .................................................... 16
Figure 8. Ball and stick models and electrostatic potential maps for category 1 compounds ......................... 19
Figure 9. (a) Fluorescence titration of DB 2324 with 5’-AAAATTT-3’ (b) Structure of DB 2324 ............... 20
Figure 10. (a) Fluorescence titration of DB 2324 with 5’-AAAGTTT-3’ (b) Structure of DB 2324 ............. 21
Figure 11. (a) Fluorescence titration of DB 2363 with 5’-AAAATTT-3’ (b) Structure of DB 2363 .......... 22
Figure 12. (a) Fluorescence titration of DB 2363 with 5’-AAAGTTT-3’ (b) Structure of DB 2363 .......... 23
Figure 13. (a) Fluorescence titration of DB 2364 with 5’-AAAATTT-3’ (b) Structure of DB 2364 .......... 24
Figure 14: (a) Fluorescence titration of DB 2364 with 5’-AAAGTTT-3’ (b) Structure of DB 2364 .......... 25
Figure 15. (a) Fluorescence titration of DB 2368 with 5’-AAAATTT-3’ (b) Structure of DB 2368 .......... 26
Figure 16. (a) Fluorescence titration of DB 2368 with 5’-AAAGTTT-3’ (b) Structure of DB 2368 .......... 27
Figure 17. (a) Circular dichroism of 5’-AAAATTT-3’ titrated with DB 2324 (b) Structure of DB 2324 . 28
Figure 18. (a) Circular dichroism of 5’-AAAGTTT-3’ titrated with DB 2324 (b) Structure of DB 2324. 29
Figure 19. (a) Circular dichroism of 5’-AAAATTT-3’ titrated with DB 2363 (b) Structure of DB 2363. 30
Figure 20. (a) Circular dichroism of 5’-AAAGTTT-3’ titrated with DB 2363 (b) Structure of DB 2363. 31
Figure 21. (a) Circular dichroism of 5’-AAAATTT-3’ titrated with DB 2364 (b) Structure of DB 2364. 32
Figure 22. (a) Circular dichroism of 5’-AAAGTTT-3’ titrated with DB 2364 (b) Structure of DB 2364. 33
Figure 23. (a) Circular dichroism of 5’-AAAATTT-3’ titrated with DB 2368 (b) Structure of DB 2368. 34
Figure 24. (a) Circular dichroism of 5'-AAAGTTT-3' titrated with DB 2368 (b) Structure of DB 2368.
Figure 25. Ball and stick models and electrostatic potential maps for category 2 compounds.
Figure 26. (a) Fluorescence titration of DB 2309 with 5'-AAAATTT-3' (b) Structure of DB 2309.
Figure 27. (a) Fluorescence titration of DB 2309 with 5'-AAAGTTT-3' (b) Structure of DB 2309.
Figure 28. (a) Fluorescence titration of DB 2325 with 5'-AAAATTT-3' (b) Structure of DB 2325.
Figure 29. (a) Fluorescence titration of DB 2325 with 5'-AAAGTTT-3' (b) Structure of DB 2325.
Figure 30. (a) Fluorescence titration of DB 2327 with 5'-AAAATTT-3' (b) Structure of DB 2327.
Figure 31. (a) Fluorescence titration of DB 2327 with 5'-AAAGTTT-3' (b) Structure of DB 2327.
Figure 32. (a) Fluorescence titration of DB 2328 with 5'-AAAATTT-3' (b) Structure of DB 2328.
Figure 33. (a) Fluorescence titration of DB 2328 with 5'-AAAGTTT-3' (b) Structure of DB 2328.
Figure 34. (a) Circular dichroism of 5'-AAAATTT-3' titrated with DB 2309 (b) Structure of DB 2309.
Figure 35. (a) Circular dichroism of 5'-AAAATTT-3' titrated with DB 2309 (b) Structure of DB 2309.
Figure 36. (a) Circular dichroism of 5'-AAAATTT-3' titrated with DB 2325 (b) Structure of DB 2325.
Figure 37. (a) Circular dichroism of 5'-AAAGTTT-3' titrated with DB 2325 (b) Structure of DB 2325.
Figure 38. (a) Circular dichroism of 5'-AAAATTT-3' titrated with DB 2328 (b) Structure of DB 2328.
Figure 39. (a) Circular dichroism of 5'-AAAGTTT-3' titrated with DB 2328 (b) Structure of DB 2328.
Figure 40. (a) Circular dichroism of 5'-AAAATTT-3' titrated with DB 2328 (b) Structure of DB 2328.
Figure 41 (a) Circular dichroism of 5'-AAAGTTT-3' titrated with DB 2328 (b) Structure of DB 2328.
Figure 42. Ball and stick models and electrostatic potential maps for category 3 compounds.
Figure 43. (a) Fluorescence titration of DB 2326 with 5'-AAAATTT-3' (b) Structure of DB 2326.
Figure 44. (a) Fluorescence titration of DB 2326 with 5'-AAAGTTT-3' (b) Structure of DB 2326.
Figure 45. (a) Fluorescence titration of DB 2348 with 5'-AAAATTT-3' (b) Structure of DB 2348.
Figure 46. (a) Fluorescence titration of DB 2348 with 5'-AAAGTTT-3' (b) Structure of DB 2348.
Figure 47. (a) Fluorescence titration of DB 2350 with 5'-AAAATTT-3' (b) Structure of DB 2350.
Figure 48. (a) Fluorescence titration of DB 2350 with 5’-AAAGTTT-3’ (b) Structure of DB 2350........59
Figure 49. (a) Fluorescence titration of DB 2351 with 5’-AAAATTT-3’ (b) Structure of DB 2351........60
Figure 50. (a) Fluorescence titration of DB 2351 with 5’-AAAGTTT-3’ (b) Structure of DB 2351........61
Figure 51. (a) Circular dichroism of 5’-AAAATTT-3’ titrated with DB 2326 (b) Structure of DB 2326. 62
Figure 52. (a) Circular dichroism of 5’-AAAGTTT-3’ titrated with DB 2326 (b) Structure of DB 2326. 63
Figure 53. (a) Circular dichroism of 5’-AAAATTT-3’ titrated with DB 2348 (b) Structure of DB 2348. 64
Figure 54. (a) Circular dichroism of 5’-AAAGTTT-3’ titrated with DB 2348 (b) Structure of DB 2348. 65
Figure 55. (a) Circular dichroism of 5’-AAAATTT-3’ titrated with DB 2350 (b) Structure of DB 2350. 66
Figure 56. (a) Circular dichroism of 5’-AAAGTTT-3’ titrated with DB 2350 (b) Structure of DB 2350. 67
Figure 57. (a) Circular dichroism of 5’-AAAATTT-3’ titrated with DB 2351 (b) Structure of DB 2351. 68
Figure 58. (a) Circular dichroism of 5’-AAAGTTT-3’ titrated with DB 2351 (b) Structure of DB 2351. 69
List of Equations

Equation 1. Beer-Lambert Law

................................................................. 3
1. Introduction

1.1 Minor Groove Binding Compounds

Errors in gene expression are responsible for numerous common human diseases\(^1,3\). A comprehensive understanding of the biochemistry helps to identify irregularities in genetic expression pathways. Beginning at DNA transcription and ending with protein transcription, each step along this pathway is a potential target for therapeutic agents that can identify abnormalities. Recently, there has been increasing amount of focus toward the development of molecules designed to target a specific sequence of DNA at the beginning of this process\(^4\). These molecules then have the ability to influence gene expression.

Chemical agents that target double-stranded DNA can do so by two different methods of interaction (Figure 1). Intercalators, such as ethidium bromide, bind to target DNA by stacking between the planar base pairs in DNA. Distinct from intercalation, groove binders target the major or minor groove of the DNA helix. In B-form DNA, the major groove is wider and deeper than the minor groove making it the preferred target for DNA binding proteins\(^5\). This leaves the narrower minor groove available for interaction with small molecules.
Figure 1. (a) Possible ways in which molecules can interact with DNA. Intercalation by polycyclic aromatic ring structures shown in red. Major groove binding agents, such as proteins, shown in green. Minor groove binding small molecules represented in light blue. (b) Major and minor groove sides of DNA base pairings with Watson-Crick hydrogen bonds represented by dashed lines.

Minor groove binding compounds are typically small molecules that have an ability to adapt to the native curvature of DNA backbone. Generally, these small molecules are heterocyclic, aromatic structures that form hydrogen bonds with DNA base pairs. Molecules that align favorably will also form van der Waals interactions with the groove, furthering binding stability. Binding sequence selectivity is often attributed to these binding patterns. Classical minor groove binding molecules such as DAPI, distamycin and Hoechst 33258 (Figure 2) have historically have shown a preference for AT base pairs. This is attributed to the higher electrostatic potential at AT rich regions, which aids in binding for cationic compounds such as the monocationic polyamide distamycin and the dicationic indole DAPI. At GC steps, the amino group of guanines protrudes into the minor groove hindering favorable stabilizing van der Waals interactions.
1.2 Molar Extinction Coefficients

Molar extinction coefficients, also known as molar absorptivity or molar attenuation coefficients, measure the strength of light absorption at a given wavelength. This intrinsic chemical property is determined by measuring absorbance using ultraviolet and visible light (UV/Vis) spectroscopy. The Beer-Lambert law allows the molar extinction coefficient to be calculated from experimental data. The Beer-Lambert law states that absorbance is equal to the concentration of a species multiplied by the path length of the cuvette and molar extinction coefficient. This implies that the absorbance is directly proportion to both the path length of the cuvette and the concentration of the sample.

\[ A = c \lambda \epsilon \]

Equation 1. Beer-Lambert Law

In Equation 1, \( c \), is the concentration of the sample, \( l \), is the path length defined as the distance the light wave travels through the cuvette, while \( A \), is the measured absorbance of the sample. The molar extinction coefficient, \( \epsilon \), can be solved for from the previously defined
variables. Once the value of \( \epsilon \) is solved for from a known concentration it can be used to determine an unknown concentration of a given chemical species\(^9\).

### 1.3 Fluorescence Spectroscopy

Light emission can provide insight to properties that are different from those properties related to light absorption\(^{10}\). When a molecule enters an excited electric state, one electron occupies a higher energy level. In order for it to return to the lower energy ground state configuration, the absorbed energy has to be released either in the form of heat or light. Luminescence is the emission of light from a substance that does not produce heat when an electron becomes excited by the absorption of a photon. Species that luminesce can do so in two processes: fluorescence or phosphorescence. To return to the ground state energy level, one of three things may occur: (1) it can fall back to the ground state from the singlet state after internal conversion, resulting in fluorescence, (2) emit a photon, (3) or it can convert to triplet state by intersystem crossing\(^{11}\). In the triplet state scenario, the electron again has three possible paths: (i) it can fall back to the ground state, resulting in phosphoresce, (ii) emit a photon (iii) or undergo a non-radiative transition to the ground level. The various types of energy level transitions are depicted in the Jabłoński diagram (Figure 3)\(^{10,11}\).
Three of the possible energy transfers do not result in luminescence. One such transfer is vibrational relaxation\textsuperscript{10,11}. Vibrational relaxation, represented as black arrows in Figure 3, is the process by which energy is transferred to other vibrational levels as kinetic energy\textsuperscript{10,11}. Internal conversion is another non-luminescent process by which the energy absorbed can be transferred to a lower energy level. It occurs when energy is transferred between overlapping vibrational and electronic energy levels and is depicted in the above Jabłoński diagram by the cyan arrow between $S_2$ and $S_1$ energy states. The final non-radiative energy conversion is an intersystem crossing. In this process, energy is transferred from a higher excited state to a lower excited state in conjunction with a change in spin orientation\textsuperscript{11}. It is depicted as a cyan arrow between $S_1$ and $T_1$ in Figure 3. This process is the rarest of all three non-radiative energy transfers and is referred to as “forbidden”\textsuperscript{11}. 

Figure 3. Jabłoński diagram\textsuperscript{12}
After intersystem crossing to the triplet state, an electron can return to the ground state by phosphorescence\textsuperscript{11}. Intersystem crossing to the triplet state occurs extremely rarely because it requires changing the orientation of a spin so that the unpaired ground state electron and excited electron are symmetric\textsuperscript{11}. This can occur in three separate ways in that the unpaired ground state electron and excited electron can both have spin up or spin down, or the sum of the paired spin states is symmetric. The observed emission rates are extremely slow for phosphorescence, on the order of $10^{-2}$ to $10^{-4}$ s\textsuperscript{11}. Fluorescence, on the other hand, occurs after singlet state transitions. The spins of the unpaired ground state electron and excited electron remain antisymmetric and the emission is considered an “allowed” transition\textsuperscript{11}. This type of transition happens rapidly, on the magnitude of $10^{-8}$ s for photon emission\textsuperscript{11}.

Fluorescence spectroscopy can be used for a number of biochemical applications including binding studies. The relative changes in fluorescence during a titration can indicate possible interactions occurring and both the binding affinity and kinetics can be calculated from this data\textsuperscript{11}. Due to the highly conjugated nature of aromatic compounds and their ability to act as chromophores, fluorescence may readily occur after photon absorption; however, even if the compound of interest does not have intrinsic fluorescence, it can still be studied using fluorescence spectroscopy using a fluorescently labeled tag\textsuperscript{13}.

1.4 Circular Dichroism

Due to their asymmetry, most biomolecules are natively optically active. Optical activity is also seen in many small molecules due to the presence of asymmetric carbon atoms and their interaction on conjugated ring systems\textsuperscript{11}. Optical activity can be measured in two ways, the optical rotatory dispersion (ORD) and the circular dichroism (CD). Passing a linearly polarized traverse wave through an asymmetric structure will cause the resultant wave to change in amplitude and
orientation in comparison to the incident wave\textsuperscript{10,11}. The amplitude of the electric field is no longer restricted to the perpendicular plane of the propagation and it creates an elliptical screw axis\textsuperscript{11}. The resultant ellipticity is measured as the arc tangent of the ratio of the minor axis to the major axis of the projected ellipse.

The orientation of the ellipse is also an indicator of optical activity. As a result of the change in the amplitude, the elliptical screw axis is no longer parallel to the incident light. At the limit of optical absorption where the axis ratio approaches zero, the resultant wave is equivalent to plane polarized light. Thus, the measurement of the orientation of the ellipse corresponds to the optical rotation. This optical rotation is measured as a function of the wavelength is called optical rotary dispersion (ORD)\textsuperscript{11}. The resultant electric field can be decomposed into the two components of circular polarization. This is useful to compare the differences in absorptions between left and right handed circularly polarized light. The difference between these two absorptions is called circular dichroism (CD)\textsuperscript{11}. The left or right-handed circularly polarized wave that is absorbed more by the optically active sample will display an increase in amplitude relative to its counterpart. This results in an elliptically polarized light. The degree of ellipticity is calculated by the CD spectrophotometer and then displayed as a function of wavelength\textsuperscript{10,11}.

Circular dichroism spectroscopy depends on producing circularly polarized light. Light waves are transverse waves comprised of electric and magnetic fields that oscillate perpendicular to the direction of travel. Polarization of transverse waves refers to the polarization of the electric component only\textsuperscript{10}. When the electric and magnetic fields are oscillating together with equivalent amplitude but differing in phase by 90°, the resultant light wave will be circularly polarized. As circular polarized light propagates along the direction of travel, the result is an electric field rotating directionally but not changing in strength\textsuperscript{11}. By passing linearly polarized light through a
quarter-wave plate at an angle of 45° to the optic axis this rotation can be achieved\textsuperscript{14}. The electric vector then emerges at an angle of 45° to the axis of propagation. It can be split into two components, the horizontal and vertical direction. The light then passes through a quarter-wave retarder. When the horizontal component is retarded one-quarter of a wave relative to vertical component, the resultant wave is right circularly polarized\textsuperscript{14}. When the vertical component is retarded one-quarter wave relative to the horizontal component the wave is left circularly polarized\textsuperscript{14}.

![Diagram of circularly polarized light]

Figure 4. Image of circularly polarized light. The blue line represents the magnetic component of light while the green line represents the electric component. The red line indicates the circularly polarized light that occurs when the magnetic and electric waves are 90° out of phase\textsuperscript{15}.

CD and ORD spectroscopy are most often used to determine biomolecule secondary structures\textsuperscript{10,11}. ORD can be used to determine chemical concentrations or to determine purity of the chiral ligand. Beta sheets and alpha helices in proteins show discrete and reproducible patterns in a CD spectrum while different DNA helical types also produce distinct patterns and thus resultant CDs can be compared in databases for structure determination\textsuperscript{16}. CD spectroscopy is not limited to use in secondary structure determination. DNA binding studies have also been
conducted using CD to discover information such as binding affinities and/or types of interactions\textsuperscript{16}.

1.5 Computational Chemistry

Although circular dichroism, fluorescence, UV-Vis spectroscopy provide critical information such as kinetics of binding, these methods are limited because they cannot provide atomistic detail. Computational chemistry is widely applied to biomolecular inquiries such as drug discovery, free energy prediction and protein folding\textsuperscript{17}. It is ideally suited for a variety of chemically related investigations, such as small molecule binding, because the computational expenses and sampling concerns usually encountered with large biomolecular systems are diminished\textsuperscript{17}. Molecular dynamics (MD) has been shown to be a promising tool in response to the increasing demand for greater detail in experimental results. Advances in MD studies have also aided in making this a reliable means for chemical inquiries. Molecular dynamics provides details both temporal and spatial evolution of systems based on Newtonian physics\textsuperscript{17}.

There are numerous methods to investigate the dynamics of biological systems. Each method depends on the potential energy function used therefore choosing the correct potential energy function is crucial to a successful simulation\textsuperscript{18}. Each of these energy functions generally describes the bonded terms using Hooke’s Law, Newtonian Mechanics, and non-bonded terms by the Lennard-Jones Potential and Coulomb’s Law\textsuperscript{17}. Each of these terms consists of a number of parameterized terms that are obtained from experimental results or quantum mechanical calculations of small molecules\textsuperscript{18}.

The set of parameters describing the potential energy function is called a force field. Choosing the appropriate force field is the first step in building a model for the system of interest.
Some of the most common force fields include AMBER ff99SB, CHARMM 27 and SYBYL\textsuperscript{17}. These force fields include parameters for the most commonly encountered biological molecules including protein amino acids and nucleic acid bases.

In the investigation of small molecules, many force fields are generally not sufficient to describe the ligand because the molecule has not been parameterized. In these cases, additional methods are necessary to parameterize the terms in the force field\textsuperscript{17}. Quantum chemical methods can be employed to determine these parameters for later use in MD simulations. One of the benefits of quantum chemistry is that no prior knowledge is necessary to study the small molecule in question. The results from the quantum mechanical calculations are then compared to experimental data to gain further insight into energetics.

As with all computational techniques, it is necessary to choose a method that can accurately reproduce known results while at the same time keep computational expenses at attainable levels. Quantum mechanical models such as Hartree-Fock, density functional theory and semi-empirical models are highly accurate, but generally extremely time consuming to run. Each of these methods seeks to solve the many-body Schrödinger equation. Historically, the solution to solving the quantum many-body system has been the Hartree-Fock equation\textsuperscript{19}. In this approximation, the electrons are considered to be non-interactive. Therefore, the resultant Hamiltonian becomes separable and solvable\textsuperscript{20}. Each electron is considered to be in its own molecular orbital, which moves in an average field of the other electrons\textsuperscript{19}. Each of these orbitals is expressed in a so-called Slater Determinant\textsuperscript{20}. This is a convenient form to express the orbitals because interchange of any of the elements of the determinant causes antisymmetry, which is the requirement of the Schrödinger equation\textsuperscript{20}. The determinant is comprised of a set of functions that describes the molecular orbitals, called a basis set. The minimal basis set only contains functions required to
accommodate all the electrons in the atom while maintaining spherical symmetry and is called the STO-3G basis set\textsuperscript{18}. There have been many expansions to this basis set including the split-valence Pople basis sets which use a set of Gaussian functions to describe the valence and outer shell electrons separately\textsuperscript{18}. The most commonly used Gaussian basis set is the 6-31G\textsuperscript{*} set which uses six Gaussian functions to describe the core orbitals, and the valence orbitals are split into three and one Gaussian functions. The star indicates that there are additional functions describing the polarizability of the d-orbitals of the molecule. Using this basis set and the Hartree-Fock approximation, fairly accurate quantum chemical predictions can be calculated including energy-minimized geometries, electron potential surfaces and torsional energy profiles\textsuperscript{18}. 
2. Purpose of Study

Compounds that selectively target DNA have supplied clinically beneficial therapeutics for a variety of diseases ranging from African sleeping sickness to cancer. Minor groove binding drugs have advantages over intercalating agents due to their increased sequence specificity and lower levels of mutagenic effects. Although a variety of these compounds have been produced and are now in use as treatments, much more information is still needed regarding binding strategies and sequence selectivity.

The research presented in this thesis has two principal objectives. First, the determination of absorption, fluorescence, and circular dichroic properties of selected minor groove binders in the presence of two DNA sequences in order to understand binding selectivity. The second aspect of the study is to perform computational calculations on the minor groove binding compounds to gain an understanding of their unbound conformations and electrostatics. Both spectroscopic and computational calculations will lead to further insight into the compound-DNA complex and binding mechanics.

In order to better determine sequence specificities, two DNA sequences, 5’-AAAATTT-3’ (A4T3) and 5’-AAAGTTT-3’ (A3GT3), were studied. In addition to the two DNA sequences, three groups of minor groove binders were selected for additional investigation. Cationic minor groove binders have been shown to be successful therapeutics. They can readily enter the cell nucleus, target specific DNA sequences and work allosterically with gene-control proteins by targeting the minor groove. Using this knowledge as a starting point, the Boykin group at Georgia State University has synthesized numerous small molecules with additional heterocycles and functional groups as “linkers” in effort to increase selective targeting. Using data from previously
described spectroscopic techniques and computational calculations, minor groove binders can be modified to selectivity target AT regions or a single GC step in an AT tract. The core group of compounds in this study contains DB 2363, DB 2364, DB 2324 and DB 2368. Each of these potential groove binders is built around a central benzimidazole linked to ionic groups via one or more phenyl rings. The second category is comprised of DB 2309, DB 2325, DB 2327 and DB 2328. These compounds have unique heterocycles incorporated as central moieties or linkers. The third group, DB 2326, DB 2351, DB 2348 and DB 2350, contains larger, symmetric potential groove binders all with the same symmetric components but with varying central linkers.

**DNA Sequences**

\[
\begin{align*}
5' & -\text{CGAAAGTTTCC} - 3' \\
3' & -\text{GCTTTCAAGT} - 5'
\end{align*}
\]

**Category 1**

![DB 2324](image1)

![DB 2363](image2)

![DB 2364](image3)

![DB 2368](image4)
Figure 5. (a) Two DNA sequences used for binding titrations. (b) Heterocyclic cationic compounds synthesized by the Boykin group at GSU. Category 1 compounds consist of a core benzimidazole with minor interior modifications. Category 2 compounds vary based upon their central moiety and incorporation of unique heterocycles. Category 3 contains large symmetric compounds with different joining groups.
3. Methods

3.1 Molecular Absorptivity

Molar absorptivity coefficients (or extinction coefficient) were obtained using a Cary 300 Bio UV-Vis spectrophotometer by Varian. The extinction coefficient of a known concentration of a compound was determined from the absorbance at its maximum wavelength by using the Beer-Lambert Law that states that the absorbance of a compound is directly proportional to the concentration of the absorbing species and the cuvette pathlength.

\[ A = \varepsilon cl \]

3.2 Fluorescence Spectroscopy

Previously determined maximum absorption wavelengths from UV-Vis spectroscopy were used for each compound in fluorescence titration analyses (Figure 7). Each compound was titrated with both 5’-AAAATTT-3’ and 5’-AAAGTTT-3’ DNA sequences in a 50 mM Tris-HCl, 100 mM NaCl, 1mM EDTA buffer, at pH 7.45. All twelve small molecules were titrated at a concentration of 1µM in a 1cm quartz cuvette for baseline reference. Compound was then titrated into the sample solution to give concentration ratios ranging from 0.2:1 to 1:1 of DNA to compound (DNA:ligand). Excitation and emissions slit widths varied among chemical compounds. All data was graphed and analyzed using Origin software²¹.
Table 1 Fluorescence spectroscopy parameters for each DB compound. Excitation wavelengths, excitation and emission slit widths for all compounds shown below.

<table>
<thead>
<tr>
<th>Compound</th>
<th>λ_max (nm)</th>
<th>Excitation slit width (nm)</th>
<th>Emission slit width (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB 2324</td>
<td>334</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>DB 2363</td>
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<td>2.5</td>
<td>2.5</td>
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<td>5</td>
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<td>2.5</td>
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<tr>
<td>DB 2325</td>
<td>388</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>DB 2327</td>
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</tr>
<tr>
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<td>325</td>
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</tr>
<tr>
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<td>5</td>
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<td>10</td>
</tr>
<tr>
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<td>304</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>DB 2351</td>
<td>302</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

Figure 6. Spectral representation of a molecule excited at one wavelength (i.e. absorbance) represented by the blue line and emission (i.e. fluorescence) represented by the red line. Peak maxima show where the hypothetical compound best absorbs and emits light. The emission curve is at a lower energy level and, thus, at a higher wavelength than the absorbance curve\textsuperscript{22}.  

\textsuperscript{22}
3.3 Circular Dichroism

CD was conducted using a Jasco J-810 spectrophotometer using a 1 cm quartz cuvette. A solution of 3 µM of DNA in a 50 mM Tris-HCl, 100 mM NaCl, 1mM EDTA buffer at pH 7.45 was used in all titration scans. The compound of interest was then added beginning with a 0.4:1 concentration ratio (ligand:DNA) and increased by increments until 3.2:1 (ligand:DNA) ratio was met. The process was repeat for both 5’-AAAATTT-3’ and 5’-AAAGTTT-3’ DNA sequences. Wavelengths ranged between 650 to 220 nm with a response speed of 1 s and a scanning speed of 50 nm/min, reproduced in triplicate and then averaged. All data was graphed and analyzed using Origin software\textsuperscript{21}.

3.4 Molecular Modeling

Electrostatic potential maps were calculated from the optimized structures using Spartan ‘14 software package (Wavefunction Inc., Irvine, CA)\textsuperscript{23}. Final geometry optimizations were calculated using the Hartree-Fock method and 6-31G* basis set.
4. Results

4.1 Molar Extinction Coefficients

Table 2. Molar extinction coefficients are listed for all compounds. Wavelength of maximum absorption for each DB compound is displayed.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Extinction Coefficient (L/M*cm))</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB 2324</td>
<td>$3.2 \times 10^4$</td>
<td>334</td>
</tr>
<tr>
<td>DB 2363</td>
<td>$3.0 \times 10^4$</td>
<td>314</td>
</tr>
<tr>
<td>DB 2364</td>
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<td>302</td>
</tr>
<tr>
<td>DB 2368</td>
<td>$4.6 \times 10^4$</td>
<td>350</td>
</tr>
<tr>
<td>DB 2309</td>
<td>$3.3 \times 10^4$</td>
<td>323</td>
</tr>
<tr>
<td>DB 2325</td>
<td>$3.6 \times 10^4$</td>
<td>388</td>
</tr>
<tr>
<td>DB 2327</td>
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<td>345</td>
</tr>
<tr>
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</tr>
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<td>$3.1 \times 10^4$</td>
<td>306</td>
</tr>
<tr>
<td>DB 2348</td>
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</tr>
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<td>DB 2350</td>
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<tr>
<td>DB 2351</td>
<td>$1.9 \times 10^4$</td>
<td>302</td>
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</tbody>
</table>
4.2 Category 1 Compounds

4.2.1 Molecular Modeling

Figure 7. Ball and stick models along with their electrostatic potential maps generated using Spartan ’14. Hartree-Fock method with a 6-31G* basis was used to determine minimized structures. Electrostatic potential surfaces are mapped using calculated values. Blue regions represent areas of positive electrostatic potential whereas areas with negative electrostatics are displayed as red. Green represents hydrophobic regions of the compound. Compounds (from top to bottom) DB 2324, DB 2363, DB 2364, DB 2368 are shown.
4.2.2 Fluorescence Titrations

DB 2324 Titrated with 5’-AAAATTT-3’

Figure 8. (a) Fluorescence titration of 1µM DB 2324 with 5’-AAAATTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 334 nm was used. The titration was run with an excitation slit width of 2.5 nm and an emission slit width of 5 nm. The black line indicates free compound, while the blue lines represent a 1:1 ratio of 5’-AAAGTTT-3’. The fluorescence intensity showed both a steady decrease in intensity and blue shift in wavelength maximum. (b) Chemical structure of DB 2324.
Figure 9. (a) Fluorescence titration of a 1µM DB 2324 with 5’-AAAGTTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 334 nm was used. The titration was run with an excitation slit width of 2.5 nm and an emission slit width of 5 nm. The black line indicates free compound while the blue lines shows a 1:1 ratio of 5’-AAAGTTT-3’. Complete fluorescence quenching appears at 1:1 binding. (b) Chemical structure of DB 2324.
DB 2363 Titrated with 5’-AAAATTT-3’

Figure 10. (a) Fluorescence titration of 1µM DB 2363 with 5’-AAAATTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 314 nm was used. The titration was run with an excitation slit width of 2.5 nm and an emission slit width of 2.5 nm. The black line indicates free compound while the blue line shows a 1:1 ratio of 5’-AAAATTT-3’. DB 2363 showed a steady decrease in fluorescence upon binding to DNA. (b) Chemical structure of DB 2363.
Figure 11. (a) Fluorescence titration of 1µM DB 2363 with 5’-AAAGTTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 314 nm was used. The titration was run with an excitation slit width of 2.5 nm and an emission slit width of 2.5 nm. The black line indicates free compound while the blue line shows a 1:1 ratio of 5’-AAAGTTT-3’. DB 2363 showed a steady decrease in fluorescence upon binding to 5’-AAAGTTT-3’. (b) Chemical structure of DB 2363.
Figure 12. (a) Fluorescence titration of 1µM DB 2364 with 5’-AAAATTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 302 nm was used. The titration was run with an excitation slit width of 2.5 nm and an emission slit width of 5 nm. The black line indicates free compound while the blue line shows a 1:1 ratio of 5’-AAAATTT-3’. With an increase in DNA concentration, fluorescence intensity decreased. (b) Chemical structure of DB 2364.
Figure 13: (a) Fluorescence titration of 1µM DB 2364 with 5’-AAAGTTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 302 nm was used. The titration was run with an excitation slit width of 2.5 nm and an emission slit width of 5 nm. The black line indicates free compounds while the blue line shows a 1:1 ratio of 5’-AAAGTTT-3’. With an increase in DNA concentrations, fluorescence intensity decreased. (b) Chemical structure of DB 2364.
Figure 14. (a) Fluorescence titration of 1µM DB 2368 with 5’-AAAATTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 350 nm was used. The titration was run with an excitation slit width of 10 nm and an emission slit width of 5 nm. The black line indicates free compound while the blue line shows a 1:1 ratio of 5’-AAAATTT-3’. DB 2368 showed little inherent fluorescence. Additions of DNA up to a 0.4:1 ratio displayed an increase in fluorescence; however, the fluorescence intensity began to decrease following higher concentrations of DNA to compound. (b) Chemical structure of DB 2368.
DB 2368 Titrated with 5’-AAAGTTT-3’

Figure 15. (a) Fluorescence titration of 1µM DB 2368 with 5’-AAAGTTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 350 nm was used. The titration was run with an excitation slit width of 10 nm and an emission slit width of 5 nm. The black line indicates free compound while the blue line shows a 1:1 ratio of 5’-AAAGTTT-3’. DB 2368 showed low inherent fluorescence. Initial additions of DNA (up to 0.6:1) displayed an increase in fluorescence; however, the fluorescence began to decrease starting at a 0.8:1 DNA to compound ratio. (b) Chemical structure of DB 2368.
4.2.3 Circular Dichroism

DB 2324 Titrated with 5'-AAAATTT-3'

Figure 16. (a) Circular dichroism results of 5'-AAAATTT-3’ titrated with DB 2324. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. DNA concentration was 3 µM. Changes in the regions past 300 nm indicate minor groove binding. Regions before 300 nm suggest that the conformation of DNA had little change. (b) Chemical structure of DB 2324 is shown below.
Figure 17. (a) Circular dichroism results of 5’-AAAGTTT-3’ titrated with DB 2324. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. DNA concentration was 3 µM. Changes in the regions past 300 nm indicate minor groove binding. The large changes in the regions prior to 300 nm suggest that DB 2324 is changing the conformation of the DNA when binding occurs. (b) Chemical structure of DB2324.
DB 2363 Titrated with 5’-AAAATTT-3’

Figure 18. (a) Circular dichroism results of 5’-AAAATTT-3’ titrated with DB 2363. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. Minor groove binding is shown in the 300 to 350 nm region for DB 2363. Microstructural changes in the DNA may occur in the region below 300 nm as a result of minor groove binding. (b) Chemical structure of DB 2363.
Figure 19. (a) Circular dichroism results of 5’-AAAGTTT-3’ titrated with DB 2363. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. Minor groove binding is shown in the region of 300 to 350 nm for DB 2363. Microstructural changes in the DNA conformation may occur upon binding DB 2363 based on spectral changes in the region below 300 nm. (b) Chemical structure of DB 2363.
DB2364 Titrated with 5’-AAAATTT-3’

Figure 20. (a) Circular dichroism results of 5’-AAAATTT-3’ titrated with DB 2364. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. Little to no changes occur in the 300 to 350 nm region indicating DB 2363 binds weakly, if at all, in the minor groove. Microstructural changes may occur in the DNA conformation upon binding DB 2364 in the region below 300 nm. (b) Chemical structure of DB 2364.
DB2364 Titrated with 5’-AAAGTTT-3’

Figure 21. (a) Circular dichroism results of 5’-AAAGTTT-3’ titrated with DB 2364. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. Very small changes in the 300 to 350 nm region indicate that DB 2363 does not bind in the minor groove. Microstructural changes may occur in the DNA conformation in the region below 300 nm. (b) Chemical structure of DB 2364.
Figure 22. (a) Circular dichroism results of 5’-AAAATTT-3’ titrated with DB 2368. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. Fluctuations in the 300 to 350 nm region indicate that DB 2368 binds to the minor groove. Large structural changes occur in the DNA conformation as shown in the spectrum below 300 nm. Peaks shown above 350 nm are due to the Cotton effect. (b) Chemical structure of DB 2368.
DB 2368 Titrated with 5’-AAAGTTT-3’

Figure 23. (a) Circular dichroism results of 5’-AAAGTTT-3’ titrated with DB 2368. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. Fluctuations in the 300 to 350 nm region indicate that DB 2368 binds to the minor groove. Microstructural changes may occur in the DNA conformation as shown in the spectrum below 300 nm. The maximum wavelength of absorption for DB 2368 was at 388 nm. Peaks shown above the 350 nm are due to the Cotton effect. (b) Chemical structure of DB 2368.
4.3 Category 2 Compounds

4.3.1 Molecular Modeling

Figure 24. Ball and stick models along with their electrostatic potential maps generated using Spartan ’14. Hartree-Fock calculations with a 6-31G* basis were used to determine minimized structures. Electrostatic potential surfaces are mapped using calculated values. Blue regions represent an area of positive electrostatic potential whereas areas with negative electrostatic interaction are displayed as red. Green areas indicate hydrophobic regions of the compound. Compounds (top to bottom) DB 2309, DB 2325, DB 2327, DB 2328 are shown.
4.3.2 Fluorescence Spectroscopy

DB 2309 Titrated with 5’-AAAATTT-3’

Figure 25. (a) Fluorescence titration of 1µM DB 2309 with 5’-AAAATTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 334 nm was used. The titration was run with an excitation slit width of 2.5 nm and an emission slit width of 2.5 nm. The black line indicates free compound while the blue line shows a concentration ratio of 1:1 for 5’-AAAATTT-3’. Addition of DNA showed a steady decrease in fluorescence. (b) Chemical structure of DB 2309.
DB 2309 Titrated with 5’-AAAGTTT-3’

Figure 26. (a) Fluorescence titration of 1μM DB 2309 with 5’-AAAGTTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 334 nm was used. The titration was run with an excitation slit width of 2.5 nm and an emission slit width of 2.5 nm. The black line indicates free compound while the blue line shows a 1:1 ratio of 5’-AAAGTTT-3’. Each addition of DNA displayed a decrease in fluorescence intensity. (b) Chemical structure of DB 2309.
Figure 27. (a) Fluorescence titration of 1µM DB 2325 with 5’-AAAATTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 388 nm was used. The titration was run with an excitation slit width of 10 nm and an emission slit width of 5 nm. The black line indicates free compound while the blue line shows a 1:1 ratio of 5’-AAAATTT-3’. Once a 0.4:1 concentration ratio of DNA to compound was met, a nominal increase in fluorescence intensity was reached to 0.8:1; however, a slight decrease in fluorescence intensity was observed at a 1:1 ratio. (b) Chemical structure of DB 2325.
Figure 28. (a) Fluorescence titration of 1µM DB 2325 with 5’-AAAGTTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 388 nm was used. The titration was run with an excitation slit width of 10 nm and an emission slit width of 5 nm. The black line indicates free compound while the blue line shows a 1:1 ratio of 5’-AAAGTTT-3’. Initial additions of DNA resulted in increasing fluorescence intensities. Once a 0.4:1 ratio of DNA to compound was met, the fluorescence intensity experienced a nominal increase with each additional aliquot added until a 1:1 ratio, which showed a decrease in fluorescence. (b) Chemical structure of DB 2325.
Figure 29. (a) Fluorescence titration of 1µM DB 2327 with 5’-AAAATTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 345 nm was used. The titration was run with an excitation slit width of 2.5 nm and an emission slit width of 2.5 nm. The black line indicates free compound while the blue line shows a 1:1 ratio of 5’-AAAATTT-3’. Initial additions resulted in increases in fluorescent intensities. Fluorescence intensities increased, until a 1:1 ratio of DNA to compound was met which resulted in a decrease in intensity. (b) Chemical structure of DB 2327.
Figure 30. (a) Fluorescence titration of 1µM DB 2327 with 5’-AAAGTTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 345 nm was used. The titration was run with an excitation slit width of 2.5 nm and an emission slit width of 2.5 nm. The black line indicates free compound while the blue line shows a 1:1 ratio of 5’-AAAGTTT-3’. Upon addition of DNA, a dramatic increase in fluorescence intensity was observed. Fluorescence intensities increased until a 0.6:1 ratio of DNA to compound was met after which decreases were observed. (b) Chemical structure of DB 2327.
Figure 31. (a) Fluorescence titration of 1µM DB 2328 with 5’-AAAATTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 325 nm was used. The titration was run with an excitation slit width of 5 nm and an emission slit width of 5 nm. The black line indicates free compounds while the blue line shows a 1:1 ratio of 5’-AAAATTT-3’. Fluorescence intensity increased with each titration of DNA added. (b) Chemical structure of DB 2328.
Figure 32. (a) Fluorescence titration of 1µM DB 2328 with 5’-AAAGTTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 325 nm was used. The titration was run with an excitation slit width of 5 nm and an emission slit width of 5 nm. The black line indicates free compound while the blue line shows a 1:1 ratio of 5’-AAAGTTT-3’. Fluorescence intensity steadily decreased with each additional aliquot of DNA. (b) Chemical structure of DB 2328.
4.3.3 Circular Dichroism

DB2309 Titrated with 5’-AAAATTT-3’

Figure 33. (a) Circular dichroism results of 5’-AAAATTTT-3’ titrated with DB 2309. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer at pH 7.4. Very small changes in the 300 to 350 nm region indicate that DB 2309 is binding in the minor groove. Minor DNA microstructural changes may occur upon binding DB 2309 as shown below 300 nm. (b) Chemical structure of DB 2309.
Figure 34. (a) Circular dichroism results of 5'-AAAATTT-3’ titrated with DB 2309. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer at pH 7.4. Very small changes in the 300 to 350 nm region indicate that DB 2309 is binding in the minor groove. DNA may experience some microstructural changes represented by the variations shown below 300 nm. (b) Chemical structure of DB 2309.
Figure 35. (a) Circular dichroism results of 5'-AAAATTT-3' titrated with DB 2325. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer at pH 7.4. Negatively induced rotation above 400 nm is indicative of intercalation. DNA microstructural changes may occur below 300 nm. (b) Chemical structure of DB 2325.
DB 2325 Titrated with 5'-AAAGTTT-3'

Figure 36. (a) Circular dichroism results of 5'-AAAGTTT-3’ titrated with DB 2325. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer at pH 7.4. Negatively induced CD above 400 nm is indicative of intercalation. DNA microstructural changes may occur below 300 nm. (b) Chemical structure of DB 2325.
Figure 37. (a) Circular dichroism results of 5’-AAAATTT-3’ titrated with DB 2327. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer at pH 7.4. Large induced changes in the spectrum in the 300 to 350 nm region indicate DB 2327 is binding in the minor groove. Negatively induced CD may indicate a secondary intercalation-binding mode. DNA shows some microstructural changes represented by the increased CD shown below 300 nm. (b) Chemical structure of DB 2327.
Figure 38. (a) Circular dichroism results of 5’-AAAGTTT-3’ titrated with DB 2327. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer at pH 7.4. Large induced CD changes in the 300-350 nm region indicate that DB 2327 is binding in the minor groove. Negative CD changes above 350 nm may indicate a secondary intercalation-binding mode. Positive induced CD in the region below 300 nm DNA suggests microstructural changes in the DNA. (b) Chemical structure of DB 2327.
DB 2328 Titrated with 5’-AAAATTT-3’

Figure 39. (a) Circular dichroism results of 5’-AAAATTT-3’ titrated with DB 2328. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer at pH 7.4. Induced CD changes in the 300 nm region indicate binding to the minor groove. Variations below 300 nm may suggest DNA experiences some microstructural changes. (b) Chemical structure of DB 2328.
Figure 40 (a) Circular dichroism results of 5’-AAAGTTT-3’ titrated with DB 2328. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer at pH 7.4. Alterations near the 350 nm region suggest DB 2328 is binding in the minor groove. DNA may experience some microstructural changes upon binding as based on changes in CD below 300 nm. (b) Chemical structure of DB 2328.
4.4 Category 3 Compounds

4.4.1 Molecular Modeling

Figure 41. Ball and stick models along with their electrostatic potential maps generated using Spartan '14. Hartree-Fock method with a 6-31G* basis was used to calculate the minimized structure. Electrostatic potential surfaces are mapped using calculated values. Blue regions represent areas of positive electrostatic potential whereas areas with negative electrostatic interaction are displayed as red. Green indicates hydrophobic regions of the compound. Compounds (top to bottom) DB 2326, DB 2348, DB 2350, DB 2351.
4.4.2 Fluorescence Spectroscopy

DB 2326 Titrated with 5’-AAAATTT-3’

Figure 42. (a) Fluorescence titration of a 1µM DB 2326 with 5’-AAAATTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 306 nm was used. The titration was run with an excitation slit width of 5 nm and an emission slit width of 5 nm. The black line indicates free compound while the blue line shows a 1:1 concentration ratio of 5’-AAAATTT-3’. DB 2326 showed little inherent fluorescence. Addition of DNA resulted in an increase in fluorescence up to 0.6:1; however, the fluorescence began to decrease at a 0.8:1 ratio (DNA to compound). (b) Chemical structure of DB 2326.
Figure 43. (a) Fluorescence titration of a 1µM DB 2326 with 5'-AAAGTTT-3'. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 306 nm was used. The titration was run with an excitation slit width of 10 nm and an emission slit width of 10 nm. The black line indicates free compound while the blue line shows a 1:1 ratio of 5'-AAAGTTT-3'. Initially, addition of DNA displayed a large increase in fluorescence; however, fluorescence began to decrease at a 0.4:1 ratio of DNA to compound. (b) Chemical structure of DB 2326.
Figure 44. (a) Fluorescence titration of a 1µM DB 2348 with 5’-AAAATTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 305 nm was used. The titration was run with an excitation slit width of 5 nm and an emission slit width of 5 nm. DB 2348 showed little inherent fluorescence; however, upon binding with DNA, the fluorescence intensity increased. Upon reaching a concentration ratio of 0.6:1 (DNA to compound), fluorescence intensity decreased. (b) Chemical structure of DB 2348.
Figure 45. (a) Fluorescence titration of a 1µM DB 2348 with 5’-AAAGTTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 305 nm was used. The titration was run with an excitation slit width of 5 nm and an emission slit width of 5 nm. DB 2348 showed little inherent fluorescence; however, upon binding with DNA, fluorescence increased. Initial titrations of DNA showed a marked increase in fluorescence intensity but upon reaching a 0.6:1 ratio of DNA to DB 2348, fluorescence intensity became stagnant. (b) Chemical structure of DB 2348.
Figure 46. (a) Fluorescence titration of a 1µM DB 2350 with 5’-AAAATTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 304 nm was used. The titration was run with an excitation slit width of 5 nm and an emission slit width of 5 nm. DB 2350 showed little to no inherent fluorescence; however, upon binding with DNA, the fluorescence intensity increased. (b) Chemical structure of DB 2350.
DB 2350 Titrated with 5’-AAAGTTT-3’

Figure 47. (a) Fluorescence titration of a 1µM DB 2350 with 5’-AAAGTTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 304 nm was used. The titration was run with an excitation slit width of 10 nm and an emission slit width of 10 nm. DB 2350 showed little inherent fluorescence; however, upon binding with DNA, the fluorescence intensity increased. (b) Chemical structure of DB 2350.
Figure 48. (a) Fluorescence titration of a 1µM DB 2351 with 5’-AAAATTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 302 nm was used. The titration was run with an excitation slit width of 10 nm and an emission slit width of 5 nm. Fluorescence intensity increased to a concentration ratio of 0.6:1 (DNA to compound) followed by a slight decrease in the intensity with higher ratios. (b) Chemical structure of DB 2351.
Figure 49. (a) Fluorescence titration of a 1µM DB 2351 with 5’-AAAGTTT-3’. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer, at pH 7.4. An excitation wavelength of 302 nm was used. The titration was run with an excitation slit width of 5 nm and an emission slit width of 5 nm. Fluorescence intensity maximum for DB 2351 experienced a blue shift upon increasing DNA concentrations and continued to decrease upon each addition of DNA. (b) Chemical structure of DB 2351.
4.4.3 Circular Dichroism

DB 2326 Titrated with 5'-AAAATTT-3’

Figure 50. (a) Circular dichroism results of 5'-AAAATTT-3’ titrated with DB 2326. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer at pH 7.4. Small increase shifts in the 300 nm region suggest that DB 2326 binds weakly in the minor groove. The DNA may experience some microstructural changes upon binding, as represented by the CD changes as shown below 300 nm. (b) Chemical structure of DB 2326.
DB 2326 Titrated with 5’-AAAGTTT-3’

Figure 51. (a) Circular dichroism results of 5’-AAAGTTT-3’ titrated with DB 2326. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer at pH 7.4. Little induced CD changes in the 300 nm region suggest that DB 2326 binds weakly in the DNA minor groove. Microstructural changes in the DNA may occur upon binding as represented in the region below 300 nm. (b) Chemical structure of DB 2326.
Figure 52. (a) Circular dichroism results of 5’-AAAATTT-3’ titrated with DB 2348. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer at pH 7.4. Changes in the 300 nm region suggest that DB 2348 binds in the DNA minor groove. Microstructural changes in the DNA may occur as represented by the changes observed in the region between 250 and 300 nm. (b) Chemical structure of DB 2348.
Figure 53. (a) Circular dichroism results of 5’-AAAGTTT-3’ titrated with DB 2348. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer at pH 7.4. DB 2348 likely binds weakly in the minor groove of DNA as indicated by subtle changes in the 300 nm region. The DNA microstructure may experience a change upon binding of DB 2348 as represented by variations in the induced CD curves in the region below 300 nm. (b) Chemical structure of DB 2348.
Figure 54. (a) Circular dichroism results of 5’-AAAATTT-3’ titrated with DB 2350. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer at pH 7.4. Weak binding of DB 2350 to the minor groove of DNA is suggested by subtle changes in the 300 nm region. The DNA microstructure may undergo some changes as represented by variations in the CD curve spectra below 300 nm. (b) Chemical structure of DB 2350.
Figure 55. (a) Circular dichroism results of 5'-AAAGTTT-3' titrated with DB 2350. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer at pH 7.4. DB 2350 binding to the minor groove of DNA likely occurs weakly as indicated by the subtle CD changes in the 300 nm region. DNA microstructure may experience a change upon binding at high concentration ratios (compound to DNA) as represented by variations in the spectral region below 300 nm. (b) Chemical structure of DB 2350.
DB 2351 Titrated with 5’-AAAATTT-3’

Figure 56. (a) Circular dichroism results of 5’-AAAATTT-3’ titrated with DB 2351. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer at pH 7.4. Minor changes in the CD 300 nm region suggest DB 2351 binds weakly to the minor groove of DNA. The DNA microstructure may undergo some change at high compound concentrations, as represented by variations in the titration curves below 300 nm. (b) Chemical structure of DB 2351.
Figure 57. (a) Circular dichroism results of 5’-AAAGTTT-3’ titrated with DB 2351. DNA concentration was 3 µM. The titration was carried out in a 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer at pH 7.4. Binding of DB 2351 to the minor groove of DNA is suggested based on changes in the 300 nm region. Microstructural changes in the DNA may occur upon addition of DB 2351 as represented by variations in the spectral region below 300 nm. (b) Chemical structure of DB 2351.
5. Results

5.1 Structure Comparison

From previously synthesized compounds by the Boykin laboratory, three groups of minor groove binders were selected for investigation using spectroscopic techniques. Each of these small molecules was designed from established modules to target specific sequences in DNA for possible use as therapeutics. Specifically, these modules are known components of minor groove binders that recognize AT tracts. Using these structures as building blocks, efforts were taken to modify the selectivity by adding various functional groups and linkers to target sequences that contain GC base pairs. The selection of molecules for this study was made based on structurally similar elements that could be easily studied using spectroscopic techniques. The core group of compounds consists of DB 2363, DB 2364, DB 2324 and DB 2368. DB 2363 is comprised of a benzimidazole moiety with an amidine at position 5 and amide at position 2. DB 2364 has the same moiety and attached functional groups as DB 2363 but the benzimidazole has been methylated at position 3. DB 2324 has a 7-azabenzimidazole as its primary moiety with a benzamide at position 2, similar to DB 2363 and DB 2364. At position 6, this molecule has an alkyl ether linker to a benzamidine. DB 2368 contains both a 7-azabenzimidazole and a benzimidazole joined at the 2 position of benzimidazole and the 6 position of 7-azabenzimidazole. At position 2 of the 7-azabenzimidazole, there is a phenol group and at position 6 of the benzimidazole there is an amidine.

The second group of small molecules, Category 2 compounds, is a derivative of the core group containing DB 2309, DB 2325, DB 2327 and DB 2328. These molecules specifically differ in their central moiety. DB 2309 is a symmetric structure comprised of a two 2-para-benzamidine-
4-methyl-thiazole linked at the 5 position of each thiazole. Similar to DB 2364, DB 2325 is also symmetric with 2 thiophenes linked at position 5. At the 2 position of each thiophene, there is a 1-methyl-5-amidino-benzimidazole. DB 2327 is structurally related to DB 2363 in that it has the same benzimidazole moiety with an amidine at position 5 and a phenyl group at position 2 and attached to the phenyl ring is a 2-amide-furan. DB 2328 differs from DB 2327 only by methylation at position 1 of benzimidazole, similar to the difference between DB2364 and DB 2363.

The last category of minor groove binders investigated, Category 3 compounds, contains four Boykin compounds that are much larger than those in the previous two groups: DB 2326, DB 2351, DB 2348 and DB 2350. Each of these structures is symmetric, only differing in the group joining the two symmetric terminal moieties. This moiety is a methylated benzimidazole with an amidine at position 5 and a phenyl ring at position 2. Linking each of these symmetric units to the central functional group are two alkyl ethers at the para position of the phenyl ring. The central functional group for DB 2326 is a pyridine. DB 2348 has a methyl-phenyl group as the central linker. The symmetric moieties in DB 2350 are separated by a tertiary amine. Similar to DB 2326, DB 2351 has a central pyridine. The difference lies in the symmetric unit with the incorporation of fluorine on the phenyl rings.

To gain further understanding about the atomic detail of these compounds, all molecules were energy minimized using a Hartree-Fock approximation with a 6-31G* basis set. It can be seen that each structure adapts to a crescent like shape after minimization, which is characteristic of known minor groove binders. Longer linking units, such as alkyl ethers, increases the flexibility of the small molecules and allows for greater diversity in the crescent configuration. This can be seen in DB 2324, DB 2325 and DB 2348. The electrostatic potential surfaces were also calculated and show the characteristic positive electrostatic regions at the amide and amino groups, as well
as around the nitrogen atoms of azabenzimidazole and benzimidazole groups. Each of these regions provides a potential location for hydrogen bond interactions, which would further enhance minor groove binding. In the second group of groove binders, the incorporation of heterocycles provides additional hydrogen bond locations and increases the number of negative electrostatic potential sights.

5.2 Category 1 Compounds

For each small molecule, fluorescence and CD measurements were recorded for varying concentrations in solution with one of the two DNA sequences of interest. DB 2363 showed strong binding for both A4T3 and A3GT3 based on the fluorescence intensity indicating no sequence selectivity. The peak emission at 390 nm decreases as the concentration of DNA increases, indicating association between the small molecule and DNA sequence. This is supported by observations taken using CD spectroscopy. In the region of compound binding (> 300 nm), there are subtle fluctuations in the CD ellipticity indicting no structural changes in the conformation of DNA.

DB 2364 also shows binding for both sequences in the fluorescence emission spectra with noticeable decreases in the peak emission at 395 nm with increasing DNA concentration. However, this is not supported by the observed CD spectra. There is no significant increase in ellipticity in the region above 300 nm with increasing concentration of the small molecule. This suggests limited changes in CD upon binding are occurring. In the region below 300 nm there are variations in peak ellipticity, which may indicate interaction with sequence A4T3. Therefore, DB 2364 has the potential to change the DNA structure of A4T3 but it may not be the result of minor groove binding. It is possible that the DB 2364 compounds area binding to the anionic DNA backbone and stacking on top of one another to form aggregates. The amide of DB 2364 has the potential to
form hydrogen bonds with the amide or amidine to stabilize dimers or multimers. This type of interaction is highly cooperative and is more favorable at higher concentrations of small molecule, which could explain the quenching seen in the fluorescence.

The intensity of the maximum peak emission at 425 nm decreases with increasing DNA solution for sequences in the presence of DB 2324 for both sequences. However, there is a more substantial decrease in emission in the A3GT3 sequence indicating selectivity for the AAAGTTT sequence with quenching upon binding to the amino group of G. The emission spectrum for DB 2324 in the presence of A4T3 is blue-shifted. This type of hypsochromic shift is seen in intercalators specifically those that bind as multimers such as the mutagenic agent\textsuperscript{24,25}. It is possible that more than one DB 2324 is interacting with the minor groove of the A4T3 sequence. The CD spectrum for DB 2324 also shows a preference for A3GT3 with a noticeable change in ellipticity at 325 nm. Additionally, there are some changes in ellipticity observed in the region below 300 nm, which may correlate to microstructural changes in the A3GT3 sequence upon binding DB 2324. These features, although present in the CD spectrum of DB 2324 in the presence of A3GT3 are absent in the CD spectrum of A4T3.

DB 2368 shows no sequence specificity and subtle changes in the fluorescence emission data; however, the CD shows a large peak ellipticity around 360 nm that would be indicative of strong binding for both sequences. The CD spectra of optically active compounds are directly correlated to their native absorbance. Therefore, in the vicinity of the absorption band, changes in the spectra that are not the result of structure can be seen\textsuperscript{11}. This effect, is called the Cotton effect, will cause either positive or negative rotational changes in the CD spectrum around the maximum absorbance of the sample\textsuperscript{11}. The strong ellipticity seen in the CD could be the result of a positive
Cotton effect due to absorption by the aromatic groups, which was observed at 356 nm. This would support the evidence seen in the fluorescence data indicating little to no binding.

5.3 Category 2 Compounds

The second group of molecules has an extended conjugated ring system and the effect it has on these chromophores is apparent in the fluorescence spectra. DB 2309 shows only one peak absorbance around 430 nm. It is the only compound that does not have a benzimidazole in this group and it is possible that this difference results in only one peak absorbance in the fluorescence spectrum. The fluorescence spectra of both sequences decrease with increasing amount of DNA indicating minor groove binding for both sequences. In the CD spectra, very small changes in the 300 to 350 nm region indicate that DB 2309 is weakly interacting with the minor groove.

DB 2325 shows two characteristic emission peaks: one at 455 nm and another at 485 nm. The fluorescence for each sequence shows increasing intensity with the addition of DNA indicating minor groove binding. In the CD spectra of both sequences there is a clear and distinctive decrease in ellipticity around 425 nm. This indicates possible intercalation upon binding. Additionally, there are changes in ellipticity around 280 nm indicating that the small molecule may influence the microstructure of both DNA sequences upon association.

DB 2327 shows a strong preference for A4T3 in the fluorescence data with two characteristic peaks: one at 380 nm and a second at 400 nm. There is a clear increase in fluorescence with increasing concentrations of DNA. On the other hand, DB 2327 only shows weak binding with A3GT3. No clear trend in emission and concentration is observed. Similar to the CD spectra of DB 2325, the ellipticity decreases above 300nm indicates possible intercalation with both sequences. The observed ellipticity could be the result of a negative Cotton effect at 345
nm, the same wavelength of observed absorption. In addition to the negative amplitude above 400 nm, there is strong increase in amplitude in the 300 nm range, which could indicate changes in the DNA microstructural conformation.

DB 2328 has two peak emissions at 380 nm and 400 nm similar to DB 2327. The fluorescence decreases with increasing concentration of A3GT3. In the fluorescence titration of DB 2328 with A4T3, however, the emission increases with increasing concentration of DNA. The decrease in fluorescence seen in the DB 2328 A3GT3 could indicate DNA minor groove binding. In contrast, hyperchromism seen in the titration of DB 2328 with A4T3 could indicate damage to the DNA double-helix structure. DB 2328 shows preferential binding to A3GT3 in contrast to the fluorescence titration with A4T3. This is supported by the CD spectrum. There is clear increase in amplitude of ellipticity at approximately 350 nm in the region of minor groove binding with A3GT3. Also, there are possible conformational changes in the DNA microstructure, as observed below 300 nm. For the A4T3 sequence, there is limited variance in the ellipticity above 300 nm and large variation in ellipticity below 300 nm. This supports the fluorescence data indicating possible structure degradation.

5.4 Category 3 Compounds

The small molecules that belong to Category 3 are larger than those in Categories 1 and 2. The effect of this change in size is a decrease in binding observed with both sequences in all four compounds. The fluorescence spectra of DB 2326 shows limited binding to both A4T3 and A3GT3 with slightly better results seen with A3GT3. This is supported by the CD spectra, which are almost superimposable. The only difference is in minor conformational shifts seen around 275 nm for the A3GT3 sequence. DB 2348 shows similar weak binding as DB 2326. Overall, there is binding for both sequences but possibly a slight preference for A3GT3. The CD supports this observation with
weak microstructural changes seen in lower wavelengths in the A3GT3 sequence and possible binding around 325 nm. DB 2350 shows no changes with A4T3 or A3GT3 in either fluorescence or CD spectra. DB 2351 is the only small molecule in this category that shows a strong possibility of binding to either sequence. Specifically, the fluorescence spectrum of DB 2351 in association with A3GT3 shows two peaks at 340 nm and 410 nm that decrease with increasing DNA concentration. Based on the CD for the A3GT3 sequence, there appears to be a slight increase in ellipticity at 320 nm indicating possible minor groove binding. The CD also shows similar changes in the region below 300 nm, which may correlate to distortions in the DNA microstructure once DB 2351 binds in the minor groove.
6. Discussion

The primary goals of this research were the determination of absorption, fluorescence and circular dichroism spectral properties for three groups of selected minor groove binders in the presence of two DNA sequences and the analysis of computational calculations and electrostatic potentials of the unbound conformations. Both spectroscopic and computational calculations led to further insight into the compound-DNA complexes and binding strategies.

Two DNA sequences, 5‘-AAAATTT-3’ (A4T3) and 5‘-AAAGTTT-3’ (A3GT3), were used in order to better determine sequence specificities. In addition to the two DNA sequences, three groups of minor groove binders were selected from previously synthesized Boykin compounds. Using spectroscopic techniques and computational calculations, modifications in the minor groove binders can be related to sequence selectivity.

The core group of compounds comprised of DB 2363, DB 2364, DB 2324 and DB 2368 contains a central benzimidazole with small derivations. Both DB 2363 and DB 2364 show potential binding for both A4T3 and A3GT3 based on the observed emission spectra. This is confirmed for DB 2363 in the CD spectra, but not for DB 2364, which shows no minor groove binding peak above 300 nm. Observing the minimized structure for both of these compounds, it would appear that both compounds would bind similarly. The only difference is a methyl group at the 1 position of DB 2364. The possible effect of this methyl group is that it does not fit in the minor groove and distorts the DNA structure. Evidence of this can be seen in the CD spectra for both A4T3 and A3GT3 in the presence of DB 2364 below 300 nm. DB 2324, an azabenzimidazole groove binder, shows indication of minor groove binding to both A4T3 and A3GT3 sequences. This is supported by the decrease of emission intensity in the fluorescence for both sequences and
a slight peak above 300 nm in the CD. There appears to be a slight preference for A3GT3 with a stronger ellipticity seen in the CD spectrum. DB 2368, containing both an azabenzimidazole and a benzimidazole, shows no sequence specificity and subtle changes in the emission data. However, the CD shows a large peak ellipticity around 360 nm, which would be indicative of strong binding for both sequences.

The second category includes DB 2309, DB 2325, DB 2327 and DB 2328 with unique heterocycles incorporated as central moieties or linkers. DB 2309 shows evidence of minor groove binding for both sequences in the fluorescence data. The CD shows limited binding for A4T3 but some binding for A3GT3 indicating possible GC step targeting. The fluorescence intensity of DB 2325 for each sequence shows increasing intensity with the addition of DNA indicating minor groove binding. Interestingly, in the CD spectra there is a clear and distinctive decrease in ellipticity around 425 nm. This indicates possible intercalation upon binding for this small molecule rather than the predicted minor groove binding. Observing the minimized structure and electrostatic potential map from the computational calculations, it is possible that one of the symmetric units intercalates, leaving the remaining portion of the small molecule solvent accessible. DB 2327 shows a strong preference for A4T3 in the fluorescence data but there is no clear trend in emission in the presence of A3GT3. Similar to the CD of DB 2325, the ellipticity at 345 nm indicates possible intercalation with both sequences. However, this could be the result of the Cotton effect given that the negative ellipticity occurs at the same wavelength of observed absorption. DB 2328 shows strong preferential binding to A3GT3, in contrast to the limited binding observed with A4T3 in the fluorescence emission spectrum. This is supported by the CD spectrum. In the CD spectrum of A3GT3 sequence, there is a clear increase in amplitude of ellipticity at approximately 350 nm in the region of minor groove binding. However, there are
distinct conformation changes in the DNA observed below 300 nm. This could be the result of the high degree of internal rotation between the phenyl ring and the benzimidazole as seen in the minimized structure of DB 2328.

The third group contains large, symmetric potential groove binders. DB 2326, DB 2351, DB 2348 and DB 2350 all have the same essential symmetric units but vary in their central linkers. The effect of this change in size results in a decrease in binding to both sequences. For DB 2326, DB 2348, DB 2350, and DB 2351, the fluorescence shows weak trends toward binding for both structures. This could indicate weak association of the DB compounds to both DNA microstructures. This is supported by the CD data. None of these structures show strong indication of minor groove binding to either sequence. In the region below 300 nm, there is evidence to suggest microstructural changes in the DNA sequences, further substantiating the idea that no minor groove binding is occurring. It is possible that these larger DB compounds require a longer DNA microsequence in order to probably study binding.
7. Conclusion

In this research, we have focused on the absorption, fluorescence, circular dichroism, electrostatic potentials and calculated minimized geometries of three groups of minor group binders in order gain further insight into minor groove binding with select sequences of DNA. Using both spectroscopic techniques and computational calculations, compounds that selectively target DNA have been studied in order to supply insight into potential, clinically beneficial therapeutics for genetic disorders. Cationic minor groove binders have been shown to be advantageous over intercalating agents because they can readily enter the cell nucleus, target specific DNA sequences and work allosterically with gene-control proteins\textsuperscript{26}. Furthermore, minor groove binding drugs have been shown to have lower levels of toxicity\textsuperscript{7}. Although a variety of these compounds have been produced and are now under study, much more information is still needed regarding binding strategies and sequence selectivity.
References


