NONCOVALENT INTERACTION OF PLATINUM PLANAR AMINE COMPOUNDS WITH TRYPTOPHAN: A STRATEGY TO INTERFERE WITH P53-MDM2 INTERACTIONS AND TARGETING RETROVIRAL ZN FINGER-DNA INTERACTION (HIV NCP7)

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NONCOVALENT INTERACTION OF PLATINUM PLANAR AMINE COMPOUNDS

WITH TRYPTOPHAN: A STRATEGY TO INTERFERE WITH P53-MDM2

INTERACTIONS AND

TARGETING RETROVIRAL ZN FINGER-DNA INTERACTION (HIV NCP7)

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

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# Table of Contents

Acknowledgements.......................................................................................................................2

List of Figures................................................................................................................................3

List of Schemes.................................................................................................................................5

List of Tables....................................................................................................................................5

List of Abbreviations.........................................................................................................................6

Chapter N°

1  Introduction..................................................................................................................................10

1.1  π stacking.................................................................................................................................10

1.2  π-π stacking in biological processes.....................................................................................13

1.3  Nucleobase Modification.........................................................................................................21

1.4  References.................................................................................................................................26

2  Platination of Nucleobases.........................................................................................................27

2.1  Abstract....................................................................................................................................27

2.2  Introduction...............................................................................................................................27

2.3  Results and Discussion.............................................................................................................30

2.4  Stacking interaction by fluorescence spectroscopy............................................................37

2.5  Experimental section................................................................................................................48

2.6  Conclusion................................................................................................................................54

2.7  References................................................................................................................................55

VITA..................................................................................................................................................57

Supplemental Information.................................................................................................................59
List of Figures

Figure 1.1: Principal orientations of aromatic-aromatic interactions.................................10

Figure 1.2: Pictorial representation of the electrostatic interactions.................................11

Figure 1.3: $\pi-\pi$ repulsion in benzene-benzene interaction if in face-to-face alignment...........12

Figure 1.4: Decrease in $\pi$-electron density for aromatic rings containing a nitrogen heteroatom within the ring........................................................................................................13

Figure 1.5: Unpaired T in the MutS co-crystal partially stacked in the DNA duplex..............15

Figure 1.6: Stacking of Phe 39 of the MutS to the unpaired T of the DNA..........................15

Figure 1.7: Three dimensional structure of the 82-nucleotide RNA-DNA complex..............17

Figure 1.8: View of complex perpendicular to the principal axis, showing stacking interactions.................................................................................................................................18

Figure 1.9: Crystal structure of p53 (green)/MDM2 (yellow and white) binding pocket. Three amino acids of p53 shown inside binding pocket, leucine, tryptophan and phenylalanine; which are essential for the binding of MDM2.................................................................20

Figure 1.10. Sequence of NCp7 showing coordinating residues in red...............................20

Figure 1.11. Amino acids capable of $\pi-\pi$ stacking interactions.......................................21

Figure 1.12. Nucleobases adenine (A), guanine (G), thymine (T), and cytosine (C).............21
Figure 1.13: Protonation, alkylation or coordination of a metal ion such as Pd(II) or Pt(II) to a nucleobases strengthens the interaction by lowering the energy of the lowest unoccupied molecular orbital of the modified nucleobases (LUMO) and improving overlap with the highest occupies molecular orbital (HOMO) in N-acetyl tryptophan………………………………………………..22

Figure 1.14: HOMO/LUMO energies for nucleobases, methylated nucleobases and metal coordinated nucleobases. Dash line is the HOMO of N-AcTrp. [Pd(dien)(1-MethylCytosine)]^{2+} (1); [Pt(dien)(1-MethylCytosine)]^{2+} (2); [Pd(dien)(9-EthylGuanine)]^{2+} (3); [Pt(dien)(9-EthylGuanine)]^{2+} (4)………………………………………………………………………..23

Figure 1.15: Platination, Pt(II), of a nucleobase enhances the interaction by lowering the energy of the LUMO of the nucleobase and improving the overlap with the HOMO of the N-acetyl tryptophan………………………………………………………………………………………..24

Figure 1.16: Correlation between association constants, determined for free 1-MeCyt and Pd/Pt-1MeCyt complexes, with the \( \Delta \varepsilon \) value………………………………………………………………………………………………………..25

Figure 2.1: Schematic structures of complexes used in this study………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………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Figure 2.10: Fluorescence spectrum of [Pt(dien)(pyridine)]NO₃ stacking with tryptophan.............................................................................................................................38

Figure 2.11: Fluorescence spectrum of [Pt(dien)(4-picoline)]NO₃ stacking with L-acetyl tryptophan.............................................................................................................................39

Figure 2.12: Fluorescence spectrum of [Pt(dien)(4-methoxypyridine)]NO₃ stacking with L-acetyl tryptophan.............................................................................................................................40

Figure 2.13: Fluorescence spectrum of Pt(dien)(4-Dimethylaminopyridine)](NO₃) stacking with L-acetyl tryptophan.............................................................................................................................42

Figure 2.14: Fluorescence spectrum of [Pt(dien)(cyanopyridine)]NO₃ stacking with L-acetyl tryptophan.............................................................................................................................43

Figure 2.15: Fluorescence spectrum of [Pt(dien)(thiazole)]NO₃ stacking with L-acetyl tryptophan.............................................................................................................................44

Figure 2.16: Fluorescence spectrum of [Pt(dien)(benzothiazole)](NO₃) stacking with L-acetyl tryptophan.............................................................................................................................46

Figure 2.17: Fluorescence spectrum of [Pt(dien)(quinoline)](NO₃) stacking with L-acetyl tryptophan.............................................................................................................................47

Figure 2.18: % fluorescence quenching of tryptophan by [Pt(dien)nucleobase]NO₃ complexes.............................................................................................................................54

List of Schemes

Page

Scheme 2.1: Platination of nucleobases.............................................................................................................................49

List of Tables

Page

Table 1: Pt(dien)L data......................................................................................................................................................53
Table 2: $^{195}$Pt coupling (J) values (Hz)………………………………………………………………..53

**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>D$_2$O</td>
<td>Deuterium Oxide</td>
</tr>
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<td>Dien</td>
<td>Diethylenetriamine</td>
</tr>
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<td>DMF</td>
<td>Dimethyl foramide</td>
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<td>eV</td>
<td>Electron Volts</td>
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<td>HNPCC</td>
<td>Nonpolyposis Colorectal Cancer</td>
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<tr>
<td>HOMO</td>
<td>Highest Occupied Molecular Orbital</td>
</tr>
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<td>ITC</td>
<td>Isothermal Titration Calorimetry</td>
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<td>Ka</td>
<td>Association constant</td>
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<tr>
<td>LUMO</td>
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<td>1-MeCyt</td>
<td>1-Methyl cytosine</td>
</tr>
<tr>
<td>MMR</td>
<td>DNA mismatch repair</td>
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<td>NCp7</td>
<td>Nucleocapsid 7 protein</td>
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<td>N-AcTrp</td>
<td>N-Acetyl Tryptophan</td>
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<td>NMR</td>
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</tr>
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<td>Phe</td>
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<td>py</td>
<td>Pyridine</td>
</tr>
<tr>
<td>p53</td>
<td>Tumor protein 53 or protein 53</td>
</tr>
<tr>
<td>RNA</td>
<td>RiboNucleic Acid</td>
</tr>
<tr>
<td>TAQ</td>
<td><em>Thermus aquaticus</em></td>
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Abstract

Non-covalent interactions involving π-π stacking play an essential role in self-assembly and molecular recognition processes such as protein folding and DNA/RNA-protein selective recognition. The knowledge gained from these studies could provide insight into possible site recognition complexes, inhibiting or mimicking protein-protein or protein-DNA interactions.

Based on molecular modeling as well as HOMO and LUMO energies, several chromophores were selected with a variety of Δε values (Δε = |ε_{HOMO,NAcTrp} − ε_{LUMO,chromophores}|), high and low, to establish a correlating trend with the modeling and experimental data. The corresponding Pt(dien) compounds were synthesized and their ability to stack to N-acetyl tryptophan was evaluated by fluorescence quench experiments. Attaching a strong electron donating/withdrawing group or extending the π system of pyridine or thiazole by means of a benzene ring (quinoline and benzothiazole) was found to enhance the π-π interaction with N-acetyl tryptophan.
Chapter 1 Introduction

1.1. π stacking

Non-covalent interactions involving π-π stacking are pivotal in biological processes and can range from reasonably small molecules to large biological systems.[1-5] They play an essential role in self-assembly and molecular recognition processes such as protein folding and DNA/RNA-protein selective recognition.[6] These π-π interactions are non-covalent intermolecular forces just as important as hydrogen bonding.[7] The term “π-π interaction” is often used indiscriminately, where it is mistakenly expected that aromatic rings will interact through π-π parallel stacking interaction, face-to-face. Aromatic rings may interact through several distinguishable arrangements: stacked, an edge or point-to-face, or T-shaped conformation (Figure 1.1).[7]

Figure 1.1. Principal orientations of aromatic-aromatic interactions[7]
Both the face-to-face and T-shaped stacking arrangements are limiting forms in aromatic interactions.[7] The electrostatic and Van der Waals interactions [dipole-dipole (electrostatic) interaction, dipole-induced-dipole interaction, and induced-dipole induced dipole (London) dispersion interactions] are the intermolecular forces for the stabilization of $\pi-\pi$ interactions between closed shell molecules.[3] Dipole-dipole (electrostatic) interactions are interactions between different permanent and static molecular charge distributions. Dipole-induced-dipole interactions are interactions between the static molecular charge distribution of group A with a proximity induced change in charge distribution of group B. Induced-dipole-induced-dipole (London) dispersion interactions are where an instantaneous dipole moment from a fluctuation electron cloud polarizes a neighboring molecule and induces in it also an instantaneous dipole.[7] (Figure 1.2)

![Diagram of electrostatic interactions]

**Figure 1.2.** Pictorial representation of the electrostatic interactions.[7]
These electrostatic and Van der Waals interactions are essentially attractive forces but are dependent on distance, with their potentials falling off rapidly with distance by $1/r^6$. [7] At very short distances repulsion becomes the main interacting force due to the overlap of the filled electron clouds of the electron shells of the molecules involved in the interaction. A prototypical model for $\pi-\pi$ interactions in aromatic systems is benzene. [8-10] With a benzene-benzene interaction the T-shaped motif is found to be the most favorable orientation due to the repulsion of the negatively charged out of plane $\pi$ electrons. (Figure 1.3)

![Figure 1.3. $\pi-\pi$ repulsion in benzene-benzene interaction if in face-to-face alignment.][7]

[5]The point- or edge-to-face, edge-on, or T-shaped orientation can be seen as the motif for arene interaction in many protein structures.[11-14] In $\pi$ systems that are extended or polarized, through substituents or heteroatoms, the conclusions drawn from benzene are considerably changed. The addition of substituents or heteroatoms disturbs the uniform charge distribution,
which results in partial atomic charges and a permanent dipole.[3, 5] This permanent dipole
induces dipole-dipole and dipole induced dipole interactions as well as affect atom-atom and out
of plane atom π-electron interactions (Figure 1.3). [3, 5] Having a nitrogen heteroatom (electron-withdrawing) within the ring decreases the π-electron density in the ring, resulting in a decrease
in the π-electron repulsion and an increase in stability of the face-to-face π stacking moiety.
(Figure 1.4)

![Diagram](image)

**Figure 1.4.** Decrease in π-electron density for aromatic rings containing a nitrogen heteroatom
within the ring.[7]

Therefore, aromatic moieties such as pyridine, bypyridines, and other aromatic nitrogen
heterocycles should produce more stable π stacked complexes.

**1.2. π-π stacking Interactions in Biological Processes**

1.2.a DNA mismatch repair

As mentioned previously, non-covalent interactions involving π-π stacking play an
essential role in self-assembly and molecular recognition processes such as protein folding and
DNA/RNA-protein selective recognition.[6] MutS, a mismatch DNA repair protein, is an
example of DNA-protein selective recognition.[15, 16] DNA mismatch repair (MMR) corrects mispaired or unpaired bases caused by DNA polymerase, increasing the overall reliability of DNA replication up to a 1,000-fold.[15, 17] Cells that contain mutations in MMR genes are characterized by the elevated rates of spontaneous mutation and instability of microsatellite repeats.[15] MMR genes can be deactivated by mutation or epigenetic process but predisposes people to nonpolyposis colorectal cancer (HNPCC) and random tumors.[18] Escherichia coli contains three MMR proteins, MutS, MutL and MutH. These MMR proteins set off a strand specific mismatch repair process that takes advantage of the briefly unmethylated state of the daughter strand, when newly synthesized. [15] The MMR protein MutS specifically recognizes mispaired or unpaired bases, up to four, and with the aid of MutL, will activate the endonuclease MutH initiating the repair process. Homologues of E. coli MutS have been found in nearly all organisms. [16] The bulk of the MutS proteins are related to the proteins that are involved with MMR, preventing homologous DNA recombination between heterologous sequences and mediating in cell death. [16, 19] The mechanism of MutS recognition of large range of mismatches embedded in a random DNA sequence remained unclear until Obmolova et al. obtained crystal structures of Thermus aquaticus (TAQ) MutS. TAQ MutS is a thermostable homologue of E. coli. TAQ MutS protein has been co-crystallized with a 21-bp hetero-duplex containing an unpaired thymidine (T).[7] (Figure 1.5)
Figure 1.5. Unpaired T in the MutS co-crystal partially stacked in the DNA duplex[20]

The unpaired base is displaced towards the minor groove by 3 Å and tilts toward the 5’ base pair.[20] The conformation is stabilized by the stacking of a phenylalanine of the MutS. (Figure 1.6)

Figure 1.6. Stacking of Phe 39 of the MutS to the unpaired T of the DNA.[20]
The phenylalanine approaches the DNA from the minor groove side and recognizes the unpaired or mismatched thymidine. It stacks onto the unpaired thymidine in order to initiate the process of repair.

1.2.b  \(\pi-\pi\) stacking interactions in stabilization of complexes

DNA enzymes are a class of catalysts that are composed of single stranded DNA that catalyze a variety of chemical reactions such as cleavage and formation of phosphoester bonds, porphyrin metallation, and oxidative cleavage of DNA.[21] The 10-23 DNA enzyme catalyses the sequence-specific cleavage of RNA[22] and, more specifically, the phosphoester bond between an unpaired purine and paired pyrimidine residue within the RNA substrate. In the case of structural stabilization, \(\pi-\pi\) interactions play an important role in the 82-nucleotide RNA-DNA complex formed by the 10-23 DNA enzyme.[23] 10-23 DNA is composed of a 15-nucleotide catalytic core, which is flanked by two substrate recognition domains. When the 10-23 DNA was crystallized with a RNA substrate it formed a complex containing five double-helical regions, two strands of DNA and two strands of RNA.[23] For each RNA substrate two DNA enzymes were bound by six nucleotides at the 5’ end of the RNA binding to one DNA enzyme and seven nucleotides at the 3’ end binding to the second DNA enzyme. (Figure 1.7)
Only three of the five helices are unique due to symmetry of the complex. Stems 1 and 2 take on an A-form geometry and stem 3, bridging stem 1 and 2, takes on a B-form. Each end of stem 3 interacts with the DNA through the stacking of threonine-adenine base pair to guanine-cytosine base pair. As a whole, the three dimensional shape of the RNA-DNA complex is stabilized by extensive base stacking and pairing of the nucleotides; all 82 of the nucleotide bases of the complex are involved in stacking intra- or intermolecularly. (Figure 1.8)[23]
1.2.c Interfering with p53-MDM2 interaction

The protein p53 has been referred to as the “guardian of the genome”. P53 works as a transcriptase factor that regulates the cell cycle and functions as a tumor suppressor. [24, 25] P53 is upregulated when the cell undergoes any kind of stress. Accumulation of p53 in the cell can trigger cell growth arrest, apoptosis, or senescence.[23] Another protein MDM2 is a negative regulator for p53. When bound to p53 MDM2 inactivates and promotes degradation.[26] The amount of the p53 and MDM2 expressed in the cell are correlated. There have been several strategies to activate the p53 pathway and utilize it as an anticancer target.[27] One strategy is to disrupt the p53-MDM2 interaction, releasing p53 from negative control.[28] One compound synthesized, called RITA, binds to p53 and disrupts the interaction between MDM2 and p53, releasing p53 to be able to accumulate in the cell nucleus and induce apoptosis.[24] The
mechanism in which it inhibits p53-MDM2 binding is not fully understood. Another class of compounds called nutlins interferes with the binding of p53 and MDM2 by binding to the MDM2 hydrophobic cavity and mimicking the interaction of the three critical p53 amino acids (Phenylalanine, Tryptophan, and Leucine).[25-27] Nutlins have the capability to penetrate cell membranes, activate the p53 pathway, and inhibit cell growth. The approach in this research was to target the amino acids tryptophan and phenylalanine on the N-terminal sequence of p53 and use platinum based molecules designed to π stack tryptophan and or phenylalanine to block the interaction between p53 and MDM2. (Figure 1.9)

**Figure 1.9.** Crystal structure of p53 (green)/MDM2 (yellow and white) binding pocket. Three amino acids of p53 shown inside binding pocket, leucine, tryptophan and phenylalanine; which are essential for the binding of MDM2.[29]

1.2.d Disruption of HIV-I zinc finger NCp7 function

Zinc fingers are one of the most abundant class of metalloproteins in the human proteome. Zinc fingers typically consist of a definite amount of amino acid residues, 30 to 40, with suitable Zn-binding sites[30]. The zinc ion (Zn$^{2+}$) is the key component of the system and
will bind to the residues in a tetrahedral arrangement; which is essential for its function. Release or substitution of the zinc ion will result in a loss of the zinc fingers function.

It was found that Zinc fingers that are used as spacers for DNA interaction are involved in the interaction with RNA[30]. One of the interactions between the Zinc finger and DNA/RNA is by $\pi$-stacking with a tryptophan residue of the zinc finger and an adenine of DNA/RNA. This $\pi$-stacking interaction with aromatic residues of the zinc finger, tryptophan and phenylalanine, and nucleobases has been seen for the HIV Nucleocapsid 7 protein (NCp7)[31]. (Figure 1.10)

Figure 1.10. Sequence of NCp7 showing coordinating residues in red. [32]

It has been established that the $\pi$-stacking interactions play an important role in selectivity and recognition between the NCp7 and its substrate, the $\Psi$ site in the viral RNA. Studies using models of the $\Psi$ site in the viral RNA showed important $\pi$-stacking interaction between aromatic nucleobases with the phenylalanine (F16) and the tryptophan (W37) in the zinc finger of NCp7[31, 33, 34]. Platinum-aromatic chromophores complexes could be used for molecular recognition of NCp7 by means of $\pi$-stacking with W37 or F16; resulting in a displacement of the zinc ion, loss of structure and loss of function. NCp7 is a C$_3$H zinc finger and the molecular recognition by C$_3$H zinc fingers differ from the most common zinc fingers found in the human body[35], C$_2$H$_2$ and C$_4$, providing a possible specific way to target for NCp7[36].
1.3. Nucleobase Modification

There are several amino acids in the human body that contain aromatic groups and are capable of π-π stacking interactions. (Figure 1.11)

Figure 1.11. Amino acids capable of π-π stacking interactions

These amino acids play an important role in DNA/RNA-protein recognition; generally stacking with cytosine and guanine, (Figure 1.12). [6, 23, 37, 38]

Figure 1.12. Nucleobases adenine (A), guanine (G), thymine (T), and cytosine (C).
Methylation of purine or pyrimidine based nucleic acids will enhance the $\pi-\pi$ stacking interactions when stacking with tryptophan.[39] This enhancement is due to the lowering of the energy of the lowest unoccupied molecular orbital (LUMO) of the $\pi$ acceptor, bringing it close in energy to the highest occupied molecular orbital (HOMO) of the $\pi$ donor. The decrease in the energy gap ($\Delta E$) between the LUMO of the $\pi$-acceptor and the HOMO of the $\pi$-donor, improves the acceptor properties toward the $\pi$-donor. (Figure 1.13)

![Figure 1.13.](image)

**Figure 1.13.** Protonation, alkylation or coordination of a metal ion such as Pd(II) or Pt(II) to a nucleobase strengthens the interaction by lowering the energy of the lowest unoccupied molecular orbital of the modified nucleobases (LUMO) and improving overlap with the highest occupied molecular orbital (HOMO) in N-acetyl tryptophan.[40]

A metal which is coordinated with the nucleic acid base will further enhance the $\pi$ stacking capability by further decreasing the gap between the HOMO of the $\pi$-donor (tryptophan) and the LUMO of the $\pi$-acceptor (metallated nucleobase).[41] Two metals that are notably used in increasing the $\pi-\pi$ stacking interactions are Pt(II) and Pd(II). [42, 43] Coordination of a nucleobase by either Pt(II) or Pd(II) will produce a complex with a higher net charge (+2), compared to just methylation (+1) of the nucleobase; producing a larger decrease in the energy of LUMO by 7-8 eV (electron volts).[41] Comparing Pt(II) to Pd(II), Pt(II) metallated
nucleobases produced a smaller energy gap between the HOMO of the \(\pi\)-donor (tryptophan) and the LUMO of the \(\pi\)-acceptor (metallated nucleobase). This indicates that coordinating a Pt(II) metal to the nucleobase will enhance the \(\pi\)-stacking interaction versus coordinating a Pd(II) metal. (Figure 1.14)

**Figure 1.14.** HOMO/LUMO energies for nucleobases, methylated nucleobases and metal coordinated nucleobases. Dash line is the HOMO of N-AcTrp. [Pd(dien)(1-MethylCytosine)]\(^{2+}\) (1); [Pt(dien)(1-MethylCytosine)]\(^{2+}\) (2); [Pd(dien)(9-EthylGuanine)]\(^{2+}\) (3); [Pt(dien)(9-EthylGuanine)]\(^{2+}\) (4).

These results suggest a novel structural design for metal coordinated complexes that are capable of recognition and probing of DNA- or RNA-protein interactions and protein-protein interactions involving tryptophan.

Previous work done by Anzellotti et al. suggested a nucleobase has enhanced capabilities to \(\pi\) stack with tryptophan when attached a platinum ion (Pt\(^{2+}\)).[44] This enhancement is a result
of lowering the energy of the LUMO in the platinated nucleobase (π acceptor), bringing it closer in energy to the HOMO of tryptophan (π donor).[44] (Figure 1.15)

Figure 1.15. Platination, Pt(II), of a nucleobase enhances the interaction by lowering the energy of the LUMO of the nucleobase and improving the overlap with the HOMO of the N-acetyl tryptophan.[40]

1.3.a Research Conducted

Based on molecular modeling as well as HOMO and LUMO energies, several chromophores were selected with a variety of \( \Delta \varepsilon \) values (\( \Delta \varepsilon = |\varepsilon_{\text{HOMO,NAcTrp}} - \varepsilon_{\text{LUMO,chromophore}}| \)), high and low, to establish a correlating trend with the modeling and experimental data. The corresponding Pt(dien) compounds were synthesized (Figure 3.1) and their ability to bind to tryptophan were evaluated by fluorescence quench experiments. Fluorescence spectroscopy can be used to monitor small changes occurring on the π-cloud of tryptophan due to π stacking interactions, and the degree of quenching in the fluorescence spectrum of tryptophan is an estimate of the strength of the π-π stacking interaction.[45, 46]

The fluorescence data can be used to calculate the association constant, \( K_a \); a mathematical constant that describes the bonding affinity between two molecules at equilibrium.
\[ \Delta F = -(1/K_a) \times (\Delta F/\text{Quencher}) + \Delta F_c \]

\(\Delta F\) is the difference between fluorescence intensities of N-Acetyl tryptophan with and without the presence of the platinated nucleobases. \(\Delta F_c\) is the difference when N-Acetyl tryptophan is completely saturated with the platinated nucleobase. In Figure 1.16 a plot of the correlation between the frontier orbital data (Figure 1.15) and experimental \(K_a\) values for 1-methylcytosine (1-MeCyt), 9-ethylguanine (9-EtGua) and their metallated analogues are shown.[40] The data shows that the \(\Delta\varepsilon\) values for the metal-nucleobase complexes are smaller for Pt(II) than Pd(II). This data is consistent with fluorescence experiments where a higher \(K_a\) was observed for Pt(II)-nucleobase vs. Pd(II)-nucleobase complexes. Thus, showing that \(\Delta\varepsilon\) values have an inverse relationship with the experimental \(K_a\) values[44]. (Figure 1.16)

**Figure 1.16.** A plot of the correlation between the frontier orbital data and available experimental \(K\pi\) values for 1-MeCyt, 9-EtGua and their metallated analogues. Points from left to right correspond to free base, Pd(II)-nucleobase, Pt(II)-nucleobase[40].
References

Chapter 2: Platination of Nucleobases

2.1. Abstract

The presence of an aromatic residue(s) such as a tryptophan or phenylalanine provides a recognition site in which can be selectively targeted by aromatic complexes; opening a plausible improved or more selective approach to protein-protein or protein-DNA interaction inhibition by means of non-covalent interactions. Platinated aromatic complexes can further enhance or mimic the $\pi$-$\pi$ interaction with aromatic residues by decreasing the energy gap between the HOMO of the $\pi$-donor (tryptophan) and the LUMO of the $\pi$-acceptor (platinated nucleobase). Fluorescence spectroscopy can be used to monitor small changes occurring on the $\pi$-cloud of tryptophan due to $\pi$ stacking interactions, and the degree of quenching in the fluorescence spectrum of tryptophan is an estimate of the strength of the $\pi$-$\pi$ stacking interaction.[45, 46]

2.2. Introduction

There are several amino acids in the human body that contain aromatic groups and are capable of $\pi$-$\pi$ stacking interactions. These amino acids play an important role in DNA/RNA-protein recognition; generally involving the amino acids cytosine, guanine, phenylalanine, and tryptophan.[6, 23, 37, 38] Methylation of purine or pyrimidine based nucleic acids will enhance the $\pi$-$\pi$ stacking interactions when stacking with tryptophan.[39] This enhancement is due to the lowering of the energy of the lowest unoccupied molecular orbital (LUMO) of the $\pi$ acceptor, bringing it close in energy to the highest occupied molecular orbital (HOMO) of the $\pi$ donor. The decrease in the energy gap ($\Delta E$) between the LUMO of the $\pi$-acceptor and the HOMO of the $\pi$-donor, improves the acceptor properties toward the $\pi$-donor. Coordination of a nucleobase by either Pt(II) or Pd(II) will produce a complex with a higher net charge (+2), compared to just
methylation (+1) of the nucleobase; producing a larger decrease in the energy of LUMO by 7-8 eV (electron volts). Comparing Pt(II) to Pd(II), Pt(II) metallated nucleobases produced a smaller energy gap between the HOMO of the π-donor (tryptophan) and the LUMO of the π-acceptor (metallated nucleobase). This indicates that coordinating a Pt(II) metal to the nucleobase will enhance the π-stacking interaction versus coordinating a Pd(II) metal.

Most approaches to protein-protein (p53-MDM2) or nucleic acid-zinc finger interaction (NCp7) inhibition have involved covalent interactions with metallated nucleobases or small organic molecules. In the case of NCp7 inhibition the approach has involved alkylation or oxidation of the cysteine residues, which results in a loss of conformation and reducing infectivity. This approach lacks in the area of selectivity, having little or no DNA selectivity and therefore causing these complexes to bind/attack in areas other than the desired target. The presence of an aromatic residue(s) such as a tryptophan or phenylalanine provides a recognition site in which can be selectively targeted by aromatic complexes; opening a plausible improved or more selective approach to protein-protein or protein-DNA interaction inhibition by means of non-covalent interactions. Anzellotti et al has used such an approach by targeting the tryptophan (W37) of the zinc finger knuckle bound to NCp7.[32] The tryptophan (W37) of the zinc finger has shown to be inserted between adjacent cytosine and guanine residues and stacking with the guanine residue. Anzellotti showed by mimicking this interaction by means of small molecules [Pt(dien)(9-EtGH)]⁺² and [Pt(dien)(5'-GMP)] you could stack with the tryptophan residue (W37); interaction measured by fluorescence spectroscopy. (Figure 2.0)
There was a slight difference between both complexes in stacking with free tryptophan and the zinc finger but both platinated complexes showed enhanced interaction over the free base counterpart. [32] Although these platinated bases were successful at mimicking the DNA-protein stacking interaction and forming an adduct between the zinc finger and the platinated complex; there was no significant disruption of the three-dimensional structure of the zinc finger. [32] Though this does provide a new avenue of investigation for selective targeting by means of π-π stacking interactions.

2.2.a Research Conducted

In this research conducted, several chromophores capable of π-π stacking interactions were investigated to assess their potential as possible protein-protein or protein-DNA interaction inhibitors. Based on molecular modeling as well as HOMO and LUMO energies, several chromophores were selected with a variety of Δε values ($\Delta\varepsilon = |\varepsilon_{\text{HOMO,NAcTrp}} - \varepsilon_{\text{LUMO,chromophore}}|$), high and low, to establish a correlating trend in stacking capability with the modeling and
experimental. The corresponding Pt(dien) compounds were synthesized (Figure 2.1) and their ability to bind to tryptophan were evaluated by fluorescence quench experiments.

2.3 Results and Discussion.

The systems examined were [Pt(dien)(L)]^{2+}, where L= quinoline (1), 4-dimethylaminopyridine (2), benzothiazole (3), 4-methoxypyridine (4), pyridine (5), 4-picoline (6), thiazole (7), 4-cyanopyridine (8), and 9-ethylguanine (9). (Figure 2.1)

![Schematic structures of complexes used in this study.](image-url)
All complexes were synthesized using [Pt(dien)NO$_3$]NO$_3$ as a starting material. $^1$H-NMR spectroscopy of Complex 1 in D$_2$O gave 6 peaks (3.0, 7.6, 7.9, 8.1, 8.3, 8.5, 9.5). The quinoline protons exhibited a shift upon platination ($\Delta\delta\approx0.7$ppm) in comparison with the free base. (Figure 2.2)

Figure 2.2. $^1$H-NMR spectroscopy of Complex 1 in D$_2$O

$^1$H-NMR spectroscopy of Complex 2 in D$_2$O gave 3 peaks ($\delta$ 3.0, 6.6, 8.0ppm). The 4-dimethylaminopyridine protons exhibited only a small shift upon platination ($\Delta\delta=0.1$ppm) in comparison with the free base; probably due to the strong electron donating group of the dimethylaminogroup donating electron density on to the already electron dense platinum metal and some of that electron density was donated back onto the nucleobase. (Figure 2.3)
Figure 2.3. $^1$H-NMR spectroscopy of Complex 2 in D$_2$O

$^1$H-NMR spectroscopy of Complex 3 in D$_2$O gave 6 peaks ($\delta$ 3.0, 7.7, 7.8, 8.1, 8.8, 9.7 ppm). The benzothiazole protons exhibited a shift upon platination ($\Delta\delta \approx$ 0.5 ppm) in comparison with the free base. (Figure 2.4)
Figure 2.4. $^1$H-NMR spectroscopy of Complex 3 in D$_2$O

$^1$H-NMR spectroscopy of Complex 4 in D$_2$O gave 4 peaks (δ 3.0, 3.9, 7.1, 8.4 ppm). The pyridine protons of Complex 4 exhibited a small shift upon platination ($\Delta\delta \approx$ 0.2 ppm) in comparison with the free base; probably due to the same reason as the 4-dimethylaminopyridine. (Figure 2.5)

Figure 2.5. $^1$H-NMR spectroscopy of Complex 4 in D$_2$O.
\(^1\)H-NMR spectroscopy of Complex 5 in D\(_2\)O gave 4 peaks (\(\delta\ 3.0, 7.5, 8.0, 8.7\) ppm). The pyridine protons exhibited a shift upon platination (\(\Delta\delta \approx 0.7\) ppm) in comparison with the free base. (Figure 2.6)

![Figure 2.6. \(^1\)H-NMR spectroscopy of Complex 5 in D\(_2\)O.](image)

\(^1\)H-NMR spectroscopy of Complex 6 in D\(_2\)O gave 4 peaks (\(\delta\ 2.4, 3.0, 7.38, 8.5\) ppm). The pyridine protons of Complex 6 exhibited a shift upon platination (\(\Delta\delta \approx 0.2\) ppm) in comparison with the free base. (Figure 2.7)
Figure 2.7. $^1$H-NMR spectroscopy of Complex 6 in D$_2$O.

$^1$H-NMR spectroscopy of Complex 7 in D$_2$O gave 4 peaks ($\delta$ 3.0, 7.75, 7.94, 9.2ppm). The thiazole protons exhibited a shift upon platination ($\Delta \delta=0.4$ppm) in comparison with the free base. (Figure 2.8)
Figure 2.8. $^1$H-NMR spectroscopy of Complex 7 in D$_2$O.

$^1$H-NMR spectroscopy of Complex 8 in D$_2$O gave 3 peaks ($\delta$ 3.0, 7.96, 9.03ppm). The pyridine protons of Complex 8 exhibited a shift upon platination ($\Delta \delta \approx 0.4$ppm) in comparison with the free base. (Figure 2.9)
2.4. Stacking Interactions by Fluorescence Spectroscopy:

Fluorescence spectroscopy can be used to monitor small changes occurring on the π-cloud of tryptophan due to π stacking interactions, and the degree of quenching in the fluorescence spectrum of tryptophan is an estimate of the strength of the π-π stacking interaction.[45, 46] Different π systems, electron donating/electron withdrawing groups, ring size and extended π systems, were examined to see if the π stacking interaction of the platinated nucleobase with tryptophan can be enhanced. When examining electron donating groups versus electron withdrawing groups, pyridine was used as a baseline for comparison with a % fluorescence quenching of 7.56. (Figure 2.10)
Figure 2.10. (a) absorbance spectrum of Pt(dien)(pyridine)(NO₃)₂ at 7mM concentration (b) Fluorescence spectrum of [Pt(dien)(pyridine)](NO₃)₂ (7mM) stacking with L-acetyl tryptophan (5µM) after excitation at 280nm. First line is the emission of just tryptophan; last line is [quencher]/[tryptophan] at 100:10; after ten additions of quencher (7mM)
It was found that adding strong electron donating/withdrawing groups to the pyridine ring would increase the π-π stacking interaction with tryptophan. Adding a weak donating group, CH₃, and a moderate donating group, OCH₃, only slightly increased the π-π stacking interaction with tryptophan. [Pt(dien)(4-picoline)]NO₃ showed a % fluorescence quenching of 7.96 (Figure 2.11) and [Pt(dien)(4-Methoxypyridine)](NO₃) showed a % fluorescence quenching of 8.15. (Figure 2.12)
Figure 2.11. (a) absorbance spectrum of [Pt(dien)(4-picoline)](NO$_3$)$_2$ at 7mM concentration (b) Fluorescence spectrum of [Pt(dien)(4-picoline)](NO$_3$)$_2$ (7mM) stacking with L-acetyl tryptophan (5µM) after excitation at 280nm. First line is the emission of just tryptophan; last line is [quencher]/[tryptophan] at 100:10; after ten additions of quencher (7mM).
Figure 2.12. (a) absorbance spectrum of \([\text{Pt(dien)(4-methoxypyridine)}](\text{NO}_3)_2\) at 7mM concentration (b) Fluorescence spectrum of \([\text{Pt(dien)(4-methoxypyridine)}](\text{NO}_3)_2\) (7mM) stacking with L-acetyl tryptophan (5µM) after excitation at 280nm. First line is the emission of just tryptophan; last line is [quencher]/[tryptophan] at 100:10; after ten additions of quencher (7mM).

The addition of a strong electron donating group, dimethylamino, to the pyridine ring greatly enhanced the \(\pi-\pi\) stacking interaction with tryptophan. \([\text{Pt(dien)(4-Dimethylaminopyridine)}](\text{NO}_3)\) showed a % fluorescence quenching of 65.4. (Figure 2.13)
Figure 2.13. (a) Absorbance spectrum of Pt(dien)(4-Dimethylaminopyridine)(NO₃)₂ at 7 mM concentration (b) Fluorescence spectrum of Pt(dien)(4-Dimethylaminopyridine)(NO₃)₂ (7 mM) stacking with L-acetyl tryptophan (5 µM) after excitation at 280 nm. First line is the emission of just tryptophan; last line is [quencher]/[tryptophan] at 100:10; after ten additions of quencher (7 mM)
The addition of a strong electron withdrawing group, cyano, to the pyridine ring greatly enhanced the $\pi-\pi$ stacking interaction with tryptophan as well. [Pt(dien)(4-cyanopyridine)](NO$_3$)$_2$ showed a % fluorescence quenching of 63.45. (Figure 2.14)

**Figure 2.14.** (a) absorbance spectrum of Pt(dien)(4-cyanopyridine)](NO$_3$)$_2$ at 7mM concentration (b) Fluorescence spectrum of Pt(dien)(4-cyanopyridine)](NO$_3$)$_2$ (7mM) stacking with L-acetyl tryptophan (5µM) after excitation at 280nm. First line is the emission of just tryptophan; last line is [quencher]/[tryptophan] at 100:10; after ten additions of quencher (7mM).
The size of the ring also has an effect on the stacking capability. Comparing the 6 member ring pyridine versus 5 member ring thiazole it was found that the 6 member ring size has more of an effect than originally expected. [Pt(dien)(pyridine)]NO₃ has shown a % fluorescence quenching of 7.56, [Pt(dien)(thiazole)]NO₃ showed a minimal % fluorescence quenching of 4.32. (Figure 2.15)
Figure 2.15. (a) absorbance spectrum of [Pt(dien)(thiazole)](NO₃)₂ at 7mM concentration (b) Fluorescence spectrum of [Pt(dien)(thiazole)](NO₃)₂ (7mM) stacking with L-acetyl tryptophan (5µM) after excitation at 280nm. First line is the emission of just tryptophan; last line is [quencher]/[tryptophan] at 100:10; after ten additions of quencher (7mM).

Extending these two π systems further improved the stacking with tryptophan. The two compounds examined were benzothiazole and quinoline, just an addition of benzene to the previous two compounds. [Pt(dien)(benzothiazole)](NO₃) showed a % fluorescence quenching of 36.24 (figure 2.16) and [Pt(dien)(quinoline)](NO₃) showed a % fluorescence quenching of 29.41. (Figure 2.17)
Figure 2.16. (a) absorbance spectrum of Pt(dien)(benzothiazole)](NO₃)₂ at 7mM concentration (b) Fluorescence spectrum of [Pt(dien)(benzothiazole)](NO₃)₂ (7mM) stacking with L-acetyl tryptophan (5µM) after excitation at 280nm. First line is the emission of just tryptophan; last line is [quencher]/[tryptophan] at 100:10; after ten additions of quencher (7mM).
Figure 2.17. (a) absorbance spectrum of $[\text{Pt(dien)(quinoline)}](\text{NO}_3)_2$ at 7mM concentration (b) Fluorescence spectrum of $[\text{Pt(dien)(quinoline)}](\text{NO}_3)_2$ (7mM) stacking with L-acetyl tryptophan (5µM) after excitation at 280nm. First line is the emission of just tryptophan; last line is [quencher]/[tryptophan] at 100:10; after ten additions of quencher (7mM).
2.5. Experimental Section

**Materials.** Quinoline, 4-Dimethylaminopyridine, Benzothiazole, 4-Methoxypyridine, Pyridine, 4-Picoline, Thiazole, 4-Cyanopyridine, 9-Ethylguanine and N-Acetyltryptophan were Alfa Aesar, Sigma Aldrich and TCI America; complex [Pt(dien)(NO₃)](NO₃) was synthesized according to reported procedures.[49]

**Nuclear Magnetic Resonance experiments.** ^1H NMR spectra were recorded on a Varian Mercury series 300 MHz Spectrometer using a 5-mm tube; chemical shifts were referenced to a residual signal in D₂O (4.79 ppm) and DMF-d₇ (8.00 ppm). ^195Pt-NMR were recorded on a Varian Mercury series 300 MHz Spectrometer using a 10-mm tube. Chemical shifts were referenced to K₂[PtCl₆]. The scanning frequency for ^195Pt nuclei was set at 64.32 MHz.

**Fluorescence Experiments.** In a typical experiment, 3 mL of N-AcTrp (5 μM) were titrated with aliquots of the corresponding quenching compound (7 mM) with a ratio of ([quencher]/[N-AcTrp]) 100-10; 10.5 mM Phosphate buffer adjusted with a few drops of HCL was used in all experiments (pH 7.0). The maximum intensity of the spectrum (ca. 357 nm) was measured for each addition, and the association constants for each system were obtained from the analysis of the Eadie-Hofstee plots.[50] Measurements were made at 20°C, and the reported % fluorescence quenching of tryptophan were averaged over a number of three different experiments. Fluorescence spectra were recorded in the range of 320-420 nm with a scan rate of 120nm/min.

Scheme 2.1. Platination of nucleobases.
**Pt(Cl₂)(DMSO₄).** Pt(Cl₂)(DMSO₂) prepared similar to a reported procedure.[51] K₂PtCl₄ was suspended in 10 mL H₂O and reacted with a 1:1 molar ratio of DMSO at room temperature for 24 hrs. Precipitate was filtered and washed with water, ethanol, and ether and dried in vacuo (84% yield). ¹H-NMR (D₂O) δ 3.65.

**[Pt(dien)Cl]Cl.** [Pt(dien)Cl]Cl was prepared similar to a reported procedure.[52] PtCl₂DMSO₂ was suspended in 120 mL methanol and reacted with a 1:1 molar ratio of diethyltriamine at reflux for 4 hrs. Solution was evaporated down to ~10 mL and 5ml of methanol was added. After this ether was added and white solid appeared. Centrifuge was used to obtain the solid. Solid was then dried in vacuo (86% yield). ¹H-NMR (D₂O) δ 2.8.

**[Pt(dien)(Quinoline)](NO₃)(1).** [Pt(dien)(Quinoline)](NO₃) was prepared similar to a reported procedure.[53] Two equivalents of AgNO₃ were added to a solution of [Pt(dien)Cl]Cl in H₂O and solution stirred overnight to remove the chloride ligands and produce the complex [Pt(dien)NO₃]NO₃ in situ. The solution was then filtered and lyophilized to produce a white solid. The white solid was dissolved in DMF and Quinoline was then added in 3-1 molar amounts and the mixture was stirred overnight. Solution was evaporated down to ~5 mL and 5 mL of methanol was added and an off white solid was obtained upon addition of ether and centrifugation. Solid dried in vacuo (50% yield). ¹H-NMR (D₂O) δ 3.0, 7.6, 7.9, 8.1, 8.3, 8.5, 9.5. Calcd for C₁₃H₂₀N₆O₆Pt: C, 28.32; H, 3.66; N, 15.24. Found: C 27.76; H, 3.45; N, 14.88%.
[Pt(dien)(4-Dimethylaminopyridine)](NO₃)(2). [Pt(dien)(4-Dimethylaminopyridine)](NO₃) was prepared similar to that of [Pt(dien)(Quinoline)](NO₃) using 4-Dimethylaminopyridine as the nucleobase and H₂O as the solvent (26% yield). ¹H-NMR (D₂O) δ 3.0, 6.6, 8.0. Calcd for C₁₁H₂₃N₇O₆Pt: C, 24.27; H, 4.26; N, 18.01. Found: C, 23.91; H, 4.89; N, 19.62%.

[Pt(dien)(Benzothiazole)](NO₃)(3). [Pt(dien)(Benzothiazole)](NO₃) was prepared similar to that of [Pt(dien)(4-Dimethylaminopyridine)](NO₃) using benzothiazole as the nucleobase (29% yield). ¹H-NMR (D₂O) δ 3.0, 7.7, 7.8, 8.1, 8.8, 9.7. Calcd for C₁₁H₁₈N₆O₆Pt: C, 23.70; H, 3.25; N, 15.08. Found: C, 23.14; H, 3.29; N, 14.26%.

[Pt(dien)(4-Methoxypyridine)](NO₃)(4). [Pt(dien)(4-Methoxypyridine)](NO₃) was prepared similar to that of [Pt(dien)(4-Dimethylaminopyridine)](NO₃) using 4-methoxypyridine as the nucleobase (24% yield). ¹H-NMR (D₂O) δ 3.0, 3.9, 7.1, 8.4. Calcd for C₁₀H₂₀N₆O₇Pt: C, 22.60; H, 3.79; N, 15.82. Found: C, 21.31; H, 3.91; N, 15.41%.

[Pt(dien)(pyridine)](NO₃)(5). [Pt(dien)(pyridine)](NO₃) was prepared similar to that of [Pt(dien)(4-Dimethylaminopyridine)](NO₃) using pyridine as the nucleobase (62% yield). ¹H-NMR (D₂O) δ 3.0, 7.5, 8.0, 8.7. Calcd for C₉H₁₈N₆O₆Pt: C, 21.56; H, 3.62; N, 16.76. Found: C, 21.28; H, 3.34; N, 15.86%.

[Pt(dien)(4-picoline)](NO₃)(6). [Pt(dien)(4-picoline)](NO₃) was prepared similar to that of [Pt(dien)(4-Dimethylaminopyridine)](NO₃) using 4-picoline as the nucleobase (48% yield). ¹H-
NMR (D$_2$O) $\delta$ 2.4, 3.0, 7.38, 8.5. Calcd for C$_{10}$H$_{20}$N$_6$O$_6$Pt: C, 23.30; H, 3.91; N, 16.31. Found: C, 22.82; H, 3.69; N, 15.39%.

**PT(dien)(Thiazole)](NO$_3$)(7).** [Pt(dien)(Thiazole)](NO$_3$) was prepared similar to that of [Pt(dien)(4-Dimethylaminopyridine)](NO$_3$) using thiazole as the nucleobase (39% yield). $^1$H-NMR (D$_2$O) $\delta$ 3.0, 7.75, 7.94, 9.2. Calcd for C$_7$H$_{16}$N$_6$O$_6$PtS: C, 16.57; H, 3.18; N, 16.56. Found: C, 16.74; H, 2.67; N, 16.07%.

**Pt(dien)(4-Cyanopyridine)](NO$_3$)(8).** [Pt(dien)(4-Cyanopyridine)](NO$_3$) similar to that of [Pt(dien)(4-Dimethylaminopyridine)](NO$_3$) using 4-cyanopyridine as the nucleobase and heating at 40°C (23% yield). $^1$H-NMR (D$_2$O) $\delta$ 3.0, 7.94, 9.0. Calcd for C$_{10}$H$_{17}$N$_7$O$_6$Pt: C, 22.82; H, 3.26; N, 18.63. Found: C, 21.93; H, 3.19; N, 18.11%.

**Pt(dien)(9-Ethylguanine)](NO$_3$)(9)** was a gift from Dr. Q.A. dePaula.

Table 1. Pt(dien)L data.

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2.6. Conclusion

Trends have been established when examining the stacking capability of platinum(II) complexes with tryptophan (figure 2.17); adding a strong electron donating/withdrawing group or extending the \(\pi\) system of pyridine can significantly enhance the \(\pi-\pi\) stacking interaction with tryptophan. This can be useful in being applied to recognition processes of proteins or DNA/RNA that contain tryptophan.
Figure 2.18. % fluorescence quenching of tryptophan by [Pt(dien)nucleobase]NO₃ complexes.
References

44. Anzellotti, A.I., Ma, E.S.; Farrell, N., Platination of Nucleobases to enhance Noncovalent
3815.
40822.
VITA

Education

- **Master of Science in Chemistry**, Virginia Commonwealth University (VCU). Richmond, VA. **GPA: 3.02**  
  Thesis Advisor: Dr. Nicholas P. Farrell

- **Bachelors of Science in Chemistry**, Salisbury University. Salisbury, MD. **GPA: 3.0**  
  Advisor: Dr. Miguel Mitchell  
  “Quaternized promazine/promethazine derivatives in inhibiting NADH II production”

Relative Work Experience

**Research Assistant**, VCU, Richmond, VA, 01/08-12/09  
- Synthesis and characterization of platinum(II) antitumor complexes.

**Teaching Assistant**, VCU, Richmond, VA, 07/07-12/09  
- Assisted in teaching General chemistry I and II, Organic I and II laboratory.

**Research Assistant**, Salisbury University, Salisbury, MD, 01/06-06/07  
- Synthesis and characterization of promazine/promethazine derivatives in inhibiting NADH II production.

Presentations and Seminars

- Aaron B. Bate, “Noncovalent Interaction of Platinum Planar Amine Compounds with Tryptophan: As a Strategy to Interfere with p53-MDM2 Interactions” poster presentation at Virginia Commonwealth University, October 2008


- Senior Research Seminar titled, “Halogen Bonding.” Salisbury University, May 2007

- Aaron B. Bate, “Synthesis and Biological Study of Quaternized Promazine and Promethazine derivatives” Salisbury University Research Conference. May 2007
• Aaron B. Bate, “Synthesis and Biological Study of Quaternized Promazine and Promethazine derivatives” Intercollegiate Student Chemists Convention, Ursinus College PA, April 2007

Publications


Awards and Scholarships

• Philip Morris first year research scholarship (2008)

• Dean’s List (2007)

• Senatorial Scholarship (2003-2007)

• State Scholarship (2003-2007)

Additional Skills

• Spectroscopic methods of characterization: UV/VIS spectroscopy, IR spectroscopy, Raman spectroscopy, Fluorescence spectroscopy, Isothermal Titration Calorimetry, Nuclear Magnetic Resonance Spectroscopy (1D and 2D).

• Computer skills: Several versions of MS-Windows, Word, Excel, PowerPoint, Photoshop.

• Visualization programs: MestReC, ChemOffice, PyMol.
Supplemental Information.

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