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## HIV-1 Integrase Multimerization By Quinoline Based Drugs

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The University of Southern Mississippi

HIV-1 Integrase Multimerization By Quinoline Based Drugs

by

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Submitted to the Honors College of  
The University of Southern Mississippi  
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of Honors Requirements

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## Abstract

Human Immunodeficiency Virus Type 1 (HIV-1), a single stranded RNA retrovirus, affects over 30,000,000 people world-wide<sup>1</sup>. The virus works by infecting and promoting destruction of CD4 immune cells. and thus, suppressing proper immune functions<sup>2</sup>. Contrary to DNA viruses, the absence of extensive proofreading mechanisms in RNA viruses makes HIV latency a major obstacle in the discovery of long-term, effective treatments. Moreover, the importance of exploring novel therapeutic targets and designing complimentary inhibitory molecules remains steadfast in HIV research<sup>3</sup>

Recently, HIV-1 Integrase (IN) multimerization, the core enzyme used for integration of the viral DNA into an invaded host chromosome, has been identified as an unexploited therapeutic target. Moreover, a class of quinoline based allosteric integrase inhibitors (ALLINs) have shown promising inhibitory effects, most notably in the assembly of inactive viral particles<sup>4</sup>. The exact mechanism of action of this class of molecules, however, remains unclear due to the multimodal role of the drug. Herein, we report potencies of six synthesized single point derivatives of 4-phenylquinoline using an innovative *in vitro* assay capable of measuring the multimerization between full length IN and constructed C-terminal domain (CTD) using Fluorescence Resonance Energy Transfer (FRET) and antibody-conjugated fluorophores. The potencies of IN multimerizing drugs were characterized by their EC<sub>50</sub> values obtained from the drug concentration vs FRET curve. Our results, complimentary to recent studies, support the proposed mechanism of action of this class of ALLINs and highlight the antiviral potential of improved quinoline-based molecule derivatives to further exploit HIV-1 IN

multimerization as a therapeutic target

Key Words: HIV-1 Integrase Multimerization, Allosteric Integrase inhibitors, Human Immunodeficiency Virus

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## List of Abbreviations:

|                  |  |
|------------------|--|
| AIDS             | acquired immunodeficiency virus        |
| ALLINI           | allosteric integrase inhibitor         |
| CTD              | C-terminal domain                      |
| CCD              | catalytic core domain                  |
| DNA              | deoxyribonucleic acid                  |
| EC <sub>50</sub> | half maximal effective concentration   |
| FDA              | Food and Drug Administration           |
| FRET             | fluorescence resonance energy transfer |
| HAART            | Highly Active Retroviral Therapy       |
| HIV-1            | Human Immunodeficiency Virus Type 1    |
| HTFR             | homogeneous time resolved fluorescence |
| IBD              | integrase binding domain               |
| IN               | integrase                              |
| LEDGF            | lens epithelium-derived growth factor  |
| LTR              | long terminal repeat                   |
| mRNA             | messenger ribonucleic acid             |
| NTD              | N-terminal domain                      |
| PIC              | pre-integration complex                |
| RNA              | ribonucleic acid                       |
| SSC              | strand transfer complex                |
| vDNA             | viral DNA                              |



## Chapter I: Introduction

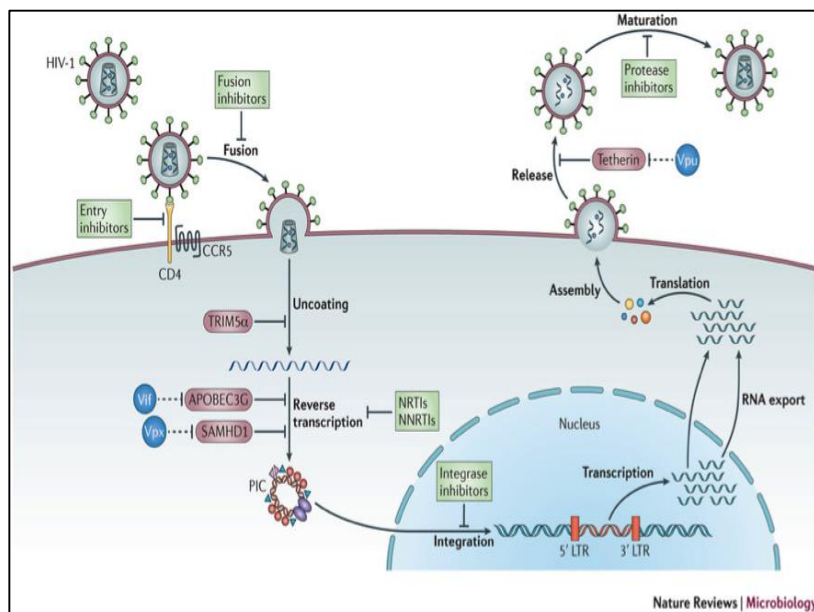
Human Immunodeficiency Virus (HIV) affects over 36 million people world-wide characterized by the progressive degradation of the immune system. If left untreated, the infection results in the late stage Acquired Immunodeficiency Syndrome (AIDS), characterized by a severe depletion of CD4<sup>+</sup> T-cells and an incapacitated immune system<sup>5</sup>. Although effective treatments have been discovered to control the spread of the virus within a patient, a potential cure has yet to be identified. The homodimer interface of HIV type 1 (HIV-1) integrase (IN), the viral enzyme central to the integration stage of the HIV-1 life cycle, has recently been identified as a viable therapeutic target<sup>6</sup>. Moreover, a novel class of quinoline based compounds has exhibited promising inhibitory effects on IN *in vitro*<sup>3,7-9</sup>. Due to the high dynamic state of IN, crystallizing and characterizing the full-length IN protein has proven difficult. Additionally, the proposed multimodal action of this class of IN inhibitors has suggested evaluation of previous experimental designs to account for the distinct mechanisms of inhibition and for the active site characterization<sup>10</sup>. Our assay was designed to quantify multimerization potencies of synthesized compounds. To conduct the assay, separate preparations of full length IN and recombinant CTD of IN were marked with Flag and Histidine tags, respectively. Complimentary fluorophore-conjugated antibodies were added to each preparation and then incubated in serial dilutions of the drugs to be assayed. The EC<sub>50</sub> values were obtained from the homogeneous time-resolved fluorescence resonance energy transfer (HTR-FRET) signal exhibited between donor and acceptor fluorophores.

## Chapter II: Literature Review

### *HIV-1 Life Cycle*

The HIV-1 life cycle occurs through a series of dependent steps involving both host and viral proteins to induce an infection. (Figure 1). Glycoproteins on the virion surface bind to the abundant receptors and coreceptors of the CD4 immune cell, allowing for subsequent fusion of the two membranes. The contents of the virion are then released into the cell where the viral proteins reverse transcribes the viral RNA, catalyzing the formation and nuclear transport of the pre-integration complex (PIC)<sup>5</sup>.

After HIV-1 Integrase inserts the viral DNA into the host chromosome, the cell becomes permanently infected and host proteins continuously transcribe and translate the incorporated genetic material into chains of HIV-1 proteins. The viral proteins then assemble into an immature HIV-1 particle, bud through the host's cell membrane, and begin maturation via proteolysis of the HIV protein chain (Figure 1)<sup>11</sup>. Inhibitory drugs are



categorized according to their mode of action within the viral life cycle. Current FDA approved drugs include entry inhibitors, fusion inhibitors, reverse transcriptase inhibitors (NRTIs & NNRTIs),

*Figure 1: HIV Life Cycle and Current Inhibitor Targets*

integrase strand transfer inhibitors (INSTIs), and protease inhibitors (PI)<sup>12</sup>. Each of these HIV-1 drugs involves the disruption of a single stage in the viral life cycle, effectively preventing further viral replication. The lack of proofreading mechanisms in retroviruses, however, allows for spontaneous mutations from host cell to host cell, and thus, HIV rapidly becomes resistant to drug treatments. Introduction of Highly Active Antiretroviral Therapy (HAART) in 1996 offered a breakthrough in HIV treatment, transforming the once universally fatal infection to a manageable chronic illness. Opposed to single-drug or dual-drug therapies, HAART targets three or more stages of the HIV life cycle, reducing the probability of mutation and eliciting a synergetic antiviral effect<sup>13</sup>. Optimal HAART treatment utilizes either a protease inhibitor (PI) or a nucleoside reverse transcriptase inhibitor (NRTI), in combination with two non-nucleoside reverse transcriptase inhibitors (NNRTIs) through an orally bioavailable drug cocktail<sup>12</sup>. Despite the success of HAART, mutations that allow for multi-drug resistance and cross resistance continues to hinder treatment. Additionally, diversity in a patient's body introduces the possibilities of insufficient drug metabolism or unmanageable side effects. These limitations require altering medication cocktail, but the narrow selection of HIV drugs continues to restrict HAART's long-term effectiveness.

#### *LEDGF/p75 Integration Factor*

Despite the discovery, the HIV-1 infection in 1983<sup>5</sup>, the role of host cellular proteins remains unclear, and only a few have been thoroughly studied and characterized. Epithelium-derived growth factor (LEDGF), an Endogenous and ubiquitous transcriptional coactivator, is one of those few and has recently been the center of attention for its

association with multiple lentiviral INs including HIV-1 IN<sup>14</sup>. The LEDGF/p75 cofactor contains a chromatin-binding domain at the N terminus and the integrase binding domain (IBD) at the C-terminus. During nuclear transport of the pre-integration complex (PIC), the LEDGF/p75 engages IN at a cleft formed between interacting CCDs, then directs the PIC to an active transcription site of the host chromosome. Using the crystal structure of LEDGF/p75, the indicated studies suggested that the cofactor acts to tether the HIV-1 PIC to cellular chromatin and is essential for initiating viral integration and replication. In a study by Christ *et al.* on IN binding, IBD was overexpressed in human cells, and the fragment efficiently competed with the endogenous LEDGF/p75 cofactor, inhibiting HIV replication and integration to nearly undetectable levels<sup>15</sup>. Complimentary to the characterization of the integrase binding domain (IBD) and the chromatin tethering regions of LEDGF/p75, studies have indicated that the cofactor increases IN stability and protects IN from degradation<sup>14</sup>. From these relevant studies, it can be deduced that integration can be performed solely by IN; LEDGF/p75 is necessary, however, for precise and consistent binding at an active transcription site. The progression of LEDGF/p75 research has, thus, introduced a number of potential therapeutic HIV targets, particularly where the LEDGF IBD contacts the IN.

#### *HIV-1 Integrase as a Therapeutic Target*

The HIV-IN protein monomer is characterized by three distinct domains: The N-terminal domain (NTD), the catalytic core domain (CCD), and the C-terminal domain (CTD). Although each domain is essential for IN to carry out its enzymatic functions, the CCD contains primary active site residues of Asp-64, Asp-116, and Glu-152. Dimerization

between two CCD is favored in its highly dynamic, inactive state. The presence of viral DNA promotes the association between IN dimers at their CCDs, thereby forming a functional tetramer to carry out its enzymatic functions<sup>16</sup>. IN catalyzes two reactions in vivo; the 3' processing of the viral DNA after the PIC to the nucleus, and the strand transfer reaction of the 3' vDNA into the host DNA. The resulting strand transfer complex (STC) contains strand discontinuation and hemi-integrated, flanking vDNA, both of which are repaired by the host's DNA polymerase, 5'-Flap endonuclease and DNA ligase, to form the stable provirus<sup>17</sup>. This stage of the HIV-1 lifecycle is irreversible, and the infected cell becomes a permanent carrier of the virus.

Studies of the LEDGF/p75 IBD interaction with IN have offered valuable data in identifying and characterizing HIV-1 IN active sites for therapeutic exploitation. The co-crystal structure of a functional IN protein accelerated identification of drug targets and complimentary inhibitory molecules. Due to its dynamic oligomeric state, however, difficulty in crystalizing the active form of IN has hindered the exploitation of IN structure/activity relationships. Nevertheless, alternative routes of protein structure identification have presented similar incentives to researchers; the reported co-crystal structure of an IN CCD dimer complexed with the LEDGF/p75 IBD<sup>18</sup> has been especially useful in advancing HIV drug discovery. In a proof-of-concept study, Christ *et al.* evaluated the available LEDGF/p75 CCD co-crystal structure as a potential antiviral target. The relevant protein to protein contact residues were then used to design small molecule inhibitors and test their effects on HIV-1 replication in an infected cell. Notably, the 2-quinolin-3-yl acetic acid-based compounds exhibited the most potency in inhibiting viral replication. In addition to reducing LEDGF/p75-mediated integration, the compounds were

found to block 3'-processing of the viral DNA ends<sup>15</sup>. Continued design and discovery of small molecules that interfere with the IN to LEDGF/p75 interaction (LEDGINs) have exemplified the promising therapeutic target. This reported class of compounds led to a deeper investigation of the specific mechanism of action. Knockdown of endogenous LEDGF/p75 led to a significant increase in potencies of the molecules *in-vivo*, suggesting competitive binding between the cofactor and the compound during the early stages of viral replication<sup>19</sup>. Although first coined as LEDGINs, literature on this class of compound has evolved to suggest a multimodal, cooperative effect during early and late stages of viral replication. In addition to inhibiting LEDGF-IN interactions, this class of molecules were shown to also inhibit LEDGF-independent IN 3' processing and strand transfer reactions. Therefore, 2-quinolin-3-yl acetic acid derivatives were, more fittingly, reclassified as Allosteric Integrase Inhibitors (ALLINIs)<sup>10</sup>. ALLINIs have been shown to effectively promote aberrant IN multimerization within newly produced virions, resulting in immature, noninfectious viral particles. Conclusively, the overwhelming evidence of ALLINIs' multimodal effect suggests a synergetic repression of HIV-1 with primary potencies occurring through IN multimerization during late stage viral replication, and secondary potencies through competitive binding with LEDGF in early stage viral replication.

## Small Molecule Inhibitors of HIV-1 IN

Currently, FDA approved drugs that target the integration stage of HIV-1 replication, as are IN strand transfer inhibitors (INSTIs), such as raltegravir and elvitegravir. Recent investigations have presented novel small molecule inhibitors that bind to a non-catalytic site of HIV-1 IN, in contrast to INSTIs. The class of tricyclic quinoline heterocycles have received considerable attention due to their potencies in the multimodal, cooperative effect by allosterically inhibiting IN catalytic activity in early stage replication as well as by promoting aberrant multimerization of IN subunits during late stage

replication.

The 2,3,4-trisubstituted aryl quinolines have been shown to exhibit inhibitory effects against HIV-1 IN. Fader *et al.* used SAR and hit-to-lead studies to report strategic substitutions of the quinoline scaffold for maximal inhibition of IN 3'

processing activity, and those propositions were especially useful in our identification and selection of the optimal quinoline scaffold for maximal multimodal potency of ALLINIs. The crystalized

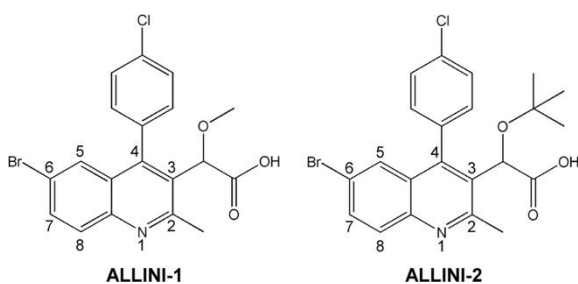


Figure 2: Structures of notable ALLINIs.

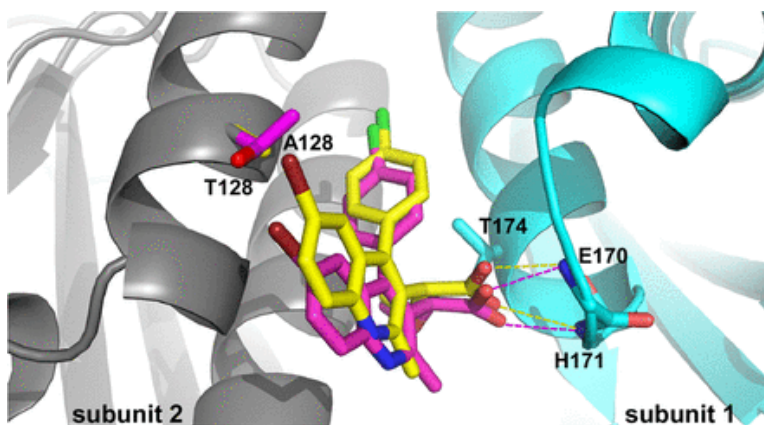


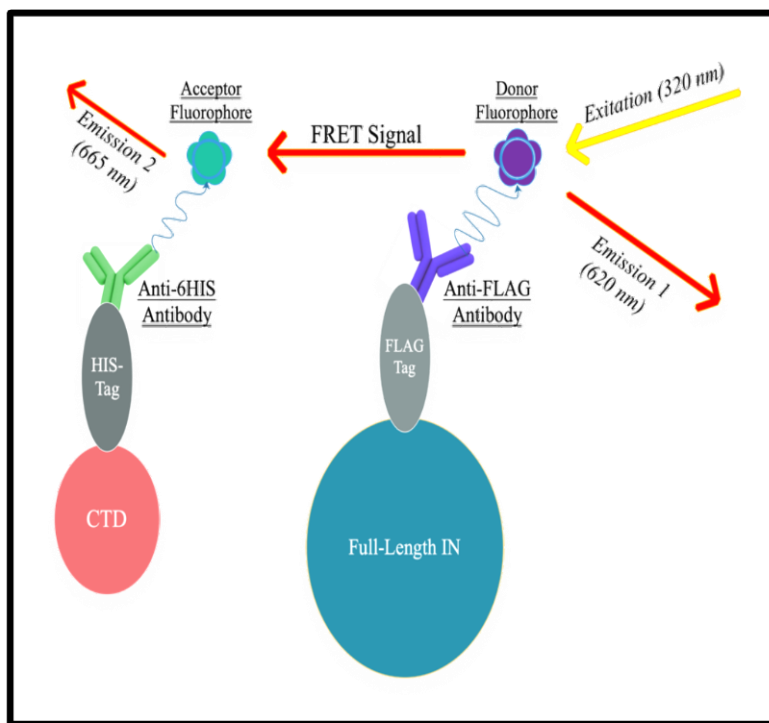
Figure 3: Three-Dimensional depiction of ALLINI-1 bound within the allosteric, hydrophobic pocket of the HIV-1 IN CCD dimer. Separate IN monomer subunits are color-coded.

IN-CCD with a bound ALLINI has also proved useful in the design of small molecule inhibitors through hinting at the mechanism in which high-order multimerization is promoted. Analysis of the protein to drug interactions revealed hydrogen bonding of the ALLINI carboxylic acid with the Glutamine and Histidine residues of the CCD and the ALLINI methoxy group with the Thr-174 residue of the same subunit. These observations indicate that the 2-methyl and 3-acetic acid moieties are crucial for the drug's secure attachment within the deep, hydrophobic LEDGF binding pocket. Inclusion of a tert-butyl substitution at the 3-carbon position of the scaffold (ALLINI-2) exhibited even stronger binding affinity and increased the multimerization potency of the inhibitor. Moreover, hydrophobic interaction occurred between the quinoline ring and the CTD of another IN subunit. These findings aided us in developing derivatives to the trisubstituted 4-arylquinoline scaffold (Figure 2) and implementing a structure-activity relationship study that examines properties of IN multimerization according to scaffold substitutions. From these preliminary investigations, optimization and derivation of the quinoline scaffold for IN multimerization was carried out.



### *HTRF-based FRET Analysis*

The Fluorescence Resonance Energy Transfer (FRET) phenomenon occurs when electron excitation of the donor fluorophore transfers its excitation energy to a nearby acceptor fluorophore. In accordance to the Förster theory, the resulting FRET signal (E) varies as the inverse sixth power of the molecular distance between donor and acceptor fluorophores. This allows for FRET to be used as a precise molecular ruler for the IN



*Figure 4: The Fluorescence Resonance Energy Transfer (FRET) signal occurs after initial excitation of Donor Fluorophore (320 nm) during IN Multimerization Assay. Resulting emission ratios of the donor (620 nm) and the acceptor (665 nm) are proportional to the FRET signal.*

multimerization assay. In characterizing the potency of different compounds, the effective concentration of the drug required to exhibit a half-maximal response ( $EC_{50}$ ) could therefore be used to compare and analyze repeated and varied experiments.

According to the reported crystal structure of an ALLINI bound to the IN-

CCD dimer interface (Figure 3), the drug binds to the LEDGF binding pocket and then promotes multimerization through hydrophobic interactions between the protruding quinoline ring and the CTD of another IN subunit. In characterizing and comparing the extent of multimerization of the synthesized drug derivatives, a previously described

homogeneous time-resolved fluorescence (HTRF)-based assay<sup>20</sup> was altered to accommodate our research design. Mixed in a binding buffer, full-length IN was tagged at the N-terminus with the FLAG epitope (FLAG-IN) and recombinant CTD of IN was tagged with the hexahistidine epitope (HIS-CTD). Incubation of the tagged protein fragments with a series dilution of the drugs to-be-tested, followed by addition of fluorophore-conjugated antibodies. Europium cryptate conjugated Anti-His<sub>6</sub> antibody and the XL665 conjugated anti-FLAG antibody enabled the indirect measurement of CCD to CTD proximities (Figure 4).

### Chapter III: Materials and Methods

*Construction of recombinant HIS-tagged CTD and FLAG-tagged IN used in the assay were prepared as previously described<sup>10</sup> and provided by the Kessl lab group. The compounds tested were synthesized under a collaborative effort with the Donahue lab and Pigza lab of the University of Southern Mississippi's College of Arts and Science.*

#### *HTR-FRET IN Multimerization Assay*

(All chemicals and substrates were purchased from Sigma-Aldrich or Fisher and were used as received unless otherwise indicated.)

A 4 mL Tris-HCl Buffer containing 3496  $\mu$ L mQ-H<sub>2</sub>O, 100  $\mu$ L Tris-HCL (1 M), 300  $\mu$ L NaCl (2 M), 8  $\mu$ L MgCl<sub>2</sub> (1 M), 40  $\mu$ L Tween-20 (10%), and 70  $\mu$ L BSA (75 mg/mL). An IN-mix solution (2 mL) was prepared by adding His tagged CTD-IN (3  $\mu$ L, 20.48 M) and Flag tagged full length IN (3  $\mu$ L, 20.48  $\mu$ M) to 2 mL of the reaction buffer and incubated for 30 minutes at room temperature. Two drugs were tested during each assay. IN-Mix (67  $\mu$ L) and 2  $\mu$ L of each drug dilution was added to a labeled microcentrifuge tube (0.5 mL) and incubated for 3.5 hours at room temperature. An antibody mix (1.0 mL) was prepared by adding fluorophore-conjugated antibodies, 7.92  $\mu$ L of anti-His<sub>6</sub>-XL665 and 5  $\mu$ L of anti-FLAG EuCryptate (Cisbio, Inc., Bedford, MA), and KF (188.7  $\mu$ L, 1 M) to the reaction buffer (745  $\mu$ L). To each microcentrifuge tube, the antibody mix (33  $\mu$ L) was added and the reactions were incubated for 2 hours at RT. In a 384 well plate, 20  $\mu$ L of each reaction was added and left to settle for 20 minutes. The FRET signal efficiency

was measured for each reaction-well twice. The average of the two readings were used to plotted and fit the dose-response curve as previously described in the previous section.

For analysis of the drug-induced IN multimerization assay, a Molecular Devices M5 plate reader was used to record the HTR-FRET signal from the corresponding reactions in the 384-well plate. As previously conducted<sup>10,20</sup>, the FRET efficiency signal was defined as a ratio between emission 2 (665 nm) and emission 1 (620 nm) multiplied by 10<sup>6</sup>. To normalize the data, Origin Software (OriginLab, Inc.) was used to plot and fit the dose-response curve for each compound using the modified Hill equation (Equation 1). From the curve,  $k^n$  indicated the EC<sub>50</sub> value for each compound. The EC<sub>50</sub> of each compound was used as the comparable measure for multimerization potency.

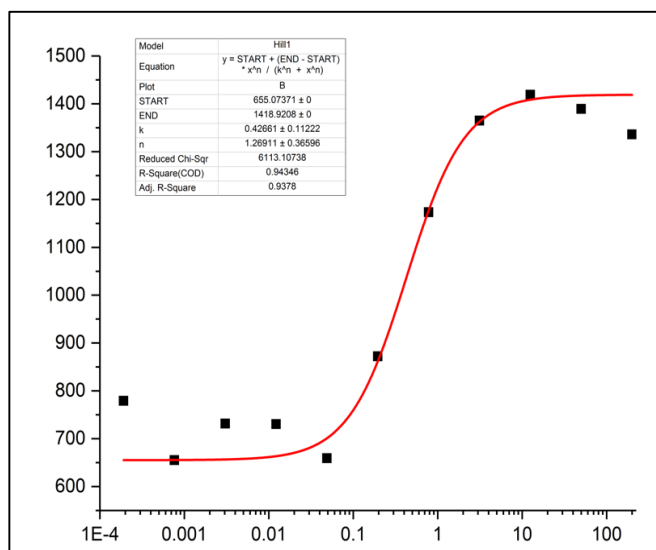
$$y = \frac{x^n}{k^n + x^n}$$

*Equation 1: The Modified Hill equation for the dose response curve is expressed, where (x) is the inhibitor concentration, (y) is the percentage of inhibition, (k) is the EC<sub>50</sub>, and (n) is the Hill slope<sup>10,21</sup>.*

## Chapter IV: Data and Results

Drug induced IN multimerization was quantified through FRET analysis between the interacting fluorophore-conjugated antibodies, anti-His<sub>6</sub>-XL665 and anti-FLAG EuCryptate. The dose-response curve (Figure 5) was obtained for each of the tested drugs.

The X-axis defines the concentration of the drug ( $\mu\text{M}$ ) and the Y-axis defines the extent of



*Figure 5: The fitted dose-response curve for antiviral multimerization activity of compound 3 is shown.*

multimerization activity. The  $\text{EC}_{50}$  value (k) was exhibited at the midpoint of the fitted-dose response curve and was characterized by the corresponding drug concentration.

The concentration of drug required for 50% effectivity in vitro ( $\text{EC}_{50}$ ) was used to characterize the potency of each compound (Table 1). In comparing different drugs, a

lower  $\text{EC}_{50}$  value deemed a drug more potent in promoting multimerization between HIV-1 IN subunits, while a higher  $\text{EC}_{50}$  deemed a drug less potent. Derivatives of tri-substituted 4-arylquinolines and their obtained  $\text{EC}_{50}$  values are shown in Table 1, columns 1-3. Column 4 presents data from the previously reported, full length IN assay on identical compounds<sup>7</sup>. Column 5 compares the results of the full-length assay with our modified assay.

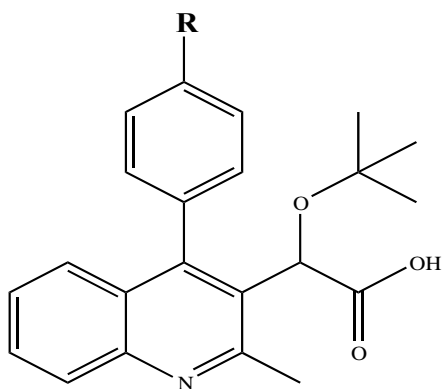


Figure 6: Para-4-phenylquinoline

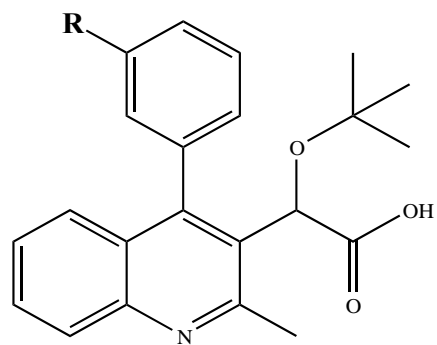


Figure 7: Meta-4-phenylquinoline

| Compound | Substitution (R)       | IN - CTD Assay<br>EC <sub>50</sub> (μM) | IN - IN Assay <sup>7</sup><br>EC <sub>50</sub> (μM) | Ratio of EC <sub>50</sub><br>(IN-CT : IN-IN) |
|----------|------------------------|---|---|--|
| <b>1</b> | Cl (Para)              | 0.86                                    | 0.10  | 8.60   |
| <b>2</b> | F (Para)               | 0.92                                    | 0.49  | 1.88   |
| <b>3</b> | CH <sub>3</sub> (Para) | 0.43                                    | 0.24  | 1.79   |
| <b>4</b> | Cl (Meta)              | 6.87                                    | 3.79  | 1.81   |
| <b>5</b> | F (Meta)               | No Inhibition                           | 2.11  | -----  |
| <b>6</b> | CH <sub>3</sub> (Meta) | 1.62                                    | 0.95  | 1.71   |

**Table 1:** Compound EC<sub>50</sub> results according to substitution (R) in the para- and meta-position of Figure 6 and Figure 7, respectively.

## Chapter V: Discussion and Conclusion

Based on the obtained multimerization data, potency of the assayed quinoline based molecules were shown to vary significantly with slight variations in molecular structure. Compounds 4 and 5 were shown to have the least effect on IN Multimerization while compound 3 was the most potent ( $EC_{50}$  of 0.43  $\mu$ M). Drugs with variations in the *meta*-position showed lower potency than the variations in the *para* position. Also, a variation from chlorine to methyl in the *para* position significantly increased potency, indicating that the position of derivatization is associated with hydrophobic interactions inside of the binding pocket.

When comparing the obtained  $EC_{50}$  values to that of the published full length IN assay<sup>7</sup>, the data indicated a parallel in compounds 2, 3, 4, 5, and 6 (Table 1). All obtained  $EC_{50}$  values were greater than those of the full-length assay, indicating less potent multimerization. Additionally, the ratio between  $EC_{50}$  values of corresponding drugs exhibited a ratio of approximately 2:1 for compounds 2, 3, 4, 5, and 6. The observed differences in multimerization, however, do not vindicate less potent inhibitors. Since our assay used identical equipment, techniques, and compounds as the published full-length assay,  $EC_{50}$  values could be reliably compared. Our assay differed in that only the CTD of IN was used rather than a full-length IN, suggesting that the absence of the NTD and CCD modulated the measured FRET signal, and thus, increased the calculated  $EC_{50}$ . This relationship supports our hypothesis that the full-length assay data was influenced by not only the drug induced multimerization, but also the consequential aggregation of additional CCD drug binding sites (Table 1).

The results of our IN-CTD assay suggested additional features of the proposed allosteric drug binding model within the CCD of IN<sup>10</sup>. Although there is strong evidence for the drug binding site being contained within the CCD dimer<sup>4,8,20</sup>, the effects of the adjacent CTD and the NTD domains on the CCD drug binding sites has not been firmly established. Substituting the HIS-CTD with a histidine tagged NTD, and then with histidine tagged CCD, would contribute to the deciphering of the HIV-1 IN protein structure/activity relationship.

Further testing on quinoline-based drug derivatives would provide valuable insight on the activity within the CCD binding site. The infinite number of possible substitutions in these novel compounds gives a promising outlook on HIV-1 IN drug discovery. The scope of possible compounds, however, also presents hindrance since the synthesis of this class of molecules has been relatively unexplored in literature. Given the immense substitution possibilities, grouping potential substitutions by their polarity and atom size could contribute in guiding future studies. The discovery and optimization of IN inhibitors may also benefit from high throughput screening to propel pharmaceutical advancements in HIV treatment, similar to the study used to identify optimal arylquinoline inhibitor scaffolds<sup>20</sup>. In continued building of the trisubstituted arylquinoline compound library, further insight on optimal binding of quinoline based drugs and analysis of their multimerization potencies can contribute to deciphering the interface of therapeutic drug interactions. Furthermore, refined and optimized ALLINIs that are successful in clinical trials could be included to the current HAART treatments of controlling an HIV-1 infection, which would reduce the possibility of multi-drug, HAART resistant HIV-1 mutants. Potent inhibitors would also add to the narrow selection of compounds in



HAART, thereby reducing the chance of a patient being incompatible with all available treatment drugs. Our contribution of multimerization potencies of the presented ALLINIs clarifies that multimerization continues to be a viable and relatively unexploited therapeutic target, and that IN inhibitors could allow for desirable clinical properties.

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