# Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format.

1. **Grantee Institution:** Drexel University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010-12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Anne Martella

4. **Grant Contact Person's Telephone Number:** (215) 895-6471

5. **Grant SAP Number:** 4100050893

6. **Project Number and Title of Research Project**: 2 - *Modulation of Capsid Stability as an Antiviral Strategy*

7. **Start and End Date of Research Project:** 1/1/2010-6/30/2011

8. **Name of Principal Investigator for the Research Project:** Simon Cocklin, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 153,500.

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

| Last Name, First Name | Position Title | % of Effort on Project | Cost |
|---|---|---|---|
| Simon Cocklin | Assistant Professor | 5% | $3,250 |
| Sandhya Kortagere | Assistant Professor | 5% | $4,000 |

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

| Last Name, First Name | Position Title | % of Effort on Project |
|---|---|---|
| None | | |

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

| Type of Scientific Equipment | Value Derived | Cost |
|---|---|---|
| None | | |

**10. Co-funding of Research Project during Health Research Grant Award Period.** Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No_____X_____

If yes, please indicate the source and amount of other funds:

**11. Leveraging of Additional Funds**

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes____X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert “not funded” in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

| A. Title of research project on grant application | B. Funding agency (check those that apply) | C. Month and Year Submitted | D. Amount of funds requested: | E. Amount of funds to be awarded: |
|---|---|---|---|---|
| 1R01AI095070-01, "Small-molecule modulation of HIV-1 capsid function." | ☒NIH<br>☐ Other federal (specify:_____)<br>☐ Nonfederal source (specify:_) | September 2010 | $1,964,266 | Not funded |

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____X_____ No_____

If yes, please describe your plans:

1. The September 2010, NIH/NIAID submission, "Small-molecule modulation of HIV-1 capsid function." Not discussed. Reviewers wanted the data presented within the proposal to be published in a manuscript and also improved efficacy of the lead compound. We are addressing both critiques and will be resubmitting.

2. We are in the process of assembling a further publication on the CA inhibitors. We plan on trying to increase the potency of the CA inhibitors before we apply for an R01.

**12. Future of Research Project.** What are the future plans for this research project?

We are preparing the data for publication. In addition, we plan on resuming the search for more potent CA inhibitors.

**13. New Investigator Training and Development**. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____ No_____X_____

If yes, how many students? Please specify in the tables below:

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| Male | | | | |
| Female | | | | |
| Unknown | | | | |
| **Total** | | | | |

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| Hispanic | | | | |
| Non-Hispanic | | | | |
| Unknown | | | | |
| **Total** | | | | |

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| White | | | | |
| Black | | | | |
| Asian | | | | |
| Other | | | | |
| Unknown | | | | |
| **Total** | | | | |

**14. Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No_____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

**15. Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_____x____ No_____ _____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

This award contributed to the formation of the Medicinal Chemistry Working Group at Drexel University, which was funded by the Office of the Vice Dean for Research to enhance the drug discovery capabilities at Drexel.

**16. Collaboration, business and community involvement.**

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes___X_____ No_____

If yes, please describe the collaborations:

- Dr. Navid Madani, Dana-Farber/Harvard Cancer Center, Harvard Medical School, Boston, MA (Antiviral testing using a single round infection assay)
- Mr. Roger Ptak, Southern Research Institute, Birmingham, AL (Antiviral testing using a multiple round infection assay)
- A limited collaboration with Eric Stavale, Assistant Director of Bioservices at the contract research organization Integrated Biotherapeutics, Inc. (Germantown, MD). This entails discounted services with a view to publishing together. (tested compounds for viral specificity)
- Drs. Ernesto Freire and Arne Schon (Department of Biology, Johns Hopkins, Baltimore, MD) (performed isothermal titration calorimetry on the compound-CA interaction)

16(B) Did the research project result in commercial development of any research products?

Yes_____ No_____x_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

**17. Progress in Achieving Research Goals, Objectives and Aims.**

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable

performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

**There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.**

**Summary of Research**

Acquired immune deficiency syndrome (AIDS), caused by the human immunodeficiency virus (HIV-1), is a global epidemic that represents one of the world's major health problems. Currently, over 40 million people are infected with HIV-1, leading to 3 million deaths from AIDS or AIDS-related causes every year. Despite recent progress in anti-HIV therapy, drug toxicity and the emergence of drug-resistant isolates during long-term treatment of HIV-infected patients necessitate the search for new targets that can be used to develop novel antiviral agents. It is very unlikely that any single inhibitor will be the sole solution to these problems. The more stages of the viral life cycle that can be antagonized, however, the more likely it is that the virus would be forced to mutate at the expense of its fitness, and the less likely that enough viral replication would occur to generate escape mutants. Given the essential roles, both structural and regulatory, of capsid (CA) within the HIV-1 life cycle, and the existence of compounds with the ability to disrupt CA-CA interactions, the capsid is a tractable therapeutic target. In this project we identified, characterized, and augmented novel small molecules designed to bind the capsid and antagonize its function. These "capsid hexamerization antagonists or agonists" could represent a new class of small-molecule capsid inhibitors, targeted at a highly conserved oligomerization surface and possess a novel mechanism of action. We believe these data not only will provide new reagents for the dissection of the basic biology of HIV-1 replication and pathogenesis but also will lay the foundation for a major new initiative in HIV-1 drug discovery.

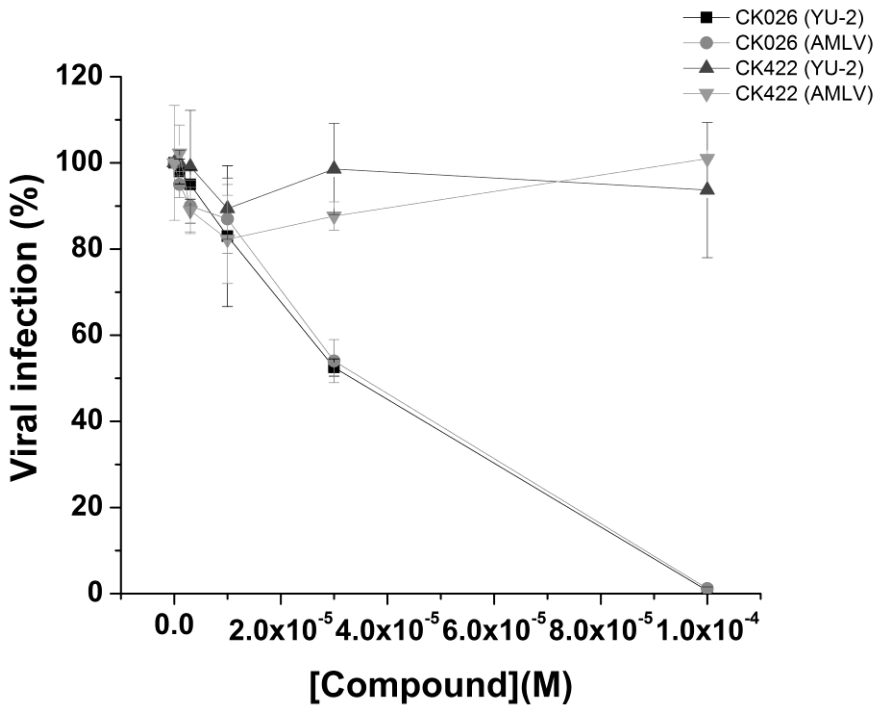
Identification of CK026 as an early-stage inhibitor of HIV-1 replication. The hybrid structure–based (HSB) method [1-5] was used to design small-molecule inhibitors targeted to the N-terminal domain–N-terminal domain (NTD-NTD) hexameric interface of HIV-1 CA. The HSB method, as the name implies, is a hybrid method combining elements of ligand-based and structure-based virtual screening strategies: using ligand-based methods to build enriched libraries of small molecules, and then employing a combined receptor–ligand pharmacophore to screen molecules

from the enriched library and to further dock the molecules to their receptor. The docked complexes are then scored based on a number of physicochemical parameters to indicate high-ranking molecules. The results of this detailed analysis of the dynamic mode of association between the receptor and ligand are then used to list candidate molecules that are suitable for biological and biochemical testing. Screening with the hybrid pharmacophore resulted in 900 hits that were filtered for drug-like properties. The molecules were also screened using principal component analysis to identify those with unique chemical cores, which resulted in ~300 hits. These molecules were then docked into the dimeric interface region of the CA monomer and scored using a GoldScore, ChemScore, and a customized scoring scheme. From the 300 docked complexes, the 25 best ranking molecules were purchased for analysis of antiviral activity using single-round infection assays. Details of the single-round infection assay have been published in detail elsewhere and the method has been routinely used for phenotypic characterization of HIV-1 envelope glycoproteins and studies of inhibitors of HIV-1 replication[6-8]. Effect on early-stage events by the compounds were determined by producing virus in the absence of compound, then exposing target cells to virus in the absence or presence of various concentrations of compounds.

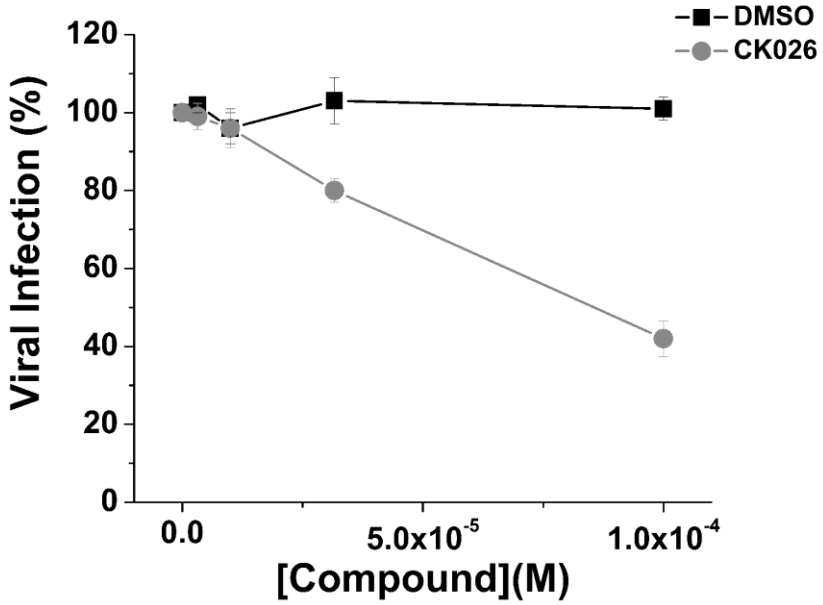
From this initial screen, one compound, 4,4′-[dibenzo[b,d]furan-2,8-diylbis(5-phenyl-1H-imidazole-4,2-diyl)]dibenzoic acid, which will be referred to as CK026, was identified as having anti-HIV-1 activity of 33.3 ± 0.31 μM on the infection of recombinant luciferase-containing HIV-1 viruses (HIV-$1_{NL4-3}$ backbone) pseudotyped with the envelope protein from HIV-$1_{YU-2}$. CK026 was found to disrupt infection at an early, post entry stage (Figure 1A) as its activity was independent of Env-mediated fusion, inhibiting HIV-1 pseudotyped with the envelope glycoprotein from amphotropic murine leukemia virus (AMLV). Although production of pseudovirions by transfection and the ability to analyze inhibition in a single-round infection are advantageous for addressing the inhibitory effect of a given compound, this type of assay cannot address the effects of multiple rounds of infection and cell-to-cell spread on the efficacy of the test compounds. We therefore sought to address whether CK026 could inhibit the replication of fully infectious virus (Figure 1B). The compound was assessed against fully infectious HIV-$1_{IIIB}$ replicating in the P4-R5 MAGI cell line. This analysis demonstrated that CK026 could inhibit the replication of this isolate with a half-maximal inhibitory concentration ($IC_{50}$) value of 89 ± 3.2 μM. The P4-R5 MAGI cell line is a HeLa derivative and is therefore not a natural target cell type, being able to support infection by HIV-1 only by overexpression of CD4, CXCR4, and CCR5. Moreover, HIV-$1_{IIIB}$ is a laboratory-adapted virus that has been multiply passaged in culture and lacks some of the accessory proteins. We therefore wished to test whether CK026 could inhibit a primary isolate, HIV-$1_{92BR030}$, replicating in primary peripheral blood mononuclear cells (PBMCs) (Figure 1C). Interestingly, the compound displayed no activity in the PBMC assay, despite being available and stable in the media over the course of the experiment.

**Figure 1**

**(A)**


CK026 (YU-2)
CK026 (AMLV)
CK422 (YU-2)
CK422 (AMLV)
Viral infection (%)
120
100
80
60
40
20
0
0.0
2.0x10-5
4.0x10-5
6.0x10-5
8.0x10-5
1.0x10-4
[Compound](M)

**(B)**


DMSO
CK026
Viral Infection (%)
120
100
80
60
40
20
0
0.0
5.0x10-5
1.0x10-4
[Compound](M)

**(C)**

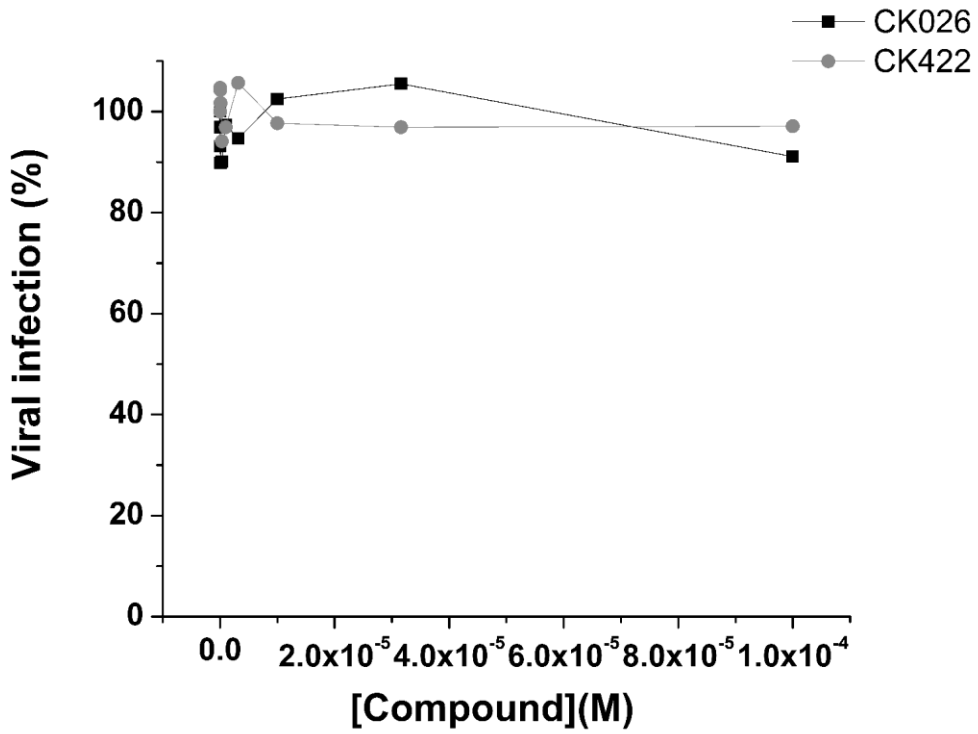



**Figure 1. Effect of compound CK026 on the replication of single- and multiple-round infectious HIV-1**. **(A)** Disruption of infection by CK026 at early and postentry stages as shown by single-round infection assays. Shown are the effects of compound CK026 on the infection of Cf2Th-CCR5 cells by recombinant luciferase-expressing HIV-1 bearing the envelope glycoprotein of the HIV-$1_{YU-2}$ strain or AMLV. Virus infection is expressed as the percentage of infection (measured by luciferase activity in the target cells) observed in the presence of compound relative to the level of infection observed in the absence of the compound. The data from three replicates are shown. $IC_{50}$ value for compound CK026 against HIV-1 was demonstrated to be 33.3 ± 0.31 µM. Compound CK422 was included as a compound control, as it has previously been determined not to have any effect on HIV-1 infection. **(B)** Effect of CK026 on replication of HIV-$1_{IIIB}$ in the primary peripheral HeLa P4-R5 MAGI cell line. **(C)** CK026 did not affect replication of HIV-$1_{92BR030}$ in primary peripheral blood mononuclear cells.

**Size reduction and optimization of CK026: identification of I-XW-053.** As compound CK026 displayed activity in single- and multiple-round infection assays using cell lines, we reasoned that the compound may be poorly permeable across the PBMC membrane. This is probably a function of its poor drug-like properties (high octanol water partition coefficient [logP] of 9.31 as determined using the weighted logP function in Jchem; ChemAxon, Budapest, Hungary) and large molecular weight (692 Da). As such, we sought to reduce the size of the compound and optimize its physicochemical properties and to improve its permeability while retaining its antiviral activity. CK026 has C2 symmetry along the central dibenzofuran ring. Docking results suggested that the proposed binding area of CK026 spans the entire NTD dimer interface including the junction between the N- and C-terminal lobes (Figure 2). Based on the docking model, the upper arm of CK026 is proposed to interact with residues Arg173, Asp166, Lys170, Tyr169, Glu180, Gln179, Ser33, and Pro34 and the lower arm with Pro38, Met39, Glu35, Lys30, and Val36. Owing to its symmetry, docking solutions indicated that either arm could occupy either of the two sites; there was no preference for one arm over the other. In order to test these docking observations, two analogues of CK026 (Scheme 1) were designed. The first, I-XW-091, is composed of the furan ring linker region attached to one arm of the parental molecule (Scheme 4), whereas the other, I-XW-053, corresponded to only the arm structure (Scheme 2). In addition, the benzoic acid moiety on CK026 was predicted from the docking pose to form hydrogen bond interactions with Gln179 and Glu180 of CA. We therefore tested this prediction, and how critical these potential interactions were to the antiviral activity of CK026, by synthesizing DMJ-I-073, a dimethyl ester variant of CK026, which removed the hydrogen-bonding capability at this region (Scheme 3). These compounds were then subjected to antiviral analysis using the single-round infection assay.

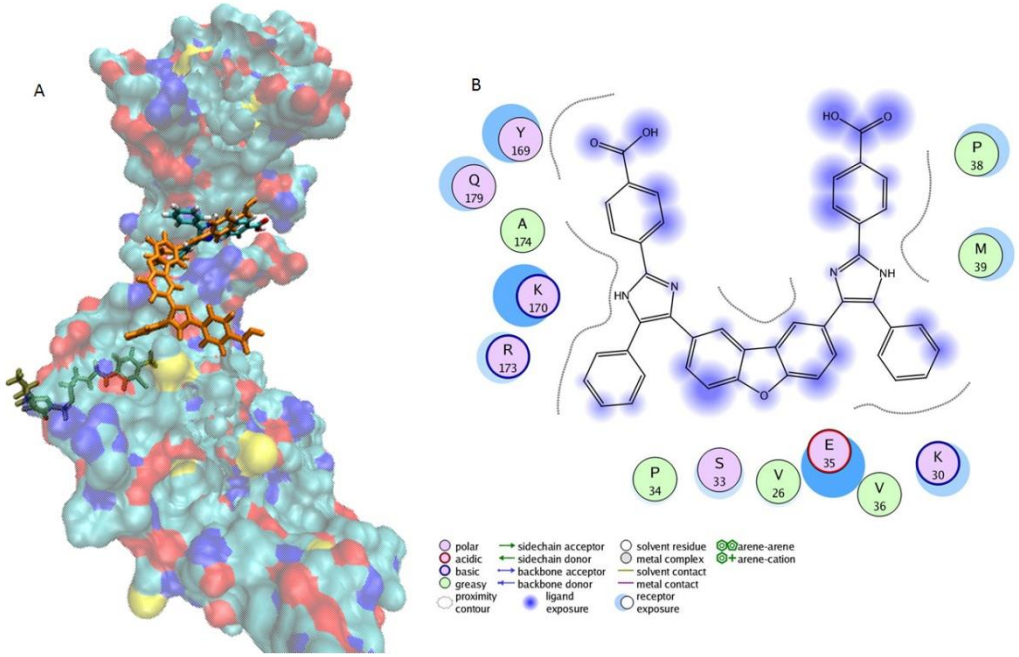
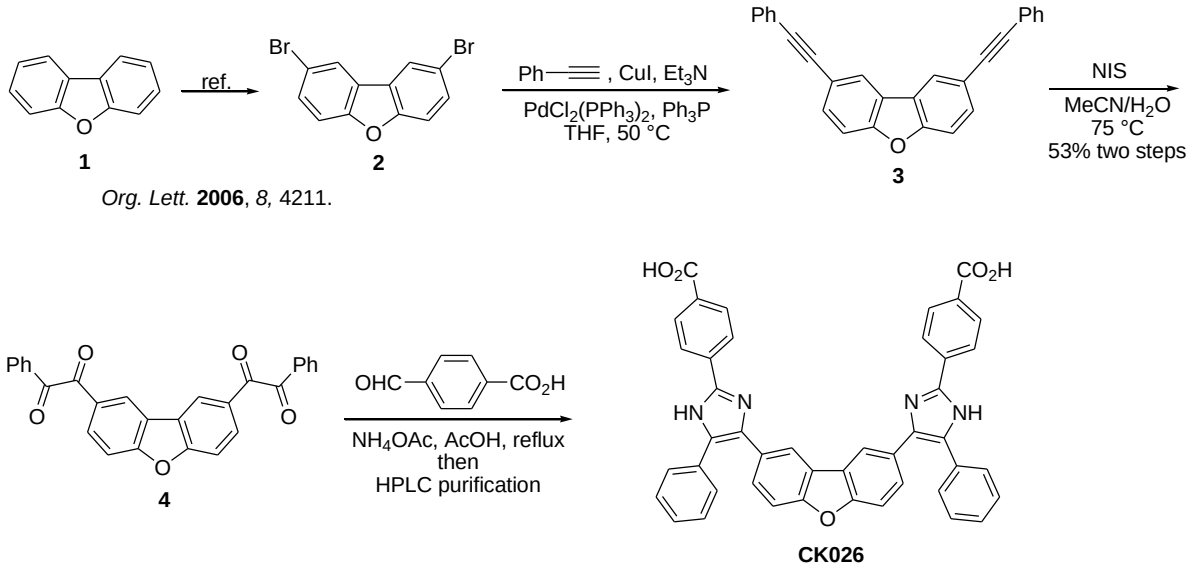
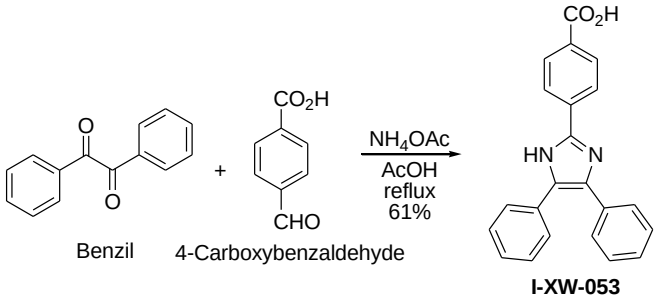



**Figure 2. Proposed binding mode of CK026 to the HIV-1$_{NL4-3}$ capsid protein**. **(A)** Surface representation of the monomeric unit of CA protein colored by atom type (C in cyan, O in red, N in blue, and S in yellow). CK026, I-XW-053, and a known CA inhibitor CAP-1 are docked to their predicted binding sites and are represented as orange, atom type-colored, and green-colored licorice models, respectively. **(B)** Proposed binding mode of CK026 in CA. The binding site residues are colored by their nature, with hydrophobic residues in green, polar residues in purple, and charged residues highlighted with bold contours. Blue spheres and contours indicate matching regions between ligand and receptors. Hydrogen-bonded interactions are shown by green arrows. The figure was generated using MOE ligX module.

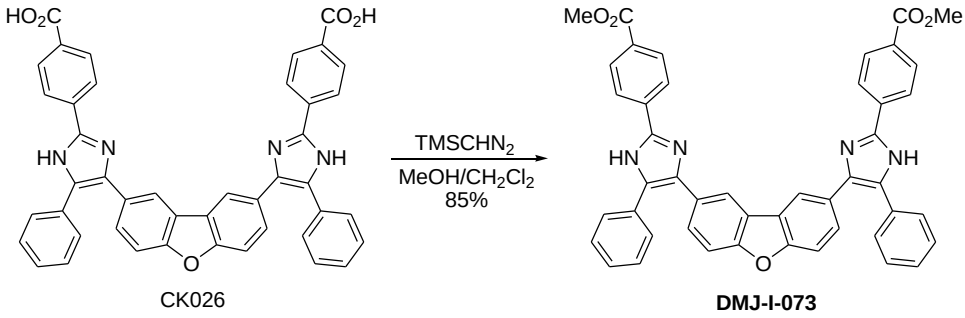
**Synthesis of compounds CK026, I-XW-053, DMJ-I-73, and I-XW-091.** Compound I-W-053 was synthesized as previously described by Fridman *et al.* [21]



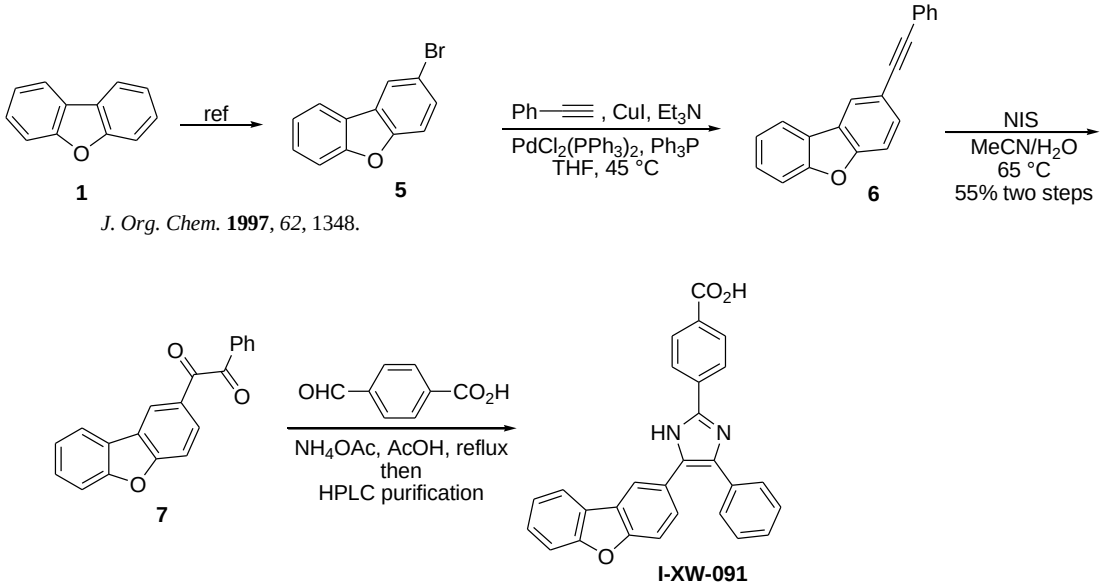

**Scheme 1**. Synthesis of diacid **CK026**.




**Scheme 2**. Synthesis of **CK026** analogue compound **I-XW-053**.[15]




**Scheme 3**. Synthesis of **CK026** analogue diester **DMJ-I-073**.




*J. Org. Chem.* **1997**, *62*, 1348.

**Scheme 4**. Synthesis of **CK026** analogue compound **I-XW-091**.

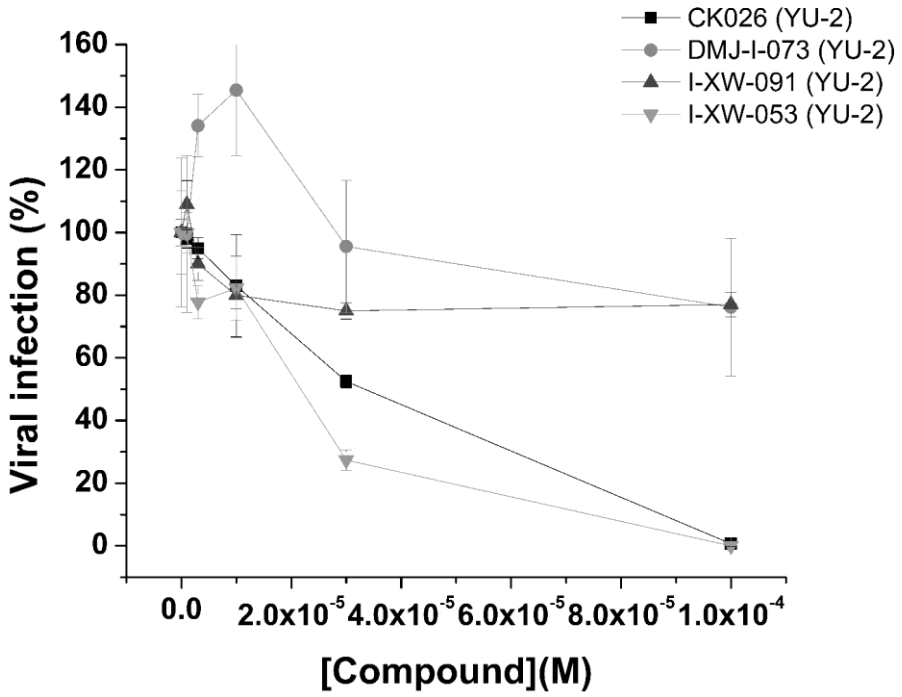



**Figure 3**. **Comparison of the effects of compounds CK026, DMJ-I-073, I-XW-091, and I-XW-053 on viral replication.** Shown are the effects of compounds CK026, DMJ-I-073, I-XW-091, and I-XW-053 on the infection of Cf2Th-CCR5 cells by recombinant luciferase-expressing HIV-1 bearing the envelope glycoprotein of the HIV-$1_{YU-2}$ strain. Virus infection is expressed as the percentage of infection (measured by luciferase activity in the target cells) observed in the presence of compound relative to the level of infection observed in the absence of the compound. The data from three replicates are shown. $IC_{50}$ value for compound I-XW-053 against HIV-1 was demonstrated to be 22.5 ± 1.1 μM.

As can be seen in Figure 3, DMJ-I-073 lost all activity in the single-round infection assay, indicating that potential hydrogen bonds formed by benzoic acid moiety in CK026 are key to its activity. Compounds I-XW-053 and I-XW-091, however, retained the activity of the parental molecule, reducing the replication of both YU-2 and AMLV (data not shown) pseudotyped viral particles. I-XW-091 could not be tested at lower concentrations due to its poor solubility in dimethyl sulfoxide (DMSO). I-XW-053, which retains the antiviral activity of CK026, represents a significant reduction in molecular size (692 vs 340 Da) and improvement in physicochemical properties (logP 9.31 vs 5.05), so we therefore decided to test I-XW-053 in the PBMC assay. As can be seen in Table 2, I-XW-053 has an $IC_{50}$ value for inhibition of the HIV-$1_{92BR030}$ isolate replicating in primary PBMCs comparable to that exhibited by the parental CK026 against HIV-$1_{IIIB}$ replicating in P4-R5 MAGI cells.

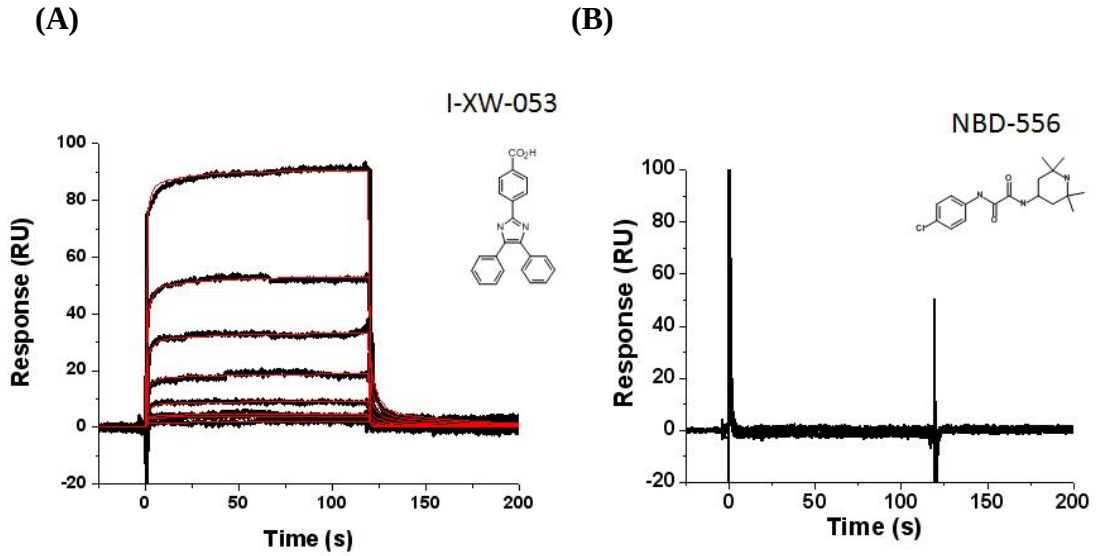



**Figure 4**. **Sensorgrams depicting the interaction of the (A) I-XW-053 and (B) NBD-556 with sensorchip-immobilized HIV-1$_{NL4-3}$ CA**. I-XW-053 at concentrations in the range 0.86–110 µM are shown. Black lines indicate experimental data, whereas red lines indicate fitting to the heterogeneous ligand model. The individual rate constants were out of the dynamic range of the instrument. The equilibrium dissociation constants are as follows: $K_{D1}$ = 66.3 ± 4.8 µM; $K_{D2}$ = 66.3 ± 5.2 µM. The chemical structures of each compound are shown inset.

<u>I-XW-053 binds to HIV-1 CA and stops its assembly in vitro.</u> Compound I-XW-053 is predicted to interact with the NTD of HIV-1 CA and thereby alter its assembly. However, it is possible that the compound exerts its action via another mechanism not involving CA. We therefore wished to establish that I-XW-053 is directed against HIV-1 CA. The direct interaction of I-XW-053 was assayed using SPR interaction analyses. Wild-type HIV-1 CA protein was purified using immobilized metal affinity chromatography (IMAC) and immobilized onto the surface of a high-capacity CM7 sensorchip. A surface to which the monoclonal antibody 17b (a generous gift from Dr. James E. Robinson, Department of Pediatrics, Tulane University Medical Center, New Orleans, LA) was immobilized was used to correct for background binding and instrument and buffer artifacts. I-XW-053 directly interacts with sensorchip-immobilized HIV-1 CA (Figure 4A)**.** In contrast, the small-molecule CD4 mimetic compound NBD-556 displayed no such interaction with HIV-1 CA, establishing the specificity of I-XW-053 for HIV-1 CA (Figure 4B). Interestingly, fitting of the SPR data indicated that the I-XW-053 interacted with HIV-1 CA with a 2:1 stoichiometry.

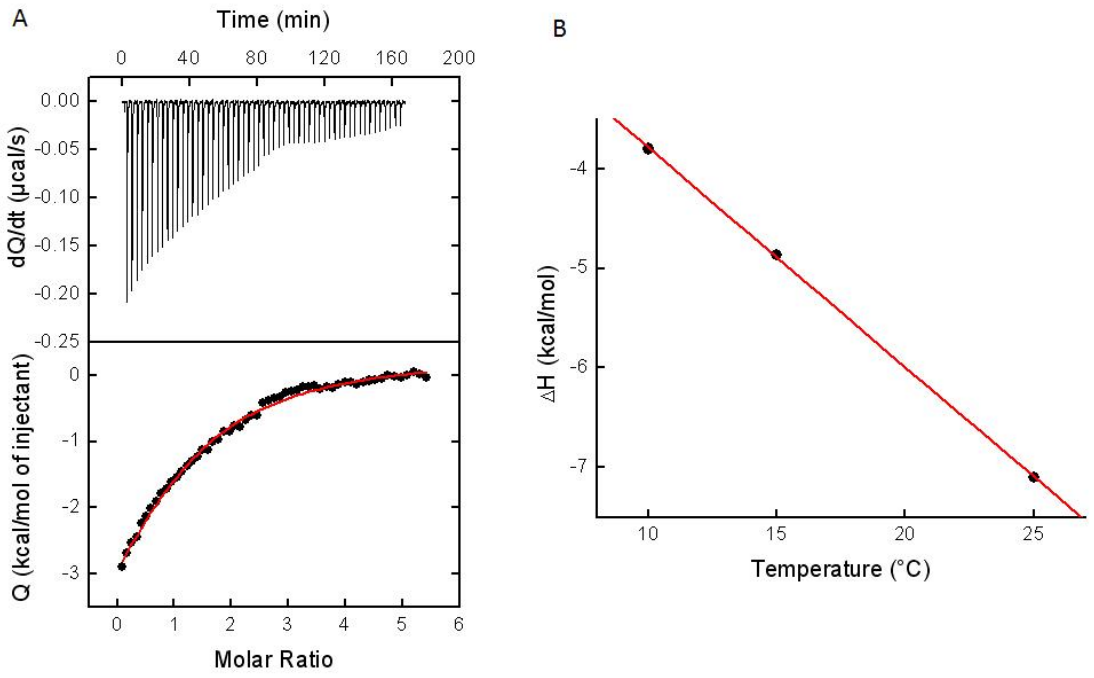



**Figure 5. (A) Calorimetric titration of HIV-1$_{NL4-3}$ CA with I-XW-053 at 25°C in Tris-HCl, 150 mM NaCl with 3% DMSO.** The concentration of CA was 35 µM, and the syringe contained I-XW-053 at a concentration of 600 µM. The experimental data fit with a binding model whereby two molecules of I-XW-053 bind to one CA, each with a binding affinity of 85 µM, which corresponds to a change in Gibbs energy of -6.6 kcal/mol. The changes in enthalpy ($\Delta H$) and entropy ($\Delta S$) are -7.3 kcal/mol and -5.0 cal/(K × mol), respectively. **(B) Temperature dependence of the enthalpy of binding of I-XW-053 to CA**. The slope corresponds to a heat capacity change of -220 cal/(K × mol).

The binding of I-XW-053 to HIV-1 CA was further characterized thermodynamically by isothermal titration calorimetry (ITC). Figure 5A shows the calorimetric titration of HIV-$1_{NL4-3}$ CA with I-XW-053 at 25°C in Tris-HCl, 150 mM NaCl with 3% DMSO (the exact buffer used for the SPR experiment). The experimental data fitted to a binding model wherein two molecules of I-XW-053 bind to one CA molecule with equal affinity, supporting the 2:1 stoichiometry obtained from SPR analysis. The affinity of the I-XW-053–CA interaction at 25°C was determined to be 85 µM, corresponding to a change in Gibbs energy of -6.6 kcal/mol per mole of I-XW-053. The changes in enthalpy ($\Delta H$) and entropy ($\Delta S$) were -7.3 kcal/mol and -5.0 cal/(K × mol), respectively, and the change in heat capacity, calculated from temperature dependence of the enthalpy, was -220 cal/(K × mol) (Figure 5B). As such, the measured thermodynamic parameters are typical for the binding of a small molecule that binds without inducing any major conformational changes in the target protein[9, 10].

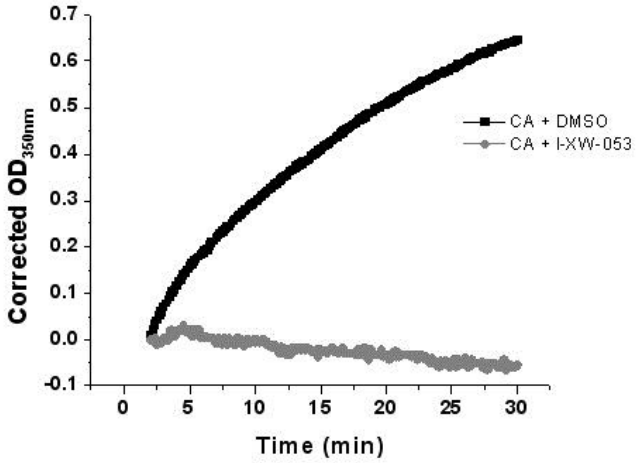



**Figure 6**. **Effect of I-XW-053 on assembly of HIV-1 CA in vitro**. CA assembly monitored by an increase in turbidity using a spectrophotometer at 350 nm. CA was used at a final concentration of 30 µM, and I-XW-053 at a final concentration of 147 µM. The presence of I-XW-053 prevents the assembly of the capsid.

Having demonstrated that I-XW-053 interacts directly with HIV-1 CA, we next sought to assess whether it affects the assembly of CA. Soluble HIV-1 CA can be triggered to assemble into tubes similar in diameter and morphology to intact cores by dilution into high-ionic-strength buffer. The kinetics of assembly can be followed by monitoring the increase in turbidity using a spectrophotometer. We therefore performed the assembly assay in the presence of I-XW-053 or DMSO alone. As shown in Figure 6, compound I-XW-053 prevented assembly of CA in vitro. Taken together, this body of data supports the docking model in which I-XW-053 binds at the interface of the two CA protomers and blocks hexamerization mediated by NTD-NTD interactions (Figure 7A).

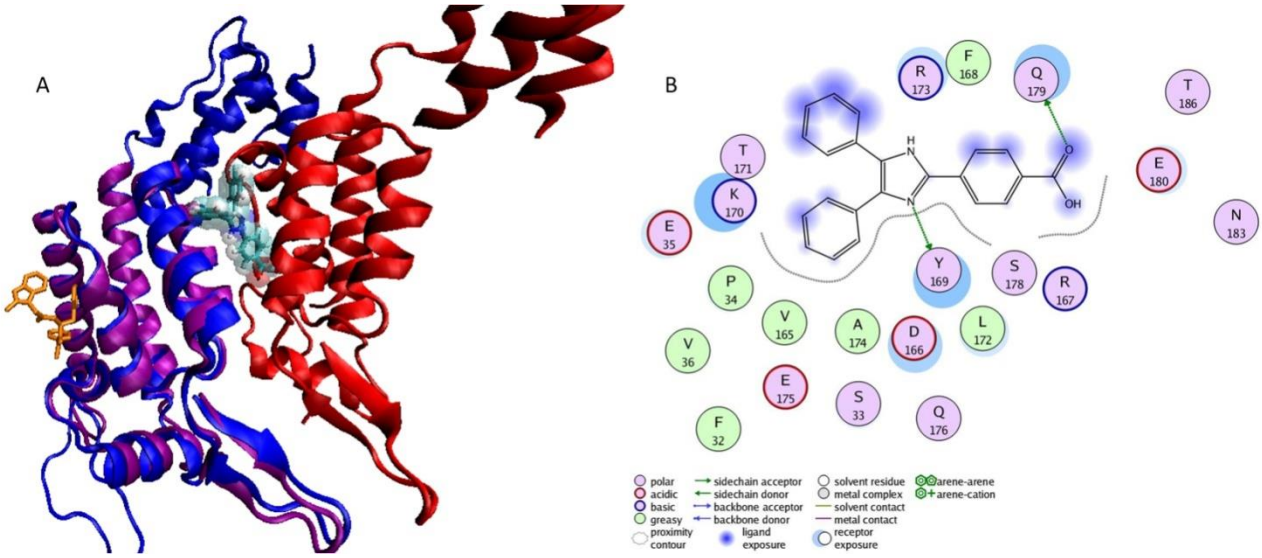



**Figure 7**. **(A) Comparison of the proposed binding site of I-XW-053 with the binding site of compound PF74.** Structural superpositioning of cocrystallized PF74 with NTD of CA protein on CA dimers. The protein is represented in cartoon model and the protomers are colored purple, green, and red. PF74 is represented in orange licorice model and I-XW-053 is docked at the proposed binding site and is represented in licorice model and colored atom type (C in cyan, N in blue, O in red, and H in white). The two binding sites are distinct and opposite to each other. The van der Waals surface model of I-XW-053 clearly shows that I-XW-053 sterically clashes with one of the CA protomers (colored red) and hence blocks the assembly of the CA protein. **(B)** I-XW-053 in the binding site of CA monomer. The binding site residues are colored by their nature, with hydrophobic residues in green, polar residues in purple, and charged residues highlighted with bold contours. Blue spheres and contours indicate matching regions between ligand and receptors. Hydrogen-bonded interactions are shown by green arrows. The figure was generated using MOE ligX module.

Mutation of residues within the NTD of CA reduces the binding of I-XW-053. The combined results from SPR and ITC studies indicated that compound I-XW-053 binds to HIV-1 CA with a 2:1 stoichiometry. This finding is consistent with results obtained from docking studies using I-XW-053 that imply that I-XW-053 could potentially interact with both upper and lower regions of the NTD. Therefore, to further investigate the potential binding site(s) of I-XW-053 on CA, we created mutations in the HIV-1 CA protein based on the docking models. Residues Ile37, Pro38, Asn139, Asp166, Tyr169, Lys170, Arg173, and Glu180 were individually mutated to alanine and their effect on the binding of a single concentration (27.5 µM) of I-XW-053 as compared with wild-type CA was assessed using SPR. From this analysis, mutation of residues Ile37, Pro38, Asn139, or Arg173 reduced the binding of I-XW-053 as compared with wild-type CA to varying degrees, with residues Ile37 and Arg173 having the most pronounced effect (a two-fold reduction or greater; Figure 8). In contrast, PF-3450074 (PF74) localizes to an opposite pocket situated on the NTD of the CA protein that is formed by helices 3, 4, 5, and 7. The

binding pocket for PF74 as determined by cocrystallization studies involves residues Asn53, Leu56, Val59, Gln63, Met66, Gln67, Leu69, Lys70, Ile73, Ala105, Thr107, and Tyr130 [13]. Our results demonstrate that the binding of I-XW-053 to HIV-1 CA is dependent on interactions with residues within the NTD and point to a site(s) of interaction not seen among previously discovered CA inhibitors (Figure 7B).

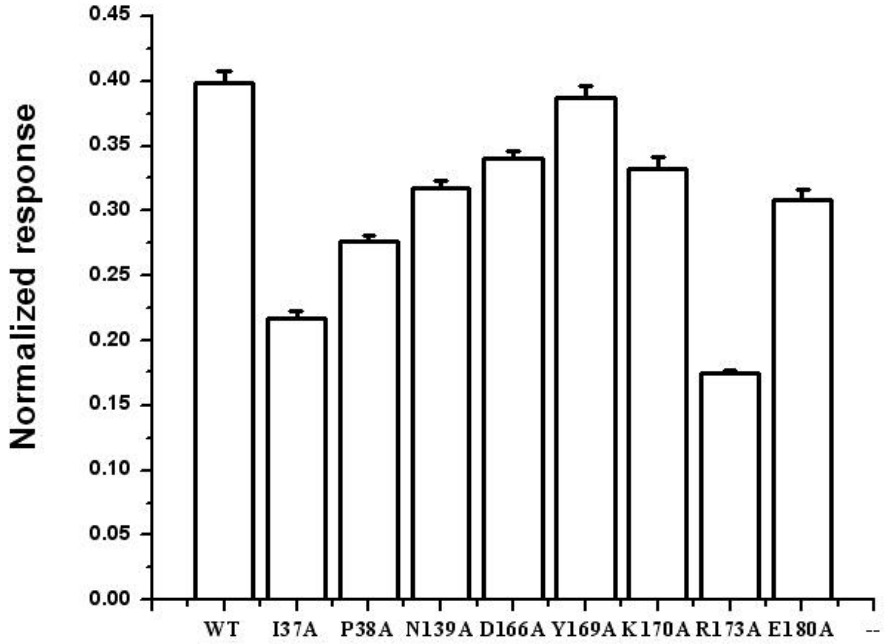



**Figure 8. Effect of mutation of capsid residues in and around the proposed I-XW-053 binding site on compound binding.** The interaction of I-XW-053 at a concentration of 27.5 µM with wild-type and mutant versions of the CA protein was assessed using SPR. To allow comparison, responses at equilibrium were normalized to the theoretical $R_{max}$, assuming a 2:1 interaction. Error bars correspond to one standard deviation.

<u>I-XW-053 is a specific inhibitor of HIV-1 replication.</u> We next sought to define the antiviral spectrum of I-XW-053. To achieve this, I-XW-053 was evaluated in cytopathic effect (CPE) assays against a panel of viruses from different classes (Table 1). I-XW-053 was evaluated against this panel of viruses up to a highest concentration of 100 µM. The compound displayed no inhibitory effects on the replication of Dengue serotypes 1-4, influenza H1N1, respiratory syncytial virus, yellow fever, Japanese encephalitis, Vaccinia, or Chikungunya viruses. Therefore, I-XW-053 appears to be specific for HIV-1, while further experiments are needed to confirm the null activity against other retroviruses.

**Table 1. Antiviral spectrum of I-XW-053**

| Virus strain* | $IC_{50}$ (µM) | $TC_{50}$ (µM) | Antiviral index ($TC_{50}/IC_{50}$) |
|---|---|---|---|
| DENV (serotypes 1-4) | > 100 | >100 | NA |
| RSV | > 100 | | NA |
| YFV | > 100 | | NA |
| JEV | > 100 | | NA |
| H1N1 | > 100 | | NA |
| Vaccinia | > 100 | | NA |
| Chikungunya | > 100 | | NA |

DENV = Dengue virus; RSV = respiratory syncytial virus; YFV = yellow fever virus 17D vaccine strain; JEV = Japanese encephalitis virus 14-14-2; H1N1 = influenza strain A/PR/8/34; NA = not applicable.
$IC_{50}$ = half-maximal inhibitory concentration; $TC_{50}$ = half toxic concentration.

**Table 2. Therapeutic spectrum of I-XW-053.**

| Virus strain* | $IC_{50}$ (µM) | $TC_{50}$ (µM) | Antiviral index ($TC_{50}/IC_{50}$) |
|---|---|---|---|
| 92UG031 | 93.6 | >100 | > 1.07 |
| 89BZ167 | 92.9 | | > 1.08 |
| 92BR030 | 68.7 | | >1.46 |
| 92BR025 | 51.0 | | >1.96 |
| 93IN101 | 48.4 | | >2.07 |
| 92UG024 | 51.8 | | >1.93 |
| CMU08 | 100 | | >1 |
| 93BR020 | 65.6 | | >1.53 |
| G3 | 9.03 | | >11.1 |
| BCF02 | 71.3 | | >1.4 |

92UG031 = HIV-1 subtype A clinical isolate; 89BZ167 = HIV-1 subtype B clinical isolate; 92BR030 = HIV-1 subtype B clinical isolate; 92BR025 = HIV-1 subtype C clinical isolate; 93IN101 = HIV-1 subtype C clinical isolate; 92UG024 = HIV-1 subtype D clinical isolate; CMU08 = HIV-1 subtype E clinical isolate; 93BR020 = HIV-1 subtype F clinical isolate; G3 = HIV-1 subtype G clinical isolate; BCF02 = HIV-1 group O clinical isolate.
$IC_{50}$ = half-maximal inhibitory concentration; $TC_{50}$ = half toxic concentration.

<u>I-XW-053 displays broad antiviral activity against multiple subtypes of HIV-1.</u> A key issue in the development of novel HIV drugs is their ability to inhibit the replication of genetically

diverse isolates, especially isolates from the most globally prevalent subtypes (A, B, and C). Therefore, the antiviral efficacy of I-XW-053 was evaluated in a standardized PBMC-based anti-HIV-1 assay with a panel of HIV-1 clinical isolates and laboratory strains from different geographic locations that included HIV-1 group M subtypes A, B, C, D, E , F, and G, as well as HIV-1 group O (Table 2). The panel included CCR5-tropic (R5), CXCR4-tropic (X4), and dual-tropic (R5X4) viruses. I-XW-053 inhibited the replication of viruses from all group M subtypes (A, B, C, D, E, F, and G) and also the group O isolate. Homology modeling of the available sequences of the isolates used in this study demonstrated a structural homology between the isolates of between 85% and 93% with reference to the crystal structure. Consistent with the antiviral analysis, I-XW-053 docked with nearly similar profiles to all the isolates, albeit with slightly better scores for the group O isolate (data not shown).

The HIV-1 CA protein plays essential roles in both the early and late stages of viral replication and has recently emerged as an attractive target for drug discovery and development. We have used the HSB method to identify small molecules that bind to the capsid NTD-NTD hexamerization interface and that are capable of disrupting HIV-1 replication at an early preintegration event. Using the HSB methodology, we obtained 900 hits by pharmacophore-based screening and filtering of a database of more than 3 million compounds. Of these 900 hits, 300 molecules belonging to different chemical cores (identified using principal component analysis on molecular descriptors derived from Molecular Operating Environment; Chemical Computing Group, Quebec, Canada) were subjected to further docking and scoring. Finally, the best ranked complexes were visually inspected for their potential not only to interact with CA but also to effectively disrupt the interaction of CA monomers with each other. Antiviral testing of the top ranked 25 compounds using a single-round infection assay identified 4,4′-[dibenzo[b,d]furan-2,8-diylbis(5-phenyl-1H-imidazole-4,2-diyl)]dibenzoic acid (CK026) as a CA-targeted compound with the ability to disrupt HIV-1 replication at a postentry, preintegration event. This compound retained the ability to inhibit fully infectious HIV-$1_{IIIB}$ replicating in P4-R5 MAGI cells but was unable to inhibit the primary isolate HIV-$1_{92BR030}$ replicating in PBMCs. We therefore reasoned that this lack of efficacy in the PBMC assay was due to poor permeability in that system due to the unfavorable physicochemical properties and the large size of the compound. Therefore, we attempted to reduce the size of the compound and improve its physicochemical properties, while retaining its antiviral activity. Based upon the docking pose of CK026 to HIV-1 CA, three analogues were synthesized: DMJ-I-073, I-XW-091, and I-XW-053. These analogues were subsequently assessed in the single-round infection assay yielding the finding that I-XW-053, the smallest of the analogues, retained all of the inhibitory capability of the parental compound. Having identified I-XW-053, we performed secondary screening using primary isolates replicating in PBMCs. Unlike the parental CK026, compound I-XW-053 could inhibit the HIV-$1_{92BR030}$ replicating in PBMCs. The combined use of SPR and ITC demonstrated a specific interaction of I-XW-053 with HIV-1 capsid and implicated residues in the NTD as being critical for this interaction. Using an in vitro CA assembly assay, we determined that I-XW-053 functioned by preventing the assembly of the capsid, consistent with the proposed mechanism of action as indicated by docking models. Moreover, compound I-XW-053 demonstrated little or no cytotoxicity over the concentration range tested (up to 100 μM); compound I-XW-053 is HIV-1 specific (showing no inhibition of a panel of nonretroviral DNA and RNA viruses; Table 1); and compound I-XW-053 possesses broad-spectrum anti-HIV-1 activity (Table 2).

Mutational analysis of potential I-XW-053-interacting residues on CA indicates that residues in the NTD and, more specifically, in the NTD-NTD interfacing region, are required for interaction of this compound with its target. This site includes residue Arg173, which is completely conserved across all HIV-1 strains. This binding region is in stark contrast to the binding sites of the other previously discovered CA inhibitors. CAP-1, a small-molecule compound initially discovered by the Summers group, was demonstrated by structural analyses to bind in a hidden pocket adjacent to the NTD-CTD interface and to prevent assembly by altering the local geometry required to make NTD-CTD interactions within the hexamer [14, 15]. This CAP-1 binding site has subsequently been targeted by other improved CAP-1 derivatives[16, 17] and has also been found to be the binding site for new, more efficacious molecules[18]. Another region of CA that has been exploited is a conserved hydrophobic cleft within the CTD. This area, initially identified as the site of interaction of the CA-I peptide, has recently been successfully targeted using small molecules[19].

Blair *et al.* [13] have also identified a novel compound through high-throughput screening, PF-3450074 (PF74), that targets CA and inhibits HIV-1 at an early stage. This compound, unlike previous CA inhibitors, apparently increased the rate of assembly of CA in solution [13]. Subsequent mechanism-of-action studies, however, demonstrated that PF74 destabilized the preformed capsid cores in vitro and exerted antiviral effects by triggering a premature uncoating of HIV-1 [20]. Intriguingly, the binding site of PF74, determined by x-ray crystallography, is a preformed pocket in HIV-1 CA bounded by helices 3, 4, 5, and 7 and situated in the NTD of the CA protein. Despite the similarities in the mechanism of action of PF74 and I-XW-053 (interaction with the NTD and destabilization of the capsid), our data suggest that the I-XW-053 binding site is distinct from that of PF74 and imply a novel mechanism in which hexamerization is physically blocked by interaction of the compound with CA. Further studies by our group are under way to determine the precise mechanism of action of I-XW-053 and to define the binding site of the compound on HIV-1 CA.

In summary, we have identified a new CA inhibitor that interacts with and functions through a novel binding site on HIV-1 CA: the NTD-NTD hexamerization interface. Although this compound is active against HIV-1 in the high micromolar range, it exhibits broad-spectrum anti-HIV activity, inhibiting all of the genetically diverse isolates tested in this study. These properties, coupled with the compound's promising thermodynamic signature and novel mechanism of action, suggest that I-XW-053 should serve as a good starting point for the development of high-efficacy analogues through optimization by medicinal chemistry approaches. The broad-spectrum activity of this compound is particularly promising, further highlighting the HIV-1 CA protein as a viable viral target with significant therapeutic potential.

**Identification of late-stage inhibitor of HIV-1 target against the CA - CK422 and analogues.**

CK422 consists of a 4-amino-isothiazole core and a single amino acid residue. The terminal amides are capped with hydrophobic cyclohexyl moieties, and the internal secondary amide bears an *o*-chlorobenzyl substituent. We sought to purchase and test analogues of CK422, in an

attempt to improve physicochemical properties and reduce the size of the molecule. Eleven analogues, all bearing a common 4-aminoisothiazole-3,5-dicarboxamide core were purchased and tested in the single-round infection assay. The effects of the compounds on preintegration, early-stage, and postintegration late-stage events in HIV-1 replication were assessed (Figure 9).

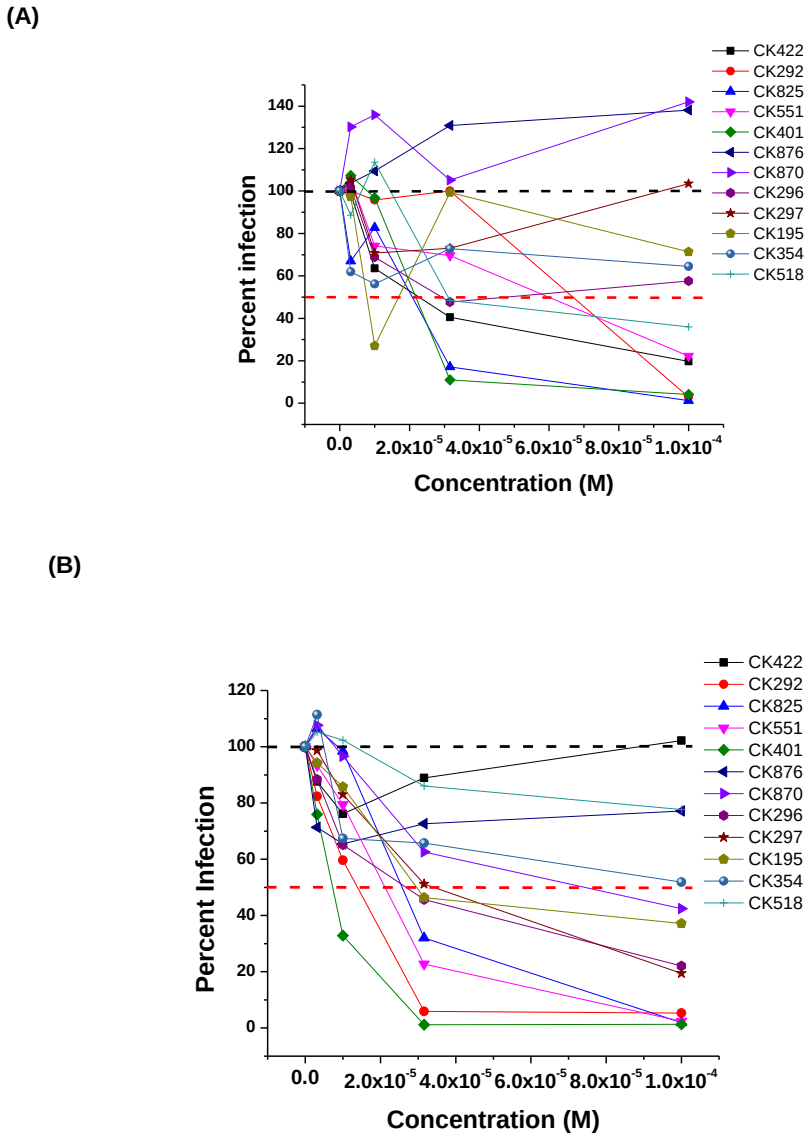



**Figure 9. Inhibition of HIV-1 infection by CK422 analogues. (A)** Effect of CK422 analogues on production of infectious virus. **(B)** Effect of CK422 analogues on the infection of recombinant luciferase-containing HIV-1 viruses (HIV-$1_{NL4-3}$ backbone) pseudotyped with the envelope protein from HIV-$1_{HxBc2}$. The U87 cell lines stably expressing CD4 and CXCR4 were used as target cells.

Compounds CK401, C292, CK551, and CK825 have emerged as lead compounds for further investigation. Incredibly pleasingly, during this limited structure–activity study we have been able to interconvert a late-stage inhibitor to an early-stage inhibitor and also to discover molecules with dual-stage inhibition, i.e., compounds capable of disrupting HIV-1 replication during *both* pre- and postintegration events. This major finding proves the initial hypothesis of the proposal, namely that by small-molecule targeting of a protein that is involved in two discrete phases of the HIV-1 replication cycle, one should be able to disrupt replication and one or both of these phases. This is the second instance by the principal investigator in which targeting of such a protein has led to the discovery of such novel, dual-stage inhibitors (Sierra et al., Small molecule disruption of early and late stages of retroviral replication: targeting the HIV-1 matrix (MA) protein; manuscript in preparation).

We have performed structure-activity-relationship studies in order to guide rationale redesign of the compound to higher potency. This initial SAR has resulted in the identification of compound CK176, which represents a 11-fold improvement in potency over I-XW-053, with no observable toxicities in primary human mononuclear cells.

**Biological evaluation of compound 1 analogues**. We have previously described compound **1** as a novel antiviral that targets a unique site on the HIV-1 CA protein and inhibits by affecting uncoating. However, compound **1** inhibits the replication of HIV-1 in the high micromolar range. Therefore, we sought to improve upon this potency whilst still retaining the novel qualities of this compound. Screening for analogues of compound **1** in commercial vendor chemical libraries resulted in the identification of 56 hit molecules with a unique set of functional groups attached to the core imidazole structure (**Table 3**). These compounds were purchased for biological testing in a PBMC-based HIV-1 replication assay as previously described.[22] We chose to evaluate the compounds against HIV-$1_{89BZ197}$ [23-26] as this isolate in our hands is one of the most refractory to inhibition using Gag-targeting small molecules [22, 27, 28]. Compound toxicity against the PBMCs was assessed in parallel using the MTT assay as previously described and was used to determine the therapeutic index (TI; $CC_{50}/IC_{50}$) of the test molecules[22]. The results of this analysis are shown in **Table 3**.

The focused library of compound **1** analogues all share an imidazole core structure. However, they could be further sub-classified into four categories: Core-1 - triphenyl imidazole core (containing 31 molecules), Core-2: an imidazol-2(3H)-ylidene core with 6 compounds, Core-3: tetrahydro phenanthro imidazole with 16 compounds and Core-4: phenanthro[9,10-d]imidazol-2(3H,4H,7H)-ylidene containing 3 molecules that had a combination of the second and third core groups. In the triphenyl imidazole core containing molecules, chemical modifications at either the *ortho*, *para* or *meta* positions on any of the three phenyl rings lead to only a modest increase in potency. These compounds also had low T.I values indicating that the increase in efficacy could be attributed to a concomitant increase toxicity of the compounds. Among the imidazol-2(3H)-ylidene core compounds, a clear SAR was observed with halogen modifications to the basal phenyl groups at the *para* positions leading to a 2-fold increase in potency and a >3 T.I value (compound **3**). Similarly, an ester group at the *ortho* position on one of the basal phenyl group lead to a 3-fold increase in potency and > 3 T.I.(Compound **14**). However, addition of large aryl or heteroaryl groups lead to no significant change in efficacy or toxicity (compound **42**) and a loss of efficacy (compound **52**). Compound **49** containing a nitro substitution on one of

the basal phenyl groups had a minor effect on both efficacy and toxicity. Among the tetrahydro phenanthrolines, halogen, nitro or thiomethyl modifications at the **para** position of the apical phenyl ring lead to an increase in potency with low toxicity values leading to an improvement in the T.I. In addition, the efficacy of these compounds was further improved with a halogen modification at the *para* position on the phenanthroline group. The nitro substitution at the para or meta position lead to a marked increase in efficacy and low toxicity as in compounds **29**, **31**, **33**, **37** and **34**. Compound **30** with the thiomethyl modification had the best efficacy but with a T.I of 3.4 indicating an increase in toxicity too. However, compound **34** demonstrated an $IC_{50}$ value of 14.2 ± 1.7 µM and no toxicity up to the highest test concentration of 100µM (**Figure 10**). The last group that had a phenanthro[9,10-d]imidazol-2(3H,4H,7H)-ylidene core structure did not show any gain in potency or T.I. The identification of compound **34** represents an 11-fold improvement in potency over the parental compound **1**.

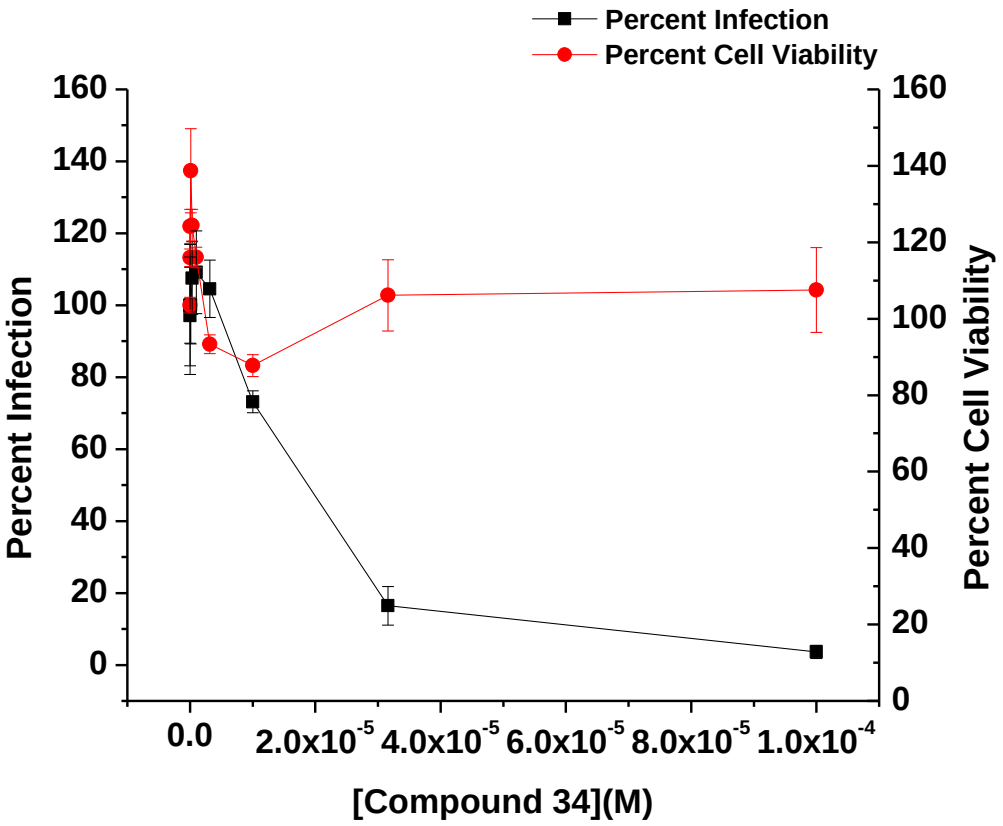



**Figure 10. The effect of compound 34 on the replication of HIV-$1_{89BZ167}$ and the viability of primary human PBMCs**. Black squares show the effect of compound **34** on the infection of primary human PBMC cells by the clade B primary isolate HIV-$1_{89BZ167}$. Virus infection is expressed as the percentage of infection (measured by reverse transcriptase activity) observed in the presence of compound relative to the level of infection observed in the absence of the compound. The data from three replicates are shown. Red circles show the effect of the compound on the viability of the PBMC and demonstrate that compound **34** is not toxic up to the highest concentration tested. The $IC_{50}$ value for compound **34** against HIV-$1_{89BZ167}$ = 14.2 ± 1.7 µM.

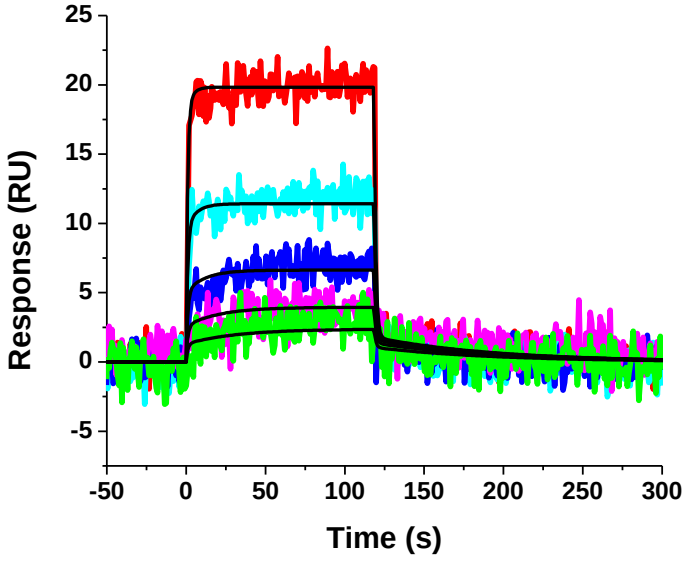



**Figure 11**. **Sensorgrams depicting the interaction of compound 34 with sensorchip-immobilized HIV-1$_{NL4-3}$ CA**. Compound **34** at concentrations in the range 18.75–300 µM are shown. Colored lines indicate experimental data, whereas black lines indicate fitting to a simple 1:1 binding model. The individual rate constants were marginally out of the dynamic range of the instrument and are therefore not reported. The equilibrium dissociation rate constant ($K_D$) for this interaction was determined to be 11.8 ± 4.7 µM.

**Direct interaction of compound 34 with HIV-1$_{NL4-3}$ CA**. Given the favorable properties of compound **34**, we next sought to demonstrate its interaction with HIV-1 CA. We therefore employed surface plasmon resonance (SPR) analysis. HIV-1$_{NL4-3}$ CA protein was purified as previously described [22] and attached to the surface of a high density GLH sensor chip (Bio-Rad Laboratories, HerculesCA) using standard amine coupling. A reference surface was created in a similar fashion using an irrelevant protein. Compound dilutions were injected simultaneously over both these surface and the responses recorded. **Figure 11** shows the resulting sensorgrams. The compound **34** - CA interaction follows a similar kinetic profile as the parental compound **1** with rapid association and rapid dissociation rates. However, unlike the parental, compound 34 appears to interact with CA with a 1:1 stoichiometry. The equilibrium dissociation constant for the compound **34** - CA interaction is approximately 5-6 fold stronger than the compound **1** - CA interaction. Moreover, there is remarkable agreement between the $IC_{50}$ values and the $K_D$ values for compound **34**.

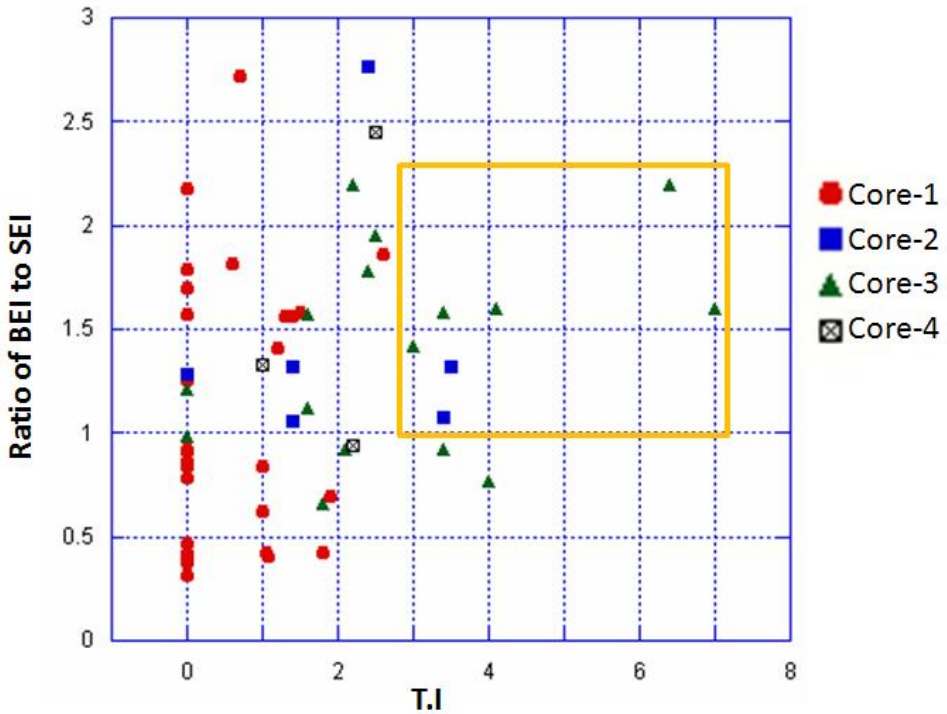



**Figure 12:** Scatter plot of the numerical values of ratio of BEI to SEI against T.I for all the molecules is shown with color coding for the various core structures.

**Structure-activity relationships**. Having demonstrated potency and maintenance of target specificity for compound **34**, we next sought to extract structure-activity relationships (SAR) from our total data. To derive the SAR of these molecules, we calculated two ligand efficiency indices, namely Binding Efficiency Index (BEI) and Surface Efficiency Index (SEI) using the experimentally derived $IC_{50}$ values and other molecular descriptors such as molecular weight (MW), p(IC50) which is computed as $-\log_{10}$(IC50) and Polar Surface Area (PSA). BEI is defined as the ratio of p(IC50) to MW and SEI as the ratio of p(IC50) to PSA per 100Å$^2$.

In determining the SAR of the compounds, the binding efficiency index (BEI) or the surface efficiency values (SEI) values were not very confirmatory. However, a ratio of BEI to SEI when plotted against T.I values (**Figure 12**) show a clear demarcation when a cut-off value of 1 and 3 for the BEI to SEI ratio and T.I values respectively are chosen. These cut-off values were chosen based on a hypothetical molecule that has an $IC_{50}$ value of 20μM, molecular weight of 500 Da and a PSA of 50 that resulted in a BEI and SEI values of 3.39. A 3-fold difference between effective versus toxic dose is a pharmacologically relevant value for T.I. Based on these cut-off values, seven molecules were chosen that included two molecules from core-2 (compounds **3** and **14**) and five from core-3 (compounds **29**, **30**, **33**, **34**, **45**). None of the molecules from core-1 or 4 were chosen under this stringent criteria suggesting that BEI, SEI and T.I can be used in this combination to derive lead compounds from a dataset that seems to have a bottomless SAR. Additionally, combining core-2 and 3 did not improve the druggability of the molecules. Based on the features described in **Figure 12**, compounds **29** and **34** were chosen as lead compounds with compound **34** as a representative member of the core.

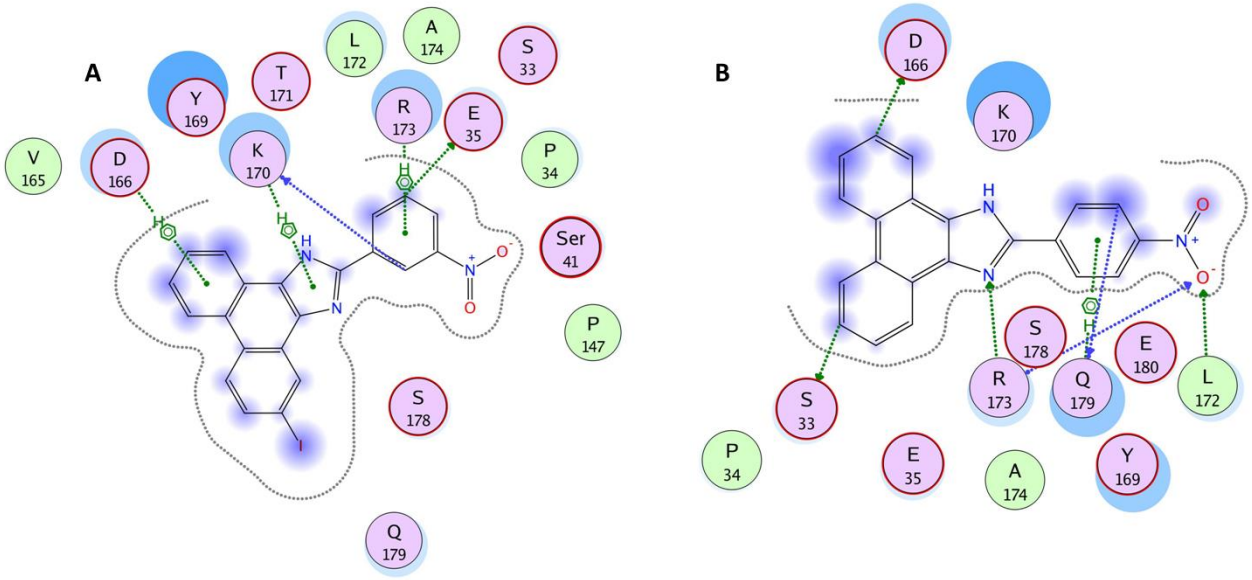



**Figure 13:** Schematic representation of the binding mode of compounds **34** (A) and **29** (B) are shown. The binding site residues are colored by their nature, with hydrophobic residues in green, polar residues in purple and charged residues highlighted with bold contours. Blue spheres and contours indicate matching regions between ligand and receptors. Hydrogen bonded interactions are shown by green arrows, ionic interactions in magenta lines and arene-H interactions in green lines extending across the aryl rings. The figures were generated using the LIGX module of MOE program.

Molecular docking studies suggested that compound 34 and 29 bind in a similar mode with conserved interactions at the binding site (**Figure 13**). The interactions include strong electrostatic interactions such as arene-cation interactions with Gln179, Lys170, Arg173, Asp166 and other residues such as Tyr169, Glu35, Ser178 and Ser41. In addition, the pocket is lined by hydrophobic residues such as Ala174, Leu172, Val165, Pro34 and Pro147 that are predicted to contribute favorably to the binding of these compounds.

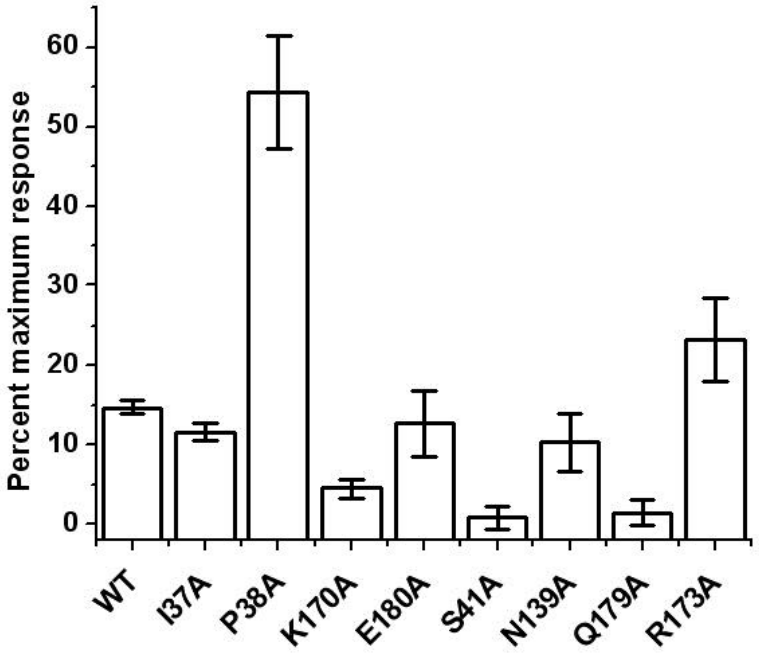
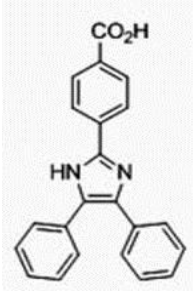
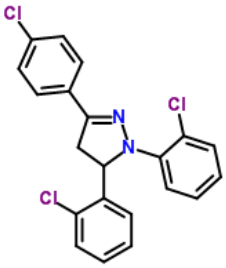
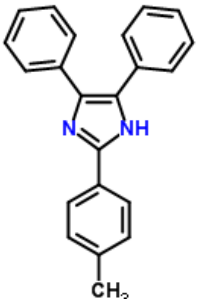
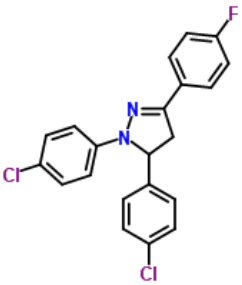
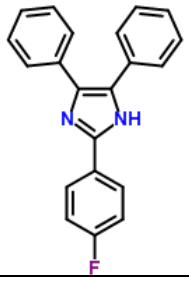
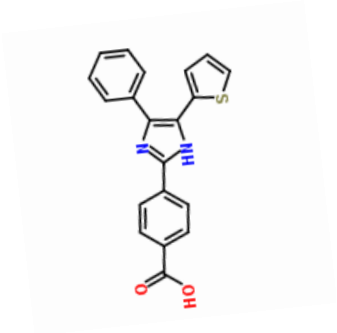
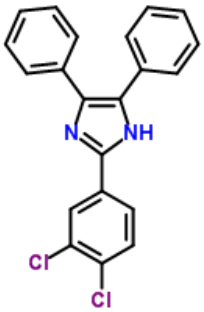
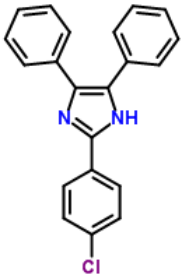


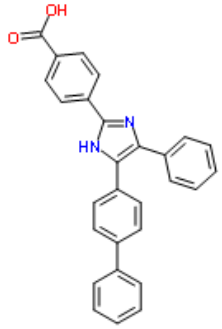
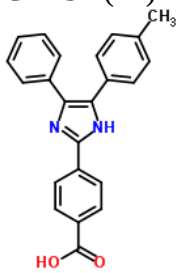
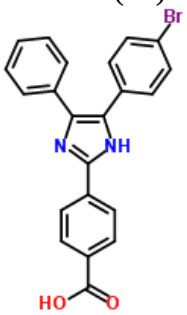
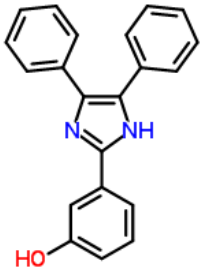

**Figure 14: Effect of mutation of capsid residues in and around the proposed compound 34 binding site on compound binding.** The interaction of compound 34 at a concentration of 50 µM with wild-type and mutant versions of the CA protein was assessed using SPR. To allow comparison, responses at equilibrium were normalized to the theoretical $R_{max}$, assuming a 1:1 interaction.

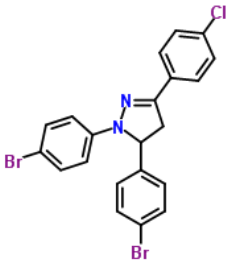
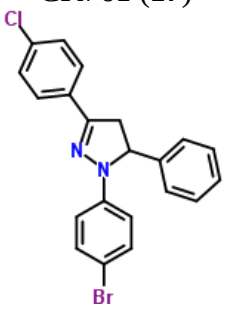
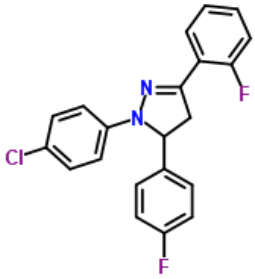
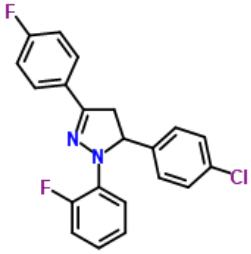
Using a combination of alanine mutagenesis and SPR, we sought to investigate this proposed binding mode. Eight mutant CA proteins containing Ile37Ala, Lys170Ala, Glu180Ala, Asn139Ala, Gln179Ala, Ser41Ala, Pro38Ala and Arg173Ala, as well as the wildtype HIV-1 NL4-3 CA protein were immobilized on a sensorchip and exposed to a single concentration of compound **34**. The results of this analysis are shown in **Figure 14** and suggest that residues Lys170, Ser41 and Gln179 when mutated to alanine lead to loss of favorable interactions with compound **34**. However, residues Arg173 and Pro38 when mutated to alanine appeared to increase the interaction of compound 34 with HIV-1 CA. From the docking model this may be due to a strengthening of arene-H interactions with the ligand. Residues Ile37, Glu180 and Asn139 when mutated to alanine did not produce any significant change in binding capacity when compared to wild type suggesting these interactions could be compensated by other residues in the binding pocket.

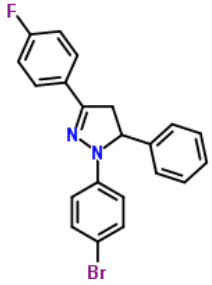
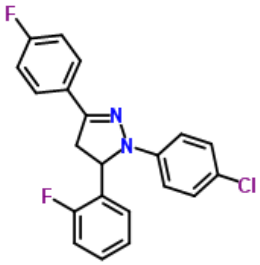
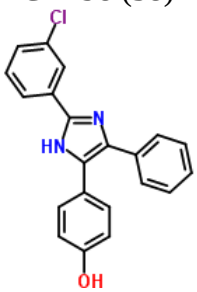
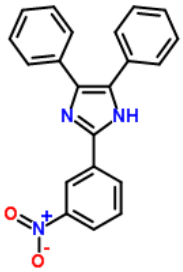
**Table 3: Antiviral activity of 1 and its analogues against HIV-$1_{89BZ167}$ replicating in PBMCs**

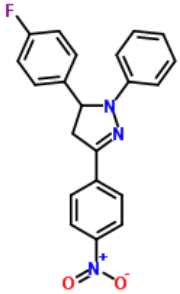
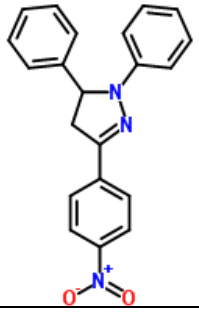
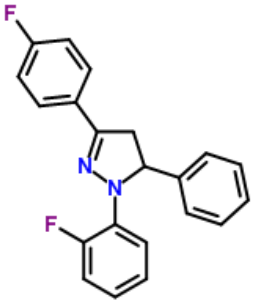
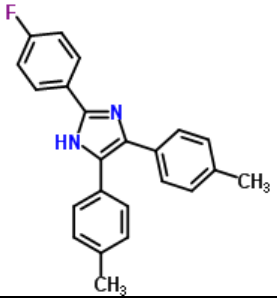
| Compound | $IC_{50}$ (µM) | BEI | PSA | Mol.wt | SEI | Therapeutic Index |
|---|---|---|---|---|---|---|
| I-XW-053 (**1**)  | 164.2 ±54 | 2.309283 | 61.69 | 340 | 1.272745 | >0.6 |
| CK599 (**2**)  | > 100 | 1.739987 | 15.6 | 401.71 | 4.480577 | NA |
| CK970 (**4**)  | > 100 | 2.251909 | 28.68 | 310.39 | 2.437134 | NA |
| CK930 (**5**)  | 55.5 ± 1.7 | 3.259376 | 15.6 | 385.26 | 8.049404 | >1.08 |

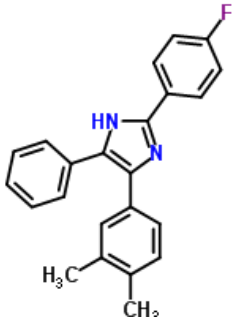
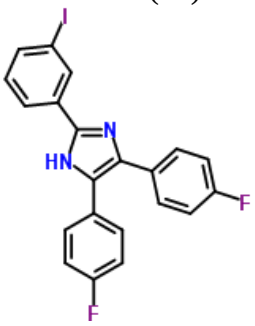
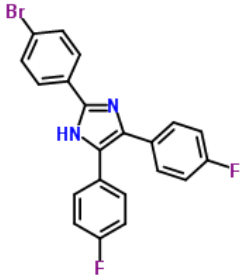
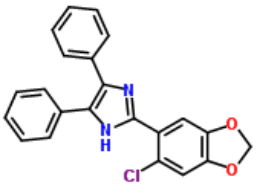
| Compound | $IC_{50}$ (µM) | BEI | PSA | Mol.wt | SEI | Therapeutic Index |
|---|---|---|---|---|---|---|
| CK543 (**6**)  | > 100 | 2.225169 | 28.68 | 314.12 | 2.437134 | NA |
| CK731 (**7**)  | 141 ±29 | 2.456065 | 94.22 | 346.40 | 0.902973 | >0.7 |
| CK663 (**8**)  | > 100 | 1.913676 | 28.68 | 365.25 | 2.437134 | NA |
| CK540 (**9**)  | > 100 | 2.112905 | 28.68 | 330.81 | 2.437134 | NA |

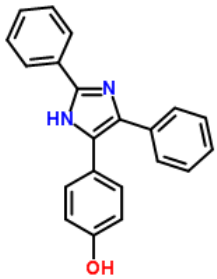
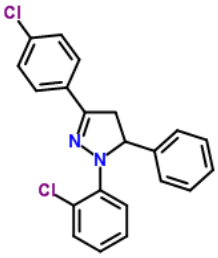
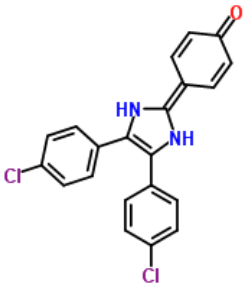
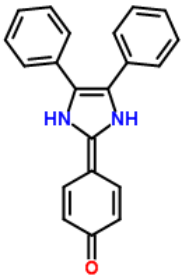
| Compound | $IC_{50}$ (µM) | BEI | PSA | Mol.wt | SEI | Therapeutic Index |
|---|---|---|---|---|---|---|
| CK750 (**10**)  | 68.2 ±0.4 | 2.800239 | 65.98 | 416.47 | 1.767529 | >1.5 |
| CK754 (**11**)  | 38.5 ±0.1 | 3.991364 | 65.98 | 354.40 | 2.143891 | >2.6 |
| CK740 (**12**)  | > 100 | 1.667112 | 65.98 | 419.27 | 1.059366 | NA |
| CK966 (**15**)  | 14.6 ±4.9 | 5.876704 | 48.91 | 312.36 | 3.753112 | 1.3 |

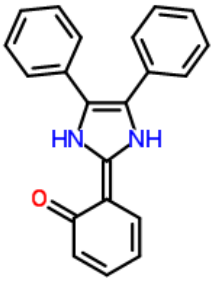
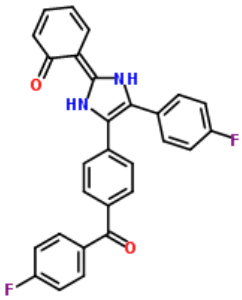
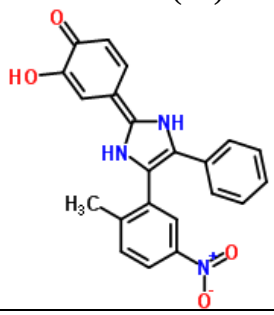
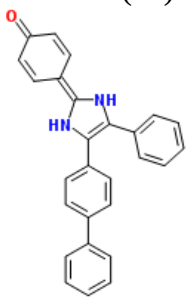
| Compound | $IC_{50}$ (µM) | BEI | PSA | Mol.wt | SEI | Therapeutic Index |
|---|---|---|---|---|---|---|
| CK775 (**16**)  | > 100 | 1.424696 | 15.6 | 490.61 | 4.480577 | NA |
| CK761 (**17**)  | > 100 | 1.697683 | 15.6 | 411.72 | 4.480577 | NA |
| CK744 (**18**)  | 95.5±0.07 | 2.765665 | 15.6 | 368.807 | 6.53844 | >1.04 |
| CK747 (**19**)  | 56.3±0.1 | 3.387928 | 15.6 | 368.807 | 8.009562 | >1.8 |

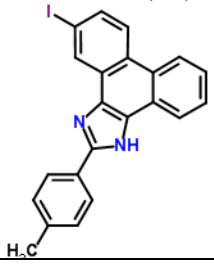
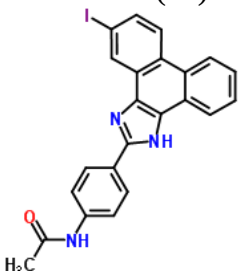
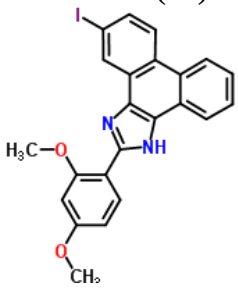
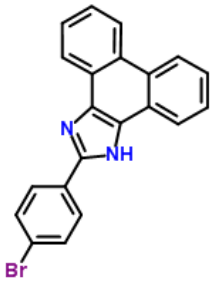
| Compound | $IC_{50}$ (µM) | BEI | PSA | Mol.wt | SEI | Therapeutic Index |
|---|---|---|---|---|---|---|
| CK926 (**20**)  | > 100 | 1.76838 | 15.6 | 395.26 | 4.480577 | NA |
| CK741 (**21**)  | > 100 | 1.895219 | 15.6 | 368.807 | 4.480577 | NA |
| CK198 (**38**)  | 16.5±0.04 | 5.139896 | 48.91 | 346.80 | 3.644482 | 1.2 |
| CK251 (**39**)  | > 100 | 2.047604 | 74.5 | 341.36 | 0.938215 | NA |

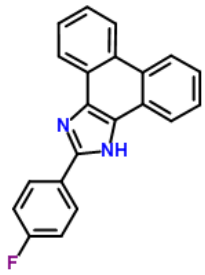
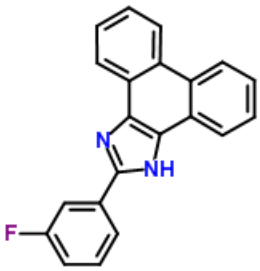
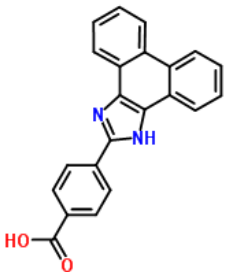
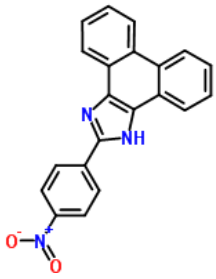
| Compound | $IC_{50}$ (µM) | BEI | PSA | Mol.wt | SEI | Therapeutic Index |
|---|---|---|---|---|---|---|
| CK721 (**43**)  | > 100 | 1.934276 | 61.42 | 361.36 | 1.138017 | NA |
| CK679 (**44**)  | > 100 | 2.035618 | 61.42 | 343.37 | 1.138017 | NA |
| CK106 (**46**)  | > 100 | 2.090471 | 15.6 | 334.36 | 4.480577 | NA |
| CK838 (**47**)  | > 100 | 2.041384 | 28.68 | 342.40 | 2.437134 | NA |

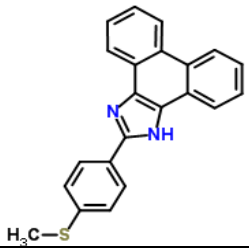
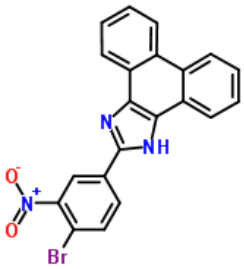
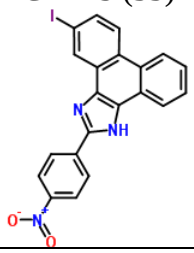
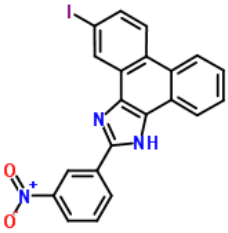
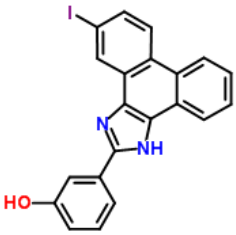
| Compound | $IC_{50}$ (µM) | BEI | PSA | Mol.wt | SEI | Therapeutic Index |
|---|---|---|---|---|---|---|
| CK088 (**48**)  | 99.6±4.2 | 2.925644 | 28.68 | 342.40 | 3.49282 | >1.0 |
| CK103 (**50**)  | 99.5±8.2 | 2.187013 | 28.68 | 458.24 | 3.494341 | >1.0 |
| CK099 (**51**)  | 12.4±0.03 | 4.636169 | 28.68 | 411.24 | 6.647763 | 1.9 |
| CK696 (**53**)  | > 100 | 1.868504 | 47.14 | 374.08 | 1.482754 | NA |

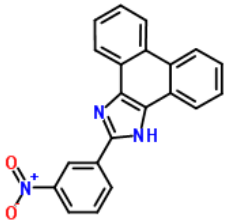
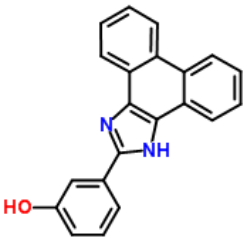
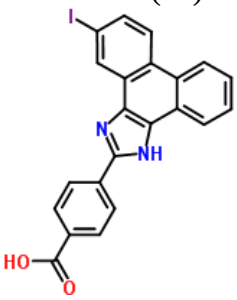
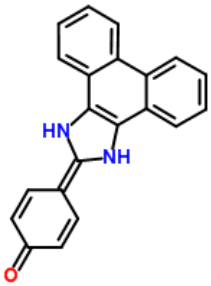
| Compound | $IC_{50}$ (µM) | BEI | PSA | Mol.wt | SEI | Therapeutic Index |
|---|---|---|---|---|---|---|
| CK184 (**54**)  | 39.8±0.1 | 4.482382 | 48.91 | 312.36 | 2.862639 | 1.4 |
| CK6026 (**55**)  | > 100 | 1.90315 | 15.6 | 367.27 | 4.480577 | NA |
| CK147 (**3**)  | 13.3 ±0.07 | 4.921045 | 41.13 | 381.25 | 4.561508 | 3.4 |
| CK967 (**13**)  | 28.6 ±0.04 | 4.941843 | 41.13 | 312.36 | 3.753061 | 1.4 |

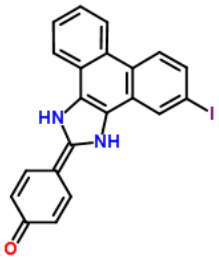
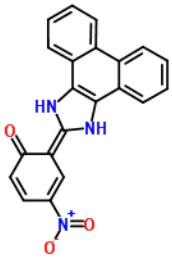
| Compound | $IC_{50}$ (µM) | BEI | PSA | Mol.wt | SEI | Therapeutic Index |
|---|---|---|---|---|---|---|
| CK688 (**14**)  | 10.9 ±2.3 | 6.28305 | 41.13 | 312.36 | 4.771635 | 3.5 |
| CK433 (**52**)  | > 100 | 1.544856 | 58.2 | 452.45 | 1.200979 | NA |
| CK669 (**49**)  | 17.8±0.1 | 4.519477 | 107.18 | 387.12 | 1.632375 | 2.4 |
| CK389 (**42**)  | 13.6±6.5 | 4.80477 | 41.13 | 388.46 | 4.537955 | 1.4 |

| Compound | $IC_{50}$ (μM) | BEI | PSA | Mol.wt | SEI | Therapeutic Index |
|---|---|---|---|---|---|---|
| CK584 (**22**)  | 13.6±4.3 | 4.297928 | 28.68 | 434.27 | 6.507884 | 1.8 |
| CK588 (**23**)  | > 100 | 1.464456 | 57.78 | 477.29 | 1.209709 | NA |
| CK690 (**24**)  | > 100 | 1.455308 | 47.14 | 480.29 | 1.482754 | NA |
| CK927 (**25**)  | 2.85±1.9 | 6.819085 | 28.68 | 373.24 | 8.874321 | 4.0 |

| Compound | $IC_{50}$ (μM) | BEI | PSA | Mol.wt | SEI | Therapeutic Index |
|---|---|---|---|---|---|---|
| CK931 (**26**)  | 5.7±3.9 | 7.185109 | 28.68 | 312.33 | 7.824704 | 3.4 |
| CK932 (**27**)  | 10.2±0.01 | 6.375948 | 28.68 | 312.33 | 6.943514 | 2.1 |
| CK936 (**28**)  | 29.8±5.3 | 4.509417 | 65.98 | 338.355 | 2.312494 | 2.5 |
| CK938 (**29**)  | 15.1±0.02 | 5.366367 | 74.5 | 339.34 | 2.444326 | >6.4 |

| Compound | $IC_{50}$ (µM) | BEI | PSA | Mol.wt | SEI | Therapeutic Index |
|---|---|---|---|---|---|---|
| CK941 (**30**)  | 1.2±0.05 | 8.57954 | 53.98 | 340.44 | 5.410928 | 3.4 |
| CK942 (**31**)  | 7.5±0.5 | 5.080668 | 74.5 | 418.24 | 2.852267 | 2.4 |
| CK175 (**33**)  | 3.1±1.0 | 5.392138 | 74.5 | 465.24 | 3.3673 | 4.1 |
| CK176 (**34**)  | 14.2±1.7 | 3.971524 | 74.5 | 465.24 | 2.48015 | >7.0 |
| CK188 (**36**)  | 12.9±0.05 | 4.331126 | 48.91 | 436.24 | 3.863035 | 1.6 |

| Compound | $IC_{50}$ (µM) | BEI | PSA | Mol.wt | SEI | Therapeutic Index |
|---|---|---|---|---|---|---|
| CK937 (**37**)  | 45.3±2.8 | 3.96034 | 74.5 | 339.34 | 1.803895 | 2.2 |
| CK944 (**41**)  | 11.3±0.3 | 6.273511 | 48.91 | 310.34 | 3.980621 | 1.6 |
| CK586 (**45**)  | 14.1±4.6 | 3.986604 | 65.98 | 464.25 | 2.805063 | >3.0 |
| CK943 (**40**)  | 15.7±7.2 | 5.813303 | 41.13 | 310.34 | 4.386337 | 1.0 |

| Compound | $IC_{50}$ (µM) | BEI | PSA | Mol.wt | SEI | Therapeutic Index |
|---|---|---|---|---|---|---|
| CK187 (**35**)  | 9.44±1.9 | 4.642004 | 41.13 | 436.24 | 4.923482 | 2.2 |
| CK945 (**32**)  | 7.1±0.05 | 6.047002 | 86.95 | 355.34 | 2.471238 | 2.5 |

NA = not applicable. Toxicity was tested up to a maximum concentration of 100 µM

***References***


1. Kortagere, S.; Krasowski, M. D.; Reschly, E. J.; Venkatesh, M.; Mani, S.; Ekins, S. Evaluation of Computational Docking to Identify PXR Agonists in the ToxCast(TM) Database. *Environ Health Perspect* **2010**.
2. Kortagere, S.; Welsh, W. J.; Morrisey, J. M.; Daly, T.; Ejigiri, I.; Sinnis, P.; Vaidya, A. B.; Bergman, L. W. Structure-based design of novel small-molecule inhibitors of Plasmodium falciparum. *J Chem Inf Model* **2010,** 50, 840-9.
3. Peng, Y.; Zhang, Q.; Arora, S.; Keenan, S. M.; Kortagere, S.; Wannemacher, K. M.; Howells, R. D.; Welsh, W. J. Novel delta opioid receptor agonists exhibit differential stimulation of signaling pathways. *Bioorg Med Chem* **2009,** 17, 6442-50.
4. Kortagere, S.; Chekmarev, D.; Welsh, W. J.; Ekins, S. Hybrid scoring and classification approaches to predict human pregnane X receptor activators. *Pharm Res* **2009,** 26, 1001-11.
5. Kortagere, S.; Welsh, W. J. Development and application of hybrid structure based method for efficient screening of ligands binding to G-protein coupled receptors. *J Comput Aided Mol Des* **2006,** 20, 789-802.
6. Madani, N.; Schon, A.; Princiotto, A. M.; Lalonde, J. M.; Courter, J. R.; Soeta, T.; Ng, D.; Wang, L.; Brower, E. T.; Xiang, S. H.; Kwon, Y. D.; Huang, C. C.; Wyatt, R.; Kwong, P. D.; Freire, E.; Smith, A. B., 3rd; Sodroski, J. Small-molecule CD4 mimics interact with a highly conserved pocket on HIV-1 gp120. *Structure* **2008,** 16, 1689-701.
7. Madani, N.; Hubicki, A. M.; Perdigoto, A. L.; Springer, M.; Sodroski, J. Inhibition of human immunodeficiency virus envelope glycoprotein- mediated single cell lysis by low-molecular-weight antagonists of viral entry. *J Virol* **2007,** 81, 532-8.

8. Si, Z.; Madani, N.; Cox, J. M.; Chruma, J. J.; Klein, J. C.; Schon, A.; Phan, N.; Wang, L.; Biorn, A. C.; Cocklin, S.; Chaiken, I.; Freire, E.; Smith, A. B., 3rd; Sodroski, J. G. Small-molecule inhibitors of HIV-1 entry block receptor-induced conformational changes in the viral envelope glycoproteins. *Proc Natl Acad Sci U S A* **2004,** 101, 5036-41.
9. Mobley, D. L.; Dill, K. A. Binding of small-molecule ligands to proteins: "what you see" is not always "what you get". *Structure* **2009,** 17, 489-98.
10. Ohtaka, H.; Freire, E. Adaptive inhibitors of the HIV-1 protease. *Prog Biophys Mol Biol* **2005,** 88, 193-208.
11. Li, Y.; Kar, A. K.; Sodroski, J. Target cell type-dependent modulation of human immunodeficiency virus type 1 capsid disassembly by cyclophilin A. *J Virol* **2009,** 83, 10951-62.
12. Tian, B.; He, M.; Tang, S.; Hewlett, I.; Tan, Z.; Li, J.; Jin, Y.; Yang, M. Synthesis and antiviral activities of novel acylhydrazone derivatives targeting HIV-1 capsid protein. *Bioorg Med Chem Lett* **2009,** 19, 2162-7.
13. Blair, W. S.; Pickford, C.; Irving, S. L.; Brown, D. G.; Anderson, M.; Bazin, R.; Cao, J.; Ciaramella, G.; Isaacson, J.; Jackson, L.; Hunt, R.; Kjerrstrom, A.; Nieman, J. A.; Patick, A. K.; Perros, M.; Scott, A. D.; Whitby, K.; Wu, H.; Butler, S. L. HIV capsid is a tractable target for small molecule therapeutic intervention. *PLoS Pathog* **2010,** 6, e1001220.
14. Kelly, B. N.; Kyere, S.; Kinde, I.; Tang, C.; Howard, B. R.; Robinson, H.; Sundquist, W. I.; Summers, M. F.; Hill, C. P. Structure of the antiviral assembly inhibitor CAP-1 complex with the HIV-1 CA protein. *J Mol Biol* **2007,** 373, 355-66.
15. Pornillos, O.; Ganser-Pornillos, B. K.; Kelly, B. N.; Hua, Y.; Whitby, F. G.; Stout, C. D.; Sundquist, W. I.; Hill, C. P.; Yeager, M. X-Ray Structures of the Hexameric Building Block of the HIV Capsid. *Cell* **2009,** 137, 1282-92.
16. Jin, Y.; Tan, Z.; He, M.; Tian, B.; Tang, S.; Hewlett, I.; Yang, M. SAR and molecular mechanism study of novel acylhydrazone compounds targeting HIV-1 CA. *Bioorg Med Chem* **2010,** 18, 2135-40.
17. Tian, B. H.; He, M. Z.; Tang, S. X.; Hewlett, I.; Tan, Z. W.; Li, J. B.; Jin, Y. X.; Yang, M. Synthesis and antiviral activities of novel acylhydrazone derivatives targeting HIV-1 capsid protein. *Bioorganic & Medicinal Chemistry Letters* **2009,** 19, 2162-2167.
18. Titolo, S.; Mercier, J.-F.; Wardrop, E.; von Schwedler, U.; Goudreau, N.; Lemke, C.; Faucher, A.-M.; Yoakim, C.; Sundquist, W.; S., M. Discovery of Potent HIV-1 Capsid Assembly Inhibitors. In *17th Conference on Retroviruses and Opportunistic Infections*, San Francisco, 2010.
19. Curreli, F.; Zhang, H.; Zhang, X.; Pyatkin, I.; Victor, Z.; Altieri, A.; Debnath, A. K. Virtual screening based identification of novel small-molecule inhibitors targeted to the HIV-1 capsid. *Bioorg Med Chem* **2011,** 19, 77-90.
20. Shi, J.; Zhou, J.; Shah, V. B.; Aiken, C.; Whitby, K. Small Molecule Inhibition of Human Immunodeficiency Virus Type 1 Infection by Virus Capsid Destabilization. *J Virol* **2010**.
21. Fridman, N.; Kaftory, M.; Eichen, Y.; Speiser, S. Crystal structures and solution spectroscopy of lophine derivatives. *Journal of Molecular Structure* **2009,** 917, 101-109.
22. Kortagere, S.; Madani, N.; Mankowski, M. K.; Schon, A.; Zentner, I.; Swaminathan, G.; Princiotto, A.; Anthony, K.; Oza, A.; Sierra, L. J.; Passic, S. R.; Wang, X.; Jones, D. M.; Stavale, E.; Krebs, F. C.; Martin-Garcia, J.; Freire, E.; Ptak, R. G.; Sodroski, J.; Cocklin, S.; Smith, A. B., 3rd. Inhibiting Early-Stage Events in HIV-1 Replication by Small-Molecule Targeting of the HIV-1 Capsid. *J Virol* **2012,** 86, 8472-81.

23. Brown, B. K.; Darden, J. M.; Tovanabutra, S.; Oblander, T.; Frost, J.; Sanders-Buell, E.; de Souza, M. S.; Birx, D. L.; McCutchan, F. E.; Polonis, V. R. Biologic and genetic characterization of a panel of 60 human immunodeficiency virus type 1 isolates, representing clades A, B, C, D, CRF01_AE, and CRF02_AG, for the development and assessment of candidate vaccines. *J Virol* **2005,** 79, 6089-101.
24. Jagodzinski, L. L.; Wiggins, D. L.; McManis, J. L.; Emery, S.; Overbaugh, J.; Robb, M.; Bodrug, S.; Michael, N. L. Use of calibrated viral load standards for group m subtypes of human immunodeficiency virus type 1 to assess the performance of viral RNA quantitation tests. *Journal of Clinical Microbiology* **2000,** 38, 1247-1249.
25. Michael, N. L.; Herman, S. A.; Kwok, S.; Dreyer, K.; Wang, J.; Christopherson, C.; Spadoro, J. P.; Young, K. K. Y.; Polonis, V.; McCutchan, F. E.; Carr, J.; Mascola, J. R.; Jagodzinsi, L. L.; Robb, M. L. Development of calibrated viral load standards for group M subtypes of human immunodeficiency virus type 1 and performance of an improved AMPLICOR HIV-1 MONITOR test with isolates of diverse subtypes. *Journal of Clinical Microbiology* **1999,** 37, 2557-2563.
26. Vahey, M.; Nau, M. E.; Barrick, S.; Cooley, J. D.; Sawyer, R.; Sleeker, A. A.; Vickerman, P.; Bloor, S.; Larder, B.; Michael, N. L.; Wegner, S. A. Performance of the Affymetrix GeneChip HIV PRT 440 platform for antiretroviral drug resistance genotyping of human immunodeficiency virus type 1 clades and viral isolates with length polymorphisms. *Journal of Clinical Microbiology* **1999,** 37, 2533-2537.
27. Zentner, I.; Sierra, L. J.; Fraser, A. K.; Maciunas, L.; Mankowski, M. K.; Vinnik, A.; Fedichev, P.; Ptak, R. G.; Martin-Garcia, J.; Cocklin, S. Identification of a small-molecule inhibitor of HIV-1 assembly that targets the phosphatidylinositol (4,5)-bisphosphate binding site of the HIV-1 matrix protein. *ChemMedChem* **2013,** 8, 426-32.
28. Zentner, I.; Sierra, L. J.; Maciunas, L.; Vinnik, A.; Fedichev, P.; Mankowski, M. K.; Ptak, R. G.; Martin-Garcia, J.; Cocklin, S. Discovery of a small-molecule antiviral targeting the HIV-1 matrix protein. *Bioorg Med Chem Lett* **2013,** 23, 1132-5.
29. Abad-Zapatero, C. Ligand efficiency indices for effective drug discovery. *Expert Opin Drug Discov* **2007,** 2, 469-88.

**18. Extent of Clinical Activities Initiated and Completed**. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

**If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed.** (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

**Note**: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

**19. Human Embryonic Stem Cell Research.** Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X___ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

**20. Articles Submitted to Peer-Reviewed Publications**.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

**Note:** The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

| Title of Journal Article: | Authors: | Name of Peer-reviewed Publication: | Month and Year Submitted: | Publication Status (check appropriate box below): |
|---|---|---|---|---|
| 1. Inhibiting Early-Stage Events in HIV-1 Replication by Small-Molecule Targeting of the HIV-1 Capsid. | Kortagere et al. | Journal of Virology | May 2012 | ☐Submitted<br>☐Accepted<br>☒Published |

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X_____ No_____

If yes, please describe your plans:

We are assembling a manuscript for publication in J. Med. Chem. Outlining the discovery of CK176 (see above for explanation).

**21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project.** Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

**22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment**. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

We have defined a new site on the now validated antiviral target, HIV-1 CA. This site could be a site of interaction of next generation drugs.

**23. Inventions, Patents and Commercial Development Opportunities.**

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance

of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No_____X___

If yes, please describe your plans:

**24. Key Investigator Qualifications.** Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. you may insert the NIH biosketch form here.

# BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| NAME | POSITION TITLE |
|---|---|
| Cocklin, Simon | Assistant Professor of Biochemistry & Molecular Biology |
| eRA COMMONS USER NAME<br>COCKLIN1 | |

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | YEAR(s) | FIELD OF STUDY |
|---|---|---|---|
| University of Newcastle upon Tyne, UK | BSc | 1997 | Genetics |
| University of Newcastle upon Tyne, UK | PhD | 2001 | Molecular Genetics |
| University of Newcastle upon Tyne, UK | Post-Doc | 2001 – 02 | Fungal Transcription |
| University of Pennsylvania, USA | Post-Doc | 2002 – 03 | HIV-1 and Cell Interactions |
| Drexel University College of Medicine, USA | Post-Doc | 2003 – 04 | HIV-1 and Cell Interactions |

## A. Personal Statement

Because of the emergence of drug resistant strains and the cumulative toxicities associated with current therapies, there remains a demand for new inhibitors of HIV-1 replication. I have a broad background in protein biochemistry and biophysics, with specific training and expertise in the application of biophysical approaches (such as surface plasmon resonance [SPR] spectroscopy and isothermal titration calorimetry) to both basic and applied infectious disease research. As a postdoctoral fellow at the University of Pennsylvania, and later at Drexel University College of Medicine, I successfully used SPR to understand the mechanisms by which peptide and small molecule HIV-1 entry inhibitors act. This work laid an excellent foundation for the pursuit of my independent research but also provided me with invaluable experience in dealing with the demands of a multidisciplinary project. In my independent research, first as a Research Assistant Professor, and now as Assistant Professor, I have continued to pursue the discovery of novel small molecule inhibitors of HIV-1 replication that target established and new therapeutic targets.

## B. Positions and Honors.

**Professional Positions:**

2004 – 2007 **Research Associate,** Department of Biochemistry, Drexel University, Philadelphia, PA

2007 – 2011 **Research Assistant Professor,** Department of Biochemistry & Molecular Biology, Drexel University College of Medicine, Philadelphia, PA

2011– Pres **Assistant Professor,** Department of Biochemistry & Molecular Biology, Drexel University College of Medicine, Philadelphia, PA

2007 – Pres **Director, Protein Production/Purification Facility**; **Co-Director, Protein-Protein Interaction Facility**, Protein Analysis Core Facility, Drexel University College of Medicine, Philadelphia, PA

**Awards:**

September, 2010 *Winner of "Bio-Rad ProteOn XPR36 Giveaway Program"*. Proposal title: Small-Molecule Inhibitors of HIV-1 Replication. Awarded a Bio-Rad ProteOn XPR36 Protein Interaction Array System, March 2011.

**Professional Activities:**
2012-present, Member, Biochemistry Graduate Admissions Committee
2012-present Director, Master's Degree in Biotechnology
2012-present Editorial Board, ISRN AIDS

## C. Select Peer-reviewed publications (reverse chronological order)

1. Deakyne, J.S., Huang, F., Negri, J., Tolliday, N., **Cocklin, S.** and Mazin, A.V. Analysis of the activities of RAD54, a SWI2/SNF2 protein, using a specific small-molecule inhibitor *Journal of Biological Chemistry, Accepted* (JBC/2013/502195)
2. Economou, N. J., **Cocklin, S.,** and Loll, P.J. 2013. High-resolution crystal structure reveals molecular details of target recognition by bacitracin. *Proceedings of the National Academy of Sciences USA*. **110:**14207-14212.
3. Economou, N.J., Zentner, I.J., Lazo, E., Jakoncic, J., Stojanoff, V., Weeks, S.D., Grasty, K.C., **Cocklin, S.,** and Loll, P.J. 2013. Structure of the complex between teicoplanin and a bacterial cell-wall peptide: use of a carrier-protein approach. *Acta Crystallogr D Biol Crystallogr* **69:**520-533.
4. Zentner, I., L. J. Sierra, A. K. Fraser, L. Maciunas, M. K. Mankowski, A. Vinnik, P. Fedichev, R. G. Ptak, J. Martin-Garcia, and **S. Cocklin**. 2013. Identification of a Small-Molecule Inhibitor of HIV-1 Assembly that Targets the Phosphatidylinositol (4,5)-bisphosphate Binding Site of the HIV-1 Matrix Protein. *ChemMedChem*. Jan 29. doi: 10.1002/cmdc.201200577
5. Checkley, M. A., B. G. Luttge, P. Y. Mercredi, S. K. Kyere, J. Donlan, T. Murakami, M. F. Summers, **S. Cocklin**, and E. O. Freed. 2013. Reevaluation of the Requirement for Tip47 in Human Immunodeficiency Virus Type 1 Envelope Glycoprotein Incorporation. *Journal of Virology*. Jan 16. [Epub ahead of print]
6. Zentner, I., L. J. Sierra, L. Maciunas, A. Vinnik, P. Fedichev, M. K. Mankowski, R. G. Ptak, J. Martin-Garcia, and **S. Cocklin**. 2013. Discovery of a small-molecule antiviral targeting the HIV-1 matrix protein. *Bioorganic & Medicinal Chemistry Letters* **23:**1132-1135.
7. Kortagere, S., Madani, N., Mankowski, M.K., Schön, A., Princiotto, A., Anthony, K., Zentner, I., Oza, A., Sierra, L.J., Passic., S.R., Wang, X., Jones, D., Carter, J., Stavale, E., Krebs, F.C., Martín-García, J., Freire, E., Ptak, R.G., Sodroski, J., **Cocklin, S.,*** and Smith, A.B. III* Inhibiting Early-Stage Events in HIV-1 Replication by Small-Molecule targeting of the HIV-1 Capsid. *Journal of Virology*. (2012) **86:**8472-8481 *corresponding authors
8. Huang, F., Mazina, O.M., Zentner, I.J., **Cocklin, S**., and Mazin, A.V. Inhibition of homologous recombination in human cells by targeting RAD51 recombinase. *Journal of Medicinal Chemistry*. (2012) **55**, 3011-3020
9. Economou, N.J., Nahoum, V., Weeks, S.D., Grasty, K.C., Townsend, T.M., Zentner, I.J., Bhuiya, M.W., **Cocklin, S.,** and Loll, P.J. A carrier protein strategy yields the structure of dalbavancin. *Journal of the American Chemical Society*. (2012) 4637-4645, doi:10.1021/ja208755j.

## BIOGRAPHICAL SKETCH

| NAME<br>Sandhya Kortagere | POSITION TITLE<br>Assistant Professor |
|---|---|
| eRA COMMONS USER NAME (credential, e.g., agency login)<br>KORTAGSA | |

EDUCATION/TRAINING

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | MM/YY | FIELD OF STUDY |
|---|---|---|---|
| Bangalore University, India | B.Sc. | 05/95 | Physics, Chemistry, Mathematics |
| Bangalore University, India | M.Sc. | 05/97 | Molecular Physics |
| National Institute of Mental Health and Neurosciences | Ph.D. | 11/03 | Molecular Pharmacology |
| Mt.Sinai School of Medicine | Postdoc | 12/05 | Physiology and Biophysics |
| Marine Biological Laboratory | Professional course | 08/12 | Biology of Parasitism |

**A. Personal Statement:** My laboratory has been involved in designing small molecule modulators/inhibitors to a number of druggable targets. We have developed a novel platform for drug discovery called the Hybrid Structure Based (HSB) method that incorporates known biochemical and structural information to screen a large library of small molecules. All modules for screening, filtering and ranking protein complexes were developed in my laboratory. My training is ideally suited to lead this project as a collaborator. A triple major in Physics, mathematics and chemistry in undergraduate followed by Masters in Physics specializing in Molecular spectroscopy and Crystallography, a graduate degree in molecular pharmacology and postdoctoral training in molecular biophysics have laid a strong foundation for my interdisciplinary scientific career. The HSB method is a novel in-silico, in-vitro iterative screening platform developed in my lab that has been successfully validated to design selective compounds that have found application as antimalarials, anti-HIV agents, atypical dopamine agonists to treat L-dopa induced dyskinesia and allosteric modulators of neurotransmitter transporters.

**B. Positions and Employment**

| | | |
|---|---|---|
| 1997-1998 | Lecturer | MES college, Bangalore, India |
| 1998-2002 | Senior Research Fellow | National Institute of Mental Health and Neurosciences, India |
| 2001-2002 | Scientist | Strand Genomics, Pvt. Ltd., Bangalore |
| 2002-2003 | Postdoctoral Fellow | Mt. Sinai School of Medicine, NY |
| 2003-2005 | Postdoctoral Associate RTS-III | Weill Cornell Medical College, NY |
| 2006-2008 | Assistant Professor | UMDNJ- Robert Wood Johnson Medical School |
| 2008-present | | Drexel University College of Medicine |

**Honors**

| | |
|---|---|
| 1990 | National Merit Scholarship, India. |
| 1996,1997 | Prof. S.B. Bondade best lecture award, Indian Physics Association. |
| 1997 | Awarded second rank in MS (Physics) Bangalore University. |
| 1998-2001.1.1.1 | Senior Research Fellowship, Indian Council for Medical Research, India. |
| 2012 | Drexel University Professional enrichment growth award |
| 2012 | Burroughs-Welcome fund fellowship to attend Molecular Parasitology course at MBL, |

MA.

**C. Selected Peer-reviewed Publications (out of 34 publications)**

**Kortagere S**, Gmeiner P, Weinstein H, Schetz JA. Certain 1,4-disubstituted aromatic piperidines and piperazines with extreme selectivity for the dopamine D4 receptor interact with a common receptor microdomain. Mol Pharmacol. 2004, 66(6):1491-9.

**Kortagere S**, Welsh WJ. Development and application of hybrid structure based method for efficient screening of ligands binding to G-protein coupled receptors. J Comput Aided Mol Des. 2006, 20(12):789-802.

**Kortagere, S.,** Krasowski, M.D. and S. Ekins. "The importance of discerning shape in molecular pharamacology". Trends Pharmacol. Sci. 30(3): 138-47, 2009.

**Kortagere S*** and Ekins S, "Troubleshooting computational methods in drug discovery", J Pharmacol Toxicol Methods. 2010, 61(2):67-75. (* corresponding author)

**Kortagere S,** Krasowski, MD, Reschly EJ, Venkatesh M, Mani S and Ekins S, "Evaluation of computational docking to identify PXR agonists in Toxcast database" Environ Health Perspect, 2010, Jun 17

**Kortagere S*,** Welsh WJ, Morrisey J, Daly TM, Ejigiri I, Sinnis P, Vaidya AB, Bergman LW, "Structure Based design of Plasmodium falciparum inhibitors" J.Chem. Inf. Model, 2010, 50(5), 840-849

**Kortagere S,** Mui E, McLeod R, Welsh WJ, "Rapid discovery of inhibitors of Toxoplasma gondii using hybrid structure based computational approach", 2011, 25(5); 403-411

Kuzhikandathil EV, **Kortagere S**. Identification and characterization of a novel class of atypical dopamine receptor agonists. Pharm Res. 2012; 29(8):2264-75.

**Kortagere S**, Madani N, Mankowski MK, Schön A, Zentner I, Swaminathan G, Princiotto A, Anthony K, Oza A, Sierra LJ, Passic SR, Wang X, Jones DM, Stavale E, Krebs FC, Martín-García J, Freire E, Ptak RG, Sodroski J, Cocklin S, Smith AB 3rd. Inhibiting early-stage events in HIV-1 replication by small-molecule targeting of the HIV-1 capsid. J Virol. 2012; 86(16):8472-81

Sacan A, Ekins S, **Kortagere S***. Applications and limitations of in silico models in drug discovery. Methods Mol Biol. 2012;910:87-124.

**Kortagere S***, Fontana AC, Rose DR, Mortensen OV*. Identification of an allosteric modulator of the serotonin transporter with novel mechanism of action. Neuropharmacology. 2013; 72:282-90.

**D. Ongoing Research Support**

Title: "Molecular Pathways targeted by pyrazole antimalarial compounds"
This project deals with using pyrazole antimalarial compounds to identify a novel molecular pathway in malaria parasite.Agency: NIH, R01, Role: collaborator – 10% effort. Period: 02/01/2012- 01/31/2016

Title: "Selective inhibitors for probing the functional role of TULA-2, a novel key regulator of Platelets"

In this project we propose to use the HSB method to design new compounds to inhibit TULA-2 phospatases Agency: AHA, Innovator award - 13IRG14230008; Role: Consultant; Period: 01/01/2013 – 12/31/2015