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Composition and Orientation of the Core Region of Novel HIV-1 Entry Inhibitors Influences Metabolic Stability

Rama Karadsheh [1], and Megan Meuser [1], Simon Cocklin [1*]

[1] Department of Biochemistry, Drexel University, 245 North 25th Street, Philadelphia, Pennsylvania, USA

* Corresponding author: sc349@drexel.edu

DREXEL UNIVERSITY College of Medicine

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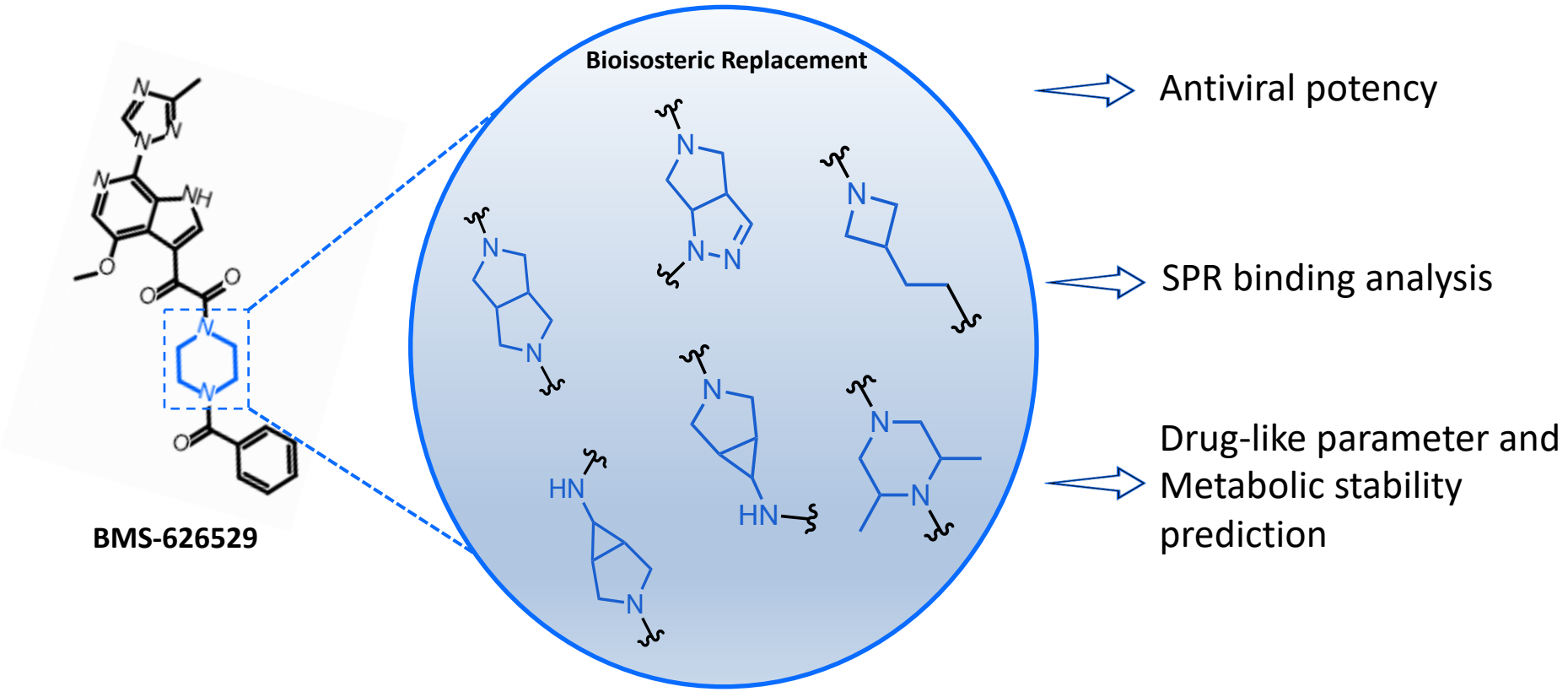
Graphical Abstract



Abstract:

Fostemsavir/temsavir is an investigational HIV-1 entry inhibitor currently in late-stage clinical trials. Although it holds promise to be a first-in-class Env-targeted entry inhibitor for the clinic, issues with bioavailability relegate its use to salvage therapies only. As such, the development of a small molecule HIV-1 entry inhibitor that can be used in standard combination antiretroviral therapy (cART) remains a longstanding goal for the field. We previously demonstrated the ability of extending the chemotypes available to this class of inhibitor as the first step towards this overarching goal. In addition to poor solubility, metabolic stability is a crucial determinant of bioavailability. Therefore, we assess the metabolic stabilities of five of our novel chemotype entry inhibitors. We found that changing the piperazine core region of temsavir alters the stability of the compound in human liver microsome assays. Moreover, we identified an entry inhibitor with more than twice the metabolic stability of temsavir and demonstrated that the orientation of the core replacement is critical for this increase. This work further demonstrates the feasibility of our long-term goal-to design an entry inhibitor with improved drug-like qualities-and warrants expanded studies to achieve this.

Keywords: HIV-1 entry inhibitor; metabolic stability; docking; antiviral; surface plasmon resonance

HIV-1 Pandemic Continues to be a Global Issue

39.0 million

People currently estimated to be living with HIV

During 2019

1.7 million

People newly infected

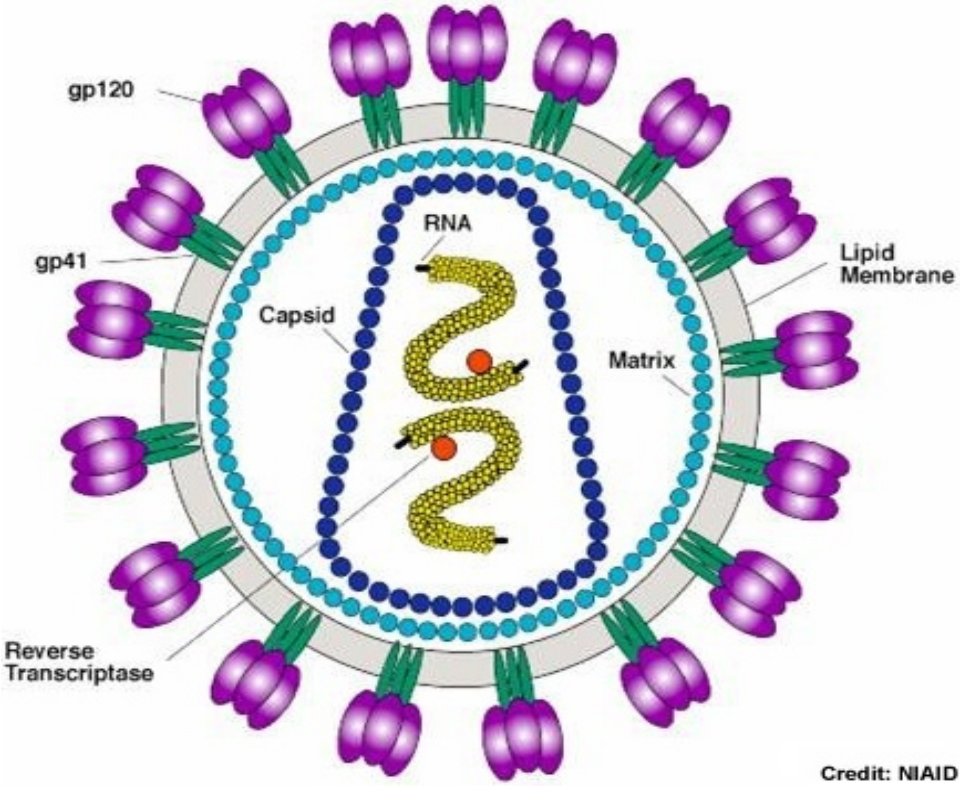
0.69 million

HIV-related deaths

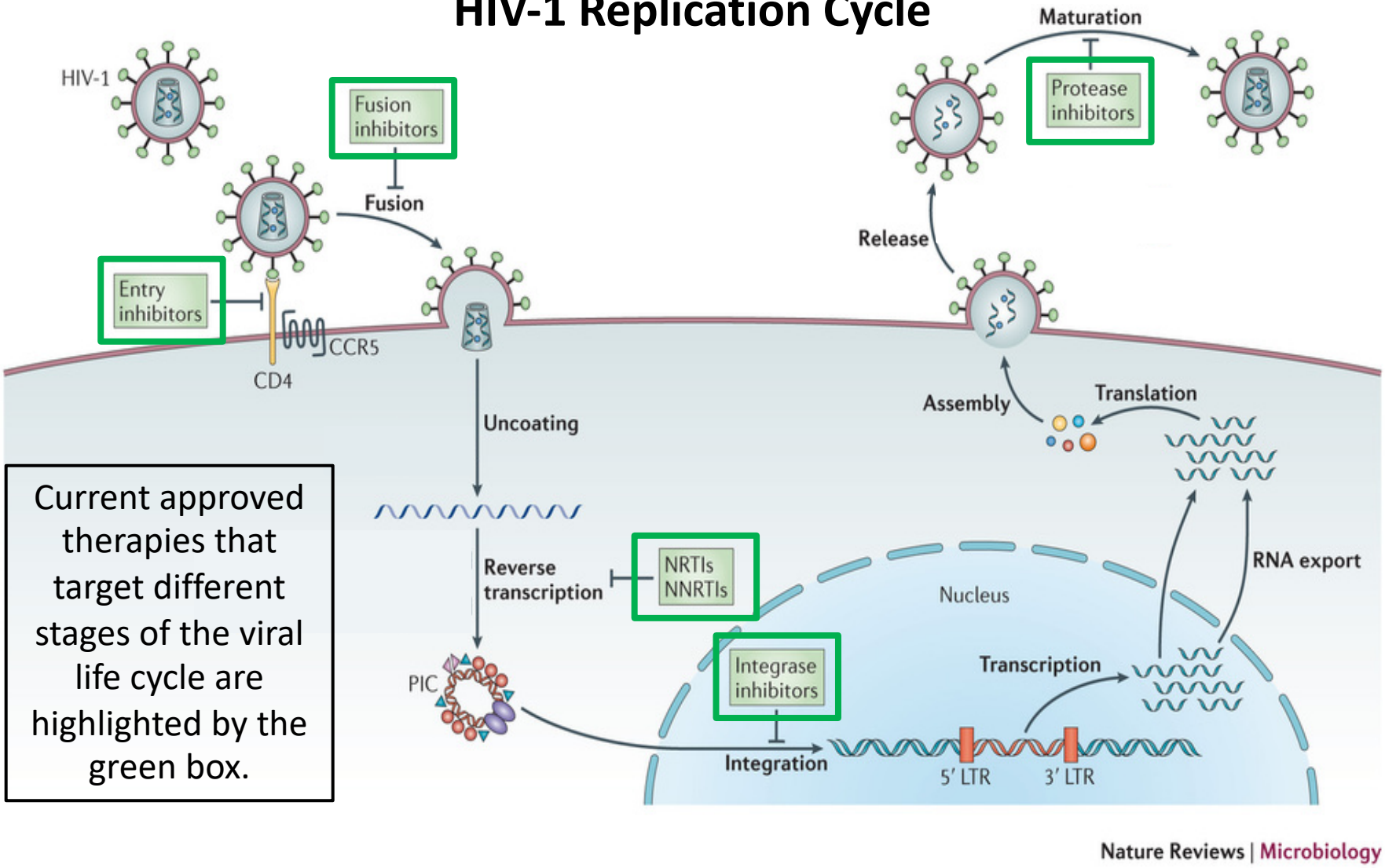
World Health Organization

HIV-1 structure

- **Env is a heterotrimer of gp120 and gp41 dimers**
  - Embedded in the lipid membrane
  - Sole viral protein on HIV-1 membrane
- Capsid houses viral genome and replication machinery
- Viral genome consists of 2 single stranded RNA molecules
- Reverse Transcriptase transcribes viral RNA to viral DNA, which is incorporated into the host cells



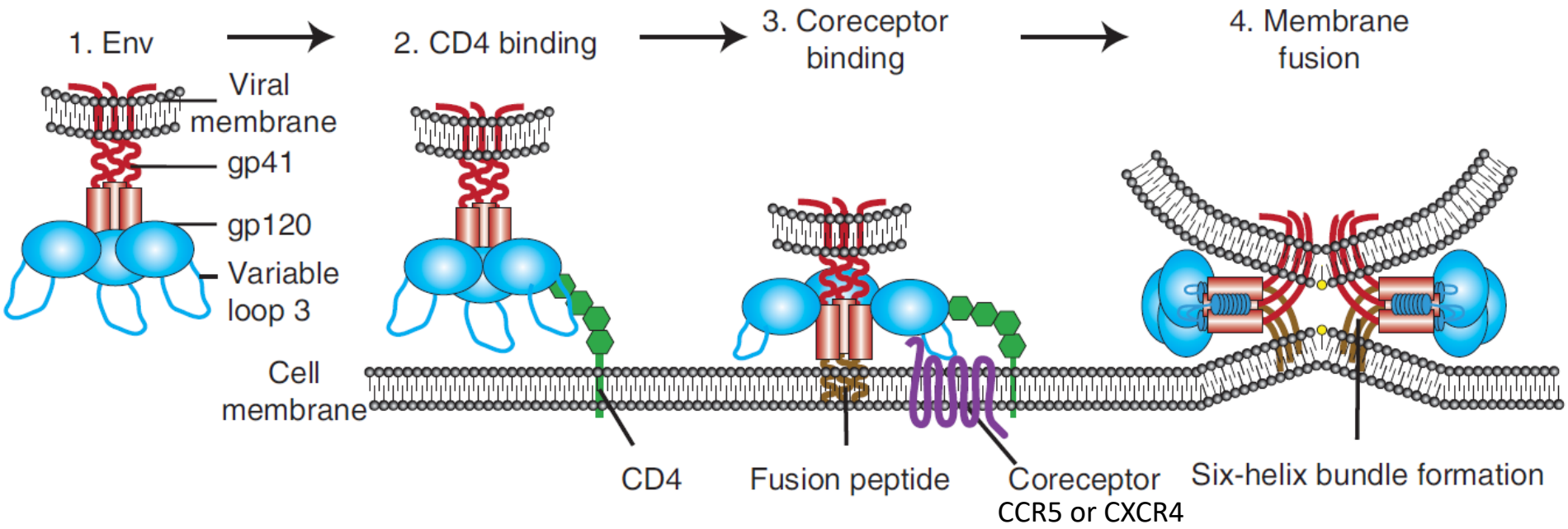
HIV-1 Replication Cycle



Current approved therapies that target different stages of the viral life cycle are highlighted by the green box.

Modified figure from *Nature Reviews Microbiology* **11**, 877–883 (2013)

A Closer Look at HIV-1 Entry



1. Env is a trimer of gp120 and gp41 heterodimers that is conformationally metastable
2. Gp120 binds the CD4 receptor
3. Conformational rearrangements promote the gp120-CD4 complex to bind the co-receptor
4. Gp41 subunit contains a fusion peptide that inserts into the host cell membrane.

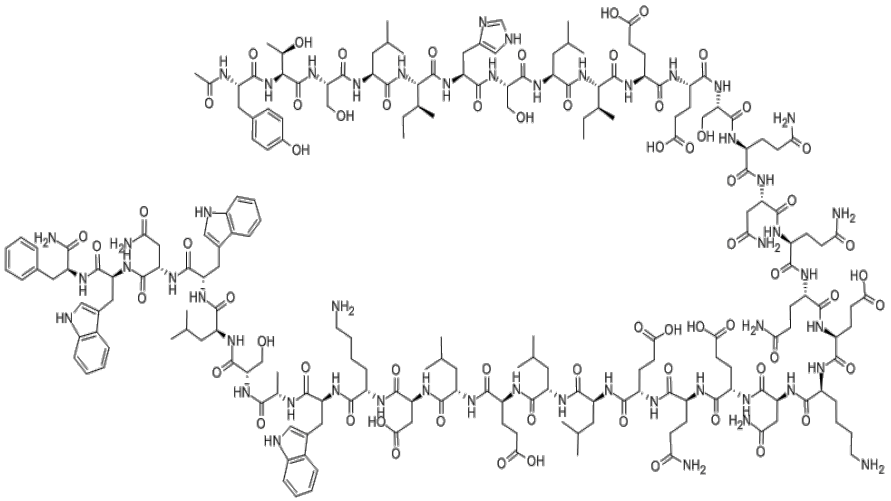
Conformational rearrangements bring the viral and host cell membrane in close proximity until fusion occurs

Wilen et al., 2012. 10.1101/cshperspect.a006866

Approved Entry Inhibitors and Their Limitations…

Enfurvitide

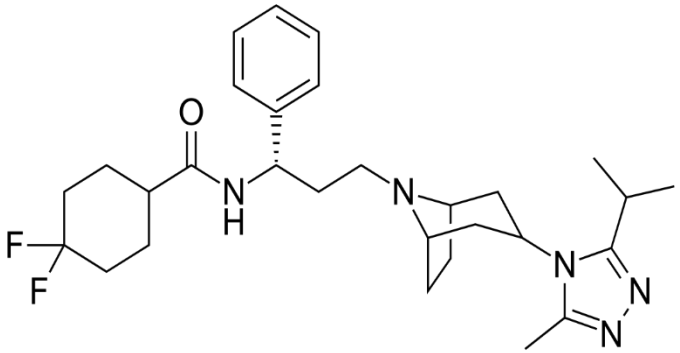
- Synthetic peptide
- Binds to gp41 to prevent fusion
- Not available orally
- Injection is necessary; however, serious reactions to local injection have been observed
- Twice daily 90 mg doses
- Expensive - $25,000 (22,251€) for 1 year of treatment



Hardy, H and Skolnik, PR. Pharmacotherapy. 2004. 24(2): 198-211.

Maraviroc

- Targets host cell
- Binds to CCR5 co-receptor to block Env binding
- Inactive against viruses that us CXCR4 co-receptor

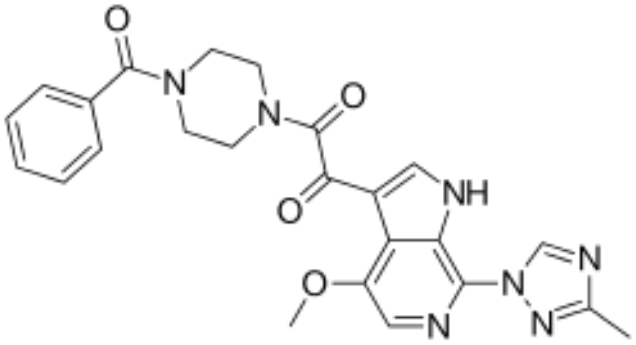


MacArthur, RD and Novak, R. Clinical Infectious Diseases 2008. 47(2):236-241.

Env-targeting Attachment Inhibitors

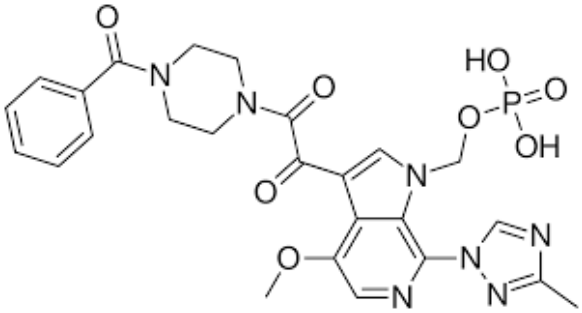
- Temsavir (BMS-626529)
  - Piperizine-based chemotype
  - Potent against many HIV-1 subtypes
  - Low solubility
  - Poor bioavailability

Temsavir



- Fostemsavir (BMS-663068)
  - Phosphooxymethyl prodrug of temsavir
  - FDA approved in July 2020
  - Suboptimal solubility after cleavage of the prodrug
  - Breadth issues against specific HIV-1 subtypes
  - Only recommended for treatment-experienced patients = limited therapeutic opportunity
  - Expensive - $7,650 for 30 day supply.

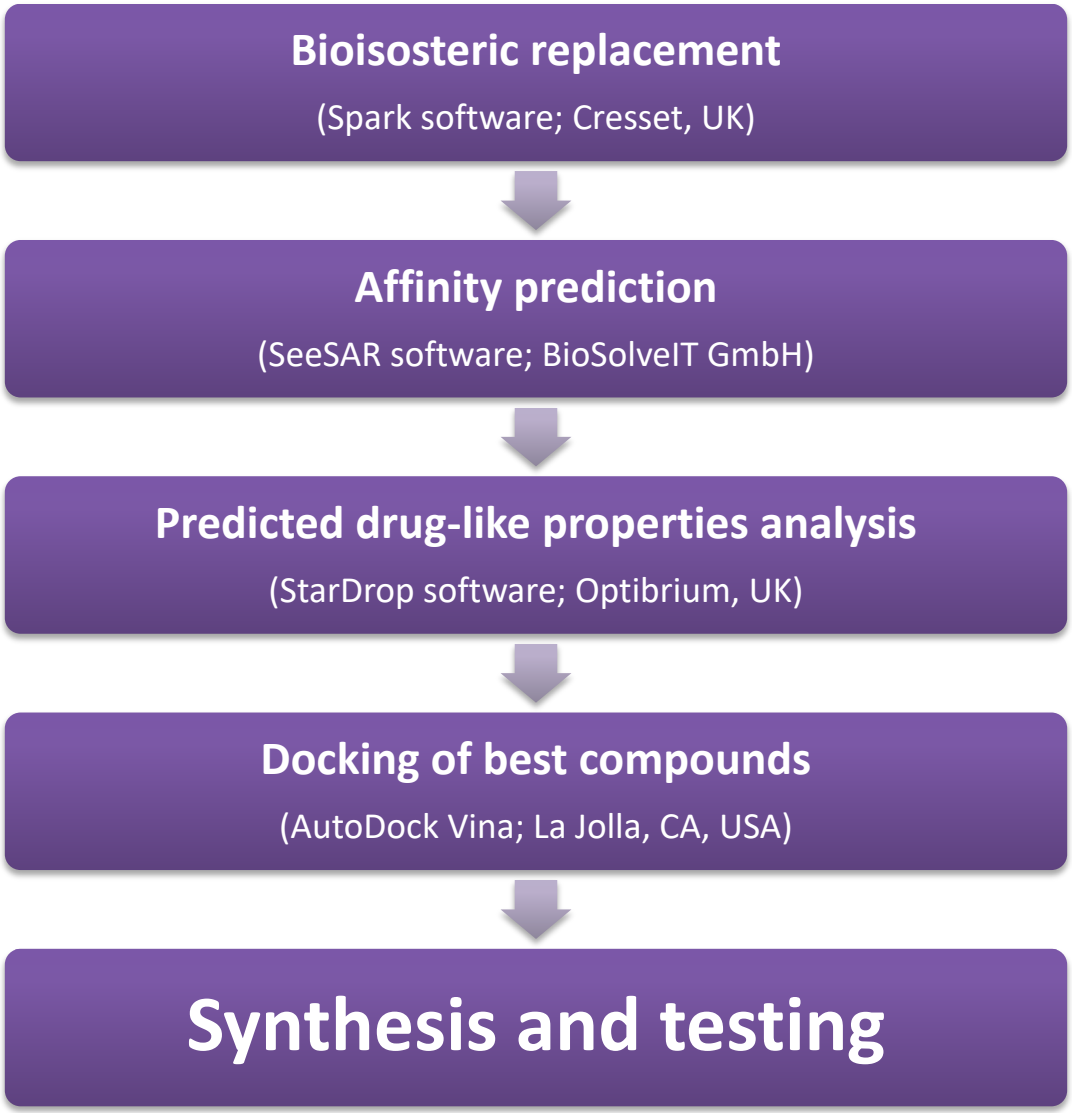
Fostemsavir



Cahn, P., Fink, V., and Patterson, P.. Curr Opin HIV AIDS. 2018. 13(4):341-345.

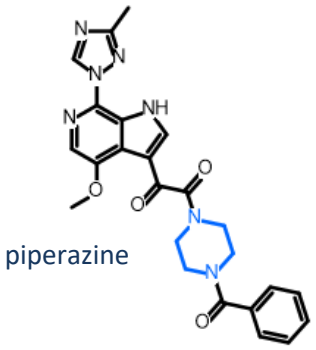
Can we design an entry inhibitor that has a similar binding site, but has better solubility and metabolic stability than BMS-626529 (Temsavir)?

Multi-step Computational Design Workflow

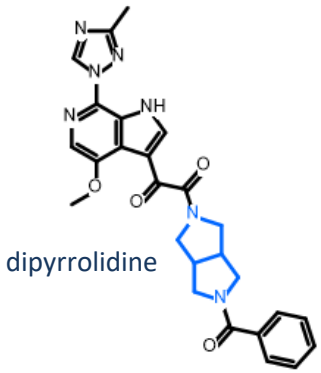


Bioisosteric Replacement Identifies Novel Scaffolds with Inhibitor Activity

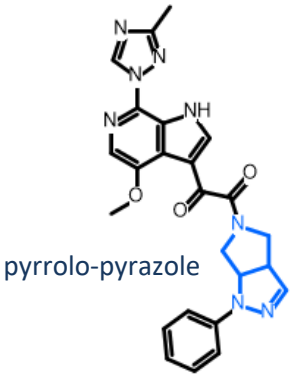
BMS-626529
(HIV-1_{B41} IC_{50} = 0.05±0.006 nM)



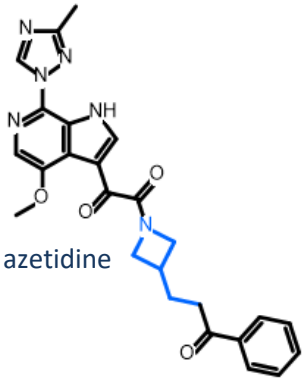
SC11
(HIV-1_{B41} IC_{50} = 2.0 ± 0.2 nM)



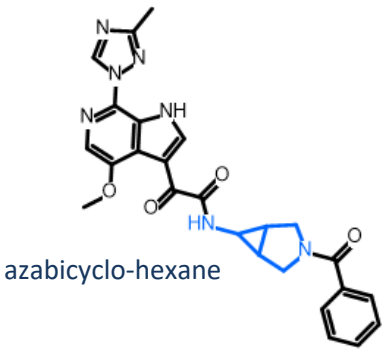
SC12
(HIV-1_{B41} IC_{50} = 6.0 ± 0.3 nM)



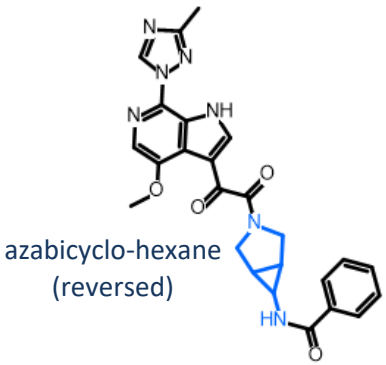
SC15
(HIV-1_{B41} IC_{50} = 7.0 ± 0.1 nM)



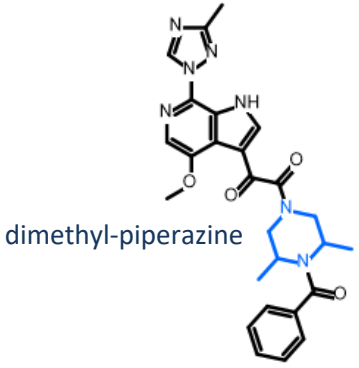
SC28
(HIV-1_{B41} IC_{50} = 35.0 ± 6.2 nM)



SC46
(HIV-1_{B41} IC_{50} = 91.5±9.8 nM)

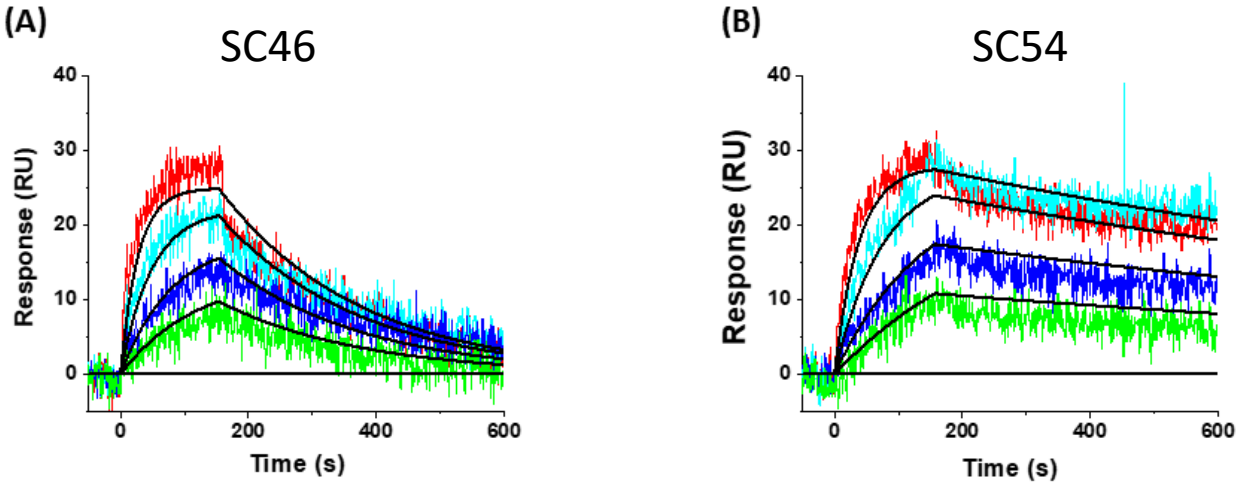


SC54
(HIV-1_{B41} IC_{50} = 0.41±0.061 nM)



SC Derivatives Retain Binding to HIV-1 B41 Env SOSIP Trimers

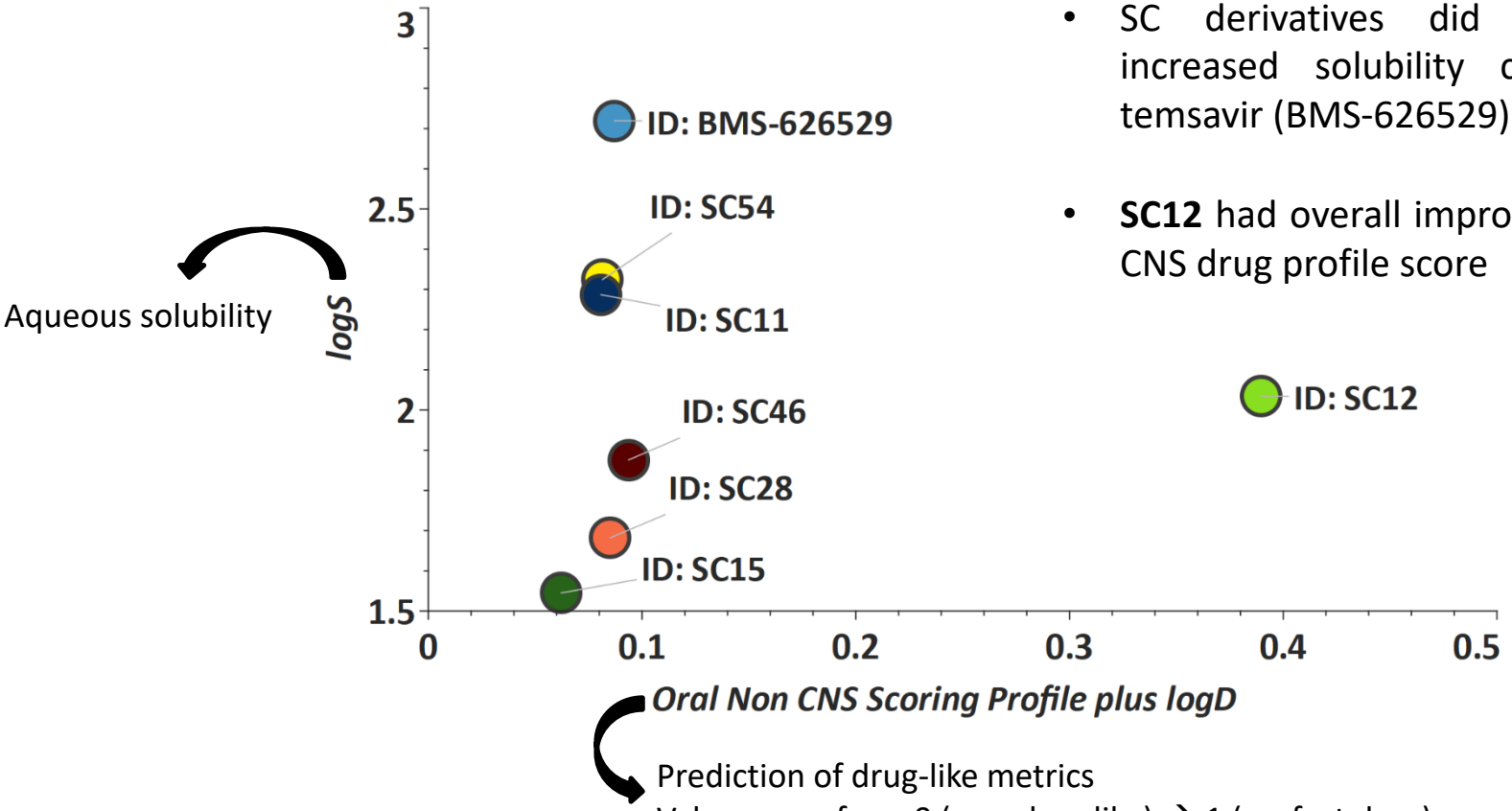
- Surface Plasmon Resonance (SPR) experiments show SC compounds bind to immobilized Env mimic (SOSIP)



- k_a = association rate
- k_d = dissociation rate
- K_D = binding affinity

Compound	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D(μM)
SC11	$3.83 \pm 1.12 \times 10^{3}$	$5.02 \pm 2.67 \times 10^{-4}$	0.131
SC12	$1.4 \pm 0.2 \times 10^{3}$	$1.2 \pm 0.05 \times 10^{-3}$	0.0901
SC15	$3.01 \pm 0.18 \times 10^{5}$	$5.44 \pm 0.67 \times 10^{-3}$	0.0181
SC28	$1.39 \pm 0.14 \times 10^{4}$	$6.99 \pm 0.43 \times 10^{-3}$	0.511
SC46	$3.22 \pm 0.203 \times 10^{3}$	$3.64 \pm 0.59 \times 10^{-4}$	1.13
SC54	$4.06 \pm 0.47 \times 10^{3}$	$1.07 \pm 0.15 \times 10^{-3}$	0.264
BMS-626529	$3.89 \pm 0.1 \times 10^{4}$	$5.9 \pm 1.16 \times 10^{-4}$	0.0152

*In silico P*rediction of Drug-like Metrics

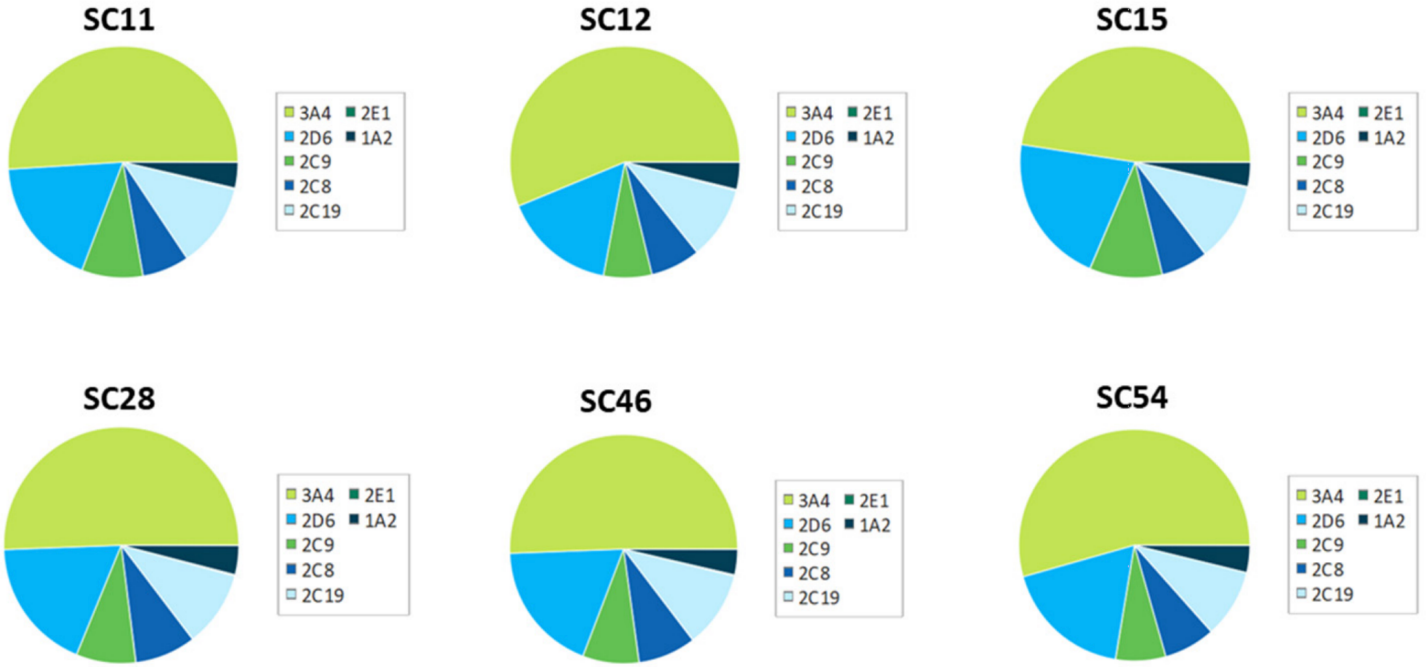


- SC derivatives did not exhibit increased solubility compared to temsavir (BMS-626529)
- **SC12** had overall improved oral non-CNS drug profile score

StarDrop 6.6 (Optibrium, Ltd., Cambridge, UK)

Computational Investigation of Metabolic Stability

- Orally administered drugs may be adversely metabolized before adequate plasma concentrations are reached
- Computational investigation of metabolic stability of SC compounds using P450 module (StarDrop 6.6 (Optibrium, Ltd., Cambridge, UK))
- SC compounds are predicted to be primarily metabolized by P450 isoform CYP3A4 (lime green)



Prediction of Metabolic Lability of SC Compounds Against CYP3A4

- CSL score reflects overall efficiency of compound metabolism by CYP34A
- Observed minimal differences of CSL scores and labile sites on SC compounds compared to BMS-626529
- This analysis assumes compounds bind to CYP3A4 with similar binding affinities
  - Predicted binding affinities were determined using HYDE (hydrogen bond and dehydration) energy scoring functions (SeeSAR 9.2)
- **SC28** and **SC46** have **greater predicted metabolic stability** compared to the other SC derivatives
- **SC12** has the **lowest predicted metabolic stability**

Compound	CSL 3A4	Labile Sites	Predicted 3A4 Affinity (M)
SC11	0.9514	3	0.004849324
SC12	0.9627	4	0.083703353
SC15	0.9564	3	0.003455543
SC28	0.9516	2	0.767515588
SC46	0.9474	3	0.166818977
SC54	0.9396	3	0.006416669
BMS-626529	0.9416	3	0.000802

Metabolic Stability Assays Using Human Liver Microsomes & Predictive Pharmacokinetic (PK) Parameters

Compound ID	k	T1/2 (min)	Clint (mL/min/mg)	Clapp (mL/min/kg)	Clh (mL/min/kg)	Eh (%)
Testosterone	0.05134	13.5	0.1027	99.005	16.639	83.19
Propranolol	0.01305	53.1	0.0261	25.170	11.145	55.72
Warfarin	0.00352	196.6	0.0070	6.797	5.073	25.37
SC11	0.07253	9.6	0.1451	139.887	17.498	87.49
SC12	0.105828	6.548348	0.211656436	204.0972779	18.21506087	91.0753
SC15	0.05268	13.2	0.1054	101.588	16.710	83.55
SC28	0.00479	144.6	0.0096	9.245	6.322	31.61
SC46	0.00760	91.2	0.0152	14.657	8.455	42.28
SC54	0.022234	31.16817	0.04446845	42.8802911	13.63870629	68.19353
BMS-626529	0.010075	68.78436	0.020149929	19.43028912	9.855514403	49.27757

- Testosterone → low stability; Propranolol → medium stability; Warfarin → high stability
- Half-lives (T½) of SC compounds exhibit a range of stabilities
- **SC28** and **SC46** have the **longest half-lives**
- **SC12** has the **shortest half-life**

Conclusions

- Successfully used bioisosteric replacement to redesign the piperazine core of BMS-626529
- Showed SC compounds retained binding to HIV-1 Env recombinant mimic via SPR analysis
- Computationally investigated and compared solubility, metabolic stability and metabolic lability of BMS-626529 with SC compound derivatives
- Concluded that replacement (and orientation) of the piperazine core influences metabolic stability
- Established a computational workflow for next-generation compounds that includes metabolic stability prediction in the design process

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