Peptidomimetic inhibitors of the Nef–Src family kinase interaction as adjuvants in an immune-directed HIV-1 cure

Antony Lurie[1], Corby Fink[1,2], Robert H.E. Hudson[3], Gregory A. Dekaban[1,2], Jimmy D. Dikeakos[1]

1. *Department of Microbiology and Immunology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada*
2. *Biotherapeutics Research Laboratory, Robarts Research Institute, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada*
3. *Department of Chemistry, University of Western Ontario, London, Ontario, Canada*

Background

- Currently, there is no practical cure for HIV-1 infection
- Cytotoxic T lymphocytes (CTLs) are critical for the function of immune-directed curative approaches, including therapeutic vaccination and shock and kill[1–2]
- The HIV-1 protein, Nef, enables HIV-1-infected cells to evade CTL killing, thereby compromising the activity of immune-directed cures[3–5]
- Nef facilitates CTL evasion by binding and activating Src family kinases (SFKs), which reduces expression of cell surface MHC-I, and in turn, inhibits viral antigen presentation (**Figure 1**)[3,6–8]
- Inhibitors of the Nef–SFK interaction may serve as adjuvants in an immune-directed HIV-1 cure by boosting anti-HIV-1 CTL killing

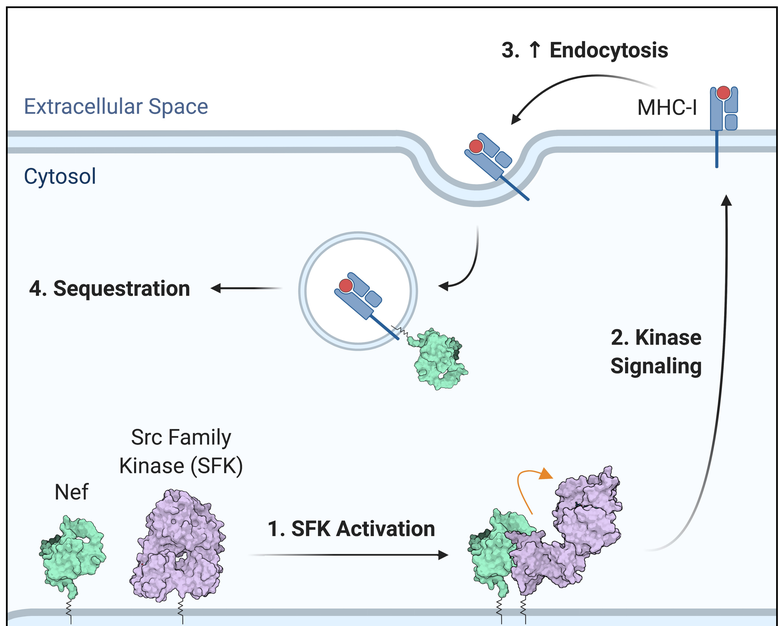

Figure 1. Mechanism for Nef-mediated MHC-I downregulation. Steps 1–2: Nef binds and activates a Src family kinase (SFK; Hck, Lyn or c-Src) to initiate an SFK-ZAP-70/Syk-PI3K signaling cascade. Step 3: Nef-mediated kinase signaling upregulates cell surface MHC-I endocytosis. Step 4: Nef binds and mistrafficks MHC-I for sequestration within the cell.

- Previously, our group identified a Nef–SFK inhibitory lead compound, termed **H3-1** (**Figure 2A**)
- Application of H3-1 in cell culture increases cell surface MHC-I levels in HIV-1-infected cells (see: Basic Sciences Oral Session #2, Corby Fink)
- **Our goal is to develop a panel of improved H3-1 analogs**

Design and Synthesis of H3-1 Analogues

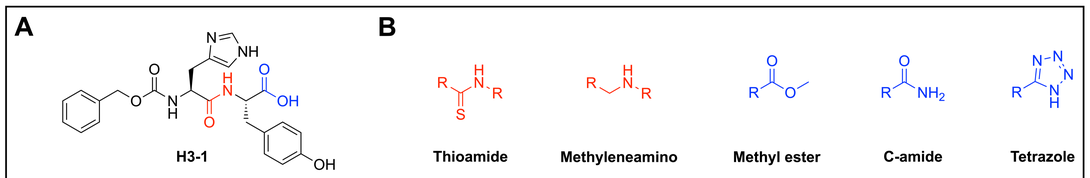

Figure 2. H3-1 and proposed replacing groups. **A)** Structure of H3-1 with the amide (*red*) and carboxyl (*blue*) groups highlighted. **B)** Proposed amide (*red*) and carboxyl (*blue*) replacing groups.

- To improve on H3-1, we used organic synthesis to generate a peptidomimetic panel of analogs incorporating substitutions of the amide (*red*) and carboxyl (*blue*) groups (**Figures 2-3**)

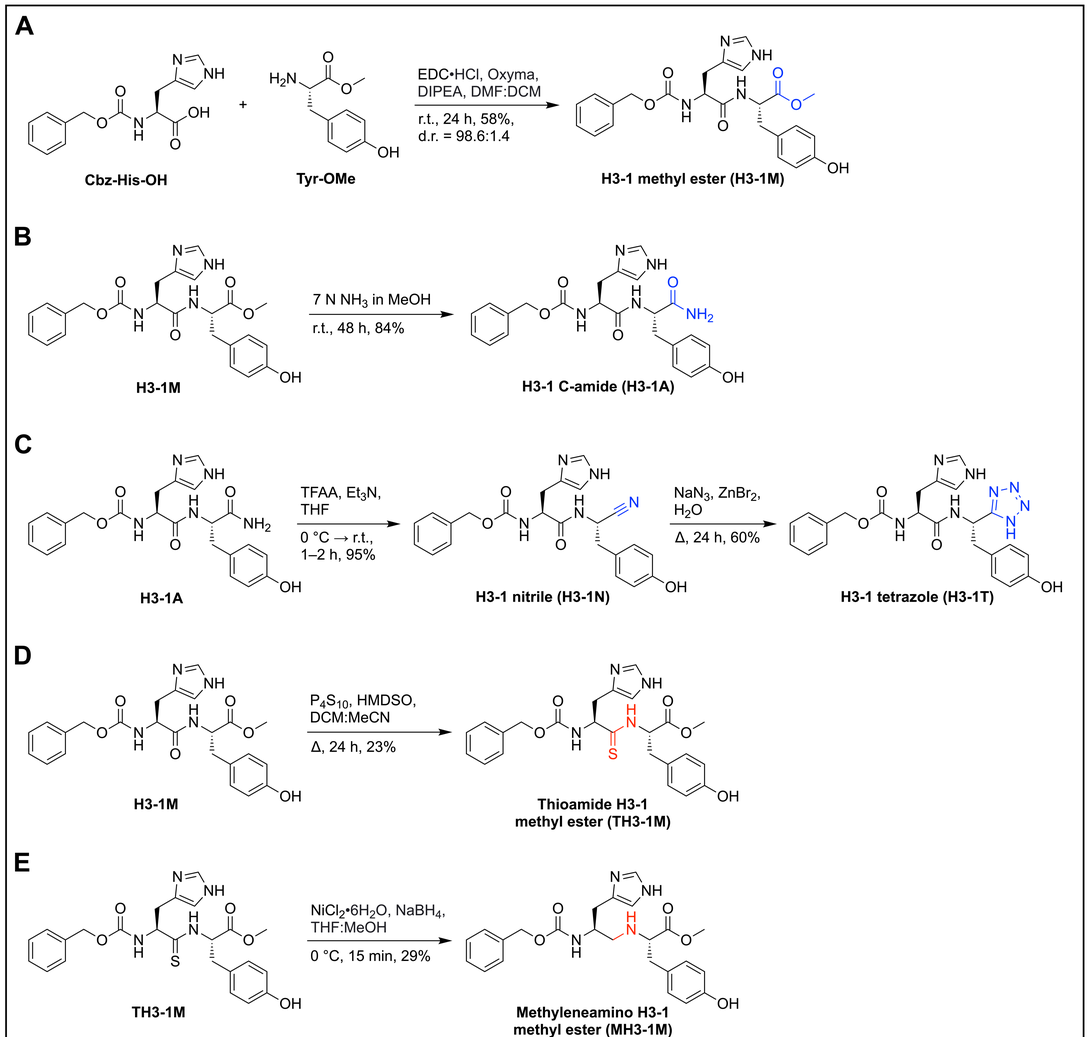

Figure 3. Syntheses of H3-1 analogs, with amide (*red*) and carboxyl (*blue*) replacing groups highlighted.

Conclusions

- Five peptidomimetic H3-1 analogs incorporating each proposed amide and carboxyl replacing group have been synthesized
- Upcoming studies will characterize each H3-1 analog on cytotoxicity, *in vivo* stability, and potency in Nef–SFK interaction inhibition and MHC-I rescue
- By identifying an improved H3-1 analog, we can test the utility of Nef–SFK inhibition in the context of an immune-directed HIV-1 cure

References

1. Kim Y, Anderson JL, Lewin SR. *Cell Host Microbe*. **2018**, *23*,14–26.

2. Collins DR, Gaiha GD, Walker BD. *Nat Rev Immunol*. **2020**, *20*, 471–482.

3. Collins KL, Chen BK, Kalams SA, *et al. Nature*. **1998**, *391*, 397-401.

4. Mujib S, Saiyed A, Fadel S, *et al. JCI Insight.* **2017**, *2*, e93684.

5. Duette G, Hiener B, Morgan H, *et al. J Clin Invest.* **2022**, *132*, e154422.

6. Schwartz O, Maréchal V, Le Gall S, *et al*. *Nat Med.* **1996**, *2*, 338–342.

7. Hung CH, Thomas L, Ruby CE, *et al. Cell Host Microbe*. **2007**, *1*, 121–133.

8. Dikeakos JD, Atkins KM, Thomas L, *et al. Mol Biol Cell.* **2010**, *21*, 3279–3292.

Acknowledgements

We thank all members of the Dikeakos and Hudson research groups for their assistance with experiments and syntheses, our collaborators, and our funding sources, the Canadian Institutes of Health Research (CIHR) and the University of Western Ontario (UWO).