Logo 1

Chemicals Targeting an HIV-1 Nef/Host Cell Kinase Complex as Novel Anti-Retroviral Compounds

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Abstract

HIV-1 has accessory proteins that are essential participants in the progression of AIDS. Nef has been identified to be an essential protein and therefore has become a target (1,2). Nef (Figure 1) forms a complex with its host cell binding partner, the Src family kinase Hck. Nef activates Hck through a mechanism that involves displacement of the SH3 domain from a negative regulatory interaction with the catalytic domain (3,4). Nef is known to influence signaling molecules, such as protein kinases (5-7). These characteristics that Nef acquire enhance viral replication as well as survival of infected cells (8-10). A high-throughput screening assay identified two classes of inhibitors of this protein-protein interaction. One class of inhibitors of HIV activity and Nef:Hck interaction was diphenylfuropyrimidines, the other 2-arylsulfonamido-3-arylaminequinoxalines. Remarkably, these agents block Nef-dependent HIV replication and show no cytotoxicity. These studies show a new and valid approach towards development of anti-HIV agents. We used organic synthesis as a means of constructing our compound, followed by purification with column chromatography, and nuclear magnetic resonance (NMR) spectroscopy and liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) to characterize its structure. Testing of the chemical will be performed in hopes of revealing its anti-HIV activity.

Method

In this project I successfully synthesised N^2,N^3--bis(4-chlorophenyl)quinoxaline-2,3-diamine. I used organic synthesis as rhw means of constructing the compound. Beginning with 2,3-dihaloquinoxaline as a starting material, palladium was the catalyst of choice for the addition of the arylamine moiety. After successfully preparing the compound, I purified the chemical. Liberation of the chemical from any contaminating substance(s) was achieved through column chromatography. To ensure that the correct chemical had been synthesized, the compound was then examined using nuclear magnetic resonance (NMR) spectroscopy and liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS).

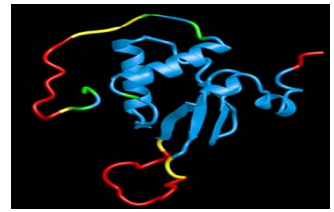


Figure 1. HIV-1 Nef Protein. (Grzesiek, S. et al., 1996)

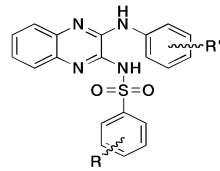


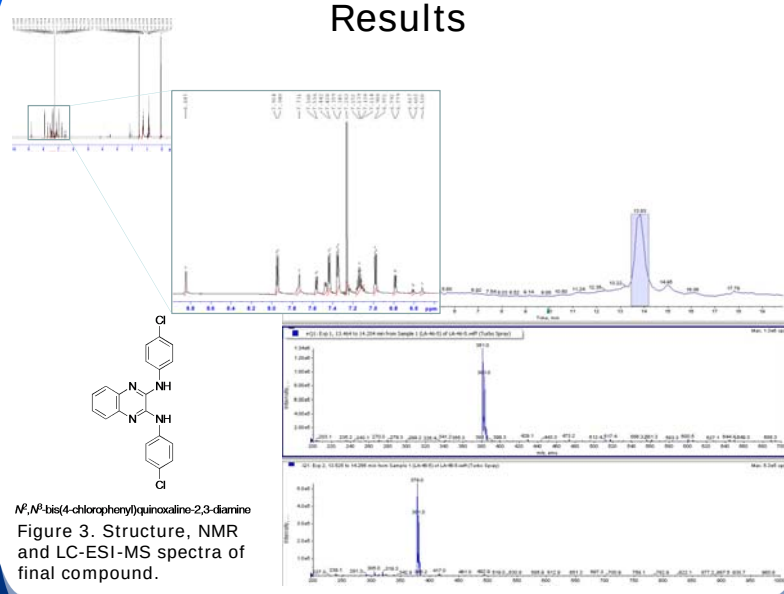
Figure 2. 2-Sulfonamido-3-arylaminequinoxaline scaffold

2-sulfonamido-3-arylaminequinoxalines

Introduction

Human immunodeficiency virus (HIV) can be acquired through different mechanism such as breast feeding, sexual intercourse, needles, blood to blood contact with open wounds, or in utero. The HIV virus attacks and conquers the immune system, subsequently resulting in AIDS. The epidemic of AIDS has resulted in many anti-retroviral drugs to manage the deadly disease. The accessory proteins that HIV-1 encompasses are essential participants in the progression of AIDS. Although the actual pathway/function has not been totally elucidated for Nef, it is known to influence several classes of signaling molecules, including immune receptors, trafficking proteins, guanine nucleotide exchange factors and protein kinases (3-5). After conducting a high-throughput screening assay, two classes of inhibitors of this protein-protein interaction were identified. One class of inhibitors of HIV activity and Nef:Hck interaction was diphenylfuropyrimidines, the other 2-arylsulfonamido-3-arylaminequinoxalines. Recent anti-retroviral drugs target enzymes such as integrase, protease, and reverse transcriptase; however, HIV mutates and many different strains exist. This new approach of inhibiting the Nef:Hck protein-protein interaction appears to be effective and may accelerate the discovery of new anti-HIV agents. Structurally similar analogs illustrate similar actions of inhibiting the replication of Nef-dependent anti-retroviral activities, therefore showing that the two scaffolds may be valuable in the investigation of HIV Nef function.

Results



N^2,N^3-bis(4-chlorophenyl)quinoxaline-2,3-diamine

Figure 3. Structure, NMR and LC-ESI-MS spectra of final compound.

Conclusion

Through a high-throughput screening test, two classes of inhibitors were found to inhibit the interaction between accessory protein Nef and its host cell binding partner, the Src family Kinase Hck. One class of inhibitor of Nef:Hck interaction was diphenylfuropyrimidines and 2-arylsulfonamido-3-arylaminequinoxalines. These substructures inhibit HIV replication, suggesting that structurally similar analogues might give comparable or even better inhibitory activity. Although a sulfonamido-containing final compound was not successfully synthesized, studies show that N^2,N^3–bis(4-chlorophenyl)quinoxaline-2,3-diamine might still be a valuable chemical in inhibiting HIV replication. NMR and mass spectrometry supported the structure assigned to the product. N^2,N^3 –bis(4-chlorophenyl)quinoxaline-2,3-diamine was isolated and crystallized and will be tested for inhibitory activity on Nef-dependent anti-retroviral activities. If this scaffold continues to provide activity against the Nef:Hck protein-protein interaction, then other Src family kinases, such as Fyn, Lyn, Lck, and c-Src, will become new targets in inhibiting HIV replication. Because HIV mutates, this new approach could possibly be a more efficient way of treating HIV.

Acknowledgements

❖UMES MARC U* STAR Program
❖Dr. Joseph Okoh MARC/MBRS Director
❖Dr. Kelly Mack MARC Co- Director
❖Dr. Jennifer Hearne Interim Co-Director
❖Dr. Billy Day
❖Dr. Vasilly Korotchenko
❖BBSI
❖The project described was supported by Award Number T34GM008411 from the National Institute of General Medical Sciences. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of General Medical Sciences or the National Institutes of Health
❖The national BBSI program (http://bbsi.eeicom.com) is a joint initiative of the NIH-NIBIB and NSF-EEC, and the BBSI @ Pitt is supported by the National Science Foundation under Grant EEC-0234002.

References

1. Fackler, O.T. & Baur, A. S. (2002) Immunity. **16**, 493-497.
2. Joseph, A. M., Kumar, M., & Mitra, D. (2005) Curr. HIV. Res. **3**, 87-94.
3. Moarefi, I., LaFevre-Bernt, M., Sicheri, F., Huse, M., Lee, C.-H., Kuriyan, J., & Miller, W. T. (1997) Nature **385**, 650-653.
4. Briggs, S. D., Sharkey, M., Stevenson, M., & Smithgall, T. E. (1997) J. Biol. Chem. **272**, 17899-17902.
5. Herna, R. G. & Saksela, K (2000) Front Biosci. **5**, D268-D283.
6. Arold, S. T. & Baur, A. S. (2001) Trends Biochem. Sci. **26**, 356-363.
7. Geyer, M., Fackler, O.T., & Peterlin, B. M. (2001) EMBO Rep. **2**, 580-585.
8. Briggs, S.D., Scholts, B., Jacqu, J. M., Swingler, S., Stevenson, M., & Smithgal, T. E. (2001) J. Biol. Chem. **276**, 25605-25611.
9. Choi, H.-J. Smithgall, T.E. (2004) J. Biol. Chem. **279,** 51668-51696.
10. Saksela, K., Cheng, G., & Baltimore, D. (1995) EMBO J. **14,** 484-491.