Bioorganic & Medicinal Chemistry Letters 26 (2016) 824–828

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Identification of a small molecule HIV-1 inhibitor that targets the capsid hexamer

Jimmy P. Xu [a], Jeffrey D. Branson [a], Rae Lawrence [b], Simon Cocklin [a,*]

[a] Department of Biochemistry & Molecular Biology, Drexel University College of Medicine, Rooms 10302-10306, 245 North 15th Street, Philadelphia, PA 19102, USA
[b] Cresset, New Cambridge House, Bassingbourn Road, Litlington, Cambridgeshire SG8 0SS, UK

ARTICLE INFO

Article history:
Received 16 October 2015
Revised 22 December 2015
Accepted 24 December 2015
Available online 25 December 2015

Keywords:
Computer-aided drug design
Field-based virtual screening
HIV-1 capsid protein
Antiviral
Surface plasmon resonance

ABSTRACT

The HIV-1 CA protein is an attractive therapeutic target for the development of new antivirals. An interprotomer pocket within the hexamer configuration of the CA, which is a binding site for key host dependency factors, is an especially appealing region for small molecule targeting. Using a field-based pharmacophore derived from an inhibitor known to interact with this region, coupled to biochemical and biological assessment, we have identified a new compound that inhibits HIV-1 infection and that targets the assembled CA hexamer.

Combination anti-HIV therapy, commonly referred to as highly active antiretroviral therapy (HAART), has led to a dramatic reduction in mortality and morbidity in patients infected with HIV. More than 25 antiretroviral drugs are currently available to treat HIV infection. These therapies are directed against the viral enzymes (reverse transcriptase, viral protease, and integrase). Drugs targeting the process of viral entry into the host cell have also been approved for clinical use. Despite these successes, current therapies for HIV-1 are limited by both the development of multidrug-resistant virus and by significant cumulative drug toxicities. Therefore, the development of new classes of antiretroviral agents with novel modes of action is highly desirable and is a driving force for the pursuit of small-molecule inhibitors of other viral targets. The HIV-1 capsid (CA) protein has recently emerged as an attractive target as it performs essential roles, both regulatory and structural.

The HIV-1 CA performs essential roles both early and late in the life cycle of HIV. The capsid is initially translated as the central region of the Gag polyprotein. As the virus buds, Gag is processed by the viral protease to produce three discrete new proteins—MA, CA, and nucleocapsid (NC)—as well as several smaller spacer peptides. After the capsid has been liberated by proteolytic processing, it rearranges into the conical core structure that surrounds the viral genome at the center of the mature virus.

The HIV-1 capsid shell is composed of about 250 CA hexamers and 12 CA pentamers, comprising about 1500 monomeric CA proteins in all. The multimers interact noncovalently to form the shell's curved surface. CA itself is composed of two domains: the N-terminal domain (CA_{NTD}) and the C-terminal domain (CA_{CTD}). Several structures of the CA have been solved;[1–7] with the most informative being those of disulfide engineered hexameric structure[8,9] and the very recent native hexameric structure.[10]

To date, the CA protein structure and stability have been demonstrated to be critical for the processes of uncoating, reverse transcription, nuclear entry, selection of the sites of integration, and assembly. Moreover, it is also important for cloaking the DNA product from intracellular immune surveillance. To achieve these functions, HIV-1 CA interacts not only with itself but with host factors including TRIM5α, cleavage and polyadenylation specific factor 6 (CPSF6), nucleoporins 153 and 358 (NUP153, NUP358), MxB, and cyclophilin A (CypA).

Several chemotypes have been found by several groups, including our own that interact with distinct pockets on the CA protein, each inhibiting by different mechanisms.[11–23] The most widely studied of these compounds, PF-3450074 (abbreviated to **PF-74**),[24] targets a pocket that is also the binding site for the host cell factors NUP153 and CPSF6.[25] Initial structural studies indicated interactions solely with the monomer[24] but recent investigations have shown that **PF-74** makes contacts between two adjacent protomers within the hexamer.[10,25,26]

* Corresponding author. Tel.: +1 215 762 7234 (office), +1 215 762 4979 (lab); fax: +1 215 762 4452.
E-mail address: scocklin@drexelmed.edu (S. Cocklin).

http://dx.doi.org/10.1016/j.bmcl.2015.12.087
0960-894X/

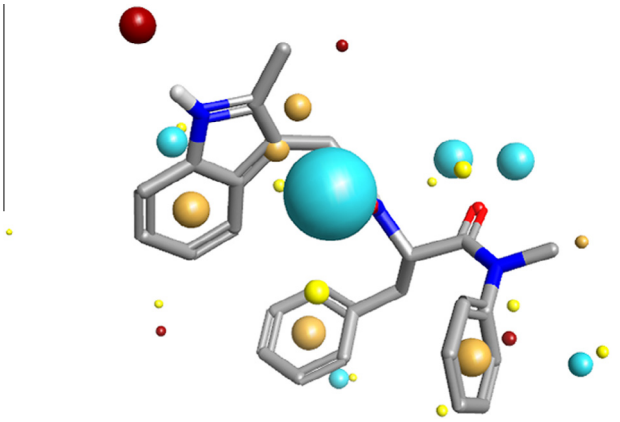

Figure 1. Calculated field point pattern of bioactive conformation of compound **PF74** extracted from PDB 2XDE and used for the in silico screen. Blue field points indicate areas where the negative electrostatics are at local maxima; red field points correspond to areas where positive electrostatics are at local maxima. Yellow field points (electrostatically neutral) represent areas where there is high accessible van der Waals surface area; and orange field points represent hydrophobic centroids (areas of high carbon density, centers of rings, near halogens). Oxygen atoms are shown in red, nitrogen in blue. The size of the field points scales with the value of the field at that point in space.

We believe that this inter-protomer pocket, which also serves as an interaction site for host cell factors, is the most attractive site on HIV-1 CA for small molecule targeting. The identification of **PF-74**, with its submicromolar affinity to this region, supports this assertion. However, **PF-74** itself is a poor drug-like chemotype, having a very peptidic nature. Therefore, in this study we sought to identify new compounds that interact in this region, inhibit HIV-1 replication (preferentially at an early post-entry stage) but are less peptide-like and more chemically tractable. Therefore, as a first step toward finding new chemical entities that function as inhibitors of the HIV-1 capsid protein, we employed field-based 3D similarity searching using Blaze (Cresset, UK) and a high-content pharmacophore[27] based on the crystal structure of **PF-74** bound to the CA-NTD (PDB ID 2XDE; Fig. 1). We have previously used this field-based pharmacophore screening approach successfully to identify new HIV-1 inhibitors.[28]

The field point pattern[29] from the conformation of **PF-74** in 2XDE was used to query a database of the 100 lowest-energy conformations of approximately 6 million commercially available compounds (600 million comparisons) using Blaze (Cresset, UK).[28,30] 1000 top scoring (50% field/50% shape) results from the Blaze field/shape-based virtual screening procedure[28,30] were compared on score and visually to the field point pattern of **PF-74**. Fifty-four compounds were chosen from this list and purchased for biological testing using the single-round infection assay.[28] The results of this analysis are shown in Figure 2.

As can be seen in Figure 2, a number of compounds were identified that demonstrated modest activity. Therefore, those compounds with activity were then screened for a direct interaction with variants of the CA protein, specifically the CA NTD, monomeric CA, and hexameric CA, using surface plasmon resonance (SPR) as previously reported.[18,23] From this analysis compound **52** (designated **H22**) was demonstrated to interact solely with the hexameric CA (Fig. 3A). The affinity of **H22** derived from this analysis was 382 ± 105 μM, which seemed to us to be larger than what we predicted from the single concentration analysis. Therefore, we determined the affinity of **PF74** using SPR and under the same conditions, and derived an affinity of 2.53 ± 1.02 μM. This is approximately 10-fold higher than the solution affinity obtained and published by Price et al.[25] and Bhattacharya et al.[26] using isothermal titration calorimetry. This may indicate that the inter-protomer pocket to which **PF74** binds, and that we believe **H22** binds to also, maybe slightly deformed due to covalent attachment to the sensor chip. Applying the 10-fold discrepancy to the K_D of **H22** would yield an equilibrium dissociation constant of 38.2 μM which more accurately reflects the level of inhibition seen in the single concentration antiviral screen.

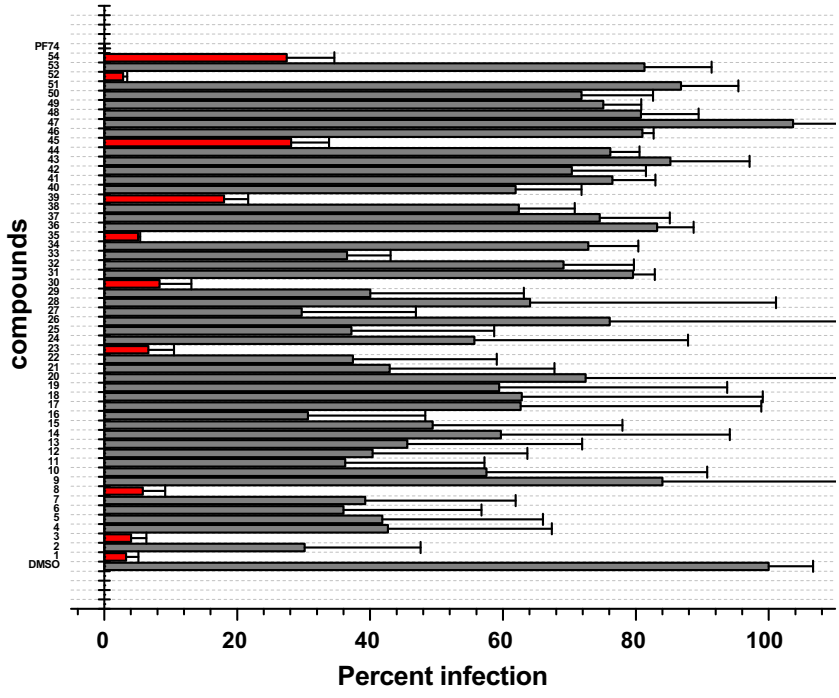

Figure 2. Single concentration (100 μM) screen of compounds identified from the Blaze procedure for activity against the infection of HIV-1. Red highlighted compounds denote those compounds that were chosen for target interaction analysis by SPR.