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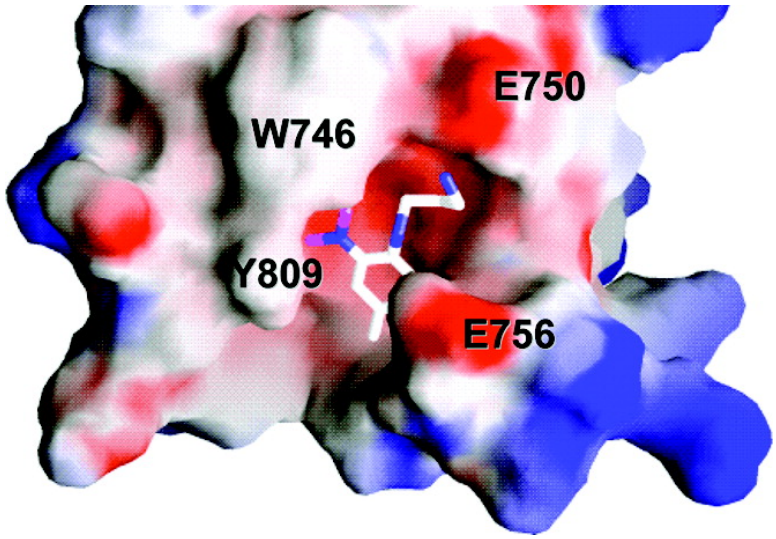

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Selective Small Molecules Blocking HIV-1 Tat and Coactivator PCAF Association

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The replication cycle of the human immunodeficiency virus (HIV) presents several viable targets for anti-HIV chemotherapy. The current anti-HIV drugs specifically target the viral reverse transcriptase, protease and integrase.[1] However, because of the development of viral drug resistance from mutations in the targeted proteins, continuous viral production by chronically infected cells contributes to HIV-mediated immune dysfunction,[2] and there is still no cure for AIDS. A rapid growing AIDS epidemic calls for new therapeutic strategies targeting different steps in the viral life cycle. Therapeutic intervention at the stage of HIV gene expression can complement the existing therapy to interfere with virus production. Transcription of the integrated HIV provirus is regulated by the concerted action between cellular transcription factors and a unique viral *trans*-activator Tat. Tat binds to a viral RNA TAR and recruits cyclin T1 and cyclin-dependent kinase 9 that hyperphosphorylates and enhances elongation efficiency of the RNA polymerase II.[3] Tat transactivation requires acetylation of its lysine 50 and recruitment of histone lysine acetyltransferase transcriptional co-activators for remodeling the nucleosome that contains the integrated proviral DNA.[4] Our recent study shows that Tat coactivator recruitment requires its acetylated lysine 50 (AcK50) binding to the bromodomain (BRD) of the coactivator PCAF,[5a] and microinjection of anti-PCAF BRD antibody blocks Tat transactivation.[5b] These data suggest that Tat/PCAF recruitment via a BRD-AcK binding is essential for HIV transcription, and this interaction serves as a new therapeutic target for intervening HIV replication.

To validate this possible new anti-HIV therapeutic target, we aim to develop small-molecule inhibitors that block Tat/PCAF binding by targeting the BRD of PCAF. Targeting a host cell protein essential for viral reproduction, rather than a viral protein, may minimize the problem of drug resistance due to mutations of the viral counterpart as observed with protease inhibitors. Here we report the development of a novel class of N1-aryl-propane-1,3-diamine compounds using a structure-based approach that binds the PCAF BRD selectively over other structurally similar BRDs.

The bromodomain, present in chromatin-associated proteins and histone lysine acetyltransferases,[6a] is an acetyl-lysine binding domain.[6b] Bromodomain/AcK binding plays an important role in control of chromatin remodeling and gene transcription.[6c] BRDs adopt the highly conserved structural fold of a left-handed four-helix bundle (αZ, αA, αB, and αC), as first shown in the PCAF BRD [6b] (Figure 1A). The ZA and BC loops at one end of the bundle form a hydrophobic pocket for AcK binding. The structure of the PCAF BRD bound to a Tat-AcK50 peptide[5a] shows that AcK50 interacts with protein residues V752, Y802 and Y809, Y47(AcK-3) with V763, and R53(AcK+3) and Q54(AcK+4) with E756,

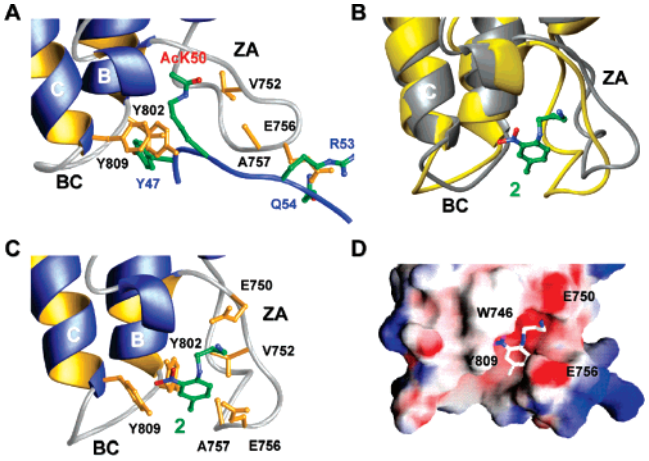

Figure 1. Structural basis of ligand recognition of the PCAF BRD. (A) Tat-AcK50 peptide recognition by the PCAF BRD. (B) Superimposition of the BRD structure in free (gray) and bound to the compound **2** (yellow). (C) Structure of the BRD/**2** complex, showing the compound **2** binding site. (D) GRASP view of the compound **2** binding pocket.

conferring a specific intermolecular association. The structures of CBP BRD/p53-AcK382 and GCN5p BRD/H4−AcK16 complexes[7] show that the residues in BRDs important for AcK recognition are largely conserved, whereas sequence variations in the ZA and BC loops enable discrimination of different binding targets. Notably, as compared to the other parts of the protein, the ZA and BC loops contain significant sequence variations with amino acid deletion or insertion, supporting the notion that different sets of residues in the ZA and/or BC loops dictate BRD ligand specificity by interacting with residues flanking the acetyl-lysine in a target protein.[6c]

To develop selective small-molecule inhibitors for blocking Tat/PCAF association, we conducted NMR-based chemical screening for the BRD by monitoring ligand-induced protein signal changes in 2D ^{15}N HSQC spectra.[8] We placed an emphasis on identifying compounds that bind selectively the BRD near but not just the AcK binding pocket, as the former may be more selective for this BRD. From screening of a few thousands of small-molecules from commercial libraries, we discovered several compounds including **1** that meet this criterion. Compound **1** binds the PCAF BRD with an affinity comparable to that of the Tat-AcK50 peptide[7a] (see below). Importantly, these compounds do not bind the structurally similar BRDs from CBP and TIF1β at millimolar concentration.

We next synthesized a series of **1** analogues to probe the structure−activity relationship (SAR) (Table 1). We assessed their binding to the PCAF BRD by measuring an IC_{50} in an ELISA assay, in which a compound competes against a biotinylated Tat-AcK50 peptide for binding to the GST-fusion BRD immobilized to

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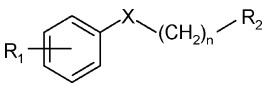
Table 1. Structure−Activity Relationship Data for Derivatives of **1**



cmpd	R_1	X	n	R_2	IC_{50} (μM)
1	2-NO_2	NH	3	$-NH_3^+$	5.1 ± 0.1
2	2-NO_2, 4-CH_3	NH	3	$-NH_3^+$	1.6 ± 0.1
3	2-NO_2, 4-CH_2-CH_3	NH	3	$-NH_3^+$	7.2 ± 0.1
4	2-NO_2, 3-CH_3	NH	3	$-NH_3^+$	5.9 ± 0.1
5	2-NO_2, 5-CH_3	NH	3	$-NH_3^+$	10.8 ± 0.1
6	2-NO_2, 4-Ph	NH	3	$-NH_3^+$	>10,000
7	2-NO_2, 4-CN	NH	3	$-NH_3^+$	34.9 ± 0.1
8	2-NO_2, 5-CN	NH	3	$-NH_3^+$	63.4 ± 0.6
9	2-CH_3, 5-NO_2	NH	3	$-NH_3^+$	77.8 ± 0.4
10	2-COO^-	NH	3	$-NH_3^+$	>10,000
11	2-$COOCH_3$	NH	3	$-NH_3^+$	>10,000
12	2-NO_2	O	3	$-NH_3^+$	125.6 ± 0.6
13	2-NO_2, 4-CH_3	O	3	$-NH_3^+$	180.0 ± 0.5
14	2-NO_2, 4-CH_3O	O	3	$-NH_3^+$	102.7 ± 0.4
15	2-NO_2, 4-Cl	O	3	$-NH_3^+$	215.1 ± 0.6
16	2-NO_2, 5-CH_3	O	3	$-NH_3^+$	203.6 ± 0.6
17	2-NO_2, 3-CH_3	O	3	$-NH_3^+$	164.8 ± 0.8
18	2-NO_2	CH_2	3	$-NH_3^+$	>10,000
19	2-NO_2	NH	4	$-NH_3^+$	145.9 ± 0.7
20	4-NO_2	NH	2	$-NH_3^+$	>2,000
21	4-NO_2	NH	4	$-NH_3^+$	>10,000
22	3-NH_2, 4-NO_2	NH	3	$-COO^-$	>2,000
23	2-NO_2, 4-Cl	NH	2	$-(OH)CH_3$	>10,000
24	2-Cl, 4-NO_2	NH	2	$-(OH)CH_3$	>10,000

glutathione-coated 96-well microtiter plate. The SAR study reveals salient features of BRD recognition of **1**. *First*, the BRD prefers a 4-methyl group on the aniline ring, which improves IC_{50} by 3-fold to 1.6 μM (**2** vs **1**). While substitution of a 4-ethyl, 3- or 5-methyl group on the aniline ring slightly weakens the binding (**3**−**5** vs. **1**), addition of a 4-phenyl group nearly abolishes the binding (**6** vs **1**). Adding a 4- or 5-cyano group weakens the binding by ∼7−12-fold (**7** and **8** vs **1**). *Second*, a 2-nitro group on the aniline ring is vital for the binding. Swapping of 2-nitro and 5-methyl causes a 7-fold reduction in binding (**9** vs **5**). Surprisingly, substitution of 2-nitro with 2-caroxylate or 2-caroxyl ester abrogates the binding (**10** and **11** vs **1**). *Third*, the functional importance of the 2-nitro is further supported by the effects of changing the NH to an O linkage in the aniline, which severely compromises the binding to the PCAF BRD (**12**−**17** vs **1**−**5**). Moreover, changing to a carbon linkage eliminates the binding (**18 vs 1**). *Fourth*, the BRD prefers an amino three-carbon aliphatic chain in **1**—a four-carbon chain reduces the binding by 30-fold (**19** vs **1**) and a two-carbon chain nearly loses the binding (**20** vs **1**). Alteration of **1** by two key elements, i.e. changing to a four-carbon chain and 4-nitro, abolishes the binding (**21** vs **1**). *Finally*, the terminal amine group is also an important functional moiety for the BRD binding (**22**−**24** vs **1**).

To understand ligand selectivity of the PCAF BRD, we solved the 3D structures of the protein bound to **1** and **2**. The two ligands are bound in the protein structure in nearly the same manner. For clarity, only the **2**-bound structure is reported here, which is similar to the free structure except for the ZA and BC loops that move closer to each other by clamping onto the ligand (Figure 1B). **2** is engulfed by residues in the ZA and BC loops outside the AcK binding pocket, blocking BRD binding to AcK of a target protein (Figure 1C). The 2-nitro group of **2** possibly forms a hydrogen bond with the phenolic −OH of Y809 and/or Y802, and the terminal $-NH_3^+$ interacts eletrostatically with the side-chain carboxylate of E750. The functional importance of the 2-nitro and the aniline NH likely results from a possible six-member ring structure formed between these two groups. However, it is not clear why substitution of 2-nitro with 2-carboxylate abrogates the BRD binding (**10** vs **1**). The aromatic ring of **2** is sandwiched between the side chains of Y802 and A757 on one side and Y809 and E756 on the other side, and the propane carbon chain is surrounded by the hydrophobic portions of the side chains of P747, E756, and V752. Finally, the 4-methyl of **2** fills a small hydrophobic cavity formed by side chains of A757, Y802, and Y809 (Figure 1D), contributing to a 3-fold increase over **1** in binding to the PCAF BRD. Notably, out of the ligand-interacting residues, only Y802 is among the conserved residues at the AcK binding site in BRDs, thus explaining the selective binding by this class of compounds to the PCAF BRD over the structurally similar CBP and TIF1β BRDs.

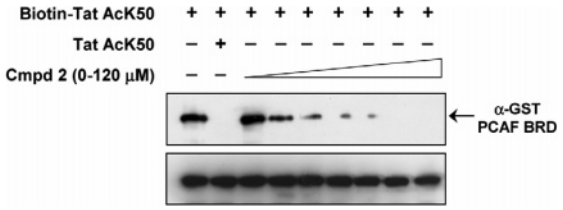


Figure 2. Inhibition of PCAF BRD/Tat-AcK50 binding by **2**. In this assay, **2** inhibits the biotinylated Tat AcK50 peptide immobilized on streptavidin−agarose by binding to the GST-PCAF BRD, as assessed by anti-GST Western blot. Lower panel indicates an equal amount of BRD used in each assay.

In summary, we have developed a class of novel small molecules that can effectively inhibit the PCAF BRD/Tat-AcK50 association in vitro by selectively binding to the BRD (Figure 2). The detailed SAR understanding of the lead compounds **1** and **2** will facilitate our efforts to optimize their affinity and selectivity by branching out to interact with the neighboring AcK binding pocket by the tethering techniques.[9] Such small-molecule inhibitors will help validate the novel anti-HIV/AIDS therapeutic strategy by targeting a cellular protein to block HIV transcription and replication.

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Supporting Information Available: Full experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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