Discovery of a PCAF Bromodomain Chemical Probe

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Abstract: The p300/CBP-associated factor (PCAF) and related GCN5 bromodomain-containing lysine acetyltransferases are members of subfamily I of the bromodomain phylogenetic tree. Iterative cycles of rational inhibitor design and biophysical characterization led to the discovery of the triazolophthalazine-based L-45 (dubbed L-Moses) as the first potent, selective, and cell-active PCAF bromodomain (Brd) inhibitor. Synthesis from readily available (1R,2S)-(−)-nor-ephedrine furnished L-45 in enantiopure form. L-45 was shown to disrupt PCAF-Brd histone H3.3 interaction in cells using a nanoBRET assay, and a co-crystal structure of L-45 with the homologous Brd P6GCN5 from Plasmodium falciparum rationalizes the high selectivity for PCAF and GCN5 bromodomains. Compound L-45 shows no observable cytotoxicity in peripheral blood mononuclear cells (PBMC), good cell-permeability, and metabolic stability in human and mouse liver microsomes, supporting its potential for in vivo use.

Bromodomains proteins (Brd) bind to acetylated lysines (KAc) through the Brd acetyllysine-binding site. Misregulation of these proteins is linked to the onset and progression of multiple disease states, such as cancer. Significant efforts have been made recently to interrogate the role of these targets through the development of chemical probes and inhibitors. Considerable work has focused on the BET family (Brd sub-family I), however non-BET Brds are increasingly receiving the attention of small molecule intervention efforts, with the disclosure of more than 10 new chemical probes/inhibitors in 2016.

The p300/CBP-associated factor, PCAF (KAT2B), is a multi-domain protein containing a single Brd, an N-terminal domain, and a histone acetyltransferase (HAT) domain. Known to associate with CBP[1] and p300[2] during transcription, misregulation of PCAF has been linked to cancer,[3] HIV infection,[4,5] and neuroinflammation.[6,7] Despite predictions of high druggability[8] and links with inflammatory disease,[9] the role of PCAF and, more specifically, contributions of the Brd in such disease states are poorly understood. The development of a small molecule modulator of PCAF Brd would provide a useful tool for interrogating this potential therapeutic target and allow for dissociation of the roles of the Brd and enzymatic domains in disease. Initial reports of PCAF Brd inhibitors were focused on disrupting interactions between the HIV-1 peptide TAT-1 and PCAF Brd.[6b,9] Wang et al. reported the first PCAF Brd inhibitor, compound 1 (PCAF IC50 1.6 μM, Figure 1), which was...
effective at disrupting HIV-1 replication (EC50 2.8 μM).[6] Further efforts made by Hu et al.[12] towards more potent compounds such as 2 were described without significant increases in potency or indication of selectivity (PCAF IC50 0.93 μM, EC50 11.5 μM, Figure 1). Additional chemotypes have been disclosed from fragment-based screening by Chaikuad et al.[8] Concurrent to this work, Constellation/Genentech reported compound 3[13] and others, which are potent PCAF inhibitors (AlphaLISA IC50 13 nM) but lack reported selectivity over other Brds (Figure 1).[7a] Despite recent developments of PCAF Brd inhibitors, a potent, selective, and cell-active chemical probe has not been reported. The work herein describes the discovery of such a probe.

Our first line of inquiry towards the first PCAF Brd chemical probe was focused on the core of non-selective Brd inhibitors, bromosporine[14] (PCAF isothermal titration calorimetry (ITC) Kd: 5 μM) and [1,2,4]triazolo[4,3-a]phthalazine[15] derivatives as starting points. Small amine substituents, as in compounds 7–9 (Table 1), were designed to extend out of the narrow PCAF pocket and target glutamic acid residues E750 and E756 at the edge of the KAc-binding pocket through amine–acid salt bridge interactions (PDB: 5FE0).[5] Commercially available 1,4-dichlorophthalazine 4 underwent a scalable (up to 20 g) tandem SAr/condensation reaction to furnish corresponding triazole intermediate 5 in good yields (Scheme 1). Significant efforts were employed to screen conditions using Pd-catalyzed couplings of 5 with various amine nucleophiles; disappointing yields or lack of reactivity were observed in all of these cases. It was found that a KI/HCl-catalyzed SAr reaction allowed for a tractable divergent synthesis of various N-linked derivatives (Scheme 1).

After the synthesis of a focused set of 20 compounds, screening conducted using a differential scanning fluorimetry (DSF) assay revealed two hits, dimethylamino compounds 7 and 10 (Table 1). It was found that compounds 8 and 9 featuring a longer amine chain were less potent. With the 2-(dimethylamino)ethyl group of compounds 7 and 10 identified as optimal substituents, a virtual library of ~12k compounds was constructed by in silico reaction of compound 5 with commercial compounds containing the 2-(dimethylamino)ethyl motif.[10] Over 60 compounds bearing a tethered 1,2-diamine motif were chosen for synthesis based on docking score, diversity, and potential for new interactions with the PCAF Brd (Table 1, compounds 11–16 and Tables S1 and S2).

Derivatives were screened for PCAF Brd affinity by ITC, leading to the discovery of compound 11 (Table 1). By ITC, the stoichiometry of binding showed that all of the activity of the racemate lay in a single enantiomer, later found to have (S)-configuration after synthesis using enantiopure building blocks (11 ITC Kd 0.30 μM, Brd/11 2:1; (S)-11 Kd 0.28 μM, Brd/(S)-11 1:1). Groups larger than a methyl substituent at R2 were detrimental to activity (compounds 12, 13) as was a bulkier N,N-diethyl substituent (compound 14). Although a phenyl substituent at R2 conferred potency to compound 10, compounds 15 and 16 with smaller methyl and ethyl groups were less potent. Compound (S)-17 featuring a trifluoromethyl group at position R1 caused a loss in activity consistent with previously reported Brd SAR of the [1,2,4]triazolo[4,3-a]phthalazines.[15]

In a DSF panel of 48 human Brds, compound (S)-11 showed binding to PCAF and GCN5 with no observable activity against other Brds (Figure S1). To improve the potency of (S)-11, it was rationalized that a combination of appropriate substituents at R1/R2 might improve the avidity of binding interactions and addition of an aryl group at R1 would
serve as a chemical handle for introduction of new functionality. The R²/R³-substituted compounds would be a hybird of the most potent analogues (S)-10 and (S)-11. Synthesis of aryly substituted compounds was achieved through a non-selective aza-Henry reaction with p-substituted benzaldehydes (Scheme 2). p-Substituted benzenediazonium salts were chosen as provisional in silico scoring of potential inhibitors suggested that α- or m-substitutions would be less tolerated for binding. Highly unstable olefins 18–24 were telescoped through a diastereoselective (d.r. 4:6:1–33:1) nitro–olefin conjugate addition furnishing racemic (S*,S*)-configured compounds 25–31, then reduced to correspondingly configured amines, 32–38, using either Pd/C- or Raney/Ni-catalyzed hydrogenation. Compounds 32–38 were isolated as single diastereomers and submitted to the aforementioned KI-catalyzed SNAP-Ar reaction (Scheme 2) to produce compounds 39–45 in low to good yields (16–79%). Racemic compounds were screened by ITC for PCAF-binding affinity (Table 2). All of the compounds showed an increase in potency compared to compound (S)-11, with the simple unsubstituted derivative 45 having highest potency.

Pleasingly, it was found following resolution by preparative chiral stationary phase HPLC, that active enantiomer L-45, which was dubbed L-Moses, showed good binding affinity for PCAF Brd (PCAF Kᵢ 126 nm, ITC). The other enantiomer D-45 showed no observable binding, implying its utility as an inactive control compound. Having achieved good potency against PCAF Brd, L-45 was then screened for selectivity against the panel of 48 human bromodomains using DSF (Figure 2B). Homologous Brd of GCN5 was the only other Brd that showed any affinity for L-45, confirmed by ITC.

\( A_{\text{eff}} = 3.6^\circ \text{C}, K_{\text{D}} 0.55 \mu \text{M} \). L-45 competitively displaced a biotinylated tool derivative, compound 46 (Supporting Information) in a homogeneous time-resolved resonance fluorescence (HTRF) assay (PCAF K, 47 nm), corresponding to exquisite selectivity over BRD4 (> 4500-fold selective).

In a cellular context, L-45 was shown to displace nuclease-enzyme-tagged PCAF-Brd from halo-tagged-H3.3 in live HEK-293 cells using the nucleaseBRET assay. [a] clogD was calculated using ChemAxon. [b] Ligand efficiency.

![Figure 2](image-url)  
**Figure 2.** A) Profile of L-45. B) L-45 is selective in a DSF assay panel of 48 Brds (black text). C) Displacement of PCAF-Brd from H3.3-nanoLuc in live HEK-293 cells using the nucleaseBRET assay. [a] clogD was calculated using ChemAxon. [b] Ligand efficiency.

![Scheme 2](image-url)  
**Scheme 2.** Synthesis of threo-substituted derivatives 39–45. Reagents and conditions: a) NH₄OAc (0.2 equiv), EtNO₂, reflux, 1:1 E/Z, quant.; b) Me₂NH (5 equiv), THF, RT, 16 h, d. 4:6:1–33:1; c) H₂ (1 atm), Pd/C (10%), MeOH, RT, 16 h, 11–15% over two steps, single diastereomer; d) H₂ (1 atm), Ra/Na (0.3 equiv), MeOH, RT, 16 h, 25–28%, over two steps, single diastereomer; e) 5 (0.8 equiv) KI (0.1 equiv), HCl (0.05 equiv), ETOH or iPrOH, reflux, 3 days 16–79%.

**Table 2:** PCAF Brd-binding affinity of compounds 39–45 measured by ITC.

| Compound | R   | Configuration | Kᵢ (nm) (ITC) |
|----------|-----|---------------|---------------|
| 39       | F   | (15°, 25°)    | 195 ± 23      |
| 40       | CO₂Me | (15°, 25°)    | 133 ± 15      |
| 41       | Me  | (15°, 25°)    | 160 ± 54      |
| 42       | Cl  | (15°, 25°)    | 223 ± 78      |
| 43       | CF₃ | (15°, 25°)    | 163 ± 117     |
| 44       | OMe | (15°, 25°)    | 179 ± 48      |
| 45       | H   | (15°, 25°)    | 168 ± 27      |
| L-45/L-Moses | H | (15°, 25°) | 126 ± 15     |
| D-45     | H   | (1R, 2R)      | Inactive      |
ligand (ITC $K_a$ 280 nm), was successfully obtained (PDB: 5TPX, Figure 3). $L$-45 bound as expected in the KAc-binding site of PfGCN5 with key interactions that include a salt bridge between E1389 (conserved in PCAF as E756) and the dimethylamino motif of $L$-45 (Figure 3A). Additional contacts are also observed in the form of an edge-to-face $\pi-\pi$ stacking interaction between W1379 (conserved in PCAF as W746) and the phenyl substituent of $L$-45 (average distance 4.5 Å); a $\pi-\pi$ stacking interaction between Y1442 (conserved in PCAF as Y809) and pyridazo ring of the triazolophthalazine motif (average distance 3.7 Å); and characteristic H-bonds from the triazolophthalazine group and N1436 residue (conserved in PCAF as N803) and a water molecule. Insolubility of substitution of $L$-45 in $R'$ and $S'$ positions (compounds 12–16, Table 1) was rationalized by the tight fit of the alkyl amine chain of $L$-45 (Figure 3B). Interestingly, K1383 in PfGCN5 is substituted with E750 in human PCAF, and as such the Plasmodium homologue features a slightly open KAc-binding site (Figure 3B). Targeting this difference may allow for design of Plasmodium-selective Brd inhibitors. As previously supported by SAR, the absolute configuration of $L$-45 was confirmed to be (1$S$,2$S$).

For the asymmetric synthesis of $L$-45, commercially available (1R,2S)-(−)-norephedrine was Boc-protected and cyclized to a sulfamidate and then directly oxidized using sodium periodate to boc-protected sulfamidate 46$^{[21]}$ extruding SO$_3$ and furnishing protected diamine 47 as a single diastereoisomer with inversion of configuration at the benzylic centre. Following a deprotection of 47 to the free amine and $S_\text{p}Ar$ with aryl chloride 5, $L$-45 was furnished in six steps as a single stereoisomer.

In conclusion, we report the discovery of $L$-45, the first nanomolar, selective, and cell-active chemical probe of the PCAF bromodomain. Iterative cycles of rational inhibitor design, in silico docking studies, and synthesis furnished $L$-45 after generation of a focused PCAF inhibitor library. $L$-45 shows a clean toxicity profile in primary PBMCs, and disrupts interactions between PCAF Brd and H3.3 in HEK293 cells, indicating cellular target engagement.

Good cell permeability in a MDCK-MDR1 assay and stability to metabolism in both human and mouse liver microsomes indicate that $L$-45, dubbed $L$-Moses, may also have utility in vivo. $L$-Moses will allow for robust interrogation of PCAF Brd inhibition and pharmacological effects in relevant diseases models. Future work will investigate the use of $L$-Moses in functional assays pertaining to PCAF-associated diseases.

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Conflict of interest

The authors declare no conflict of interest.

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[1] a) C. H. Arrowsmith, J. E. Audia, C. Austin, et al., Nat. Chem. Biol. 2015, 11, 536–541; b) C. H. Arrowsmith, C. Bountra, P. V. Fish, et al., Nat. Rev. Drug Discovery 2012, 11, 384–400; c) P.
Filippakopoulos, S. Knapp, *Nat. Rev. Drug Discovery* **2014**, *13*, 337–356.

[2] http://www.thesgc.org/chemical-probes.

[3] a) P. Filippakopoulos, J. Qi, S. Picaud, et al., *Nature* **2010**, *468*, 1067–1073; b) J. M. Garnier, P. P. Sharp, C. J. Burns, *Expert Opin. Ther. Pat.* **2014**, *24*, 185–199.

[4] a) N. H. Theodoulou, N. C. O. Tomkinson, R. K. Prinjha, et al., *ChemMedChem* **2016**, *11*, 477–487; b) M. Moustakim, P. G. K. Clark, D. A. Hay, et al., *Med. Chem. Commun.* **2016**, *7*, 2246–2264.

[5] a) B. S. Gerstenberger, J. D. Trzupek, C. Tallant, et al., *J. Med. Chem.* **2016**, *59*, 4800–4811; b) P. Bamborough, H. A. Barnett, I. Becher, et al., *ACS Med. Chem. Lett.* **2016**, *7*, 552–557; c) J. Bennett, O. Fedorov; C. Tallant, et al., *J. Med. Chem.* **2016**, *59*, 1642–1647; d) A. Unzue, M. Xu, J. Dong, et al., *J. Med. Chem.* **2016**, *59*, 1350–1356; e) C. L. Sutherell, C. Tallant, O. P. Monteiro, et al., *J. Med. Chem.* **2016**, *59*, 5095–5101; f) W. S. Palmer, G. Poncet-Montange, G. Liu, et al., *J. Med. Chem.* **2016**, *59*, 1440–1454; g) W. Palmer, P. Jones, G. Liu, et al., University of Texas System, USA, *2016*, p. 166; h) L. J. Martin, M. Koegl, G. Bader, et al., *J. Med. Chem.* **2016**, *59*, 4462–4475; i) T. D. Crawford, V. Tsai, E. M. Flynn, et al., *J. Med. Chem.* **2016**, *59*, 5391–5402; j) O. B. Cox, T. Krojer, P. Collins, et al., *Chem. Sci.* **2016**, *7*, 2322–2330; k) P. Chen, A. Chaikuad, P. Bamborough, et al., *J. Med. Chem.* **2016**, *59*, 1410–1424; l) A. Chaikuad, S. Lang, P. E. Brennan, et al., *J. Med. Chem.* **2016**, *59*, 1648–1653; m) P. Bamborough, C. W. Chung, E. H. Demont, et al., *Angew. Chem. Int. Ed.* **2016**, *55*, 11382–11386; *Angew. Chem.* **2016**, *128*, 11554–11558.

[6] a) A. J. Bannister, T. Kouzarides, *Nature* **1996**, *384*, 641–643; b) V. V. Ogryzko, R. L. Schiltz, V. Russanova, et al., *Cell* **1996**, *87*, 953–959.

[7] a) L. Krüdenier, K. Lee, D. F. Tough, et al., Glaxo Group Limited, UK, *2014*, p. 38; b) B. K. Albrecht, A. Cote, T. Crawford, et al., Genentech, Inc., USA, *Constellation Pharmaceuticals*, Inc., USA, *2016*, p. 179; c) B. K. Albrecht, A. Cote, T. Crawford, et al., Genentech, Inc., USA, *Constellation Pharmaceuticals*, Inc., USA, *2016*, p. 95.

[8] a) S. Mujtaba, Y. He, L. Zeng, et al., *Mol. Cell* **2002**, *9*, 575–586; b) V. C. Quy, S. Fantano, G. Rossetti, et al., *Biology* **2012**, *1*, 277–296; c) Q. Wang, R. Wang, B. Zhang, et al., *MedChemComm* **2013**, *4*, 737–740; d) A. Dorr, V. Kiernan, A. Pedal, et al., *EMBO J.* **2002**, *21*, 2715–2723.

[9] M.-M. Zhou, G. Gerona-Navarro, Y. Rodriguez-Fernandez, et al., *Jehan School of Medicine at Mount Sinai, USA, 2015*, p. 87.

[10] L. R. Vidler, N. Brown, S. Knapp, et al., *J. Med. Chem.* **2012**, *55*, 7346–7359.

[11] a) A. J. N. M. Bastiaansen, M. M. Ewing, H. C. de Boer, et al., *Arterioscler. Thromb. Vasc. Biol.* **2013**, *33*, 1902–1910; b) W.-G. Deng, Y. Zhu, K. K. Wu, *Blood* **2004**, *103*, 2135–2142.

[12] P. Hu, X. Wang, B. Zhang, et al., *ChemMedChem* **2014**, *9*, 928–931.

[13] B. K. Albrecht, D. J. Burdick, A. Cote, et al., Genentech, Inc., USA, *Constellation Pharmaceuticals*, Inc, USA, *2016*, p. 117.

[14] a) http://www.thesgc.org/chemical-probes/Bromosporine; b) S. Picaud, K. Leonards, J.-P. Lambert, et al., *Sci. Adv.* **2016**, *2*, e1600760.

[15] O. Fedorov, H. Lingard, C. Wells, et al., *J. Med. Chem.* **2014**, *57*, 462–476.

[16] M. A. C. Neves, M. Tostov, R. Abagyan, *J. Comput.-Aided Mol. Des.* **2012**, *26*, 675–686.

[17] P. L. Southwick, J. E. Anderson, *J. Am. Chem. Soc.* **1957**, *79*, 6222–6229.

[18] https://www.chemaxon.com/library/pka-and-logp-property-prediction-and-training/.

[19] A. L. Hopkins, C. R. Groom, A. Alex, *Drug Discovery Today* **2004**, *9*, 430–431.

[20] T. Machleidt, C. C. Woodroofe, M. K. Schwinn, et al., *ACS Chem. Biol.* **2015**, *10*, 1979–1984.

[21] R. E. Meléndez, W. D. Lubell, *Tetrahedron* **2003**, *59*, 2581–2616.

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