The “Gatekeeper” Residue Influences the Mode of Binding of Acetyl Indoles to Bromodomains

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Supporting Information

ABSTRACT: Small-molecule hits for the bromodomains of CREBBP and BAZ2B have been identified by scaffold hopping followed by docking of a set of ~200 compounds containing the acetyl indole scaffold. Chemical synthesis of nearly 30 derivatives has resulted in ligands of representatives of three subfamilies of human bromodomains with favorable ligand efficiency. The X-ray crystal structures of three different bromodomains (CREBBP, BAZ2B, and BRPF1b) in complex with acetyl indole derivatives reveal the influence of the gatekeeper residue on the orientation of small-molecule ligands in the acetyl lysine binding site.

INTRODUCTION

Acetylation of lysine residues is an important post-translational modification of histone proteins that contributes to the regulation of chromatin structure and transcription.1,2 Bromodomains are protein modules with four-helix bundle topology that specifically recognize (“read”) acetylated lysine residues, as well as butyryllysine and crotonyllysine,3 and are considered protein targets of interest for the development of chemical probes and clinical tools for the treatment of cancer, inflammation, and other diseases.4−8

The BET (bromodomain and extra terminal) subfamily (BRD2/3/4/T) has been widely addressed, and as a consequence, several potent and selective inhibitors have been developed, some of which are currently undergoing clinical trials for the treatment of NUT midline carcinoma (NMC), solid tumors, leukemia, lymphoma, hematological malignancies, atherosclerosis, and type II diabetes.7,9,10 In contrast, the specific function and potential pharmacological relevance of other bromodomains, including CREB binding protein (CREBBP), E1A binding protein p300 (EP300), BRD7/9, and bromodomain adjacent to zinc finger domain (BAZ2B), are much less understood, and thus, small-molecule inhibitors will be valuable tools for unraveling their biological roles.

The bromodomains of EP300 and CREBBP, which belong to the same subfamily and share 96% sequence identity,11 play important roles in DNA replication and repair, cell growth and cell cycle regulation, and genomic stability.12,13 As an example, EP300 and CREBBP are able to acetylate p53 on its K382 residue through the histone acetyl transferase (HAT) domain upon extracellular stress or DNA damage, and they are also known to specifically bind to acetylated p53 via their bromodomain module.14 As a consequence, changes in the p53-dependent activation of target genes result in cell cycle arrest, senescence, or apoptosis.15−17 On one hand, chromosome translocations resulting in gene fusions containing CREBBP or EP300 have been linked to leukemias and lymphomas.18,19 On the other hand, CREBBP and EP300 are mutated in solid tumors and B-cell lymphoma, suggesting they possess a tumor-suppressing role.13,20 Thus, because both oncogene or tumor suppressor roles have been reported for CREBBP and EP300,13,20 the development of chemical probes will be instrumental for the analysis of their biological function(s).

The bromodomains of BRD7 and BRD9 belong to the same subfamily and share 72% sequence identity.22 Both BRD7 and BRD9 are part of the SWI/SNF (SWItch/Sucrose Non-Fermentable) chromatin remodelling complex, which plays a key role in the regulation of gene expression.23−25 Recent reports have linked BRD9 to oncology, including non-small cell lung26 and cervical cancer.27 Its paralogue, BRD7, is frequently downregulated in cancer28−30 and is able to regulate the tumor suppressor protein p53.31−33 The bromodomain of BRPF1b (bromodomain-PHD finger protein 1b) belongs to the same subfamily as BRD7 and BRD9. Despite the function of the BRPF1b bromodomain not being yet fully understood, the availability of BRPF1b ligands might help in the elucidation of its role.34−36

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BAZ2B is another bromodomain-containing protein whose role in physiology and disease is not clear. Biophysical screening of a library of 1300 fragments resulted in the identification of 10 small molecules that bind in the micromolar range to BAZ2B.37 Recently, the first submicromolar selective CREBBP,11,38,39 BAZ2B,40,41 and BRD7/922,42,43 inhibitors have been reported.

Here we present the result of a combined scaffold hopping and docking approach that has permitted the discovery of acetyl indoles as ligands of the bromodomains of CREBBP, BAZ2B, BRPF1b, and/or BRD9, which belong to three different subfamilies that lie outside of the BET bromodomain subfamily. A comparative analysis of four crystal structures of bromodomain/acyetyl indole complexes shows the importance of the so-called gatekeeper residue with respect to the binding mode of the ligand.

## RESULTS AND DISCUSSION

### In Silico Screening by Scaffold Hopping and Docking.

In the past few years, our groups have successfully identified several low micromolar to nanomolar kinase44–47 and bromodomain39,48,49 inhibitors by high-throughput virtual screening campaigns. In this work, we decided to dock a small subset of compounds containing a moiety identified by scaffold hopping (see Experimental Section). First, the ZINC all-now library was decomposed into approximately 600000 fragments retaining key functional groups. These fragments were queried by the indolizine fragment A, which is present in the potent BAZ2B ligand GSK2801 (1a)41 and more recently has been identified in BRD7 and BRD9 ligands.22 (Capital letters are used to label generic chemical blueprints. 1a–i correspond to commercially available compounds. 1–50 represent the synthetic intermediates and self-made products.)

The acetyl indole B was identified as the top-ranking fragment with an activity-oriented fingerprint similarity of 0.975 with respect to A. The high degree of similarity is due to almost identical geometry and connecting vectors in fragments A and B (Figure 1).

![Figure 1: GSK2801 (1a), a nanomolar chemical probe for BAZ2A and BAZ2B bromodomain.](image)

In a second step, we retrieved ~200 commercially available compounds containing fragment B. As in our previous fragment-based virtual screening approach,46,48,49 the retrieved compounds were docked into crystal structures of the targets, namely, the bromodomains of CREBBP [Protein Data Bank (PDB) entry 4A9K] and BAZ2B (PDB entry 3Q2F). An in-house-developed program for automatic docking was used.48,50–52 The docking poses were subsequently rescored by a transferable scoring function (see Experimental Section).45,50–54 Finally, 14 molecules were selected for experimental validation by means of a competition binding assay.55,56 At a concentration of 50 μM, seven compounds showed significant competition [i.e., a percentage of residual binding of CREBBP to the acetylated histone peptide of <70% with respect to the DMSO control (Figure 2)], which corresponds to a hit rate of 50% for the in silico screening approach based on scaffold hopping and docking. The most active compound, 1b, exhibits an equilibrium dissociation constant (Kd) of 20 μM for CREBBP. Using the same threshold of 70% as for CREBBP, the hit rate for BAZ2B was 29% (Figure 2).

Interestingly, at a concentration of 50 μM, only compound 1g shows a significantly higher affinity for BAZ2B than for CREBBP.

#### Binding Mode of Compound 1b: Validation by X-ray Protein Crystallography.

The opposite selectivity toward CREBBP and BAZ2B observed for compounds 1b and 1g (Figure 2) prompted us to study their binding mode. While we could not determine the structure with compound 1g, the crystal structure of CREBBP in complex with compound 1b was determined at 2.0 Å resolution (Figure 3, green, PDB entry 4T8S), which revealed an overall binding mode essentially identical to the docked pose of compound 1b (Figure 3, blue).

The binding of compound 1b in CREBBP is characterized by a lipophilic sandwich of its bicyclic core between residues Phe1111, Val1174, and Ala1164 on one side and Val1115, Leu1120, and Ile1122 on the other side of the binding pocket. The carbonyl oxygen of the acetyl indole acts as the acetylated lysine mimic and is engaged in hydrogen bonding interactions with the side chains of the conserved Asn1168 (BC loop) and Tyr1125 (ZA loop), where the latter is bridged by a water molecule. Another four water molecules present at the bottom of the pocket are conserved. In addition, there is a water molecule bridging the dihydro-pyrazole ring and the guanidinium group of Arg1173 (Figure 3).

#### Assessing the Affinity Difference between Indolizine and Indole Ligands.

Compound 1a is reported to be a potent ligand for the BAZ2B bromodomain with a Kd of 136 nM.42 To study the influence on the binding affinity of the position of the nitrogen atom in our hit compound 1b, we decided to synthesize the indole analogue of indolizine 1a. Commercially available 1H-indol-5-ol (1) was transformed into compound 3 via alkylation of the phenol moiety with 1-iodopropane, followed by introduction of an N-benzenesulfonyl group and bromination at C3 in the presence of molecular bromine (Scheme 1). Bromo indole 3 was then coupled to 2-(methylsulfonyl)phenyl]boronic acid, affording compound 4 in moderate yield. Removal of the sulfonyl group under basic conditions preceded the incorporation of the acetyl group to give the indole analogue of compound 1a (5).

Interestingly, a 24-fold reduction in binding affinity toward BAZ2B was observed for acetyl indole 5 with respect to 1a [IC50 values of 8.55 and 0.36 μM, respectively, determined by AlphaScreen (see the Supporting Information)], which indicates that the position of the nitrogen atom in the double-ring system is crucial.

#### Synthesis.

We decided to focus our derivatization campaign on compounds 1b and 1g as ligands. Compound 1b was selected because of its ligand efficiency for CREBBP (0.34 kcal/mol per heavy atom) and the availability of the crystal structure (Figure 3), while compound 1g was chosen because of its selectivity toward BAZ2B (Figure 2).
The synthesis of CREBBP hit 1b is shown in Scheme 2. The carboxylic acid of commercially available 2-(1H-indol-3-yl)acetic acid (6) was transformed into the corresponding methyl ester. Acetylation of the N atom afforded indole 7. Deprotonation of 7 in the presence of in situ-generated lithium diisopropylamide (LDA) followed by reaction with acetic anhydride or 4-methoxyphenylacetic anhydride delivered intermediate 8 or 9, respectively. Cyclization in the presence of hydrizine hydrate afforded hit compound 1b and derivative 10 in moderate yields (Scheme 2).

Hit compound 1b was then reacted with a variety of acid and sulfonyl chlorides, affording disubstituted (11−18) as well as monosubstituted dihydro-pyrazole derivatives (19−22). Two more derivatives were prepared: upon condensation of 1,2-dibromoethane with compound 1b in the presence of K2CO3, derivative 23 was obtained. The reaction of 1b with p-methoxybenzyl bromide in the presence of NaH afforded O-alkylated product 24 (Scheme 3).
Finally, we decided to incorporate the propoxy group at position 5 of the indole, for which a similar protocol was applied to \(1H\)-indol-5-ol (1), delivering the final products 27 and 28 (Scheme 4).

**Synthesis of Derivatives of Compound 1g.** The common intermediate for the synthesis of derivatives of ligand 1g was obtained from the commercially available non-natural amino acid 29 in four steps with a 20% overall yield (Scheme 5). Condensation of intermediate 32 with diverse commercially available anilines afforded acetyl indole intermediates 33–38, which were then acylated in the presence of diverse acid chlorides, affording compounds 39–46. Four additional derivatives were prepared by cleaving the methoxy substituent in the presence of BBr3 to produce phenol derivatives 47–50 (Scheme 5).

**Biophysical Characterization. Compound 1b and Its Derivatives.** Compound 1b was screened against a panel of 10 different bromodomains using a thermal shift assay (Table 1 and Table S2 in the Supporting Information). This initial screening revealed BRD9 bromodomain as a potential off-target for compound 1b with a shift in the melting temperature of 1.3 °C, which translated into a \(K_d\) of 5.3 μM, as measured by a competition binding assay,\(^{55,56}\) and a ligand efficiency (LE) value of 0.38 kcal/mol per heavy atom. This result is in line with the recent work of Brennan and co-workers, who

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**Scheme 2**

\[\begin{align*}
6 & \xrightarrow{a} 7 \\
& \xrightarrow{b} 8: R = \text{Me} \\
& \xrightarrow{c} 10: R = \text{Rac}
\end{align*}\]

**Reagents and reaction conditions:** (a) (i) MeOH, H₂SO₄, 25 °C, 1 h, 94%; (ii) Ac₂O, DMAP, Et₃N, THF, 25 °C, 17 h, 90%; (b) (iPr)₂NH, n-BuLi, THF, −75 °C, 1 h, then, acetic anhydride or 4-methoxyphenylacetic anhydride, −78 °C, 2–4 h; (c) hydrazine hydrate, camphoric acid, EtOH, toluene, 93 °C, 30 min–1 h, 29–51% over two steps. Abbreviation: DMAP, 4-dimethylaminopyridine.

**Scheme 3**

\[\begin{align*}
23 & \xrightarrow{b} 11-22 \\
& \xrightarrow{c} 24
\end{align*}\]

**Reagents and reaction conditions:** (a) R-Cl, Et₃N, DCM, 0–25 °C, 5–12 h, 24–89%; (b) 1,2-dibromoethane, K₂CO₃, DMF, 80 °C, 7 h, 41%; (c) PMB-Br, NaH, TBAI, DMF, 0–25 °C, 1.5 h, 23%. Abbreviations: PMB, 4-methoxybenzyl ether; TBAI, tetrabutylammonium iodide.
developed BRD7 and BRD9 nanomolar potent indolizine derivatives starting from the BAZ2B inhibitor 1a. Importantly, no activity of compound 1b was observed for the tested BET family members, BRD4(1) and BRD4(2) (see the Supporting Information).

Because of the involvement of the acetyl group of compound 1b in hydrogen bonds with the conserved Asn1168 and Tyr1125 residues of CREBBP (Figure 3), we decided to maintain this moiety and introduce the main modifications at the dihydro-pyrazole ring to gain affinity for CREBBP.

On the outset, we decided to modulate the interactions of the solvent-exposed NH groups of the dihydro-pyrazole ring. To do so, mono- and disubstituted amide and sulfonamides that could establish new interactions with the surrounding amino acid residues, including the characteristic Leu1109 and Ile1122 and Leu1120 in CREBBP and Ala54 and Ile53 in BET family members, BRD4(1) and BRD4(2) (see the Supporting Information).

Reagents and reaction conditions: (a) (i) chloroacetic acid, K2CO3, H2O, 90 °C, 16 h; (ii) Ac2O, 25 °C, 30 min, then 37% HCl for 12 h, 33% over two steps; (b) Ac2O, Et3N, reflux, 30 min, 89%; (c) Na2SO3, reflux, 2 h, 83%; (d) method I: aniline, AcOH, reflux, 1–8 h, 26–56%; method II: aniline, pTSA, toluene, reflux, 2.5–4 h, 50–52%; (e) for MeCOCl, toluene, reflux, 12 h, 36–78%; if R4 = aryl, R4COCl, toluene, Et3N, DMAP, 100 °C, 1 h, 13–21%; (f) BBr3, DCM, 0–25 °C, 1–12 h, 47–84%. Abbreviation: pTSA, p-toluenesulfonic acid.

In an effort to form π-stacking interactions with Arg1173 in CREBBP,11,38 and residues Phe43 and Phe44 in BRD9, aromatic substituents, some of them electron-rich, were incorporated (compounds 14–18, 20, and 21). Compound 14 yielded the most active ligand toward BRD9 with a KD of 3.5 μM. Interestingly, the presence of a p-methoxybenzoate substituent in compound 20 resulted in a 2-fold improvement of binding affinity with a thermal shift of 3.3 °C in CREBBP and a KD of 9.3 μM. At the same time, compound 20 retained the activity toward BRD9 with a thermal shift of 5.1 °C and a KD of 6.3 μM. The presence of a morpholine ring at the same position (derivative 22) could only slightly improve the binding affinity for CREBBP with a KD of 12 μM.

A dihydro-pyrazolo-oxazole ring was installed (compound 23), which allowed us to revert the hydrogen bond donor capacity of the dihydro-pyrazole ring bearing two NH groups to a hydrogen bond acceptor-fused ring. Remarkably, compound 23 showed a KD of 6.2 μM in CREBBP and retained the LE of the initial hit (0.34 kcal/mol per heavy atom), which makes it an attractive lead for further optimization. On the other hand, compound 24 exhibited a drop in the thermal shift, probably due to steric clashes of the p-methoxybenzyl group.

We then aimed to establish new interactions with the hydrophobic residues located on top of the binding site, Ile1122 and Leu1120 in CREBBP and Ala54 and Ile53 in BRD9, by substitution of the indole moiety at position 5 with a propoxy group (Table 1, compounds 27 and 28), a modification that has proven to be successful in our previous work and in compound 1a. Unfortunately, the presence of the propoxy substituent retained an activity of ~10 μM.

Importantly, with the exception of compounds 14 and 23, all derivatives displayed ΔTm values of <0.6 °C for BRD4(1), one of the most promiscuous bromodomains.11,38,57

**Compound 1g and Its Derivatives.** Intrigued by the selectivity difference observed in the hit compound 1g toward BAZ2B (Figure 2), we decided to examine its binding mode more closely. As we could not obtain crystals of the complex of compound 1g with BAZ2B, several analogues were synthesized (Table 2), aiming not only to obtain a crystal structure of the complex but also to improve the binding affinity for BAZ2B. The chloroacetamide moiety was replaced by metabolically more stable amides in all derivatives to avoid covalent binding to the protein.
The affinity of the synthesized derivatives 39–50 for BAZ2B was assessed by an AlphaScreen competition binding assay at a compound concentration of 50 μM. Compound 47, bearing an o-methyl and m-hydroxy substituent at the benzene ring, showed a 54% reduction in the magnitude of the signal relative to the negative control DMSO in the AlphaScreen binding assay, which translated into a $K_D$ of 23 μM and a LE value of 0.27 kcal/mol per heavy atom. Upon substitution of the acetyl substituent at R4 with a m-CF3-benzoate group, an IC50 value of 27 μM in the AlphaScreen assay and a $K_D$ of 39 μM (BROMOscan) were measured for compound 50.

Crystal structures of the most potent derivatives, 47 and 50, in complex with BAZ2B were determined at 1.71 and 1.78 Å resolution, respectively. Compounds 47 and 50 (Figure 4A,B) have essentially identical binding modes in BAZ2B. As in the binding mode of hit 1b in CREBBP (Figure 3), the N-acetyl substituent of the indole moiety of compounds 47 and 50 is engaged in hydrogen bonds with the side chain of the conserved Tyr1901 and Asn1944, where the hydrogen bond with Tyr1901 is bridged by a water molecule. An additional hydrogen bond is formed between the carbonyl group of the acetamide and the backbone NH of Asn1894 in the ZA loop.

Table 1. Evaluation of Compounds 10–24, 27, and 28 Derived from 1b

<table>
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<tr>
<th>Cmpd</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>$\Delta T_m (^\circ C)$</th>
<th>$K_D (\mu M)$</th>
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<tr>
<td>1b</td>
<td>H</td>
<td>H</td>
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<td>0.3 (0.1)</td>
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</tr>
<tr>
<td>10</td>
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<td>H</td>
<td>H</td>
<td>Me</td>
<td>0.1 (0.1)</td>
<td>5.3</td>
</tr>
<tr>
<td>11</td>
<td>-SO2Me</td>
<td>-SO2Me</td>
<td>H</td>
<td>Me</td>
<td>2.2 (0.2)</td>
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<td>H</td>
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<td>13</td>
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<td>H</td>
<td>H</td>
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</tr>
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<td>H</td>
<td>H</td>
<td>Me</td>
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<td>Me</td>
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<tr>
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<td>6.2</td>
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<tr>
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<tr>
<td>27</td>
<td>H</td>
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<td>9.8</td>
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<tr>
<td>28</td>
<td>H</td>
<td>Opri</td>
<td>Me</td>
<td>0.4 (0.2)</td>
<td>&gt;50</td>
<td></td>
</tr>
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$^a$Median value of the shift in the melting temperature. The total number of measurements for each compound and bromodomain was between 7 and 24. Ligand and protein concentrations were 100 and 2 μM, respectively. Standard errors of the mean are given in parentheses. The similar thermal shift values measured with the CREBBP bromodomain and its paralogue, EP300, are consistent with the fact that identical residues are present in the acetyllysine binding site of both proteins. $^b$$K_D$ values were determined by a competition binding assay in duplicate. Dose–response data and fitting curves can be found in the Supporting Information. Dashes indicate data not acquired.
Table 2. Evaluation of Compounds 39−50 Derived from 1g

<table>
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<th>Cmpd</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>Kᵣ(M)(μM)</th>
<th>%Ctrl BAZ2B(μM)</th>
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<td>1g</td>
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<td>H</td>
<td>H</td>
<td>O</td>
<td>&gt;50</td>
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<td>39</td>
<td>H</td>
<td>CN</td>
<td>H</td>
<td>Acetyl</td>
<td>-</td>
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<td>41</td>
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<td>H</td>
<td>F</td>
<td>Acetyl</td>
<td>-</td>
<td>91.4</td>
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<td>Acetyl</td>
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<td>Acetyl</td>
<td>-</td>
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<tr>
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<td>Acetyl</td>
<td>-</td>
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<td>88.4</td>
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<td>Acetyl</td>
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<tr>
<td>50</td>
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<td>H</td>
<td>OH</td>
<td>Acetyl</td>
<td>39</td>
<td>29.8 (IC₅₀ = 27 μM)</td>
</tr>
</tbody>
</table>

¹Kᵣ values were determined by a competition binding assay in duplicate. Dose−response data and fitting curves are in the Supporting Information. ²Percentage of the measured signal (i.e., percentage binding of acetylated histone peptide to BAZ2B) relative to the negative control at a compound concentration of 50 μM. Lower values indicate stronger binding of the compounds. The AlphaScreen competition binding assay was performed at Reaction Biology. Dashes indicate data not acquired.

The o-methyl substituent of the phenol ring provides sufficient steric hindrance to block the conformation of compounds 47 and 50 with their hydroxyphenyl ring pointing toward Trp1887, which is the first residue of the WPF segment in BAZ2B. The additional trifluoromethylbenzoate of compound 50 points toward the solvent (Figure 4B), which explains the similar affinity for BAZ2B of compounds 47 and 50 (Table 2).

As mentioned above, the bromodomains of BRD9 and BRF1/3 belong to the same subfamily. Aiming to obtain further structural information, we took advantage of the availability in our laboratory of crystals of the apo state of the bromodomain of BRF1b that were soaked in a solution of compound 1b. In this way, we determined the crystal structure of the complex of compound 1b with BRF1b at a resolution of 1.35 Å (PDB entry 5D7X). Interestingly, the size of the so-called gatekeeper residue, small (Val1174 in CREBBP) and large (Phe714 in BRF1b), has a noticeable influence on the orientation of the indole moiety (Figure 4C). Furthermore, the presence of the bulkier Phe714 residue in the BRF1b bromodomain has a strong effect on the orientation of the dihydro-pyrazole ring that is rotated by ~180° in BRF1b with respect to the complex with CREBBP. Despite these structural differences, compound 1b has a very similar affinity for the bromodomains of CREBBP and BAZ2B with Kᵣ values of 20 and 15 μM, respectively (see the Supporting Information).

The superposition of the X-ray structures of compound 1b in CREBBP and BAZ2B with compound 47 in BAZ2B provides additional evidence of the influence of the gatekeeper residue for ligand binding (Figure 4, D). Interestingly, the 6-membered ring of the indole moiety of compound 47 is located between the indole of compound 1b in BRF1b and CREBBP because the gatekeeper residue in BAZ2B (Ile1950) is smaller than the one in BRF1b (Phe714) and larger than that in CREBBP (Val1174). It is important to note that in these three crystal structures (viz., the 1b/CREBBP, 1b/BRF1b, and 47/BAZ2B complexes) there are no crystal contacts in the binding site that could affect the orientation of the acetyl indole scaffold. Moreover, the structure of BAZ2B is essentially identical in the complex with compounds 47 and 50, and the same is observed for the structures of CREBBP in the complex with compound 1b and a previously reported acetyl benzene ligand (PDB entry 4TQN) that differ only by a small rigid-body displacement of the ZA loop. It is also interesting to compare with the orientation of the scaffold of the BAZ2B inhibitor 1a. The same progressive tilting of the indole emerges from the structural superposition of the complex of BAZ2B and the nanomolar inhibitor 1a (PDB entry 4RVR) with the crystal structures of the hit compound 1b in CREBBP and BRF1b (Figure 4E).

Overall, these structural data suggest that acetyl indole mimics the acetylated lysine side chain in its rather unspecific binding to bromodomains. In addition, the precise orientation of the indole double-ring system and the substituent at its position 3 are influenced by the size of the gatekeeper residue that is different in different bromodomains.

**CONCLUSIONS**

We have discovered in silico a series of small-molecule antagonists of the bromodomains of CREBBP and BAZ2B by docking ~200 compounds containing an acetyl indole moiety, which we had identified by scaffold hopping from a potent BAZ2B ligand. Considering that only 14 compounds were tested in vitro (by a competition binding assay), the hit rates of the in silico screening based on scaffold hopping and docking are 50 and 29% for CREBBP and BAZ2B, respectively. One of the original hits (compound 1b) has equilibrium dissociation constants of 20, 15, and 5.3 μM for CREBBP, BRF1b, and BRD9, respectively. With a relatively small derivatization campaign (i.e., ~30 derivatives), we were able to improve the affinity for CREBBP and BAZ2B and maintain the ligand efficiency (0.34 kcal/mol per heavy atom for both hit 1b and derivative 23 in CREBBP) or even slightly improve it (from <0.26 kcal/mol for hit 1g to 0.27 kcal/mol per heavy atom for compound 47 in BAZ2B). The crystal structures of three acetyl indole derivatives in complex with three different bromodomains confirm the binding mode predicted by docking with the acetyl oxygen of the ligand involved as a hydrogen bond acceptor in two hydrogen bonds with the conserved Asn side chain in the BC loop and a structural water that acts as bridge to the conserved Tyr of the ZA loop. Moreover, the X-ray structures show that the size of the gatekeeper side chain (Val1174, Ile1950, and Phe714 in the bromodomains of CREBBP, BAZ2B, and BRF1b, respectively) influences the orientation of the indole moiety. This structural information can be used to further improve the selectivity for a single bromodomain target or a small subset of bromodomains sharing the same gatekeeper residue.
Scaffold Hopping. We have used an activity-oriented fingerprint that consists of (1) chemical features such as hybridization states and different types of hydrogen bonding donors and acceptors, (2) the two-dimensional topology index as a way to reflect the spatial arrangement of such features, and (3) three-dimensional shape descriptors (Figure S1 of the Supporting Information). A similarity coefficient is computed between two fingerprints ranging from 0 to 1, with 1 being the highest degree of similarity, where the two molecules are not necessarily identical. As such, scaffold hopping resembles pharmacophore mapping but distinguishes itself by using a molecular fingerprint.

Docking and Scoring. The genetic algorithm-based program for flexible ligand docking has been described in previous applications. Approximately 20 docking poses for each compound were first minimized by the CHARMM program and subsequently ranked by a transferable scoring function.

Assays. Thermal shift measurements were taken as previously described. Thermal shift assays detect, by a fluorescent dye, the increase in the thermal stability of a protein in the presence of a ligand. BROMOscan technology is a competition experiment that uses an immobilized ligand and a DNA-tagged bromodomain protein. Compounds that bind to the bromodomain of interest will prevent binding of the bromodomain to the immobilized ligand. The amount of bromodomain captured on the solid support is then quantified by qPCR, and dissociation constants are calculated.
AlphaScreen assays consist of a donor bead that is able to transfer singlet oxygen to an acceptor bead that is in the proximity, and as a result, the acceptor bead emits a luminescent/fluorescent signal. In the presence of a bromodomain ligand, the donor/acceptor complex is disrupted, leading to a loss of singlet oxygen transfer and loss of the fluorescent signal. Further details about the assays can be found in the Supporting Information.

X-ray Crystallography. The His-tagged human bromodomains of CREBBP (residues 1081−1097), BRPF1b (residues 626−740), and BAZ2B (residues 1858−1972) were expressed in Escherichia coli. The purification procedures are reported in the Supporting Information. The inhibitors were soaked into apo crystals of the bromodomains of BRPF1b and BAZ2B, while the structure of the complex of compound 1b and CREBBP was obtained by coocrystallization as described in the Supporting Information. Data collection and refinement statistics are listed in Table S1.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01757.

General procedures for shaping hopping, synthesis and characterization, biophysical and biological evaluation of final compounds, and X-ray crystal structure refinement data (PDF)

Accession Codes

The PDB entries for CREBBP and BRPF1b in complex with the hit compound 1b are 4TS8 and SD7X, respectively. Coordinates and structure factors for the BAZ2B bromodomain in complex with compounds 47 and 50 have been deposited in the PDB as entries 5E73 and 5E74, respectively.

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Notes

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ABBREVIATIONS USED

BAZ2B, bromodomain adjacent to zinc finger domain 2B; BET, bromodomain and extra terminal domain; BRD2, -3, -4, -7, and -9, bromodomain containing 2, 3, 4, 7, and 9, respectively; BRD4(1), first/second bromodomain of BRD4; BRPF1b, bromodomain and PHD finger containing 1; CBP, CREB binding protein; CREBBP, CREB binding protein; DMAP, 4-dimethylaminopyridine; DME, 1,2-dimethoxyethane; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; EP300, E1A binding protein p300; HAT, histone acetyltransferase; LE, ligand efficiency; NMC, NUT midline carcinoma; NUT, nuclear protein in testis; PHD, plant homeodomain; PMB, 4-methoxybenzyl ether; SWI/SNF, switch/sucrose nonfermentable; TBAH, tetra-n-butylammonium bromide; TBAHS, tetrabutylammonium hydrogen sulfate; TBAI, tetra-n-butylammonium iodide

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