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Dual Inhibition of TAF1 and BET Bromodomains from the BI-2536 Kinase Inhibitor Scaffold.

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KEYWORDS: Bromodomain, Kinase Inhibitor, Epigenetics, BI-2536

ABSTRACT: Recent reports have highlighted the dual bromodomains of TAF1 (TAF1(1,2)) as synergistic with BET inhibition in cellular cancer models, engendering interest in TAF/BET polypharmacology. Here, we examine structure activity relationships within the BI-2536 PLK1 kinase inhibitor scaffold, previously reported to bind BRD4. We examine binding by this ligand to TAF1(2), and apply structure guided design strategies to discriminate binding to both the PLK1 kinase and BRD4(1) bromodomain while retaining activity on TAF1(2). Through this effort we discover potent dual inhibitors of TAF1(2)/BRD4(1), as well as biased derivatives showing marked TAF1 selectivity. We resolve X-ray crystallographic datasets to examine the mechanisms of the observed TAF1 selectivity, and to provide a resource for further development of this scaffold.

Featured in 46 human proteins, the bromodomain is the largest class of binding module, or "reader domain", for acetylated lysines (KAc), a pervasive modification in epigenetic control.[1] The development of JQ1 and related probes for the Bromodomain and Extra Terminal Domain (BET) subfamily first demonstrated the bromodomain's chemical tractability, competively occupying the hydrophobic KAc pocket, which is formed by a rim of loops between domain's common four-α-helical bundle structure. These probes have provided widely used tools for the study of BET function, in particular BRD4's role as a master transcriptional coactivator, as well as broad therapeutic investiations of BET inhibition in disease.[2-4] Subsequent clinical translations are currently underway.[5-7] Broadly ensuing efforts to extend bromodomain probe development have now succeeded in providing selective tools for nearly all existing subfamilies, enabling direct study of domain specific contributions to protein function.[8]

Several recent studies have reported small molecule ligands for the dual bromodomain containing TAF1.[9-13] As the largest of approximately 13 Tata-binding protein (TBP) associated factors "TAFs" that assemble to comprise the general transcription factor IID (TFIID),[14-15] TAF1 scaffolds the pioneer factor for general eukaryotic transcription.[16-18] In-vitro transcription and temperature sensitive mutant experiments have implied that TAF1 may be dispensable for basal transcription,[19] but directly mediates transcription downstream of RB,[20] B-Myb,[21] and C-Jun,[22] and is also critical to transcription downstream of key cell cycle drivers.[23-24] Despite these roles, no direct phenotypes of TAF1 bromodomain inhibition have been reported, suggesting that as recently observed for other non-BET bromodomain targets, existing probes may not dramatically impact protein function.[25-27] However, recent studies have suggested that TAF1 may collaborate with BRD4 to control cancer cell proliferation in a bromodomain dependent manner, potentially through cooperating transicriptional effects.[10] Indeed, two structurally dissimilar TAF1 inhibitors have now been independently demonstrated to potentiate the activity of BET inhibition in cultured cancer models.[10, 13] These findings have engendered interest in compounds that may offer dual BRD4/TAF1 inhibition. Toward this objective, we herein report structure activity relationships (SAR) toward dual TAF1/BRD4 inhibitors, as well as TAF1-biased derivatives, from a single kinase inhibitor derived scaffold.

In the course of our own studies on bromodomain ligand development, we reported the first small molecule ligand for TAF1, a mid-nanomolar binder accessed through biased multicomponent library synthesis.[9] However, further exploration of the compound's dimethylisoxazole warhead revealed a challenging BRD4 bias. Among other chemotypes previously described were several kinase inhibitor scaffolds,[28-29] examples of which have now seen in-depth study and development as polypharmacological agents.[30-31] In particular, previous work suggested that the PLK1 kinase inhibitor BI-2536 harbored activity on TAF1 through broad phage display (Ember, et al) and melt temperature (TM) shifts panels (Ciceri, et al) .[28-29, 32]

To assess the ability of BI-2536 to bind TAF1(2), we first profiled the compound in dose via competitive phage display (DiscoverX), confirming activity on the second

bromodomain of TAF1 (TAF1(2)) (170nM) in addition to BRD4(1) (24 nM) (Table S1). To facilitate further exploration of SAR within TAF1(2), we implemented an AlphaScreen assay leveraging a biotin functionalized derivative of BI-2536 (SI, Synthetic Procedures A). Guided by previously published BRD4 cocrystal structures, we sought to determine the influence of substituents buried within the acetyl-lysine recognition pocket, as well as those positioned within the "ZA channel", a known hotspot for bromodomain selectivity (Fig. 1).[1, 8] Within both TAF1(2) and BRD4(1), the imbedded methyl amide acetyl lysine mimetic showed high sensitivity to modification (Table 1; **2**,**3**). Modifications of the ZA alkoxy substituent (**4**-**7**) were better accommodated. Truncation of the buried asymmetric ethyl group to a methyl substituent (**8**,**9**) improved potency, while substitution of the solvent projected 4-aminomethylpipiridine solubilizing group was also well tolerated (Table S2).

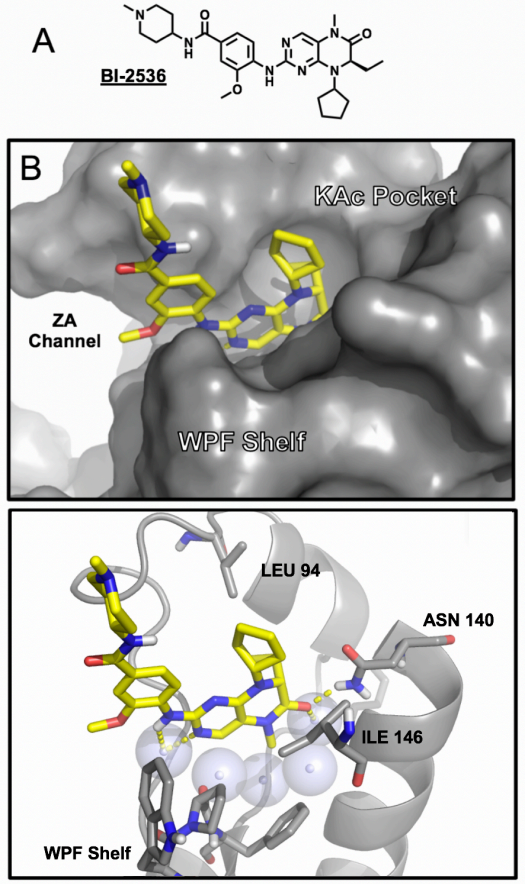
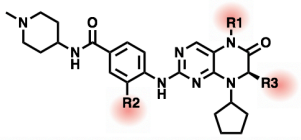


Figure 1. **A)** Chemical structure of BI-2536. **B)** Surface representation of BI-2536-BRD4(1) cocrystal structure (4O74)[29]. **C)** Cartoon cutaway of view as in (B) showing conserved waters (spheres), key residues (sticks) and H-bonding (dashed).

We next considered that the potent cytostatic activity arising from the PLK1 kinase activity of this scaffold could obscure biological studies of bromodomain inhibition. We therefore sought to alter the H-bonding capabilities of the imbedded aminopyrimidine hinge-binding motif, a well-known approach for disrupting kinase binding (Table 2).[33]

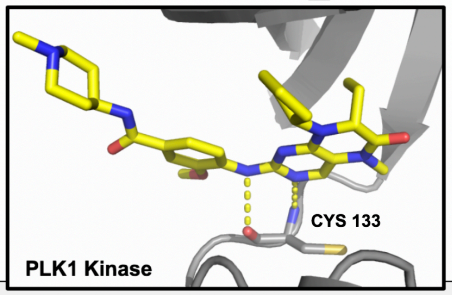
Table 1. Substitutions to the BI-2536 KAc mimetic (R_1), ZA-channel projecting alkoxy substituent (R_2), and asymmetric ethyl (R_3).



Cmpd	R1	R2	R3	TAF1(2)[a] (μM)	BRD4(1)[a] (μM)
1 BI-2536	Me	OMe	Et	0.16	0.15
2	Et	OMe	Et	0.41	0.69
3	H	OMe	Et	>10	>10
4	Me	H	Et	0.15	0.28
5	Me	O-*i*Pr	Et	0.21	0.063
6	Me	O-*cyclo*Bu	Et	0.11	0.062
7	Me	O-*cyclo*Pent	Et	0.10	0.033
8	Me	H	Me	0.087	0.068
9	Me	O-*i*Pr	Me	0.32	0.22

[a] AlphaScreen IC_{50}s from quadruplicate measurement.

Table 2. Kinase inactivation by aminopyrimidine hinge-binding motif replacement. Inset image: cartoon cutaway of BI-2536 bound to PLK1 (2RKU) showing hinge binding H-bonding (dashed) to key Cys residue backbone (sticks).



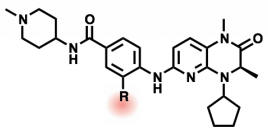
Cmpd	X	Y	TAF1(2)[a] (μM)	BRD4(1)[a] (μM)	PLK1[b] (μM)
BI2536	NH	N	0.16	0.15	0.005
10	NMe	N	>10 uM	0.10	0.73
11	O	N	>10 uM	0.62	>10 uM
12	NH	CH	0.45	0.15	>10 uM

[a] AlphaScreen IC_{50}s from quadruplicate measurement.
[b] Z-Lyte assays (Invitrogen) with [ATP] of K_M for each kinase, duplicate measurement.

Replacement of the key hydrogen bond donating NH with NMe as we previously reported,[34] or with O,[35] largely abolished kinase activity, but resulted in both cases in dramatic and preferential loss of TAF1 bromodomain activity (versus BRD4) (**10**,**11**). As this NH engages in water-mediated H-bonding within BRD4, we considered that a similar interaction may underlie an imporant contribution to TAF1 affinity (Table 2, Fig. 1C). We therefore applied an alternative strategy of removing hinge H-bond accepting to the pyrimidine core through N to CH substitution,[36] and were pleased to find that this approach successfully diminished activity against the PLK1 kinase while better retaining TAF1 affinity (Table 2; **12**).

Advancing this bromodomain selective scaffold, we sought to assess further avenues toward potency on TAF1, as well as to identify strategies to bias for TAF1 activity relative to BRD4. Introducing the favored asymmetric ethyl to methyl substitution into the kinase-selective pyridopiperazone scaffold resulted in a potent dual inhibitor of TAF1(2) (16 nM IC50) and BRD4(1) (37 nM IC50) (Table 3; **13**). Unexpectedly however, previously examined substitutions of the methoxy ZA substituent, particularly H replacement, were no longer favored, potentially reflecting rearrangement of the water mediated interaction network involving this substituent (**14**).[29] To better understand the binding mode of this series, we obtained an X-ray cocrystal structure of **15** bound to TAF1(2). Interestingly, the resolved structure contains two protein molecules per unit cell, with **15** showing a conserved core binding mode, but exhibiting two dissimilar poses of the substituted 4-aminobenzamide tail (Fig. 2, 6P38).

Table 3. Alkoxy substituent SAR for the kinase selective dihydropyridopyrazinone scaffold.



Cmpd	R	TAF1(2)[a] (μM)	BRD4(1)[a] (μM)
13	**OMe**	0.016	0.037
14	**H**	0.34	0.19
15	**O-*i*Pr**	0.089	0.13
16	**O-*cyclo*Pent**	0.031	0.048

[a] AlphaScreen IC_{50}s from quadruplicate measurement.

To further probe the ZA SAR in light of the apparent crystallographic flexibility, we undertook to study the role of tail group positioning in TAF1(2) binding. We thus examined substituents that would preclude ligand planarity, alternative tail compositions including addition of sulfonamide or methylene spacers which might allow improved "WPF shelf" access as in related efforts,[37] or which might

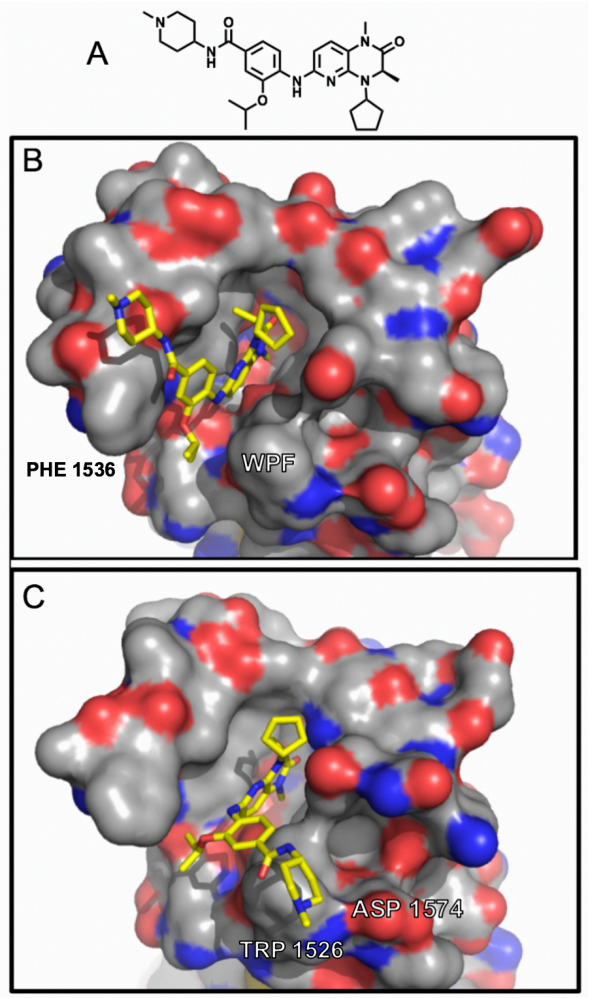
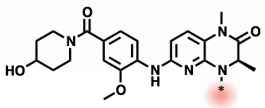


Figure 2. A) Chemical structure of **15**. **B)** Cocrystal structure of **15** and TAF1(2) showing observed binding mode (**I**). **C)** Observed binding mode (**II**) (6P38).

engage **Asp1524**, as well as direct aryl tail appendages (Table S3). We observed broadly diminished affinity across these analogs, suggesting that while the parent methoxy-*N*-(1-methylpiperidin-4-yl)benzamide tail group likely samples a variety of conformations, its poses are nonetheless well positioned to contribute considerably to affinity. Key interactions likely include offset pi-stackingand backbone H-bon bonding to **Phe1536** in Pose (**I**), and T-stacking with **Trp1526** in Pose (**II**). We note however, the influence of crystal packing contacts within the subcell featuring Pose (**II**) (Fig. S2).

We next turned our attention to examine SAR within the dihydropyridopyrazone warhead, with a particular interest in TAF1-biasing modifications. Here, a neutral solubilizing tail was implemented to facilitate purifications; although we noted a cost in affinity against TAF1 (93nM for **17** vs 16nM for **13**). We anticipated that TAF1's **Val1547** might better accommodate bulky replacements to the proximal cyclopentane moiety (Table 4; **17**-**22**) relative to BRD4's equivalently placed **Leu94**. Of this series, expansion to a six membered ring best improved TAF1 bias, with tetrahydropyran (THP) substitution further enhancing this effect (Table 4; **19**,**20**). Further considering

Table 4. Cycloalkyl moiety expansions.



Cmpd	R1	TAF1(2)[a] (μM)	BRD4(1)[a] (μM)
17		0.093	0.047
18	 [b]	0.30	0.14
19		0.072	0.15
20		0.038	0.14
21		0.24	0.22
22		0.72	0.24

[a] AlphaScreen IC_{50}s from quadruplicate measurement.
[b] Racemic

that **Leu94** of BRD4 packs between the cyclopentyl and asymmetric ethyl substituents of BI-2536, we envisioned that more severe steric discrimination of BRD4 might be achieved by linking these positions through this space via 4-carbon bridgehead (Fig. 3). Gratifyingly the resulting tricyclic warhead-containing compound **23** gave a 66-fold improvement (vs. parent **17**) in measured selectivity (BRD4 IC50/TAF IC50).

Alongside cycloalkyl modifications, we concurrently investigated substitutions to the warhead's pyridine ring (**24-30**), as well as replacement of the embedded methyl amide with extended acyl mimetics following the approach of Crawford et al (**29, 30**) (Table 5).[11] Although we found removal of the pyridyl nitrogen to be disfavored, we noted that 7-methoxy analog **27** showed largely abrogated BRD4 activity alongside only minor losses in TAF1 affinity relative to its parent analog (**24**). This SAR is consistent with the observations of Bennett et al,[37] who found that methoxy derivatization at an analogous position of a related bicyclic TRIM24 inhibitor also diminished BRD4 binding. Alternatively, we found that the extended 1-butenyl analog **30** also promoted TAF1 bias, as reported within an alternative pyrrolopyridinone scaffold.[11] As discussed previously, this permissiveness likely relates to the ability of TAF1 to favorably rearrange the conserved water network, in line with its ability to recognize natural lysine butyrylation and crotonylation.[11,38]

Encouraged by their performance, we selected the tricyclic core and N4-THP modifications for further examination. Combining these features with the preferred BI-2536

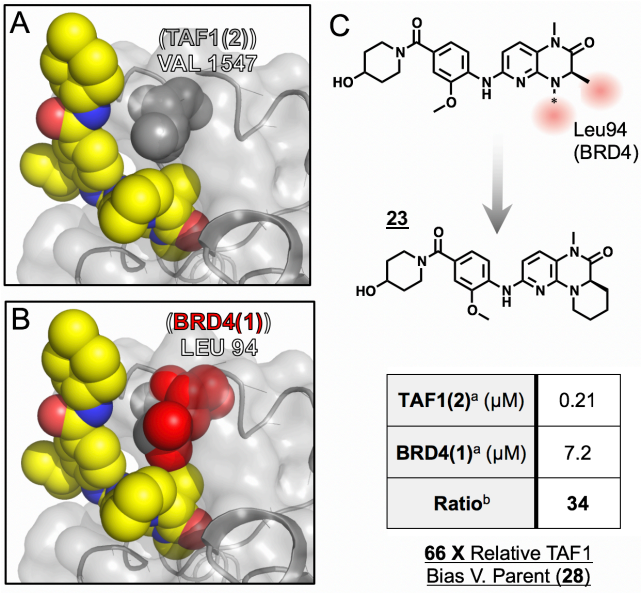
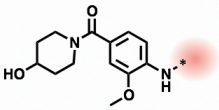
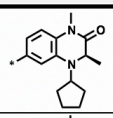
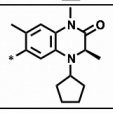
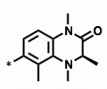
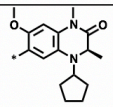
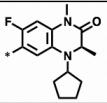
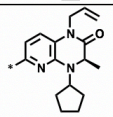
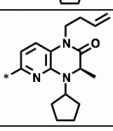


Figure 3. A) TAF1(2)-**15** cocrystal structure (6P38) showing proximal **Val1547** (gray spheres). **B)** Structural alignment (Pymol) of BRD4 (4O74) showing proximal **Leu94** (red spheres). **C)** Schematic of tricyclization approach. Inset table: [a]AlphaScreen IC_{50}s from quadruplicate measurement, and [b]IC_{50} Ratio: (BRD4(1)/TAF1(2)).

Table 5. Bicyclic core exploration.



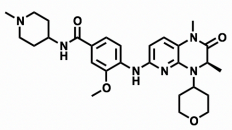
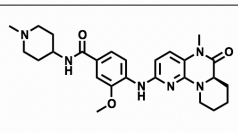
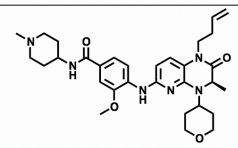
Cmpd	R1	TAF1(2)[a] (μM)	BRD4(1)[a] (μM)
24		0.58	0.11
25		>10 uM	2.1
26		0.98	>10 uM
27		0.75	7.3
28		4.6	0.68
29		1.7	4.5
30		0.57	4.4

[a]AlphaScreen IC_{50}s from quadruplicate measurement.

tail group confirmed the respective contributions of the THP group to improved potency (**31**), and tricyclization to TAF1 selectivity (**32**) (Table 6). As THP featuring compound **31** offers attractive dual potency against both TAF1 and BRD4, it was prioritized for assessment of family-wide selectivity. Profiling via the commercial phage-display BROMOscan (DiscoveRx) assay confirmed potent displacement to be largely restricted to TAF1(2) and the BET family bromodomains, with additional activity observed against the family IV bromodomain BRPF1 (Table S4).

To investigate whether structural features identified in this study might be favorably combined, we prepared analog **33** featuring both 1-butenyl and THP functionality. We were pleased to observe that this analog showed marked TAF1 bias, while still retaining considerable potency, as well as PLK1 inactivity (Table 6, Table S5). To pursue further structural insight into the selectivity promoted by analogs **32** and **33**, we resolved X-ray cocrystal structures of binding modes within TAF1(2) (Fig. 4, 6P39, 6P3A) The obtained **32** cocrystal reveals the cycloalkyl bridge to be asymmetrically projected as anticipated, effecting the intended steric contact with **Val1574** (Fig. 4 A,B). Indeed, an accommodating rearrangement of this side chain orientation is observed relative to the acyclic analog shown in Figure 2, supporting that increased burden of equivalent the **Leu94** underpins diminished binding within BRD4(1). Contrastingly, within the **33** cocrystal, we observe a distinct mode of bromodomain discrimination by projection of the 1-butenyl modification into the floor of the KAc binding pocket (Fig. 4 C,D). Here, structural comparisons reveal remarkable coherence to the 1-butenyl pose adopted in previous work (Fig. S3).[11] In addition to a nearly identical alkyl chain conformation, we note also close coherence of proximal residues, including shelf substituent **Phe1528**, backstop residue **Ile1575**, and pocket residues **Tyr1589**("gatekeeper")/**Tyr1541**, while in contrast distal ZA residues (**Trp1526**, **Phe1536**) show significant discrepancy (Fig. S3). Although the current structural resolution does not permit assignment of water positions, these data strongly imply an equivalent

Table 6. Lead Compounds.

Cmpd	R1	TAF1(2)[a] (μM)	BRD4(1)[a] (μM)	IC50 Ratio
31		< 0.01	0.049	> 4.9
32		0.063	2.7	42
33		0.082	> 10	> 120

[a]AlphaScreen IC_{50}s from quadruplicate measurement.
[b]Fold selectivity toward TAF1 given by IC_{50} ratio: BRD4(1)/TAF1(2).

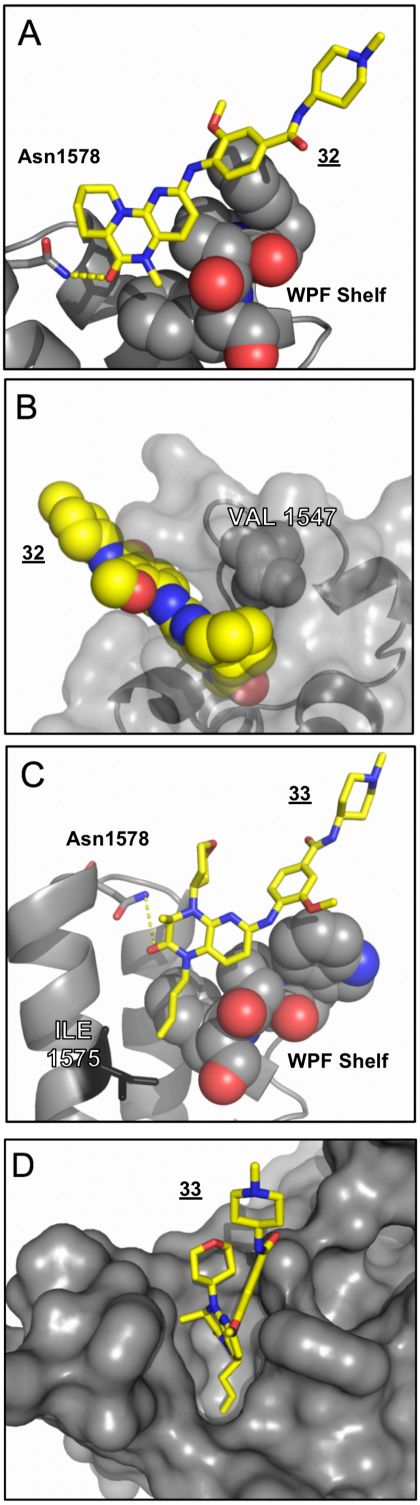


Figure 4. Cocrystal structures of **32** (**A,B**), and **33** (**C,D**) (6P39, 6P3A) showing binding modes to TAF1(2). **A)** Cutaway of **32** binding within the KAc pocket, showing H-bonding (dashes) to conserved Asn (sticks), and packing against WPF shelf (spheres). **B)** Transparent surface view of **32** (spheres) packing against **Val1547** (spheres). **C)** Cutaway of **33** binding within the KAc pocket, showing H-bonding (dashes) to conserved Asn (sticks), packing against WPF shelf (spheres), and position of "backstop" residue **Ile1575** (sticks) **D)** Surface representation of **33** showing extended conformation of 1-butenyl group into the floor of the KAc pocket.

rearrangement within the conserved water network as observed previously.[11] Together, these structural datasets provide a resource for structure-based approaches toward kinase inactive dual (BRD4(1)/TAF1(2)) and TAF1 selective compound development. Here, we highlight **31** as a potent dual lead compound for TAF1/BRD4 inhibition (Table 6). Toward TAF1 selectivity, we report three independent approaches: 3 to 4-position cyclization (**23**, **32**), 7-position ZA channel methoxy projection (**27**), and methyl to 1-butenyl amide substitution as an extended acyllysine mimetic strategy (**30**, **33**). Conversely, we find the NMe analog **10** to be over 100-fold selective for BRD4 over TAF1 by IC50 ratio, offering a potential starting point toward improving the selectivity of BET inhibitors based on this scaffold.

Because of its role in transcription initiation, marked effects on cell cycle, and potentially selective effects on amplified gene expression, TAF1 is an attractive target for both basic and translational research. Further, the multiple purported enzymatic activities make TAF1 an excellent candidate for targeted degradation approaches.[39-43] To this end, the compounds disclosed in this study offer new and advanced starting points for TAF1 probe development. In addition, previous studies of TAF1-BRD4 crosstalk have also outlined functional synergy of BRD4 and TAF1.[10] Accordingly, the potent dual BRD4 / TAF1 inhibitors reported herein may offer single agent efficacy in these contexts, and merit further biological investigation in this regard.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Supplemental Data (PDF)
Experimental Procedures (PDF)
Synthetic Procedures (PDF)

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Notes

J.E.B. is now an executive and shareholder in Novartis AG.

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ABBREVIATIONS

KAc, Acetyl-lysine; BRD, Bromodomain; BET, Bromodomain and extra-terminal domain; TBP, Tata-Binding Protein; RB, Retinoblastoma Protein; SAR, Structure-activity Relationships; TM, Thermal-Melt; THP, tetrahydropyran.

REFERENCES

1. Filippakopoulos, P.; Picaud, S.; Mangos, M.; Keates, T.; Lambert, J. P.; Barsyte-Lovejoy, D.; Felletar, I.; Volkmer, R.; Müller, S.; Pawson, T.; Gingras, A. C.; Arrowsmith, C. H.; Knapp, S., Histone recognition and large-scale structural analysis of the human bromodomain family. *Cell* **2012,** *149*, 214-231.
2. Filippakopoulos, P.; Qi, J.; Picaud, S.; Shen, Y.; Smith, W. B.; Fedorov, O.; Morse, E. M.; Keates, T.; Hickman, T. T.; Felletar, I.; Philpott, M.; Munro, S.; McKeown, M. R.; Wang, Y.; Christie, A. L.; West, N.; Cameron, M. J.; Schwartz, B.; Heightman, T. D.; La Thangue, N.; French, C. a.; Wiest, O.; Kung, A. L.; Knapp, S.; Bradner, J. E., Selective inhibition of BET bromodomains. *Nature* **2010,** *468* (7327), 1067-1073.
3. Shi, J.; Vakoc, C. R., The Mechanisms behind the Therapeutic Activity of BET Bromodomain Inhibition. *Molecular Cell* **2014,** *54* (5), 72-736.
4. Shu, S.; Polyak, K., BET bromodomain proteins as cancer therapeutic targets. *Cold Spring Harbor symposia on quantitative biology* **2016,** *81*, 123-129.
5. Theodoulou, N. H.; Tomkinson, N. C.; Prinjha, R. K.; Humphreys, P. G., Clinical progress and pharmacology of small molecule bromodomain inhibitors. *Current Opinion in Chemical Biology* **2016,** *33*, 58-66.
6. Herait, P. E.; Berthon, C.; Thieblemont, C.; Raffoux, E.; Magarotto, V.; Stathis, A.; Thomas, X.; Leleu, X.; Gomez-Roca, C.; Odore, E.; Roumier, C.; Bourrdel, F.; Quesnel, B.; Zucca, E.; Michallet, M.; Christian, R.; Cvitkovic, E.; Rezai, K.; Preudhomme, C.; Facon, T.; Palumbo, A.; Dombret, H., Abstract CT231: BET-bromodomain inhibitor OTX015 shows clinically meaningful activity at nontoxic doses: interim results of an ongoing phase I trial in hematologic malignancies. AACR: 2014.
7. Stathis, A.; Zucca, E.; Bekradda, M.; Gomez-Roca, C.; Delord, J.-P.; Rouge, T. d. L. M.; Uro-Coste, E.; de Braud, F.; Pelosi, G.; French, C. A., Clinical response of carcinomas harboring the BRD4–NUT oncoprotein to the targeted bromodomain inhibitor OTX015/MK-8628. *Cancer discovery* **2016,** *6* (5), 492-500.
8. Theodoulou, N. H.; Tomkinson, N. C. O.; Prinjha, R. K.; Humphreys, P. G., Progress in the Development of non-BET Bromodomain Chemical Probes. *ChemMedChem* **2016,** *11* (5), 477-487.
9. McKeown, M. R.; Shaw, D. L.; Fu, H.; Liu, S.; Xu, X.; Marineau, J. J.; Huang, Y.; Zhang, X.; Buckley, D. L.; Kadam, A.; Zhang, Z.; Blacklow, S. C.; Qi, J.; Zhang, W.; Bradner, J. E., Biased Multicomponent Reactions to Develop Novel Bromodomain Inhibitors. *Journal of Medicinal Chemistry* **2014,** *57* (21), 9019-9027.
10. Sdelci, S.; Lardeau, C.-H.; Tallant, C.; Klepsch, F.; Klaiber, B. o. r.; Bennett, J.; Rathert, P.; Schuster, M.; Penz, T.; Fedorov, O.; Superti-Furga, G.; Bock, C.; Zuber, J.; Huber, K. V. M.; Knapp, S.; ller, S. M. u.; Kubicek, S., Mapping the chemical chromatin reactivation landscape identifies BRD4-TAF1 crosstalk. *Nature chemical biology* **2016,** *12* (7), 504-510.
11. Crawford, T. D.; Tsui, V.; Flynn, E. M.; Wang, S.; Taylor, A. M.; Côté, A.; Audia, J. E.; Beresini, M. H.; Burdick, D. J.; Cummings, R.; Dakin, L. A.; Duplessis, M.; Good, A. C.; Hewitt, M. C.; Huang, H.-R.; Jayaram, H.; Kiefer, J. R.; Jiang, Y.;

Murray, J.; Nasveschuk, C. G.; Pardo, E.; Poy, F.; Romero, F. A.; Tang, Y.; Wang, J.; Xu, Z.; Zawadzke, L. E.; Zhu, X.; Albrecht, B. K.; Magnuson, S. R.; Bellon, S.; Cochran, A. G., Diving into the Water: Inducible Binding Conformations for BRD4, TAF1(2), BRD9, and CECR2 Bromodomains. *Journal of Medicinal Chemistry* **2016,** *59* (11), 5391-5402.

12. Bouché, L.; Christ, C. D.; Siegel, S.; Fernández-Montalván, A. E.; Holton, S. J.; Fedorov, O.; ter Laak, A.; Sugawara, T.; Stöckigt, D.; Tallant, C.; Bennett, J. M.; Monteiro, O. P.; Díaz-Sáez, L.; siejka, p.; Meier, J.; Puetter, V.; Weiske, J.; Müller, S.; Huber, K. V. M.; Hartung, I. V.; Haendler, B., Benzo-isoquinoline-diones As Potent and Selective Inhibitors of BRPF2 and TAF1/TAF1L Bromodomains. *Journal of Medicinal Chemistry* **2017,** *60* (9), 4002-4022.

13. Wang, S.; Tsui, V.; Crawford, T. D.; Audia, J. E.; Burdick, D. J.; Beresini, M. H.; Côté, A.; Cummings, R.; Duplessis, M.; Flynn, E. M.; Hewitt, M. C.; Huang, H.-R.; Jayaram, H.; Jiang, Y.; Joshi, S.; Murray, J.; Nasveschuk, C. G.; Pardo, E.; Poy, F.; Romero, F. A.; Tang, Y.; Taylor, A., M; Wang, J.; Xu, Z.; Zawadzke, L. E.; Zhu, X.; Albrecht, B. K.; Magnuson, S. R.; Bellon, S.; Cochran, A. G., GNE-371, a Potent and Selective Chemical Probe for the Second Bromodomains of Human Transcription-Initiation-Factor TFIID Subunit 1 and Transcription-Initiation-Factor TFIID Subunit 1-like. *Journal of medicinal chemistry* **2018,** *61* (20), 9301-9315.

14. Dynlacht, B. D.; Hoey, T.; Tjian, R., Isolation of coactivators associated with the TATA-binding protein that mediate transcriptional activation. *Cell* **1991,** *66* (3), 563-576.

15. Struhl, K.; Moqtaderi, Z., The TAFS in the HAT. *Cell* **1998,** *94*, 1-4.

16. Segall, J.; Matsui, T.; Roeder, R., Multiple factors are required for the accurate transcription of purified genes by RNA polymerase III. *Journal of Biological Chemistry* **1980,** *255* (24), 11986-11991.

17. Van Dyke, M. W.; Roeder, R. G.; Sawadogo, M., Physical analysis of transcription preinitiation complex assembly on a class II gene promoter. *Science* **1988,** *241* (4871), 1335-1338.

18. Buratowski, S.; Hahn, S.; Guarente, L.; Sharp, P. A., Five intermediate complexes in transcription initiation by RNA polymerase II. *Cell* **1989,** *56* (4), 549-561.

19. Wang, E. H.; Tjian, R., Promoter-selective transcriptional defect in cell cycle mutant ts13 rescued by hTAFII250. *Science* **1994,** *263* (5148), 811-814.

20. Shao, Z.; Ruppert, S.; Robbins, P. D., The retinoblastoma-susceptibility gene product binds directly to the human TATA-binding protein-associated factor TAFII250. *Proceedings of the National Academy of Sciences of the United States of America* **1995,** *92* (April), 3115-3119.

21. Bartusel, T.; Klempnauer, K.-H., Transactivation mediated by B-Myb is dependent on TAF(II)250. *Oncogene* **2003,** *22*, 2932-2941.

22. Lively, T. N.; Ferguson, H. a.; Galasinski, S. K.; Seto, A. G.; Goodrich, J. a., c-Jun Binds the N Terminus of Human TAFII250 to Derepress RNA Polymerase II Transcription in Vitro. *Journal of Biological Chemistry* **2001,** *276* (27), 25582-25588.

23. Sekiguchi, T.; Yoshida, M. C.; Sekiguchi, M.; Nishimoto, T., Isolation of a human X chromosome-linked gene essential for progression from G1 to S phase of the cell cycle. *Experimental Cell Research* **1987,** *169* (2), 395-407.

24. Sekiguchi, T.; Noguchi, E.; Hayashida, T.; Nakashima, T.; Toyoshima, H.; Nishimoto, T.; Hunter, T., D-type cyclin expression is decreased and p21 and p27 CDK inhibitor expression is increased when tsBN462 CCG1/TAFII250 mutant cells arrest in G1 at the restrictive temperature. *Genes to Cells* **1996,** *1* (7), 687-705.

25. Vangamudi, B.; Paul, T. A.; Shah, P. K.; Kost-Alimova, M.; Nottebaum, L.; Shi, X.; Zhan, Y.; Leo, E.; Mahadeshwar, H. S.; Protopopov, A.; Futreal, A.; Tieu, T. N.; Peoples, M.; Heffernan, T. P.; Marszalek, J. R.; Toniatti, C.; Petrocchi, A.; Verhelle, D.; Owen, D. R.; Draetta, G.; Jones, P.; Palmer, W. S.; Sharma, S.; Andersen, J. N., The SMARCA2/4 ATPase Domain Surpasses the Bromodomain as a Drug Target in SWI/SNF-Mutant Cancers: Insights from cDNA Rescue and PFI-3 Inhibitor Studies. *Cancer Research* **2015,** *75* (18), 3865-3878.

26. Remillard, D.; Buckley, D. L.; Paulk, J.; Brien, G. L.; Sonnett, M.; Seo, H. S.; Dastjerdi, S.; Wühr, M.; Dhe-Paganon, S.; Armstrong, S. A.; Bradner, J. E., Degradation of the BAF complex factor BRD9 by heterobifunctional ligands. *Angewandte Chemie International Edition* **2017,** *56* (21), 5738-5743.

27. Gechijian, L. N.; Buckley, D. L.; Lawlor, M. A.; Reyes, J. M.; Paulk, J.; Ott, C. J.; Winter, G. E.; Erb, M. A.; Scott, T. G.; Xu, M.; Hyuk-Soo, S.; Dhe-Paganon, S.; Kwiatkowski, N. P.; Perry, J. A.; Qi, J.; Gray, N. S.; Bradner, J. E., Functional TRIM24 degrader via conjugation of ineffectual bromodomain and VHL ligands. *Nature chemical biology* **2018,** *14* (4), 405.

28. Ciceri, P.; Müller, S.; O'Mahony, A.; Fedorov, O.; Filippakopoulos, P.; Hunt, J. P.; Lasater, E. a.; Pallares, G.; Picaud, S.; Wells, C.; Martin, S.; Wodicka, L. M.; Shah, N. P.; Treiber, D. K.; Knapp, S., Dual kinase-bromodomain inhibitors for rationally designed polypharmacology. *Nature chemical biology* **2014,** *10* (april), 305-12.

29. Ember, S. W. J.; Zhu, J.-y.; Olesen, S. H.; Martin, M. P.; Becker, A.; Berndt, N.; Georg, G. I.; Schonbrunn, E., Acetyl-lysine binding site of bromodomain-containing protein 4 (BRD4) interacts with diverse kinase inhibitors. . *ACS Chemical Biology* **2014,** *9* (5), 1160-1171.

30. Ember, S. W.; Lambert, Q. T.; Berndt, N.; Gunawan, S.; Ayaz, M.; Tauro, M.; Zhu, J.-Y.; Cranfill, P. J.; Greninger, P.; Lynch, C. C.; Benes, C. H.; Lawrence, H. R.; Reuther, G. W.; Lawrence, N. J.; Schonbrunn, E., Potent dual BET bromodomain-kinase inhibitors as value-added multitargeted chemical probes and cancer therapeutics. *Molecular cancer therapeutics* **2017,** *16* (6), 1054-1067.

31. Divakaran, A.; Talluri, S. K.; Ayoub, A. M.; Mishra, N. K.; Cui, H.; Widen, J. C.; Berndt, N.; Zhu, J.-Y.; Carlson, A. S.; Topczewski, J. J.; Ernst, S. K.; Harki, D. A.; Pomerantz, W. C., Molecular Basis for the N-Terminal Bromodomain-and-Extra-Terminal-Family Selectivity of a Dual Kinase–Bromodomain Inhibitor. *Journal of medicinal chemistry* **2018,** *61* (20), 9316-9334.

32. Steegmaier, M.; Hoffmann, M.; Baum, A.; Lénárt, P.; Petronczki, M.; Krššák, M.; Gürtler, U.; Garin-Chesa, P.; Lieb, S.; Quant, J.; Grauert, M.; Adolf, G. R.; Kraut, N.; Peters, J. M.; Rettig, W. J., BI 2536, a Potent and Selective Inhibitor of Polo-like Kinase 1, Inhibits Tumor Growth In Vivo. *Current Biology* **2007,** *17*, 316-322.

33. Gray, N. S.; Wodicka, L.; Thunnissen, A.-M. W.; Norman, T. C.; Kwon, S.; Espinoza, F. H.; Morgan, D. O.; Barnes, G.; LeClerc, S.; Meijer, L.; Kim, S.-H.; Lockhart, D. J.; Schultz, P. G., Exploiting chemical libraries, structure, and genomics in the search for kinase inhibitors. *Science* **1998,** *281* (5376), 533-538.

34. Koblan, L. W.; Buckley, D. L.; Ott, C. J.; Fitzgerald, M. E.; Ember, S. W. J.; Zhu, J.-y.; Liu, S.; Roberts, J. M.; Remillard, D.; Vittori, S.; Zhang, W.; Schonbrunn, E.; Bradner, J. E., Assessment of Bromodomain Target Engagement by a Series of BI2536 Analogues with Miniaturized BET-BRET. *ChemMedChem* **2016,** *11* (23), 2575-2581.

35. Chen, L.; Yap, J. L.; Yoshioka, M.; Lanning, M. E.; Fountain, R. N.; Raje, M.; Scheenstra, J. A.; Strovel, J. W.; Fletcher, S., BRD4 Structure–Activity Relationships of Dual PLK1 Kinase/BRD4 Bromodomain Inhibitor BI-2536. *ACS Medicinal Chemistry Letters* **2015,** *6* (7), 764-769.

36. Hu, J.; Wang, Y.; Li, Y.; Xu, L.; Cao, D.; Song, S.; Damaneh, M. S.; Wang, X.; Meng, T.; Chen, Y.-L.; Shen, J.; Miao, Z.; Xiong, B., Discovery of a series of dihydroquinoxalin-2(1H)-ones as selective BET inhibitors from a dual PLK1-BRD4 inhibitor. *European Journal of Medicinal Chemistry* **2017,** *137*, 176-195.

37. Bennett, J.; Fedorov, O.; Tallant, C.; Monteiro, O.; Meier, J.; Gamble, V.; Savitsky, P.; Nunez-Alonso, G. a.; Haendler, B.; Rogers, C.; Brennan, P. E.; Müller, S.; Knapp, S., Discovery of a Chemical Tool Inhibitor Targeting the Bromodomains of TRIM24 and BRPF. *Journal of Medicinal Chemistry* **2016,** *59* (4), 1642-1647.

38. Flynn, E. M.; Huang, O. W.; Poy, F.; Oppikofer, M.; Bellon, S. F.; Tang, Y.; Cochran, A. G., A Subset of Human Bromodomains Recognizes Butyryllysine and Crotonyllysine Histone Peptide Modifications. *Structure* **2015,** *23* (10), 1801-1814.

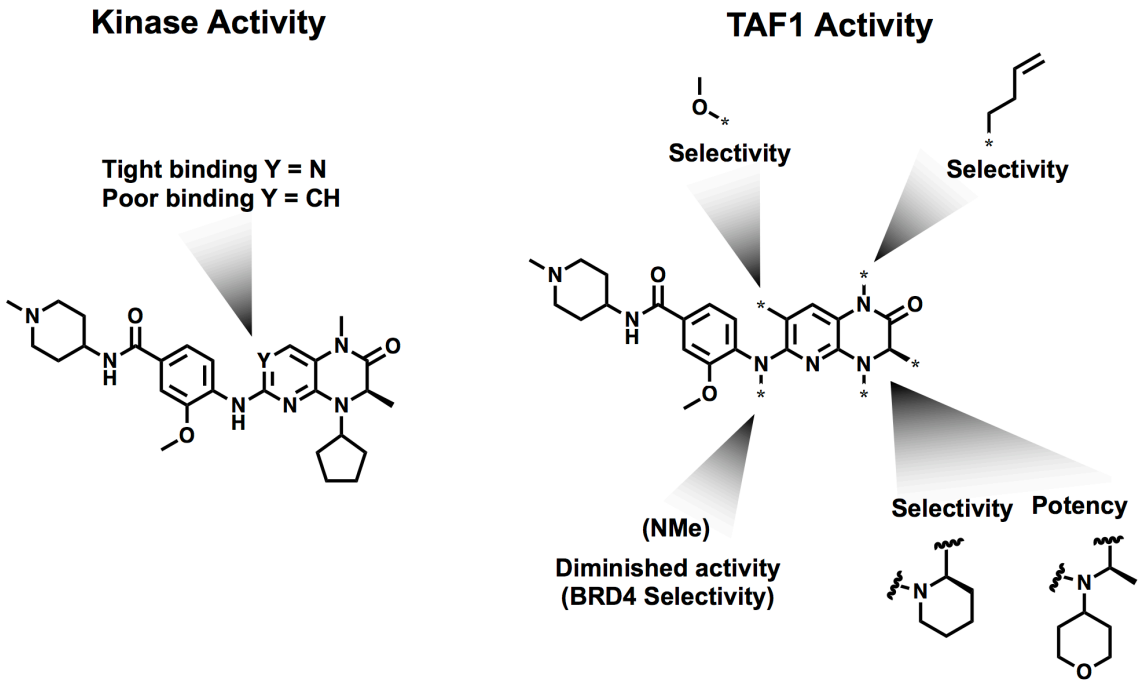
39. Dikstein, R.; Ruppert, S.; Tjian, R., TAF II 250 Is a Bipartite Protein Kinase That Phosphorylates the Basal Transcription Factor RAP74. *Cell* **1996,** *84*, 781-790.

40. Mizzen, C. A.; Yang, X.-j.; Kokubo, T.; Brownell, J. E.; Bannister, A. J.; Owen-hughes, T.; Workman, J.; Wang, L.; Berger, S. L.; Kouzarides, T.; Nakatani, Y.; Allis, C. D., The TAF II 250 Subunit of TFIID Has Histone Acetyltransferase Activity. *Cell* **1996,** *87* (7), 1261-1270.

41. Pham, A.-D.; Sauer, F., Ubiquitin-activating/conjugating activity of TAFII250, a mediator of activation of gene expression in Drosophila. *Science* **2000,** *289* (5488), 2357-2360.

42. Winter, G. E.; Buckley, D. L.; Paulk, J.; Roberts, J. M.; Souza, A.; Dhe-Paganon, S.; Bradner, J. E., Phthalimide conjugation as a strategy for in vivo target protein degradation. *Science* **2015,** *348* (6241), 1376-1381.

43. Brien, G. L.; Remillard, D.; Shi, J.; Hemming, M. L.; Chabon, J.; Wynne, K.; Dillon, E. T.; Cagney, G.; Van Mierlo, G.; Baltissen, M. P.; Vermeulen, M.; Qi, J.; Frohling, S.; Gray, N. S.; Bradner, J. E.; Vakoc, C. R.; Armstrong, S. A., Targeted degradation of BRD9 reverses oncogenic gene expression in synovial sarcoma. *Elife* **2018,** *7*, e41305.


Kinase Activity
Tight binding Y = N
Poor binding Y = CH
TAF1 Activity
Selectivity
Selectivity
(NMe)
Diminished activity
(BRD4 Selectivity)
Selectivity
Potency