Novel brd4 inhibitors with a unique scaffold exhibit antitumor effects

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Abstract. Since bromodomain containing 4 (brd4) has been considered as a prominent cancer target, numerous attempts have been made to develop potent brd4 bromodomain inhibitors. The present study provided a novel chemical scaffold which inhibited brd4 activity. Mid-throughput screening against brd4 bromodomain was performed using alpha-screen and homogeneous time-resolved fluorescence assays. Furthermore, cell cytotoxicity and xenograft assays were performed to examine if the compound was effective both in vitro and in vivo. As a result, it was revealed that compounds having naphthalene-1,4-dione scaffold inhibited the binding of bromodomain to acetylated histone. The compounds with naphthalene-1,4-dione had cytotoxic effects against the Ty82 cell line, a NUT midline carcinoma cell line, whose proliferation is dependent on brd4 activity. A10, one of the compounds with naphthalene-1,4-dione scaffold, also exhibited tumor growth inhibition effects in the xenograft assay. In addition, the compounds exhibited cytotoxic effects against gastric cancer cell lines which were resistant to I-BET-762, a BET bromodomain inhibitor. In conclusion, the novel scaffold to suppress brd4 activity was effective against cancer cells both in vitro and in vivo.

Introduction

In epigenetics, lysine acetylation has been considered as a key step of post-translational modifications (1,2). Histone acetyltransferases (HATs) and histone deacetylases (HDACs) functions as ‘writers’ and ‘erasers’ respectively by controlling acetyl mark of histone lysine residue (3). For this acetylation to be involved in gene expression, we need a ‘reader’ to recognize acetylated histone. Bromodomain is one of the best known modules to recognize and bind to acetylated histones (4). In 1992, bromodomain, a protein module containing approximately 110 amino acids, was identified as a lysine acetylation reader in Drosophila melanogaster study (5). In the human genome, there are 46 bromodomain-containing proteins, many of which are HATs, HAT-associated proteins, helicases, ATP-dependent chromatin remodeling complexes, transcriptional coactivators, and nuclear scaffolding proteins. Among them, brd4, one of the BET (bromodomain and extra-terminal proteins) proteins, was revealed to play a crucial role in NUT midline carcinoma (NMC) (6). In the majority of NMC patients, NUT gene, which is located on chromosome 15q14, is fused with BRD4 or BRD3, creating BRD4-NUT fusion proteins. As knockdown of BRD4-NUT in NMC caused significant decrease in BRD4-NUT positive cell proliferation (7), brd4 has been highlighted as a powerful therapeutic target for NMC. In addition, brd4 knockdown in AML cell lines causes downregulation of c-myc expression as to induce cell death (8). Subsequent studies demonstrated that most of the leukemic and lymphoma cells die by brd4 inhibition (9). For this reason, many studies have been conducted to develop potent bromodomain inhibitors (10). At present, approximately 40 papers relevant to BET inhibitors have been published, and 16 inhibitors are on-going in clinical trial.

Here, we performed mid-throughput screening to discover a new brd4 bromodomain inhibitor. We setup two biochemical assays, alpha-screen and Homogeneous Time Resolved Fluorescence (HTRF), we got hit compound which exhibits excellent efficacy in vitro and in vivo assay.

Materials and methods

Cell culture. Ty82, and MKN7 cell lines were obtained from JCRB cell bank (Japan). SNU638, SNU719, SNU668, SNU216,
MKN45, MKN74 and MKN1 cell lines were obtained from Korean cell line Bank (Korea). All cell lines were cultured with RPMI-1640 supplemented with 10% fetal bovine serum.

**Molecular cloning and protein expression, and purification.** Brd4 cDNA was provided by Dr. Stefan Knapp from the University of Oxford. N-terminal GST-tagged and C-terminal His-tagged BD1 (GST-HisBD1) was expressed in E. coli and purified. BD1 spans 47-170 amino acids. The pGEX 6P-1 vector was digested with EcoRI and Xhol restriction enzymes. BD1 PCR was performed with the BD1_Forward primer (5'-ATC TAG GAA TTC CCC CCA GAG ACC TCC AAC CC-3') and BD1_Rev primer (5'-ATC TAG CTC GAG TTA GTG GTG GTG GTG GTG GTG TTC GTC GCC CGC AAG CTC GTG TTC TTC TGT GGG TA-3'). BL21 Star (DE3) was transformed and induced by 0.1 mM IPTG at 18°C. The cells were lysed with lysozyme (1 mg/ml) and sonicated in lysis buffer (50 mM NaH$_2$PO$_4$, 300 mM NaCl, 10 mM imidazole, and adjusted pH to 8.0 by NaOH) and centrifuged at 8,000 rpm for 30 min. The supernatant was incubated with Ni-NTA beads (Qiagen) for 2 h at 4°C and proteins were eluted with elution buffer (50 mM NaH$_2$PO$_4$, 300 mM NaCl, 250 mM imidazole, and adjusted pH to 8.0 by NaOH). Purified His-tag proteins were further purified by size exclusion chromatography on a superdex 16/600 Hilo column (GE Healthcare) using buffer (50 mM Tris-HCl pH 7.4, 200 mM NaCl)

**Alpha-screen biochemical assay.** The alpha-screen assay was performed in accordance with the manufacturer’s protocol (PerkinElmer), by using a buffer (50 mM HEPES, 100 mM NaCl, 0.1% BSA, pH 7.4 supplemented with 0.05% CHAPS) and OptiPlate™-384 plate (PerkinElmer). Briefly, 2.5 µl of compound solution and 5 µl of peptide solution [SGRGK(Ac)GGK(Ac)GLGK(Ac)GGAK(Ac)RHRK-biotin] were added to 5 µl of glutathione-S-transferase (GST) and His-tagged BD1 in OptiPlate™-384 plate. Streptavidin-coated donor beads and anti-GST alpha-screen acceptor beads were added under low-light condition. Plate was incubated at 25°C for 60 min using a Thermomixer C (Eppendorf), and read using a Fusion-Alpha™ Multilabel Reader (PerkinElmer). The alpha-screen results were confirmed by using alpha-screen TruHit kits (PerkinElmer).

**HTRF assay.** The HTRF assay was performed in 384-well black polystyrene plate, flat bottom, low flange, non-binding surface (Corning) in assay buffer (50 mM HEPES (pH 7.0), Na$_2$PO$_4$ 0.02%, 0.01% BSA, Orthovanadate 0.1 mM) and OptiPlate™-384 plate (PerkinElmer). Briefly, 2.5 µl of compound solution and 5 µl of peptide solution [SGRGK(Ac)GGK(Ac)GLGK(Ac)GGAK(Ac)RHRK-biotin] were added to 5 µl of glutathione-S-transferase (GST) and His-tagged BD1 in OptiPlate™-384 plate. Streptavidin-coated donor beads and anti-GST alpha-screen acceptor beads were added under low-light condition. Plate was incubated at 25°C for 60 min using a Thermomixer C (Eppendorf), and read using a Fusion-Alpha™ Multilabel Reader (PerkinElmer). The alpha-screen results were confirmed by using alpha-screen TruHit kits (PerkinElmer).

**Statistical analysis.** Data are presented as the mean ± standard error in 6 mice for each group. Statistical analysis was conducted using GraphPad Prism 6 software (GraphPad Software). Statistical comparisons between vehicle-treated and compound-treated groups were performed using two-way ANOVA with Dunnett’s multiple comparisons test. P<0.01 was considered to indicate a statistically significant difference (n=6). The statistical analysis used in an experiment is described in the figure legend.

**Results**

*Mid-throughput screening using alpha-screen assay.** We performed MTS to identify molecules that have inhibitory activity on brd4 bromodomain with the compound library provided by Korea Chemical Bank (Daejeon, South Korea).
We setup 2 different biochemical assays, alpha-screen assay and HTRF. In this study, we regarded the compound hitting only one biochemical assay as false positive, and the compound hitting both biochemical assays as true hit. The workflow of MTS is shown in Fig. 1A. As a result, we found 1 compound, called as HIT-A, showing inhibition in both biochemical assays (Fig. 1B). The IC\textsubscript{50} of HIT-A is 1.29 µM in alpha-screen assay, and 0.48 µM in HTRF assay (Fig. 1C).

c-myc is known to be highly controlled by brd4 activity, so we checked the c-myc level after compound treatment to judge whether our hit compound is effective in cells (8). The cellular c-myc level was decreased by HIT-A in Ty82 cell line (Fig. 1D). These data demonstrate that HIT-A inhibits brd4 activity in biochemical assay and in cellular assay.

Study on HIT-A derivatives. Out of 400K compounds deposited in Korea Chemical Bank, there are 16 compounds similar to HIT-A in structure. We have conducted both biochemical assays and cell cytotoxic assay with all of these compounds. Interestingly, O-linked compounds (A1, A6, HIT-A, A8, A10, A12 and A14) showed inhibition in both biochemical assays, whereas non O-linked compound (A2, A3, A4, A5, A7, A9, A11, A13 and 1701) showed inhibition only in alpha-screen, not in HTRF assay (Fig. 2). Because NUT midline carcinoma (NMC) cell lines have a NUT-BRD4 fusion protein by chromosome translocation, the proliferations of NMC cell lines are dependent on brd4 activity (7). Ty82 is one of the NMC cell lines, and also has BRD4-NUT fusion protein (11). Cell cytotoxic assay shows that only O-linked compounds exert cytotoxic effect on Ty82 cells. Non O-linked compounds don't exert any cytotoxic effect on Ty82 cells. This means that only O-linked compounds, not non O-linked, are true brd4 bromodomain inhibitor, and non O-linked compounds are false positive.

In vivo xenograft assay using Ty82. To determine whether our hit compounds exhibit tumor growth inhibition, we conducted an \textit{in vivo} xenograft assay. Ty82 cells were implanted in nude mice and allowed to grow to 200 mm\textsuperscript{3} in size. Subsequently, HIT-A and A10 were administered orally at daily doses of 100 mpk. Tumor volumes were measured for 28 days. As shown in Fig. 3, A10 compound effectively inhibited tumor growth. No weight loss was shown in the mice administered with the new bromodomain inhibitors (Fig. 3B). These results suggest that our compound is a potent bromodomain inhibitor with a unique scaffold \textit{in vivo}.

\textit{Inhibitory effect of our compound on gastric cancer cell}. Brd4 bromodomain inhibitor is known to suppress the proliferation of hematological cancer cell. However, it is not well known if bromodomain inhibitor is effective in solid tumor such as gastric cancer cell. Here, we performed cytotoxic assay with various gastric cancer cell lines (Fig. 4). A7, which doesn't inhibit bromodomain and is similar to hit compound in structure, didn't suppress the proliferation of any gastric cancer cell lines tested. I-BET-762 exerts inhibition only on limited cell lines. However, our hit compounds, A10 and HIT-A, show cytotoxic effect on all the gastric cancer cell lines tested.

To see the c-myc protein level, we performed western blotting with the cell lysates treated with compounds (Fig. 5). SNU-638, MNK-45 and Ty82 cells were treated with A10 or I-BET-762. As we expected, c-myc in Ty82 cell line is downregulated by A10. I-BET-762 downregulated the c-myc level in SNU-638 to which I-BET-762 had cytotoxic effect. I-BET-762 did not downregulate the c-myc level of MKN-45 to which I-BET-762 had no cytotoxic effect. Interestingly, although A10 compound had cytotoxic effect to both SNU-638 and MNK-45, it downregulated the c-myc level only in SNU-638. It means that A10 compound has another cytotoxic mechanism other than c-myc signaling in MKN-45.

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Figure 1. A novel bromodomain inhibitor identified through MTS. (A) Bromodomain inhibitor screening steps in the present study. (B) Structure of HIT-A, which exhibits the efficient inhibitory effect, identified in mid-throughput screening. (C) Inhibitory activity of HIT-A \textit{in vitro} biochemical assays, including an alpha-screen assay and ELISA. (D) Ty-82 cells were treated with HIT-A for 18 h, and cell lysates were collected for western blotting to see c-myc level. MTS, mid-throughput screening; HTRF, homogeneous time-resolved fluorescence.

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**A**

MTS with AlphaScreen assay

First Enzyme assay

HTRF Second Enzyme assay

c-myc western blot in Ty82 cell line

**C**

| % Inhibition at 17.6 µM in AlphaScreen | 98.51 |
| IC\textsubscript{50} in alpha-screen | 1.29 µM |
| IC\textsubscript{50} in HTRF assay | 0.48 µM |

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**D**

| Compound | DMSO | 0.03 µM | 0.1 µM | 0.3 µM | 1 µM | 3 µM |
|-----------|-------|---------|--------|--------|------|------|
| c-myc | | | | | | |
| β-actin | | | | | | |
Compound screening such as high-throughput or mid-throughput screening is a key step of the early stage in drug development, to identify molecules which have activity on specific targets. However, this step is easily weakened by a high incidence of false-positives, which are not active toward the biological target of interest, but active in an assay (12). False positives result from the compound interference in assay system (13). These compound interference can be produced solely by compounds themselves, such as fluorescent compounds, or by their interaction with biological components in assay system (14). One of the powerful method to solve this problem is to use orthogonal assay systems (15-17). Here, we setup 2 orthogonal assays for bromodomain inhibitor screening, alpha-screen and homogeneous time resolved fluorescence assay. In this study, alpha-screen was used as primary screening assay for MTS. We identified more than 70 hits in alpha-screen assay. Subsequently, HTRF assay revealed that HIT-A compound is a true hit among 70 hits. To confirm HIT-A is a true hit, we checked c-myc expression level after HIT-A treatment in Ty82 cell line. Jang et al (18) reported that c-myc promoter is regulated by brd4 protein. Yang et al (19) also reported that c-myc expression is clearly impaired by brd4 knockdown. JQ-1, the first brd4 inhibitor, has demonstrated significant downregulation of c-myc protein (9). Therefore, various brd4 inhibitors were confirmed to be true hits by showing suppression of c-myc expression in cancer cell lines (20-23). Western blot data shows that HIT-A compound downregulates c-myc expression in Ty82 cell line, which means that HIT-A is a true hit. With 16 derivatives of HIT-A, we performed both biochemical assays. Interestingly, O-linked compounds exert inhibition in both assay systems, Figure 2. Structure-activity relationship of HIT-A derivatives. The IC₅₀ (µM) values obtained in alpha-screen, HTRF and cell cytotoxic assays of 15 compounds in Ty82 cells are summarized. HTRF assay was performed for the compounds which exhibited inhibitory activities in the alpha-screen assay. HTRF, homogeneous time-resolved fluorescence.

Discussion

Compound screening such as high-throughput or mid-throughput screening is a key step of the early stage in drug development, to identify molecules which have activity on specific targets. However, this step is easily weakened by a high incidence of false-positives, which are not active toward the biological target of interest, but active in an assay (12). False positives result from the compound interference in assay system (13). These compound interference can be produced solely by compounds themselves, such as fluorescent compounds, or by their interaction with biological components in assay system (14). One of the powerful method to solve this problem is to use orthogonal assay systems (15-17). Here, we setup 2 orthogonal assays for bromodomain inhibitor screening, alpha-screen and homogeneous time resolved fluorescence assay. In this study, alpha-screen was used as primary screening assay for MTS. We identified more than 70 hits in alpha-screen assay. Subsequently, HTRF assay revealed that HIT-A compound is a true hit among 70 hits. To confirm HIT-A is a true hit, we checked c-myc expression level after HIT-A treatment in Ty82 cell line. Jang et al (18) reported that c-myc promoter is regulated by brd4 protein. Yang et al (19) also reported that c-myc expression is clearly impaired by brd4 knockdown. JQ-1, the first brd4 inhibitor, has demonstrated significant downregulation of c-myc protein (9). Therefore, various brd4 inhibitors were confirmed to be true hits by showing suppression of c-myc expression in cancer cell lines (20-23). Western blot data shows that HIT-A compound downregulates c-myc expression in Ty82 cell line, which means that HIT-A is a true hit. With 16 derivatives of HIT-A, we performed both biochemical assays. Interestingly, O-linked compounds exert inhibition in both assay systems, Figure 2. Structure-activity relationship of HIT-A derivatives. The IC₅₀ (µM) values obtained in alpha-screen, HTRF and cell cytotoxic assays of 15 compounds in Ty82 cells are summarized. HTRF assay was performed for the compounds which exhibited inhibitory activities in the alpha-screen assay. HTRF, homogeneous time-resolved fluorescence.

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Figure 3. In vivo xenograft assay. Ty82 cells were implanted into nude mice and allowed to grow to 200 mm³. Vehicle or 100 mpk bromodomain inhibitors were orally administered daily. (A) Tumor sizes were measured every 2-3 days throughout the treatment period using calipers. Results are presented as the mean ± SEM. Statistical comparisons between Vehicle-treated and compound-treated groups were performed using two-way ANOVA with Dunnett’s multiple comparisons test. *P≤0.01 (n=6). (B) Body weights were measured every 2-3 days throughout the treatment period.
however, non O-linked compounds exert inhibition only in alpha-screen. We anticipated that only O-linked compounds are real hit, because they exerted inhibition in both assay systems. As we expected, cell cytotoxic assay with Ty82, which is addicted to bromodomain activity, showed that only O-linked compounds exerted cytotoxicity in Ty82. This data reflects that our orthogonal assay system is very effective to remove false positives. In vivo assay, one of the hit derivatives, A10, showed excellent tumor growth inhibition without body weight change. We tested whether our hit compound is working on gastric cancer cells. O-linked compounds, A10 and HIT-A, shows cytotoxic effect against gastric cancer cells. Because
A7, which didn’t inhibit brd4 at all, exerted no cytotoxicity, it is sure that the cytotoxic effect of O-linked compound is due to the bromodomain inhibition. In addition, O-linked compounds showed cell growth inhibition even in I-BET-762-resistant cell lines, MKNA5, SH-10-TC, SNU668, MKN7 and SNU216. Therefore, we anticipate that our compound is more powerful for cancer therapy than I-BET-762.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

YHK and CHP confirm the authenticity of all the raw data. CHP developed this project. YHK performed the mid-throughput screening and found the HIT compound. JKE and MYY performed the enzyme assay to measure the inhibitory activities. MK performed the cell-based assay. HKL and COL performed and analyzed the in vivo experiment. KYJ and MJY synthesized the compounds. YK performed the cell-based assay. SUC analyzed the data. CHP designed the study, planned and performed the experiments, analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Animal experiments were approved by the Laboratory Animal Care and Use Committee of Korea Research Institute of Chemical Technology (Daejeon, South Korea).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Yang XJ and Seto E: Lysine acetylation: Codified crosstalk with other post-translational modifications. Mol Cell 31: 449-461, 2008.

2. Kouzarides T: Chromatin modifications and their function. Cell 128: 693-705, 2007.

3. Jenuwein T and Allis CD: Translating the histone code. Science 293: 1074-1080, 2001.

4. Sanchez R, Meslamani J and Zhou MM: The bromodomain: From epigenome reader to druggable target. Biochim Biophys Acta 1839: 676-685, 2014.

5. Tamkun JW, Deuring R, Scott MP, Kissinger M, Pattattuci AM, Kaufman TC and Kennison JA: brahma: A regulator of Drosophila homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. Cell 68: 561-572, 1992.

6. French CA: NUT midline carcinoma. Cancer Genet Cytoenet 203: 16-20, 2010.

7. French CA, Ramirez CL, Kolmakova J, Hickman TT, Cameron MJ, Thyne ME, Kutolek JL, Toretsky JA, Tadavarty AK, Kees UR, et al: BRD-NUT oncoproteins: A family of closely related nuclear proteins that block epithelial differentiation and maintain the growth of carcinoma cells. Oncogene 27: 2237-2242, 2008.

8. Zuber J, Shi J, Wang E, Rappaport AR, Herrmann H, Sison EA, Magoon D, Qi J, Blatt K, Wunderlich M, et al: RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukemia. Nature 478: 524-528, 2011.

9. Mertz JA, Conery AR, Bryant BM, Sandy P, Balasubramanian S, Mele DA, Bergeron L and Sims RJ III: Targeting MYC dependence in cancer by inhibiting BET bromodomains. Proc Natl Acad Sci USA 108: 16669-16674, 2011.

10. Brand M, Measures AR, Wilson BG, Cortopassi WA, Alexander R, Hoss M, Hewings DS, Rooney TP, Paton RS and Conway SJ: Small molecule inhibitors of bromodomain-acetyl-lysine interactions. ACS Chem Biol 10: 22-39, 2015.

11. French CA, Miyoshi I, Kubonishi I, Griek HE, Perez-Atayde AR and Fletcher JA: BRD4-NUT fusion oncogene: A novel mechanism in aggressive carcinoma. Cancer Res 63: 304-307, 2003.

12. Thorne N, Auld DS and Inglese J: Apparent activity in high-throughput screening: Origins of compound-dependent assay interference. Curr Opin Chem Biol 14: 315-324, 2010.

13. Simeonov A, Yasgar A, Klumpp C, Zheng W, Shafiq N, Oppermann U, Austin CP and Inglese J: Evaluation of micro-parallel liquid chromatography as a method for HTS-coupled active verification. Assay Drug Dev Technol 5: 815-824, 2007.

14. Shapiro AB, Walkup GK and Keating TA: Correction for interference by test samples in high-throughput assays. J Biomol Screen 14: 1008-1016, 2009.

15. Weber E, Rothenaigner I, Brandner S, Hadian K and Schorpp K: A high-throughput screening strategy for development of RNF8-Ubc13 protein-protein interaction inhibitors. SLAS Discov 22: 316-323, 2017.

16. Carter DM, Specker E, Przygoda J, Neuenschwander M, von Kries JP, Heinemann U, Nazare M and Gohike U: Identification of a novel benzimidazole pyrazolone scaffold that inhibits KDM4 lysine demethylases and reduces proliferation of prostate cancer cells. SLAS Discov 22: 801-812, 2017.

17. Dahlin JL, Nissink JW, Strasser JM, Francis S, Higgins L, Zhou H, Zhang Z and Walters MA: PAINS in the assay: Chemical mechanisms of assay interference and promiscuous enzymatic inhibition observed during a sulfhydryl-scavenging HTS. J Med Chem 58: 2091-2113, 2015.

18. Jang MK, Mohizuki K, Zhou M, Jeong HS, Brady JN and Ozato K: The bromodomain protein Brd4 is a positive regulatory component of P-TEFb and stimulates RNA polymerase II-dependent transcription. Mol Cell 19: 523-534, 2005.

19. Yang Z, He N and Zhou Q: Brd4 recruits P-TEFb to chro.

20. Weber E, Rothenaigner I, Brandner S, Hadian K and Schorpp K: A high-throughput screening strategy for development of RNF8-Ubc13 protein-protein interaction inhibitors. SLAS Discov 22: 316-323, 2017.

21. Dahlin JL, Nissink JW, Strasser JM, Francis S, Higgins L, Zhou H, Zhang Z and Walters MA: PAINS in the assay: Chemical mechanisms of assay interference and promiscuous enzymatic inhibition observed during a sulfhydryl-scavenging HTS. J Med Chem 58: 2091-2113, 2015.

22. Jang MK, Mohizuki K, Zhou M, Jeong HS, Brady JN and Ozato K: The bromodomain protein Brd4 is a positive regulatory component of P-TEFb and stimulates RNA polymerase II-dependent transcription. Mol Cell 19: 523-534, 2005.

23. Yang Z, He N and Zhou Q: Brd4 recruits P-TEFb to chro.