HHS Public Access

Author manuscript

Expert Opin Ther Targets. Author manuscript; available in PMC 2024 May 14.

Published in final edited form as:

Expert Opin Ther Targets. 2023 January ; 27(1): 1–7. doi:10.1080/14728222.2023.2175317.

Bromodomain-containing protein 4 (BRD4): A key player in inflammatory bowel disease and potential to inspire epigenetic therapeutics

Zonghui Ma[a], **Andrew A. Bolinger**[a], **Jia Zhou**[a,*], **Bing Tian**[b,*]

[a]Chemical Biology Program, Department of Pharmacology and Toxicology University of Texas Medical Branch, Galveston, TX 77555, USA;

[b]Department of Internal Medicine, University of Texas Medical Branch, Galveston, TX 77555, USA.

Abstract

Introduction: The prevalence of inflammatory bowel diseases (IBDs) is increasing worldwide, and this class of chronic inflammatory disorders severely threaten human health. Epigenetic regulator bromodomain-containing protein 4 (BRD4) is critical in controlling gene expression of IBD-associated inflammatory cytokine networks. BRD4 is also tightly associated with many other diseases, such as airway inflammation and fibrosis, cancers, infectious diseases and central nervous system disorders.

Areas Covered: This review briefly summarized the critical role of BRD4 in the pathogenesis of IBD and the current clinical landscape of bromodomain and extra terminal domain (BET) inhibitor development. The challenges and opportunities as well as future directions of targeting BRD4 inhibition for IBD were also discussed.

Expert Opinion: Targeting BRD4 with potent and specific inhibitors may offer novel effective therapeutics for IBD patients, particularly those who are refractory to anti-TNFα therapy and IBD-related profibrotic. Developing highly specific BRD4 inhibitors for IBD medications may help erase the drawbacks of most current pan-BET/BRD4 inhibitors, such as off-target effects, poor oral bioavailability, and low gut mucosal absorbance. Novel strategies such as combinatorial therapy, BRD4-based dual inhibitors and proteolysis targeting chimeras (PROTACs) may also have great potential to mitigate side effects and overcome drug resistance during IBD treatment.

Keywords

Bromodomain-containing protein 4 (BRD4); inflammatory bowel diseases (IBD); Ulcerative colitis (UC); Crohn's disease (CD); epigenetics; nuclear factor-κB (NFκB); drug target; anti-inflammatory therapy

*CONTACT: Dr. Jia Zhou, Chemical Biology Program, Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas 77555, United States, jizhou@utmb.edu; Dr. Bing Tian, Department of Internal Medicine, University of Texas Medical Branch, Galveston, Texas 77555, United States, bitian@utmb.edu.

1. Introduction

The debilitating chronic intestinal disorders Crohn's disease (CD) and ulcerative colitis (UC) are two major subtypes of inflammatory bowel diseases (IBDs) [1]. Ulcerative colitis is commonly seen in the colon, with superficial mucosal inflammation that may extend contiguously, leading to serious health issues such as ulcerations, toxic megacolon, severe bleeding, and fulminant colitis. Compared to UC, CD may affect any part of the entire digestive tract, often in a noncontiguous manner, and is featured with transmural inflammation that may result in complications such as fibrotic strictures, fistulas, and abscesses [1]. IBD is reported to affect approximately 1.6 million Americans and has been recognized as a chronic inflammatory disease widely known with symptom recurrence, significant disease burden, and morbidity [2]. Furthermore, long-term persistence of chronic colonic inflammation and fibrosis associated with IBD represents one of the major risk factors contributing to colonic neoplastic transformation and the development of colitis-associated colon cancer, responsible for 10~15% of IBD-associated deaths [3, 4]. The chronic mucosal inflammation in IBD is linked to the persistent hyperactivation of innate and adaptive immune cells that produce high levels of pro-inflammatory cytokines, including tumor necrosis factor α (TNFα), interleukin-6 (IL-6), interleukin-17A (IL-17A), interferon-γ (IFN-γ), and interleukin-13 (IL-13) resulting in intestinal tissue damage [5, 6]. A great deal of interest has been focused on maximizing the probability of treatment success for IBD, but gaps remain due to the lack of efficient medications and a comprehensive understanding of its pathophysiology. While showing only limited efficacy to relieve some symptoms [7], the currently available small molecule drugs such as 5-aminosalicylate (5-ASA) have some side effects, such as flatulence, abdominal pain, nausea, diarrhea, headache, dyspepsia, and nasopharyngitis and other concerns like unknown exact drug targets and action modes [8, 9, 10, 11]. The FDA-approved monoclonal antibodies such as adalimumab and infliximab, known as TNFα blockers, are more effective and have been prescribed as the mainstay of medications for severe IBD. However, anti-TNFα therapy is associated with systemic immunosuppression, and ~40% of patients are non-responsive [12, 13]. In our recent comprehensive review paper [14], we have summarized the advances in the target-based drug discovery and pre-clinical or clinical development of novel small molecules towards IBD therapeutics that have been designed to target biologically relevant signaling pathways involved in mucosal inflammation, such as intracellular enzymes (e.g., Janus kinases, receptor-interacting protein, phosphodiesterase 4, and IκB kinase), G protein-coupled receptors (e.g., S1P, CCR9, CXCR4, CB2), integrins, and inflammasome mediators (NLRP3). Recently, we have identified the epigenetic regulator BRD4 as an overlooked yet significant regulator of inflammation in the gastrointestinal (GI) tract and a promising epigenetic therapeutic target for treatment of IBD patients [14, 15, 16].

2. The role of BRD4 during the pathogenesis of IBD

Approximately 110 amino acids make up the evolutionarily conserved protein-protein interface known as a bromodomain, which recognizes and binds acetylated lysine residues in histones and many other relevant protein partners [15, 16, 17]. All bromodomain and extra terminal domain (BET) members share two highly sequence conserved bromodomains, BD1 and BD2 [17]. Consisting of four related proteins (a.k.a. BRD2, BRD3, BRD4, and

BRDT), the BET family members act as epigenetic readers with broad specificity and critical functions on transcriptional activation [15, 16, 17]. Among the BET proteins, BRD4 plays a prominent role in the development and progression of cardiovascular, metabolic, and inflammatory diseases [15, 16, 17]. Therefore, BET family proteins, especially BRD4, have emerged as promising epigenetic therapeutic targets for various human diseases [15, 16, 18, 19, 20, 21, 22, 23, 24].

BRD4 is an epigenetic regulator of chromatin structure and controls the expression of therapeutically important and IBD-relevant inflammatory gene networks by recruiting transcription factors to form mediator complexes [14, 15, 17]. Disrupting the protein-protein interactions between BRD4 and acetylated lysine residues in histones and transcription factors blocks cell proliferation in cancer and inflammatory cytokines production in acute and chronic airway inflammation [15, 25, 26, 27, 28, 29, 30, 31]. Chronic mucosal inflammation in IBD results in the hyperactivation of innate and adaptive immune cells, which produce high levels of inflammatory cytokines, including TNFα, IL-6, and IL-17A, resulting in intestinal tissue damage [5, 6]. We and others reported that BRD4 regulates the production of key inflammatory cytokines implicated in CD and UC, such as TNF-α and type 1, 2, and 17 inflammatory cell responses (IL-1, IL-6, IFN-γ, and IL-13) [32, 33]. Furthermore, BRD4-mediated increase of inflammatory cytokines given their central role in the activation of NFκB [17] plays a critical role in the pathogenesis of IBD (Figure 1) [34].

BRD4 is an essential transcriptional coactivator of the NFκB activity, which is critically involved in regulating pro-inflammatory cytokine gene expression as an important component of the positive transcriptional elongation factor (P-TEFb) complex [17, 35] (Figure 1). BRD4 is a key molecule that is required for the stabilization of NFκB binding on the promoters of relevant inflammatory genes, activation of important markers such as RNA-Pol II, and H3K122Ac formation to permit high levels of inflammatory gene expressions in cellular events by sentinel immune cells, epithelial cells, and fibroblasts [17, 31, 36, 37, 38, 39, 40] (Figure 1). We found that the abundance of BRD4 activation marker H3K122Ac significantly increased in human IBD (both UC and CD) patient samples in comparison with healthy controls. BRD4 regulates the expression of multiple inflammatory genes of therapeutic relevance to IBD through direct interactions with acetylated lysine of proteins, including histones [41, 42, 43]. BRD4 was also known to regulate mouse Th17 cell differentiation positively, and its inhibition significantly downregulates Th17 cell activity [30, 44]. A similar observation was reported concerning the Th2 cytokine IL-13 [44, 45]. Oncostatin M (OSM) is a key protein of the IL-6 cytokine family generated by several innate and adaptive immune cells in IBD, which is now considered critical to the pathogenesis of IBD [46]. A high expression level of OSM is strongly believed to contribute to the therapeutic failure of TNFα blockers. Our results revealed that BRD4 is an essential molecule for OSM-associated signaling to activate immune cells and fibroblasts [47]. Taken together, targeting BRD4 inhibition may open a new avenue to develop novel medications to overcome OSM-mediated anti-TNFα resistance in IBD treatment. In addition, pro-inflammatory activation of fibroblasts has been widely known to play an essential role in the extracellular matrix (ECM) remodeling process during profibrotic changes in IBD [48, 49]. Although there is significant progress in our understanding of fibrosis, which is believed to be secondary to intestinal inflammation in IBD, developing

novel drugs that are also effective for attenuating fibrosis remains an unmet medical need. Accumulating evidence supports that targeting BRD4 with potent and target-specific small molecules may offer a unique approach to suppress profibrotic molecule productions as well as the consequent ECM remodeling process in gastrointestinal mucosa associated with chronic IBD.

3. Development of BRD4 inhibitors as a novel therapeutic strategy for IBD.

Over the past decade, the number of small-molecule BET inhibitors has expanded dramatically [15, 16, 50, 51]. Since the discovery of the first two BET inhibitors, GSK525762 and JQ1, various new BET inhibitors have been developed [15, 16, 50, 51]. They serve a multitude of functions in various tumors, diabetes, chronic kidney failure, coronary artery disease, and other infectious diseases for drug discovery and development [14, 15, 16, 17, 50, 51]. Some of them, such as RVX-208/Apabetalone, AZD5153, ABBV-075, BMS-986158, CPI-0610, GSK525762, OTX-015, PLX51107, INCB054329, INCB057643, ODM-207, CC-90010, and FT-1101 and TEN-010 (chemical structures shown in Figure 2) have been enrolled into different phases of human clinical trials (Table 1) [15, 16, 50, 51].

The majority of current clinical advancement of BET inhibitors remains in the early stages [15, 16, 50, 51]. Also, various side effects from pan-BET inhibitors have been reported in different studies [15, 16, 50, 51]. A major limitation of pharmacological approaches to modulating BRD4 is that most of the previous inhibitors are pan-BET inhibitors and have narrow or no selectivity (e.g., JQ1 and Phase III candidate RVX-208 with almost identical IC_{50}s for other BET family proteins), which may result in potential toxicity and off-target effects [15, 50, 51, 52, 53]. While several BRD4 inhibitors are currently available in clinical development, primarily for anticancer, their use for such a complex chronic disease as IBD was taken with caution due to the lack of specificity, low oral bioavailability, and/or gut mucosal absorbance [14, 15, 50, 51]. The abovementioned issues prompted our team to develop new BRD4 inhibitors with high specificity, low GI toxicity, and superior bioavailability/absorption when given orally. Currently, strategies for developing highly specific BRD4 inhibitots include structure-based drug design, high throughput screen (HTS), and repurposing of approved and clinical drugs, *etc*. Utilizing structure-based drug design facilitated by the Protein Data Bank (PDB) information including crystal co-complex binding modes, we identified three series of proprietary highly potent and specific BRD4 inhibitors (ZL0454 [54, 55], ZL0590 [56], and ZL0516 [57]) with nanomolar IC_{50} values as our lead compounds (Figure 2). We found that compared to generic NFκB inhibitors (e.g., IKK inhibitors), BRD4 inhibitors, including ZL0454, ZL0590, ZL0516, reduce the inflammatory response but do not affect the anti-apoptotic response. Consequently, BRD4 inhibition is much safer, better tolerated, and not associated with epithelial apoptosis, late-stage inflammation, or delayed wound healing.

Our novel BRD4 inhibitors demonstrate greater potency and BRD4 specificity than many others being advanced into the clinic, exemplified in Table 1 and compared in our models [14, 15, 54, 55, 56, 57]. We observed that BRD4 inhibitor ZL0454 can block NFκB activation and inflammatory responses (TNF-α, RelA, CCL2, IL-6, IFN-γ, IL-17A, Tbet,

and RORc) in human IBD (both CD and UC) biopsy tissues. Furthermore, we found that after administration of BRD4 inhibitors, including ZL0454, ZL0590, ZL0516, normalization of the tissue architecture and dramatic reduction in the inflammatory score were achieved in three mouse IBD models, namely dextran sulfate sodium (DSS)-induced acute colitis, oxazolone (OXA)-induced chronic colitis, and Cbir1 T cell transfer chronic colitis. Also, after treatment with BRD4 inhibitors, a dramatic reduction in the colonic expressions of inflammatory cytokines, particularly TNFα, IL-6, and IL-17A, was observed in all these mouse IBD models. Furthermore, these BRD4 inhibitors blocked the upregulation of BRD4 activity and NFκB activation in the colon of the colitis mice. In addition, BRD4 inhibitors such as ZL0516 and ZL0590, displayed extremely strong safety profiles in both *in vitro* and *in vivo* studies. Acute toxicity, abnormal manifestations and death were not observed at two single high doses (100 mg/kg; 300 mg/kg) during the toxicity studies. Collectively, this new therapeutic strategy may effectively treat IBD patients, particularly those who are refractory to anti-TNFα therapy (e.g., anti-TNFα antibodies) and treatment of IBD-related profibrotic processes [46, 58, 59, 60].

4. Conclusion

In this review, we summarize the critical roles of BRD4 in the pathogenesis of inflammatory bowel disease and the current clinical landscape of BET/BRD4 inhibitor drug discovery and development. Targeting BRD4 could be an attractive approach to abrogate inflammation and/or fibrosis in IBD. BRD4 is a major epigenetic regulator of inflammation in the GI tract, which is critical in controlling the expression of IBD-relevant inflammatory cytokine networks. BRD4-mediated increase of inflammatory cytokines is due to its vital role in the activation of NFκB, which plays a critical role in the pathogenesis of IBD. BRD4 inhibition will disrupt its interactions with acetylated histone lysine residues, thereby blocking the pathological activation of the BRD4-mediated signaling, leading to the suppression and reversing of colonic inflammation and fibrosis in IBD. This new therapeutic strategy may offer novel epigenetic therapy as an effective treatment of IBD patients, particularly those who are refractory to anti-TNFα therapy and treatment of IBD-related profibrotic processes.

5. Expert opinion

As one important class of chronic inflammatory disorders, the prevalence of IBD is steadily increasing worldwide, severely threatening global human health. To date, a comprehensive understanding of IBD pathogenesis remains to be realized. Even though considerable advances have been achieved in drug discovery and development for IBD treatment in recent decades, there still exists a tremendous gap with unmet clinical needs for effective IBD therapy. Therefore, exploring more effective therapeutic options for IBD patients, especially targeting novel distinct drug targets, is urgently needed to overcome the drawbacks of currently available therapies and attract increasing attention in the relevant research fields.

BRD4 is an epigenetic regulator of chromatin structure, playing a prominent role in the development and progression of human cancer, cardiovascular, metabolic, and inflammatory diseases. Thus, BRD4 has emerged as a promising epigenetic target for various human diseases. BRD4 controls the gene expression of IBD-relevant inflammatory cytokine

networks by recruiting key transcription factors to form important mediator complexes. As reported by our group and others, BRD4 regulates the production of key inflammatory cytokines implicated in both CD and UC (e.g., TNF-α) and type 1, 2, and 17 inflammatory cell responses (e.g., IL-1, IL-6, IFN-γ, and IL-13). Furthermore, BRD4 plays a central role in the activation of NFκB, increasing inflammatory cytokines, which was tightly associated with the pathogenesis of IBD. Therefore, BRD4 inhibition, especially potent and specific small-molecule BRD4 inhibitors, disrupts the critical protein-protein interactions (PPIs) between BRD4 protein and acetylated lysine or other protein partners in the epigenetic machinery and may pave the avenue for the treatment of inflammation-related diseases, including IBD. Over the past decade, numerous small-molecule BET/BRD4 inhibitors have been discovered and developed. Although some BET/BRD4 inhibitors have advanced into clinical trials, as shown in Table 1, most of them remain in the early stage. Most of these inhibitors are pan-BET inhibitors owing to a lack of subtype and/or domain selectivity. A diverse range of side effects has been reported for pan-BET inhibitors due to the lack of selectivity in different studies, for example, thrombocytopenia, testis toxicity, gastrointestinal toxicity, *etc.*, which potentially prevents the assessment of the full potential of their mode of action. Moreover, low oral bioavailability and/or gut mucosal absorbance hamper their clinical application advancement in disease models. Therefore, developing potent and domain-selective BRD4 inhibitors with good druggability is imperative to help mitigate the risks in clinical trials and other pharmacological studies.

Based on continued devoted efforts, some more relatively domain-selective BRD4 inhibitors have been discovered and reported. Novel BRD4 inhibitors with high potency and specificity, such as ZL0454, ZL0590, and ZL0516, reduce the inflammatory response but do not affect the anti-apoptotic response, suggesting that they are much safer and better tolerated than generic NFκB inhibitors. Selective BRD4 inhibitors such as ZL0454 *ex-vivo* in IBD inflamed tissue explants blocked NFκB activation and inflammatory responses in human IBD (CD and UC) biopsy tissues. ZL0454 can normalize the tissue architecture and dramatically reduce the inflammatory score in IBD animal models. Moreover, ZL0454 can dramatically reduce the colonic expressions of inflammatory cytokines, especially TNFα, IL-6, and IL-17A. Furthermore, ZL0454 can block the upregulation of BRD4 activity and NFκB activation in the colon of colitis mice. This suggests that potent and selective BRD4 inhibitors may serve as a promising epigenetic therapeutic strategy, offering effective treatment opportunities for IBD patients, especially those who are refractory to anti-TNFα therapy and IBD-related profibrotic.

Some kinase inhibitors have been repurposed as novel BRD4 inhibitors with advantages such as high potency, and excellent oral bioavailability. In recent years, other novel strategies, such as developing combinatorial therapy, dual inhibitors and protein degraders like proteolysis targeting chimeras (PROTACs), have been widely applied for targeting BRD4 protein inhibition and degradation. Combinatorial therapy and dual inhibitors exhibit synergistic effects, which contribute to improving the therapeutic potency and overcoming drug-resistence [61]. Especially, the protein degrdation technology including molecular glue and PROTAC degraders has been booming in recent years, acting as an effective tool for inducing target protein degradation at a catalysis amount through the ubiquitin-proteasome system (UPS) [62]. As reported, PROTACs targeting BRD4 may decrease the side effects

by lowering the administration dose and improving target protein specificity. Moreover, PROTACs may also serve as useful pharmacological tools for exploring the roles of BRD4 protein in human diseases. In short, developing BRD4-based dual inhibitors and protein degraders may offer unique target-based therapeutic opportunities for treating various human diseases, including IBD.

Funding

This work was partially supported by Crohn's & Colitis Foundation Entrepreneurial Investing (EI) Initiative award (JZ and BT), Litwin IBD Pioneers Program Award (BT and JZ) from the Crohn's & Colitis Foundation of America as well as the John D. Stobo, M.D. Distinguished Chair Endowment Fund (JZ).

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

References

1. Chang JT. Pathophysiology of Inflammatory Bowel Diseases. N Engl J Med. 2020 Dec 31;383(27):2652–2664. [PubMed: 33382932]
2. Henson MA, Phalak P. Microbiota dysbiosis in inflammatory bowel diseases: in silico investigation of the oxygen hypothesis. BMC Syst Biol. 2017 Dec 28;11(1):145. [PubMed: 29282051]
3. Eaden JA, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. Gut. 2001 Apr;48(4):526–35. [PubMed: 11247898]
4. Eaden J Review article: colorectal carcinoma and inflammatory bowel disease. Alimentary pharmacology & therapeutics. 2004 Oct;20 Suppl 4:24–30.
5. Strober W, Fuss IJ. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. Gastroenterology. 2011 May;140(6):1756–67. [PubMed: 21530742]
6. Slebioda TJ, Kmiec Z. Tumour necrosis factor superfamily members in the pathogenesis of inflammatory bowel disease. Mediators of inflammation. 2014;2014:325129. [PubMed: 25045210]
7. Akobeng AK, Gardener E. Oral 5-aminosalicylic acid for maintenance of medically-induced remission in Crohn's Disease. Cochrane Database Syst Rev. 2005 Jan 25(1):CD003715.
8. Peyrin-Biroulet L, Lemann M. Review article: remission rates achievable by current therapies for inflammatory bowel disease. Aliment Pharmacol Ther. 2011 Apr;33(8):870–9. [PubMed: 21323689]
9. Ye B, van Langenberg DR. Mesalazine preparations for the treatment of ulcerative colitis: Are all created equal? World journal of gastrointestinal pharmacology and therapeutics. 2015 Nov 06;6(4):137–44. [PubMed: 26558148]
10. Wang Y, Parker CE, Feagan BG, et al. Oral 5-aminosalicylic acid for maintenance of remission in ulcerative colitis. Cochrane Database Syst Rev. 2016 May 9;2016(5):CD000544. [PubMed: 27158764]
11. Murray A, Nguyen TM, Parker CE, et al. Oral 5-aminosalicylic acid for maintenance of remission in ulcerative colitis. Cochrane Database of Systematic Reviews 2020;2020(8).
12. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. The New England journal of medicine. 2005 Dec 08;353(23):2462–76. [PubMed: 16339095]
13. Ma C, Evaschesen CJ, Gracias G, et al. Inflammatory bowel disease patients are frequently nonadherent to scheduled induction and maintenance infliximab therapy: A Canadian cohort study. Canadian journal of gastroenterology & hepatology. 2015 Aug-Sep;29(6):309–14. [PubMed: 26069894]

14. Li Y, Chen J, Bolinger AA, et al. Target-Based Small Molecule Drug Discovery Towards Novel Therapeutics for Inflammatory Bowel Diseases. Inflamm Bowel Dis 2021 Nov 15;27(Suppl 2):S38–S62. [PubMed: 34791293]
15. Liu Z, Wang P, Chen H, et al. Drug Discovery Targeting Bromodomain-Containing Protein 4. J Med Chem. 2017 Jun 8;60(11):4533–4558. [PubMed: 28195723]
16. Liang D, Yu Y, Ma Z. Novel strategies targeting bromodomain-containing protein 4 (BRD4) for cancer drug discovery. Eur J Med Chem. 2020 Aug 15;200:112426. [PubMed: 32502863]
17. Wang N, Wu R, Tang D, et al. The BET family in immunity and disease. Signal Transduct Target Ther. 2021 Jan 19;6(1):23. [PubMed: 33462181]
18. Belkina AC, Denis GV. BET domain co-regulators in obesity, inflammation and cancer. Nature reviews Cancer. 2012 Jun 22;12(7):465–77. [PubMed: 22722403]
19. Zhu J, Gaiha GD, John SP, et al. Reactivation of latent HIV-1 by inhibition of BRD4. Cell reports. 2012 Oct 25;2(4):807–16. [PubMed: 23041316]
20. Li Z, Guo J, Wu Y, et al. The BET bromodomain inhibitor JQ1 activates HIV latency through antagonizing Brd4 inhibition of Tat-transactivation. Nucleic acids research. 2013 Jan 7;41(1):277–87. [PubMed: 23087374]
21. Korb E, Herre M, Zucker-Scharff I, et al. BET protein Brd4 activates transcription in neurons and BET inhibitor Jq1 blocks memory in mice. Nature neuroscience. 2015 Oct;18(10):1464–73. [PubMed: 26301327]
22. Duan Q, McMahon S, Anand P, et al. BET bromodomain inhibition suppresses innate inflammatory and profibrotic transcriptional networks in heart failure. Science translational medicine. 2017 May 17;9(390):eaah5084. [PubMed: 28515341]
23. Niu Q, Liu Z, Alamer E, et al. Structure-guided drug design identifies a BRD4-selective small molecule that suppresses HIV. J Clin Invest. 2019 Jul 22;129(8):3361–3373. [PubMed: 31329163]
24. Brasier AR, Zhou J. Validation of the epigenetic reader bromodomain-containing protein 4 (BRD4) as a therapeutic target for treatment of airway remodeling. Drug Discov Today 2020 Jan;25(1):126–132. [PubMed: 31733396]
25. Bachu M, Dey A, Ozato K. Chromatin Landscape of the IRF Genes and Role of the Epigenetic Reader BRD4. Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research. 2016 Jul;36(7):470–5. [PubMed: 27379869]
26. Chen J, Wang Z, Hu X, et al. BET Inhibition Attenuates Helicobacter pylori-Induced Inflammatory Response by Suppressing Inflammatory Gene Transcription and Enhancer Activation. Journal of immunology. 2016 May 15;196(10):4132–42.
27. Ding N, Hah N, Yu RT, et al. BRD4 is a novel therapeutic target for liver fibrosis. Proceedings of the National Academy of Sciences of the United States of America. 2015 Dec 22;112(51):15713–8. [PubMed: 26644586]
28. Xiao Y, Liang L, Huang M, et al. Bromodomain and extra-terminal domain bromodomain inhibition prevents synovial inflammation via blocking IkappaB kinase-dependent NF-kappaB activation in rheumatoid fibroblast-like synoviocytes. Rheumatology. 2016 Jan;55(1):173–84. [PubMed: 26324948]
29. Schmidt SF, Larsen BD, Loft A, et al. Acute TNF-induced repression of cell identity genes is mediated by NFkappaB-directed redistribution of cofactors from super-enhancers. Genome research. 2015 Sep;25(9):1281–94. [PubMed: 26113076]
30. Cheung K, Lu G, Sharma R, et al. BET N-terminal bromodomain inhibition selectively blocks Th17 cell differentiation and ameliorates colitis in mice. Proc Natl Acad Sci U S A. 2017 Mar 14;114(11):2952–2957. [PubMed: 28265070]
31. Tian B, Yang J, Zhao Y, et al. BRD4 Couples NF-kappaB/RelA with Airway Inflammation and the IRF-RIG-I Amplification Loop in Respiratory Syncytial Virus Infection. Journal of virology. 2017 Mar 15;91(6): doi: 10.1128/JVI.00007-17.
32. Wu X, Qi J, Bradner JE, et al. Bromodomain and extraterminal (BET) protein inhibition suppresses human T cell leukemia virus 1 (HTLV-1) Tax protein-mediated tumorigenesis by inhibiting nuclear factor kappaB (NF-kappaB) signaling. J Biol Chem. 2013 Dec 13;288(50):36094–105. [PubMed: 24189064]

33. Gibbons HR, Mi DJ, Farley VM, et al. Bromodomain inhibitor JQ1 reversibly blocks IFN-gamma production. Sci Rep. 2019 Jul 16;9(1):10280. [PubMed: 31311960]
34. Pasparakis M Role of NF-kappaB in epithelial biology. Immunol Rev. 2012 Mar;246(1):346–58. [PubMed: 22435565]
35. Huang B, Yang XD, Zhou MM, et al. Brd4 coactivates transcriptional activation of NF-kappaB via specific binding to acetylated RelA. Mol Cell Biol. 2009 Mar;29(5):1375–87. [PubMed: 19103749]
36. Yang J, Tian B, Brasier AR. Targeting Chromatin Remodeling in Inflammation and Fibrosis. Advances in protein chemistry and structural biology. 2017;107:1–36. [PubMed: 28215221]
37. Ijaz T, Jamaluddin M, Zhao Y, et al. Coordinate activities of BRD4 and CDK9 in the transcriptional elongation complex are required for TGFbeta-induced Nox4 expression and myofibroblast transdifferentiation. Cell death & disease. 2017 Feb 9;8(2):e2606. [PubMed: 28182006]
38. Tian B, Zhao Y, Sun H, et al. BRD4 mediates NF-kappaB-dependent epithelial-mesenchymal transition and pulmonary fibrosis via transcriptional elongation. Am J Physiol Lung Cell Mol Physiol. 2016 Dec 1;311(6):L1183–L1201. [PubMed: 27793799]
39. Tian B, Zhao Y, Kalita M, et al. CDK9-dependent transcriptional elongation in the innate interferon-stimulated gene response to respiratory syncytial virus infection in airway epithelial cells. Journal of virology. 2013 Jun;87(12):7075–92. [PubMed: 23596302]
40. Brasier AR, Tian B, Jamaluddin M, et al. RelA Ser276 phosphorylation-coupled Lys310 acetylation controls transcriptional elongation of inflammatory cytokines in respiratory syncytial virus infection. Journal of virology. 2011 Nov;85(22):11752–69. [PubMed: 21900162]
41. Zeng L, Zhou MM. Bromodomain: an acetyl-lysine binding domain. FEBS letters 2002 Feb 20;513(1):124–8. [PubMed: 11911891]
42. Dey A, Chitsaz F, Abbasi A, et al. The double bromodomain protein Brd4 binds to acetylated chromatin during interphase and mitosis. Proceedings of the National Academy of Sciences of the United States of America. 2003 Jul 22;100(15):8758–63. [PubMed: 12840145]
43. Chiang CM. Brd4 engagement from chromatin targeting to transcriptional regulation: selective contact with acetylated histone H3 and H4. F1000 biology reports 2009;1:98. [PubMed: 20495683]
44. Cheung KL, Zhang F, Jaganathan A, et al. Distinct Roles of Brd2 and Brd4 in Potentiating the Transcriptional Program for Th17 Cell Differentiation. Mol Cell. 2017 Mar 16;65(6):1068–1080 e5. [PubMed: 28262505]
45. Tian B, Yang J, Zhao Y, et al. BRD4 Couples NF-kappaB/RelA with Airway Inflammation and the IRF-RIG-I Amplification Loop in Respiratory Syncytial Virus Infection. J Virol. 2017 Mar 15;91(6).
46. West NR, Hegazy AN, Owens BMJ, et al. Oncostatin M drives intestinal inflammation and predicts response to tumor necrosis factor-neutralizing therapy in patients with inflammatory bowel disease. Nature medicine. 2017 May;23(5):579–589.
47. Ray S, Zhao Y, Jamaluddin M, et al. Inducible STAT3 NH2 terminal mono-ubiquitination promotes BRD4 complex formation to regulate apoptosis. Cellular signalling. 2014 Jul;26(7):1445–55. [PubMed: 24657799]
48. Valatas V, Filidou E, Drygiannakis I, et al. Stromal and immune cells in gut fibrosis: the myofibroblast and the scarface. Ann Gastroenterol. 2017;30(4):393–404. [PubMed: 28655975]
49. Lawrance IC, Rogler G, Bamias G, et al. Cellular and Molecular Mediators of Intestinal Fibrosis. J Crohns Colitis 2017 Dec 4;11(12):1491–1503. [PubMed: 25306501]
50. Petretich M, Demont EH, Grandi P. Domain-selective targeting of BET proteins in cancer and immunological diseases. Curr Opin Chem Biol. 2020 Aug;57:184–193. [PubMed: 32741705]
51. Shorstova T, Foulkes WD, Witcher M. Achieving clinical success with BET inhibitors as anti-cancer agents. Br J Cancer. 2021 Apr;124(9):1478–1490. [PubMed: 33723398]
52. Filippakopoulos P, Qi J, Picaud S, et al. Selective inhibition of BET bromodomains. Nature. 2010 Dec 23;468(7327):1067–73. [PubMed: 20871596]

53. Picaud S, Wells C, Felletar I, et al. RVX-208, an inhibitor of BET transcriptional regulators with selectivity for the second bromodomain. Proceedings of the National Academy of Sciences of the United States of America. 2013 Dec 03;110(49):19754–9. [PubMed: 24248379]

54. Liu Z, Tian B, Chen H, et al. Discovery of potent and selective BRD4 inhibitors capable of blocking TLR3-induced acute airway inflammation. Eur J Med Chem. 2018 May 10;151:450–461. [PubMed: 29649741]

55. Tian B, Liu Z, Yang J, et al. Selective Antagonists of the Bronchiolar Epithelial NF-kappaB-Bromodomain-Containing Protein 4 Pathway in Viral-Induced Airway Inflammation. Cell Rep. 2018 Apr 24;23(4):1138–1151. [PubMed: 29694891]

56. Liu Z, Li Y, Chen H, et al. Discovery, X-ray Crystallography, and Anti-inflammatory Activity of Bromodomain-containing Protein 4 (BRD4) BD1 Inhibitors Targeting a Distinct New Binding Site. J Med Chem. 2022 Feb 10;65(3):2388–2408. [PubMed: 34982556]

57. Liu Z, Chen H, Wang P, et al. Discovery of Orally Bioavailable Chromone Derivatives as Potent and Selective BRD4 Inhibitors: Scaffold Hopping, Optimization, and Pharmacological Evaluation. J Med Chem. 2020 May 28;63(10):5242–5256. [PubMed: 32255647]

58. Amemiya S, Yamaguchi T, Sakai T, et al. Structure-Activity Relationship Study of N(6)-Benzoyladenine-Type BRD4 Inhibitors and Their Effects on Cell Differentiation and TNF-alpha Production. Chemical & pharmaceutical bulletin 2016;64(9):1378–83. [PubMed: 27581642]

59. Zou Z, Huang B, Wu X, et al. Brd4 maintains constitutively active NF-kappaB in cancer cells by binding to acetylated RelA. Oncogene. 2014 May 01;33(18):2395–404. [PubMed: 23686307]

60. Castellucci M, Rossato M, Calzetti F, et al. IL-10 disrupts the Brd4-docking sites to inhibit LPS-induced CXCL8 and TNF-alpha expression in monocytes: Implications for chronic obstructive pulmonary disease. The Journal of allergy and clinical immunology. 2015 Sep;136(3):781–791 e9. [PubMed: 26044852]

61. Feng L, Wang G, Chen Y, et al. Dual-target inhibitors of bromodomain and extra-terminal proteins in cancer: A review from medicinal chemistry perspectives. Med Res Rev. 2022 Mar;42(2):710–743. [PubMed: 34633088]

62. Wang P, Zhou J. Proteolysis Targeting Chimera (PROTAC): A Paradigm-Shifting Approach in Small Molecule Drug Discovery. Curr Top Med Chem. 2018;18(16):1354–1356. [PubMed: 30306871]

Article highlights

- Inflammatory bowel diseases (IBDs) are chronic intestinal disorders that severely threaten human health.
- Epigenetic regulator BRD4 is critical in controlling expression of IBD-associated inflammatory cytokine networks.
- The abundance of BRD4 activation marker H3K122Ac is significantly increased in human IBD (UC and CD) patient samples *vs.* healthy controls.
- Targeting BRD4 may offer a novel therapeutic strategy for treating IBD patients.
- Potent and specific BRD4 inhibitors may be helpful to avoid or mitigate the side effects of most pan-BET inhibitors.
- Novel strategies such as developing dual inhibitors and proteolysis targeting chimeras (PROTACs) may provide unique opportunities for targeting BRD4 towards epigenetic therapeutics against various human diseases, including IBD.

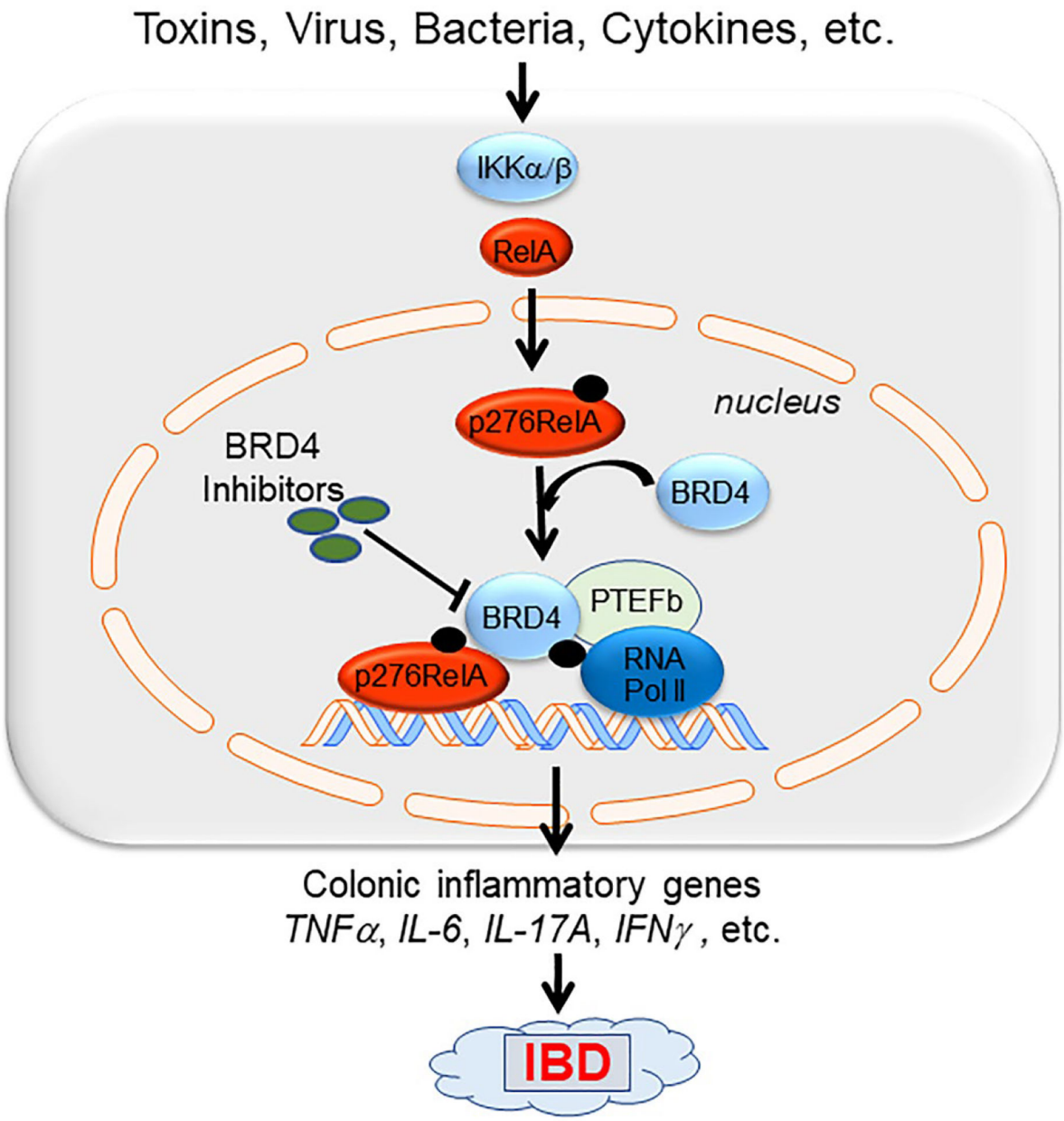

Figure 1. BRD4 is a key player during the pathogenesis of IBD and a unique drug target for novel epigenetic therapeutics of IBD including both UC and CD.

BRD4 is a major epigenetic regulator of inflammation in the gastrointestinal (GI) tract which is critical in controlling the expression of IBD-relevant inflammatory cytokine networks. BRD4-mediated increase of inflammatory cytokines is due to its vital role in the activation of NF-κB, which plays a critical role in the pathogenesis of IBD. BRD4 inhibition disrupts its interactions with acetylated histone lysine residues and other key protein partners in the epigenetic machinery, thereby blocking the pathological activation of the BRD4-mediated signaling, suppressing and reversing colonic inflammation and fibrosis in IBD.

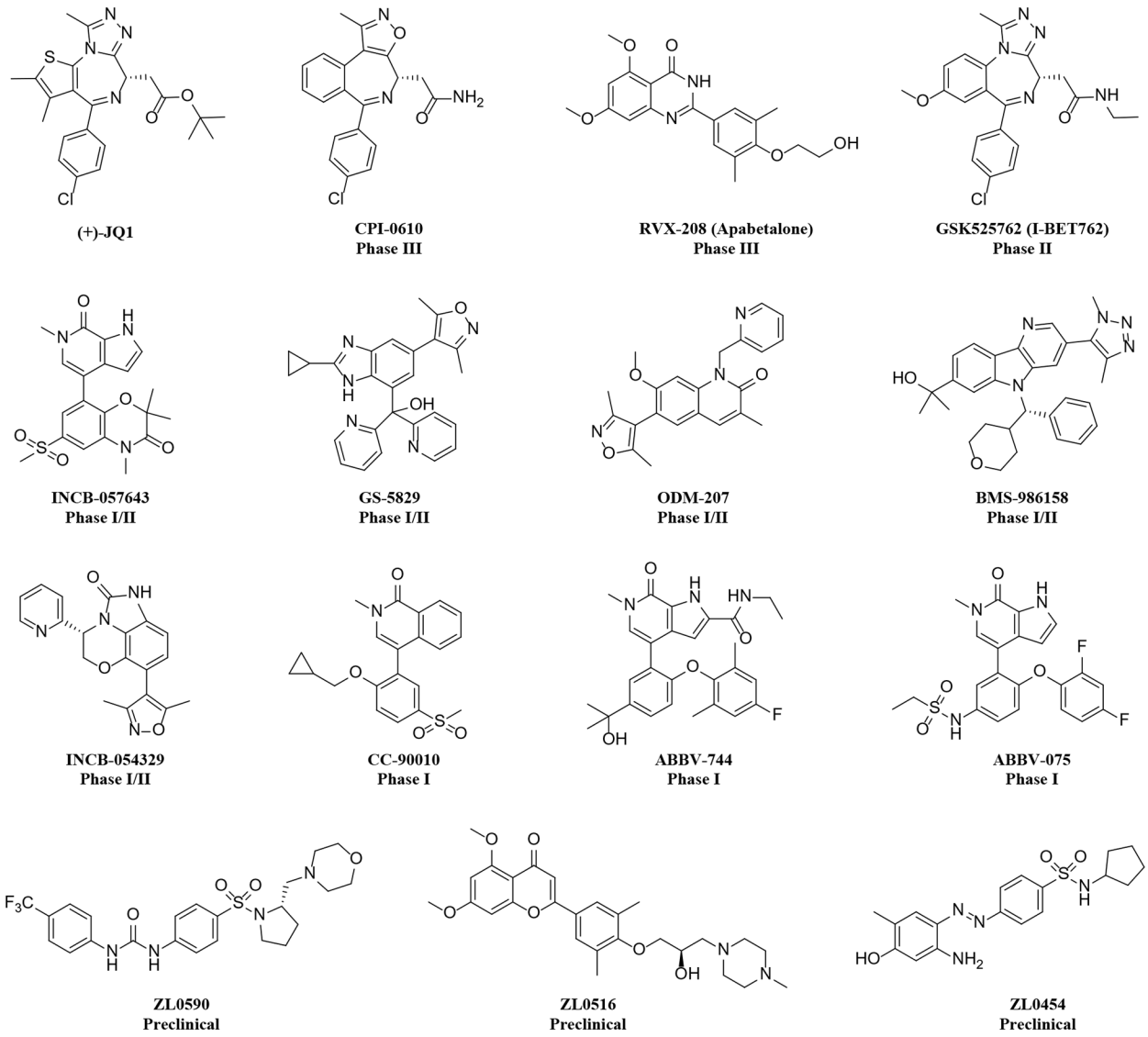

Figure 2. Chemical structures of the powerful pharmacological tool compound (+)-JQ1 and selected representative BET/BRD4 inhibitors in clinical trials and pre-clinical trials.

Table 1.

Representative BET/BRD4 Inhibitors in Clinical Trials [a]

BRD4 Inhibitors	Pharmaceuticals	Phase Stage	Indications	NCT Identifier
CPI-0610	Constellation	III	Myelofibrosis	NCT04603495
		I/II	Myelofibrosis; Hematological Malignancies	NCT02158858
		I	Advanced Malignancies	NCT05391022
RVX-208	Resverlogix	III	Type 2 Diabetes Mellitus; Coronary Artery Disease	NCT02586155
GSK525762	GlaxoSmithKline	II	Relapsed, Refractory Hematologic Malignancies	NCT01943851
OTX015	Oncoethix GmbH	II	Glioblastoma Multiforme	NCT02296476
		I/II	Acute Myeloid Leukemia	NCT02303782
INCB-057643	Incyte	I/II	Solid Tumors; Advanced Malignancies; Metastatic Cancer	NCT02959437
		I	Myelofibrosis; Other Advanced Myeloid Neoplasms	NCT04279847
PLX-2853[b]	Plexxikon	I/II	Gynecologic Neoplasms; Epithelial Ovarian Cancer	NCT04493619
		I/II	Metastatic Castration-resistant Prostate Cancer	NCT04556617
GS-5829	Gilead	I/II	Breast Cancer	NCT02983604
		I/II	Metastatic Castrate-Resistant Prostate Cancer	NCT02607228
ODM-207	Orion	I/II	Solid Tumors	NCT03035591
BMS-986158	Bristol-Myers Squibb	I/II	Myelofibrosis	NCT04817007
		I/II	Multiple Myeloma	NCT05372354
		I/II	Advanced Solid Tumors; Hematologic Malignancies	NCT02419417
INCB-054329	Incyte	I	Pediatric Cancer	NCT03936465
		I/II	Solid Tumors; Hematologic Malignancy	NCT02431260
AZD5153	AstraZeneca	I/II	Sarcomas; Malignant Peripheral Nerve Sheath Tumor	NCT05253131
		I/II	Acute Myeloid Leukemia	NCT03013998
PLX51107	Plexxikon	I/II	Acute Graft Versus Host Disease; Steroid Refractory Graft Versus Host Disease	NCT04910152
		I	Acute Myeloid Leukemia; Myelodysplastic Syndrome	NCT04022785
CC-90010	Celgene	I	Astrocytoma; Glioblastoma	NCT04047303
		I	Solid Tumors; Non-Hodgkin's Lymphomas	NCT03220347
		I	Glioblastoma	NCT04324840
		I	Pediatric Cancer	NCT03936465
		I	Small Cell Lung Carcinoma	NCT03850067
ABBV-744	AbbVie	I	Myelofibrosis	NCT04454658
		I	Acute Myeloid Leukemia	NCT03360006
BI-894999	Boehringer Ingelheim	I	Neoplasms; NUT Carcinoma	NCT02516553
ABBV-075	AbbVie	I	Breast Cancer; Non-Small Cell Lung Cancer; Acute Myeloid Leukemia; Multiple Myeloma; Prostate Cancer; Small Cell Lung Cancer; Non-Hodgkins Lymphoma	NCT02391480
FT-1101[b]	Forma Therapeutics	I	Myelofibrosis	NCT04480086
		I	Acute Myeloid Leukemia; Acute Myelogenous Leukemia; Myelodysplastic Syndrome; Non-Hodgkin Lymphoma	NCT02543879
TEN-010	Hoffmann-La Roche	I	Acute Myeloid Leukemia; Myelodysplastic Syndromes	NCT02308761
		I	Solid Tumors; Advanced Solid Tumors	NCT01987362

[a] Data collected from www.clinicaltrials.com on October 10, 2022.

[b] Structures are not disclosed.