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Anticancer benzimidazole derivatives as inhibitors of epigenetic targets: a review article

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Cancer is one of the leading causes of morbidity and mortality worldwide. One of the primary causes of cancer development and progression is epigenetic dysregulation, which is a heritable modification that alters gene expression without changing the DNA sequence. Therefore, targeting these epigenetic changes has emerged as a promising therapeutic strategy. Benzimidazole derivatives have gained attention for their potent epigenetic modulatory effects as they interact with various epigenetic targets, including DNA methyltransferases, histone deacetylases and histone methyltransferases. This review provides a comprehensive overview of benzimidazole derivatives that inhibit different acetylation and methylation reader, writer and eraser epigenetic targets. Herein, we emphasize the therapeutic potential of these compounds in developing targeted, less toxic cancer therapies. Presently, some promising benzimidazole derivatives have entered clinical trials and shown great advancements in the fields of hematological and solid malignancy therapies. Accordingly, we highlight the recent advancements in benzimidazole research as epigenetic agents that could pave the way for designing new multi-target drugs to overcome resistance and improve clinical outcomes for cancer patients. This review can help researchers in designing new anticancer benzimidazole derivatives with better properties.

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1 Introduction

Cancer is a complex disease caused by genetic and epigenetic alterations and remains a leading cause of death worldwide.[1–3] Conventional cancer therapies using alkylating agents, antimetabolites, antimicrotubular agents, antibiotics, hormones, plant alkaloids, vinca alkaloids, epipodophyllotoxins, anthracyclines, taxanes, corticosteroids, and kinase inhibitors are toxic to cancer; however, they also exhibit toxicity to normal cells, leading to some serious side effects in patients and sometimes even death.[4] There are different problems related to conventional cancer therapies, such as resistance to therapy, ineffectiveness, and the growth and reproliferation of cancerous cells if cytotoxic therapy is stopped.[5] Therefore, epigenetics has entered the field of cancer treatment with great advancements and effectiveness. Epigenetics is the study of inheritance and reversible modes originating from chemical modifications or additions to DNA without causing any changes in the primary DNA sequence.[6–8] Modifications in epigenetic regulations are the primary cause of cancer initiation, progression, and metastasis. Chromatin is a DNA and histone protein complex that is crucial in packaging DNA within the cell nucleus. Moreover, it is a dynamic structure that responds to reversible epigenetic alterations. Histones are responsible for DNA condensation and organization, making DNA accessible for replication, transcription, and repair.[7] Epigenetic modifications are regulated through three types of epigenetic proteins, *i.e.*, writers (add an epigenetic mark), readers (respond to the epigenetic mark), and erasers (remove the epigenetic mark) (Table 1).[9] These modifications include histone modification, DNA methylation and non-coding RNA (ncRNA) mutation or abnormal expression. Histone modifications include histone acetylation, methylation, phosphorylation, ubiquitination, and citrullination.[10,11] Histone acetylation is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), leading to chromatin opening and condensation, respectively.[12] Acetylation of H3 and H4 histone proteins is responsible for the relaxed conformation of chromatin by neutralizing the lysine positive charge and preventing the electrostatic interaction of histones with DNA.[13] Histone methyltransferases consist of lysine methyltransferase (KMT) and arginine methyltransferase (PRMT) families, which are responsible for various biological processes (cell cycle regulation, cell division and proliferation, survival, and genome stability) and RNA metabolism (RNA biogenesis, processing, splicing, gene expression, chromatin organization, translation, and microtubule-associated metabolic processes), respectively.[14] KMT therapeutic targets include EZH2, DOT1L, and G9a.[15] Lysine demethylase enzymes (KDMs)

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Table 1 Summary of epigenetic modifications

Type of modification	Place	Writer	Eraser	Reader
Acetylation	Histone	GNAT, P300/CBP, and MYST	Class I (HDAC 1, 2, 3, and 8), Class IIa (HDAC 4, 5, 7, and 9), Class IIb (HDAC 6 and 10), Class IV (HDAC 11), and Class III or sirtuins (SIRT1 to SIRT7)	Bromodomains
Methylation	Lysine	DOT1L, EZH2, G9a, and MLL1	Lysine-specific demethylase 1 (LSD1, BHC110 or KDM1), lysine-specific demethylase 2 (LSD2), and jumonji domain-containing protein (JMJD)	Plant homeodomains (PHDs), WD40 repeat (WDR) domains, royal superfamily domains
	Arginine	Type I PRMT enzymes (PRMT1, -2, -3, -4, -6, and -8), type II enzymes (PRMT5 and PRMT9), and type III enzymes (PRMT7)	PAD4 and JMJD6	Tudor domains, WD40 repeat (WDR) domains, and plant homeodomains (PHDs)
	DNA	DNMT1, DNMT3a, and DNMT3b	Ten-eleven translocation (TET)	Methyl-CpG binding domain (MBD) family, Kaiso family, and SET- and ring finger-associated (SRA) domain family

are divided into three families including lysine-specific demethylase 1 (LSD1 or KDM1A), lysine-specific demethylase 2 (LSD2) and Jumonji C domain-containing demethylases (JmjC). The JmjC members were identified as KDM 2–7.[16] PRMTs are divided into type I (PRMT1, 2, 3, 4, 6, and 8), II (PRMT5 and 9), and III (PRMT7), while arginine demethylases include JMJD6 and PAD4.[17] Phosphorylation, a critical histone modification, involves adding a phosphate group by kinases.[18] DNA methylation is primarily mediated by DNMT1, DNMT3a, and DNMT3b, which transfer the methyl group provided by SAM[10] by covalent addition of a methyl group to the fifth carbon of the cytosine base,[19] and DNA demethylase enzymes (erasers) including ten-to-eleven translocation (TET) proteins.[20] Non-coding RNA (ncRNA) does not encode proteins but is involved in regulating a variety of biological processes in cancer progression, such as proliferation, apoptosis, migration, and invasion.[10] ncRNA includes long non-coding RNAs (lncRNA) and small non-coding RNAs such as miRNA, snoRNAs, siRNA and piRNA.[21] Epigenetic dysregulation can cause developmental disorders, autoimmune diseases, neurodegenerative diseases, psychological disorders, and cancer. In cancer, it plays critical roles in cancer stages such as carcinogenesis, metastasis, and drug resistance development, and thus can be a potential preventive and diagnostic marker.[2,22]

Benzimidazole is a bicyclic aromatic organic compound consisting of a benzene ring fused to a five-membered imidazole ring at the 4- and 5-positions of the imidazole ring. Its IUPAC name is 1*H*-benzimidazole and it is also known as 1,3-benzodiazole, benzoglyoxaline, iminazole, and imidazole.[23–25] It is a weak base with sufficient NH-acidic to be soluble in aqueous alkali and has p*K*a values of 5.3 and 12.3 for pK_{a1} and pK_{a2}, respectively. It appears as white tabular crystals with a molecular weight of 118.14 g mol^{-1}.[23,26] The benzimidazole nucleus is a promising scaffold with different biological activities. This importance began when Woolley discovered the structural similarity of 2-aminobenzimidazole with purine[27] and when 5,6-dimethylbenzimidazole was discovered as a degradation product of vitamin-B_{12}.[28] In 1872, Hoebrecker was the first to synthesize the benzimidazole ring through the reduction and dehydration of 2-nitro-4-methylacetanilide (Fig. 1), and its tautomerism.[29] Nowadays, the benzimidazole ring can be synthesized from *o*-phenylenediamine through condensation with aldehydes, ketones, and carboxylic acids and *via* the rearrangement of benzodiazepinones, quinoxaline derivatives, and 1,2,3-triazole.[30] The 1, 2 and/or 5(or 6) positions of the benzimidazole ring are the most important positions to be substituted to obtain different biologically active compounds.[31] Substituted benzimidazole can act as antimicrobial, antituberculosis, antiviral, antiulcer, anti-inflammatory, antidiabetic, anticonvulsant, antihypertensive, antimalarial, proton pump inhibitor, anti-HIV and anticancer agents.[23,24,32,33] Benzimidazole has multiple mechanisms to act as an anticancer agent

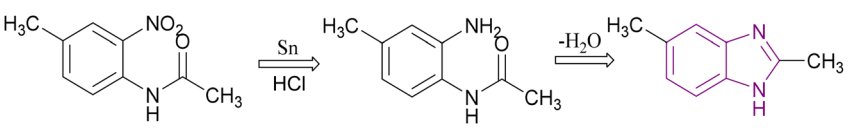


Fig. 1 Synthesis of benzimidazole derivative by Hoebrecker.

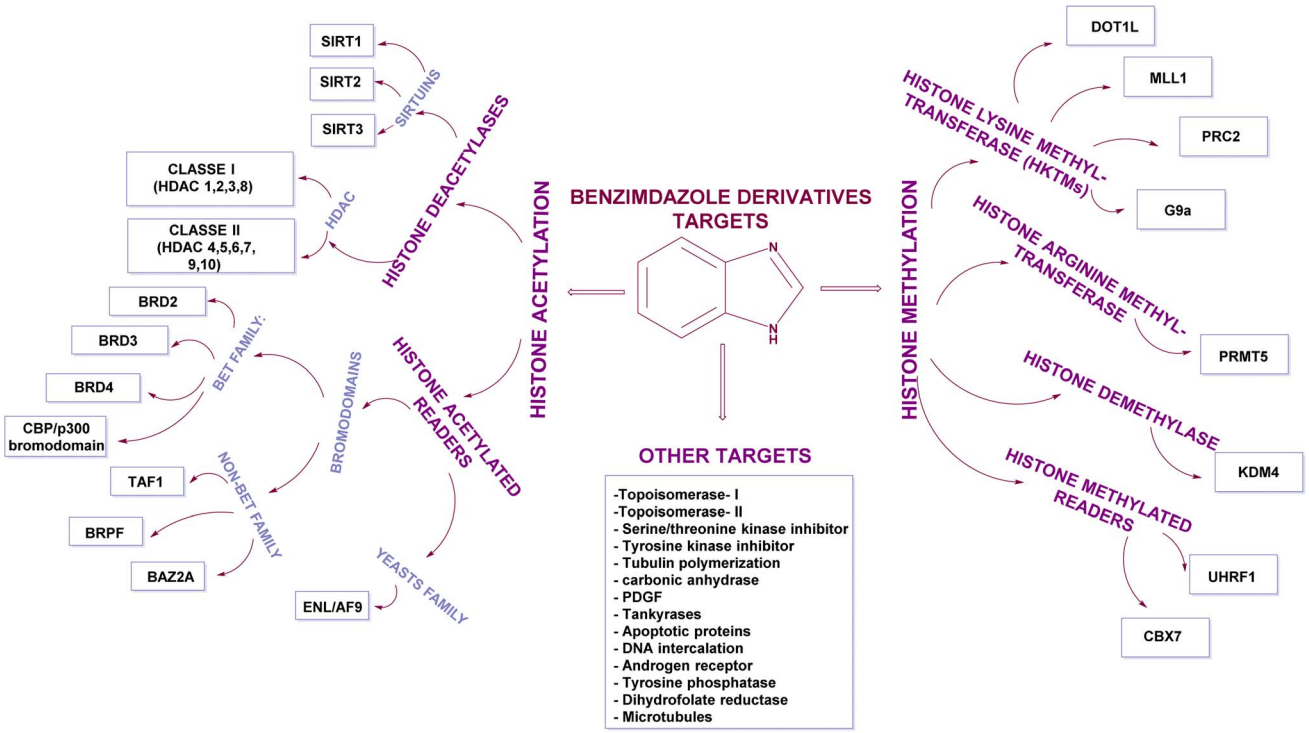


Fig. 2 Representation of various targets of 1*H*-benzimidazolederivatives having anticancer effects.

through the modulation of many epigenetics targets, as summarized in Fig. 2, and many other targets such as protein kinase CK2 inhibitors, topoisomerase inhibitors, tyrosine kinase inhibitors, antiangiogenic agents, checkpoint kinase 2 inhibitors, antiproliferative inhibitors, hypoxia-inducible factor inhibitors, interleukin-2-inducible T-cell kinase inhibitors, JNK inhibitors, dual KSP and aurora-A kinase inhibitors, and thioredoxin reductase inhibitors.[34] Consequently, many benzimidazole derivative drugs have been approved by the FDA, such as albendazole, mebendazole, and thiabendazole as anthelmintics, anti-psychotic drugs such as pimozide,[35] omeprazole and pantoprazole as proton pump inhibitors,[36] astemizole as an antihistaminic, enviradine as an antiviral, candesartan and telmisartan as antihypertensives[37] and binimetinib, bendamustine, and selumetinib as anticancer agents (Fig. 3).[38]

There are leading review articles in the field of epigenetics and benzimidazole derivatives acting as cytotoxic agents. However, a collection of the benzimidazole derivatives that inhibit epigenetic targets is currently lacking. Thus, in this review, we aim to provide a comprehensive overview of all the benzimidazole derivatives that inhibit different epigenetic targets, as summarized in Table 2, such as histone deacetylases (HDACs), histone acetylated readers (CBP/P300 bromodomain, TAF1 bromodomain, BRPF bromodomain, and ENL), lysine methyl transferases (DOT1L, MLL1, PRC2, and G9a), arginine methyl transferases (PRMT), histone demethylases (JMJD and peptidyl arginine deiminases), histone methylated readers (UHRF1 and CBX7), and ubiquitin-specific protease 5 (USP5) and how their structure–activity relationship (SAR) affects their anti-proliferative activity.

2 Benzimidazole compounds acting on histone modifications

2.1 Acetylation

The reversible addition and removal of the acetyl group in lysine is controlled by the activity of two characteristic enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs). Their actions lead to the formation of either the loose form of chromatin known as euchromatin or the condensed form known as heterochromatin, respectively, as shown in Fig. 4. HATs (writers) include the Gen-5-related *N*-acetyltransferase (GNAT) family, the p300 cAMP-responsive element-binding protein-binding protein (p300/CBP) family, and the MOZ/YBF2/SAS2/TIP60 (MYST) family. Regarding the CBP/p300 family, it consists of a HAT domain, a cysteine-rich motif, and a bromodomain, where the latter is considered one of the lysine-acetylated readers whose inhibition is involved in the treatment of cancer.[39,40] The amino groups of the lysine residues in the N-terminal tails of histone and non-histone proteins, including tubulin and several transcription factors (*e.g.*, p53, CREB, and NF-κB), are deacetylated by HDACs (erasers). The opened chromatin structure allows the cellular DNA to be more accessible to DNA-damaging agents. Accordingly, HDAC inhibitors are considered promising anticancer targets, given that the hypoacetylation of histones by HDAC leads to the development of several types of cancers.[41–45] Fortunately, many benzimidazole derivatives have been found to act on most of these targets, showing great efficacy and potency when tested on cancer cell lines.

2.1.1 Histone deacetylases (HDACs) (erasers). One of the most studied epigenetic targets is HDACs, which consist of rim, wall, and zinc-binding domain (ZBD) regions. Additionally, some isoforms contain extra regions such as the foot pocket in HDAC1-3, lower pocket in HDAC IIa, and side pocket in HDAC8 (Fig. 5a).[46] This family could be inhibited by HDAC inhibitors, which possess a pharmacophore comprised of a ZBG (hydroxamic acid and benzamide, thioester, thiol-based, or hydrazide-based derivative),[47] a connecting unit (amide, triazole, urea, and semicarbazide group), a linker (aliphatic chain, aromatic ring, and vinyl-aromatic), and a cap (Fig. 5b). ZBG interacts with the catalytic zinc ion at the bottom of the active binding site of enzymes, the connecting unit joins the cap to the linker and improves the surface interactions, increasing the potency,[48] the linker occupies the long and narrow channel between the catalytic pocket and the outer surface of the enzyme, with the optimum length of around 5–6 carbons in linear or cyclic forms attached to the cap *via* a connection unit,[49] and the cap group is located in the rim of the pocket and is involved in hydrophobic interactions with the residues on the outer surface of the enzyme, blocking the entrance of the HDAC active pocket.[50]

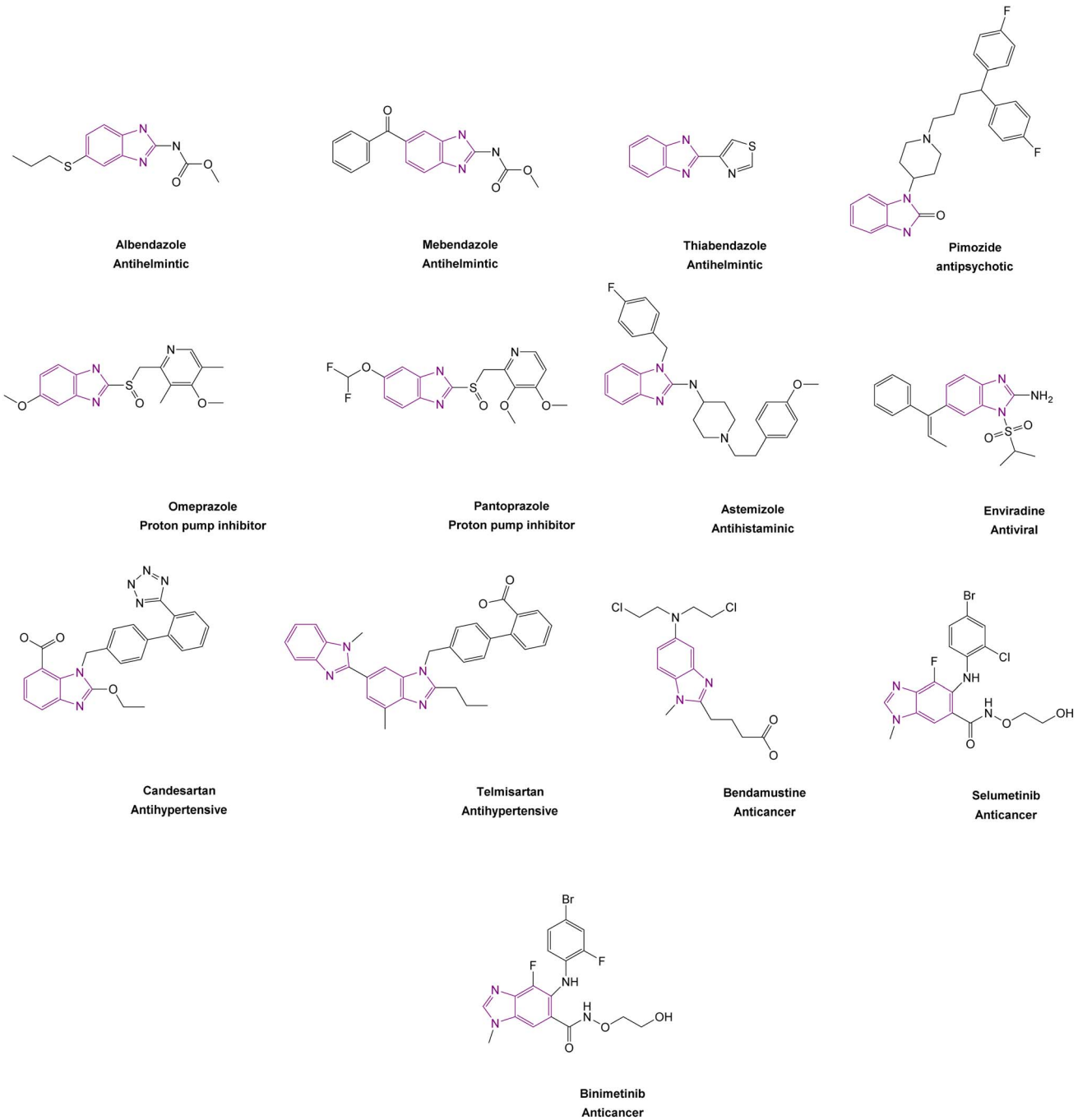


Fig. 3 Structures of FDA-approved benzimidazole derivative drugs.

Table 2 Representatives of benzimidazole derivatives as epigenetic modulators

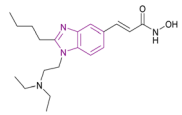
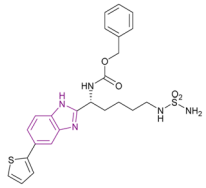
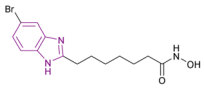
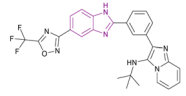
Benzimidazole derivative	Chemical structure	Epigenetic target	Mechanism of action	$IC_{50}/EC_{50}/K_d/PIC_{50}$ (μM)	Tested cancer cell lines	Key findings	Ref.
Pracinostat (**2**)		Pan-HDAC inhibitor	Enhances histone acetylation	HDAC1/2/3/8/4/5/7/9/6/10/11, IC_{50} = 0.028/0.027/0.019/0.048/0.016/0.021/0.104/0.024/0.247/0.023/0.024	MV4-11 Ramos PC-3 A2780 COLO 205	It has been investigated in phase I and II clinical trials for treating hematologic malignancies and solid tumours. It has excellent oral bioavailability, safety, long duration of action, and good ADME. However, it has dose-dependent mild myelosuppression and gastrointestinal side effects	68 and 69
Compound **10**		HDAC1 and HDAC6	Enhances histone acetylation	HDAC1/HDAC6, IC_{50} = 0.20/0.48	293T cell line	Its PK profile showed metabolic stability and a $t_{1/2}$ of 1.2 h. However, it has poor bioavailability because of the sulfamide unit	73
Compound **12**		Pan-HDAC inhibitor	Enhances histone acetylation	(HDAC1/2/3/4/5/6/7/8/9/10/11, IC_{50} = 0.0202/0.0341/0.0064/0.279/0.0025/0.0019/0.313/0.282/0.0011/0.003/0.0334	Skov-3 MiaPaCa H295R Mdamb231 H69 H23	Less toxic side effects than vorinostat as well as its *in vivo* studies using the human pancreatic cancer MiaPaCa xenograft mouse model, resulted in 74% tumour growth inhibition	74
Compound **14**		HDAC5 and SIRT	Enhances histone acetylation Downregulates 7 SIRT enzymes Induces cell cycle arrest at G1/S phase Reduces the expression of the oncogenic protein c-Myc, the anti-apoptotic genes BCL3 and BCL2, cyclin-dependent kinases (CDK2, CDK4, and CDK6), and cell cycle regulators (E2F1 and RB1 genes) but increases the expression of the	HDAC5 IC_{50} = 0.31	NA[a]	It can be beneficial in treating solid tumours and can be effective in treating sirtuin-associated diseases, including cancer, HIV, metabolic disorders, and neurological diseases	75

Table 2 (*Contd.*)

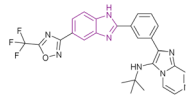
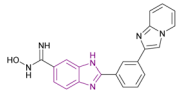
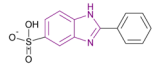
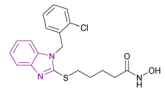
Benzimidazole derivative	Chemical structure	Epigenetic target	Mechanism of action	$IC_{50}/EC_{50}/K_d/PIC_{50}$ (μM)	Tested cancer cell lines	Key findings	Ref.
Compound **15**		HDAC5	proapoptotic proteins caspases 3 and 7 Enhances histone acetylation Induces cell cycle arrest at G1/S phase Reduces the expression of the oncogenic protein c-Myc, the anti-apoptotic genes BCL3 and BCL2, cyclin-dependent kinases (CDK2, CDK4, and CDK6), and cell cycle regulators (E2F1 and RB1 genes) but increases the expression of the proapoptotic proteins caspases 3 and 7 Enhances histone acetylation Downregulates 7 SIRT enzymes	$IC_{50} = 0.30$	NA MCF7 breast cancer cells The non-small cell lung cancer cells A549	It can be beneficial in treating solid tumours	75
Compound **16**		HDAC1, HDAC2 and SIRT	Reduces the expression of the oncogenic protein c-Myc, the anti-apoptotic genes BCL3 and BCL2, cyclin-dependent kinases (CDK2, CDK4, and CDK6), and cell cycle regulators (E2F1 and RB1 genes) but increases the expression of the proapoptotic proteins caspases 3 and 7	HDAC1/HDAC2, $IC_{50} = 0.55/0.4$		It can be beneficial in treating solid tumours and can be effective in treating sirtuin-associated diseases, including cancer, HIV, metabolic disorders, and neurological diseases	75
Ensulizole (**18**)		HDAC3	Enhances histone acetylation	NA	HCT116	Selective to HDAC3	94
Compound **20**		HDAC6	Enhances histone acetylation and caused dose-dependent increase in the level of tubulin acetylation	$IC_{50} = 0.09$	MCF-7	Potent anticancer agent for selective HDAC6 inhibition and deserves further investigation	96

Table 2 (*Contd.*)

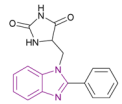
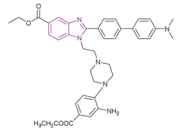
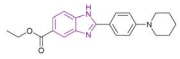
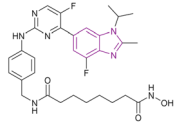
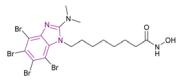
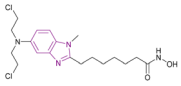
Benzimidazole derivative	Chemical structure	Epigenetic target	Mechanism of action	$IC_{50}/EC_{50}/K_d/PIC_{50}$ (μM)	Tested cancer cell lines	Key findings	Ref.
Compound **21**		HDAC6	Enhances histone acetylation	IC_{50} = 0.09735	MOLT-4	A Mannich base that fits perfectly fitting inside the binding site of HDAC6 enzyme	97
Compound **24**		SIRT1, SIRT2, and SIRT3	Inhibits SIRT1, 2 and 3 deacetylase activity	SIRT1/2/3, IC_{50} = 10.22/2.92/10.02	NA	Good inhibitory activity against SIRT2	103
BZD9L1 (**31**)		SIRT1 and SIRT2	Inhibits SIRT1, and SIRT2 deacetylase activity and with a dose-dependent reduction in cell survival and migration	SIRT1/SIRT2, IC_{50} = 42.9/9.0)	HCT116 HT-29-CRC	It is highly selective and nongenotoxic and has no effect on cell cycle distribution or cellular senescence	107
Roxyl-zhc-84 (**33**)		CDK/HDAC dual targeting	Enhances histone acetylation Inhibits cyclin-dependent kinase Thus, it downregulates the antiapoptotic protein BCL2, upregulated p21 expression, induced caspase-3 cleavage, and caused cell cycle arrest at the G1 phase	(HDAC1/2/3/4/5/6/7/8/9/10/11, IC_{50} = 0.026/0.52/0.056/<10/<10/0.0059/<10/<10/<10/0.073/6.8)	MDA-MB-231 MDA-MB-468 OVCAR-5	It is sensitive to solid tumours with good kinase selectivity and a favourable pharmacokinetic profile. It outperformed vorinostat or abemaciclib in the *in vivo* tests using both the 4T1 and MDA-MB-468 mouse models	114
Compound **36**		Multiple (HDAC1/HDAC6/CK2)	Enhances histone acetylation	HDAC1/HDAC6/CK2, IC_{50} = 13.7/8.98/5.89	SK-OV-3 Jurkat HCT-116 MCF-7 HL-60/vinc HL-60/adr HL-60	It has high cytotoxic activity, proapoptotic capability, mitochondria-targeting, and multidrug-circumventing properties	41
Tinostamustine (**38**)		DNA/HDAC dual targeting	Alkylates DNA Enhances histone acetylation Triggers apoptosis Reduces the expression of genes that control DNA repair	HDAC1/2/3/4/5/6, IC_{50} = 0.017/0.01/0.025/0.0064/0.107/0.072	HL60 Daudi cells	It was well tolerated with advanced solid tumours as had been showed in an open-label phase I/II study (NCT03345485). Also, it was well tolerated in relapsed/refractory (R/R) Hodgkin lymphoma (HL) patients as in cohort-expansion stage of a phase I study. The main side effects were	115–119

Table 2 (*Contd.*)

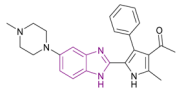
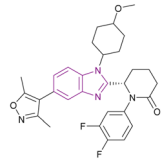
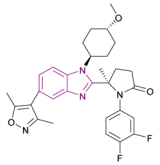
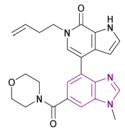
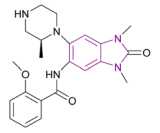
Benzimidazole derivative	Chemical structure	Epigenetic target	Mechanism of action	$IC_{50}/EC_{50}/K_d/PIC_{50}$ (μM)	Tested cancer cell lines	Key findings	Ref.
						haematological and thrombocytopenia, leading to treatment discontinuation	
Compound **42**		BRD4 (BD1)	Inhibits BET protein binding to acetylated histones Blocks transcriptional activity Induces cell cycle arrest at G0/G1 phase through upregulation of p21 and apoptotic marker-cleaved PARP and downregulation of c-Myc	$IC_{50} = 0.0501$	NA	It might be a potential candidate for the treatment of AML and some solid tumours. It has good metabolic stability and good oral bioavailability	129
Inobrodib (**48**)		CBP/p300	Blocks histone acetylation	CBP/p300, K_d = 0.0013/0.0017	−22Rv1 -VCaP	It has entered phase I/II clinical trials for treatment of AML, non-Hodgkin lymphoma, and MM (NCT04068597) and it is promising for the treatment of advanced prostate cancer	132, 135 and 136
Compound **51**		p300 bromodomain	Inhibits the binding to acetylated lysine residues on histone	$IC_{50} = 0.060$	NA	It overcomes the defect of Inobrodib **48** defect of its high efflux rate and it is metabolically stable and has no hERG risk, indicating its safety for the heart	139
GNE-371 (**52**)		TAF1(2)-bromodomain	Inhibition of bromodomain-acetylated lysine binding	$IC_{50} = 0.01$	NA	Exhibits antiproliferative synergy with JQ1	140
GSK6853 (**54**)		BRPF1 bromodomain	Inhibits BRPF1 bromodomain binding to acetylated lysine residues on histones	$pIC_{50} = 8.1$	NA	Suitable for *in vivo* studies	142

Table 2 (*Contd.*)

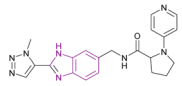
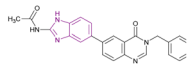
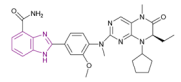
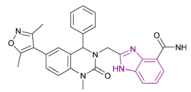
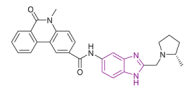
Benzimidazole derivative	Chemical structure	Epigenetic target	Mechanism of action	$IC_{50}/EC_{50}/K_d/PIC_{50}$ (μM)	Tested cancer cell lines	Key findings	Ref.
Compound **58**		BAZ2A bromodomain	Prevent bromodomain of BAZ2A from binding to acetylated lysine residues on histones	IC_{50} = 10	NA	NA	143
Compound **60**		BRD4/PI3K/ Aurora A	Inhibits BET protein binding to acetylated histones Blocks the transcriptional activity Upregulates the pro-apoptotic proteins cleaved-PARP and cleaved-caspase 9, while the anti-apoptotic protein Bcl-2 is downregulated Induces cell cycle arrest in G2/M phase	PI3K IC_{50} = 0.01312 and Aurora A IC_{50} = 0.01019	A549 H1975 HCC827	It has selectivity towards cancer cells superior to normal cells and provides better safety, enhanced efficacy, and delayed drug resistance	128
Compound **63**		PARP1/BRD4/ BRD4BD1/ BRD4BD2	Inhibits BET protein binding to acetylated histones Blocks the transcriptional activity Blocks Single-Strand Break (SSB) repair	PARP1/BRD4/ BRD4BD1/ BRD4BD2, IC_{50} = 0.013/0.101/0.075/ 0.157	SW1990 cells Breast cancer MDA-MB-231	It can be beneficial in the treatment of advanced pancreatic cancer	144
Compound **65**		PARP1/BRD4	Inhibits BET protein binding to acetylated histones Blocks the transcriptional activity Blocks Single-Strand Break (SSB) repair Thus, arrests the cell cycle at G0/G1 and G2/M phases, increases DNA damage, and reverses olaparib-induced adaptive resistance	PARP1/BRD4, IC_{50} = 0.049/0.202	CFPAC-1 Capan-1 SW1990 MDAMB-231	The inhibitory effects of compound **65** at a dose of 30 mg kg^{-1} on SW1990 xenograft tumour growth were more significant than that of JQ1, olaparib, and their combination	145
Compound **68**		ENL	Blocks YEATS domain binding to acetylated lysine. Downregulates the expression of target gene MYC	IC_{50} = 0.0154	MDA-MB-468 HCT116 THP-11 MOLM-13 MV4-11	It is a therapeutic target in leukemogenesis with exceptional target engagement in cellular context	148

Table 2 (*Contd.*)

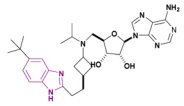
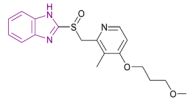
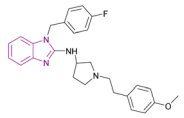
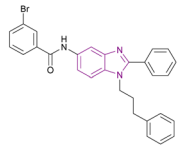
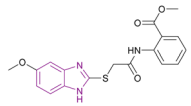
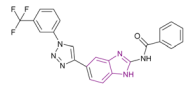
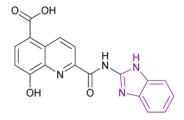
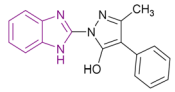
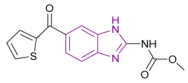
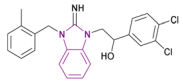
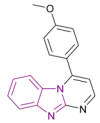
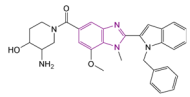
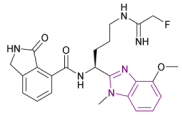
Benzimidazole derivative	Chemical structure	Epigenetic target	Mechanism of action	$IC_{50}/EC_{50}/K_d/PIC_{50}$ (μM)	Tested cancer cell lines	Key findings	Ref.
EPZ-5676 (**70**)		DOT1L	Inhibits H3K79 methylation Decreases HOXA9 and MEIS1 mRNA	—	MV4-11	It has recently entered clinical evaluation as a therapeutic agent for MLL-rearranged leukemia	159,160
Rabeprazole (**71**)		MLL1-WDR5	Disrupting the MLL1-WDR5 interaction Significantly reduces MLL1's ability to methylate H3K4 Downregulates the expression of Hoxa9 and Meis1 genes	$IC_{50} = 0.49$	MLL-AF4 MLL-AF9	More modifications are needed to repurpose the scaffold of substituted 2-pyridyl methyl/sulfinyl benzimidazole to eliminate proton pump inhibitory effects in the future	161
DC-PRC2in-01 (**77**)		EZH2-EED	Inhibits the interaction of EZH2-EED by binding to EED Arrests cell cycle at the G0/G1 phase	$IC_{50} = 4.21$	SU-DHL-4 Karpsa-422 Pfeiffer cell lines	A promising chemical scaffold for further development	163
GA001 (**78**)		G9a	Blocks H3K9 methylation Induces autophagy and apoptosis Activates the tumour suppressor p53 by inhibiting G9a, which regulates p21 activation	$IC_{50} = 1.32$	MCF-7 T-47D MDA-MB-231 MDA-MB-468 MDA-MB-435	It can help in breast cancer therapy	164
Compound **83**		PRMT5	Inhibits arginine demethylation	$IC_{50} = 0.33$	MV4-11	It can be beneficial in treatment of leukemia	166
Compound **84**		PRMT5	Inhibits arginine demethylation	$IC_{50} = 4.11$	MCF7 DU145 PC3 HepG2 HCT116	It has prominent anticancer potency	169
Compound **88**		JMJD2A	Increases H3K9me2 and H3K9me3 levels	$IC_{50} = 19.0$	MCF-7 A549	A good starting point for the development of new JmjC inhibitors	172

Table 2 (*Contd.*)

Benzimidazole derivative	Chemical structure	Epigenetic target	Mechanism of action	$IC_{50}/EC_{50}/K_d/PIC_{50}$ (μM)	Tested cancer cell lines	Key findings	Ref.
Compound **90**		KDM4	Increases H3K9 methylated levels	$IC_{50} = 13$	LnCaP cells -DU145 cells -PC3 cells	It reduced the expression of genes regulated by the androgen receptor	173
Nocodazole (**91**)		UHRF1	Gradual loss of DNA methylation	NA	NA	NA	175
MS351 (**93**)		CBX7ChD	Disrupting recognition of H3K27me3	$K_d = 23.8$	NA	NA	177
Compound **94**		USP5	Accumulating free polyubiquitin chains and disrupting ubiquitin homeostasis	$K_d = 0.47$	MYCN-amplified MYCN-non-amplified neuroblastoma cells	Selective towards cancer cell lines	178
Compound **96**		PAD4	Blocking citrullination of histones	NA	4T1 cells	The MTT assay showed potent activity	180
Compound **97**		PAD2	Blocking citrullination of histones	$EC_{50} = 0.4$	NA	May be suitable for treating diseases in which PAD2 activity is dysregulated	181

[a] NA, not available.

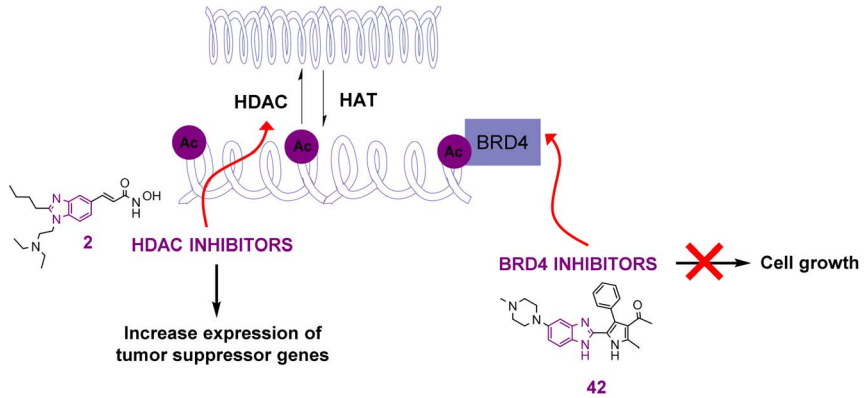


Fig. 4 Effects of benzimidazole derivatives act as HDAC inhibitors and histone acylated reader (BRD4) on cancer cell growth and gene expression.

The pentacoordinate zinc ion binds to Asp178 (O δ 1), His180 (N δ 1), and Asp267 (O δ 1), whereas the acetyl moiety (carbonyl oxygen) of the substrate and a water molecule occupy the other two coordination sites. The *N*-acetyl group is attacked by a water molecule (a nucleophile) when HDAC is activated. The tetrahedral oxyanion intermediate, formed through the nucleophilic attack by water on the carbonyl, collapses to generate the free lysine and acetate products.[51,52] Hydroxamic acid (a ZBG) forms an electrostatically favourable bidentate binding mode with two oxygen atoms to attach to the catalytic zinc cation, leaving the amide NH to create an extra H-bond connection with a histidine.[53]

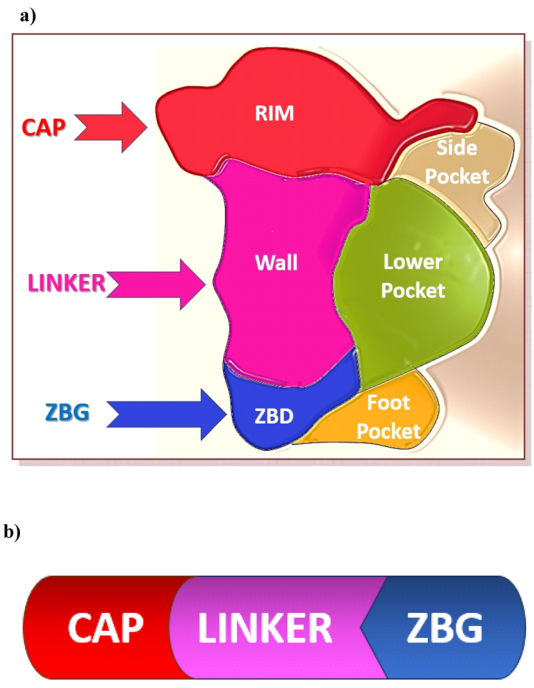


Fig. 5 (a) HDAC isoforms structures showing rim, wall, and zinc binding groups with the additional subpockets called foot pocket in HDAC1-3, side pocket in HDAC8, and lower pocket in the HDACIIa isoforms. (b) Pharmacophore of the HDAC inhibitors.

The FDA-approved HDAC inhibitors include vorinostat (SAHA), belinostat (PXD-101), romidepsin (FK-228), panobinostat (LBH589), and chidamide (Fig. 6). However, they are all pan-HDAC inhibitors, inhibiting all the HDAC isoforms, except romidepsin and chidamide, which are HDAC 1–3 selective inhibitors. There is variation in the length of HDAC isoforms; the shortest is HDAC11, with 347 amino acids, while the longest is HDAC6, with 1215 amino acids.[52] HDACs can be classified into two groups, group I (zinc-dependent amidohydrolases) and group II (NAD^+-dependent hydrolases). Group I consists of classes I (isoforms 1, 2, 3, and 8), IIa (isoforms 4, 5, 7, and 9), IIb (isoforms 6 and 10), and IV (HDAC 11). Group II consists of class III or sirtuins (SIRTs), which comprise SIRT1 to SIRT7.[54] In Class I HDACs, a second metal-binding site was discovered around 7 Å away from the zinc ion; depending on the salt present during crystallization, this site may be a potassium, calcium, or sodium ion.[55]

HDAC inhibitors act as anticancer agents by activating either the extrinsic pathway (death receptors and ligands up-regulation) or intrinsic apoptosis (pro-apoptotic upregulation and anti-apoptotic factors down-regulation). Consequently, HDAC inhibition leads to cell cycle arrest, inhibition of differentiation and proliferation, apoptosis, and necrosis.[43,56]

In addition, HDAC inhibitors target and/or modulate the expression of non-histone proteins such as transcription factors and regulators, chaperones, DNA repair enzymes, nuclear import regulators, inflammation/immune response mediators, and structural proteins.[57] Thus, they play a promising role in neurodegenerative disorders,[58,59] cardiovascular diseases,[60] viral infections,[61,62] and cancer.[48,63–65]

2.1.1.1 Benzimidazole derivatives as pan-HDAC inhibitors. In the following paragraphs, we elaborate on the group of benzimidazole derivatives 1–8 that function as pan-HDAC inhibitors (Fig. 7).

The screening of a natural compound library of 3719 compounds using a fluorescence-based HDAC activity assay showed that **1** is a potent non-hydroxamic pan-HDAC inhibitor. Wegener *et al.* showed that it has antiproliferative activity against embryonic childhood cancer such as neuroblastoma and medulloblastoma when tested in MYCN single-copy cells

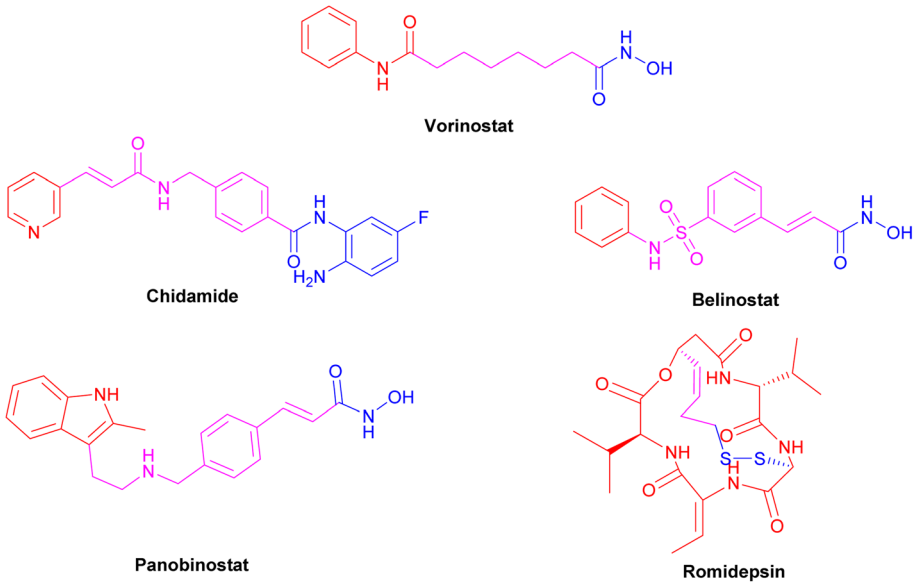


Fig. 6 FDA-approved HDAC inhibitors.

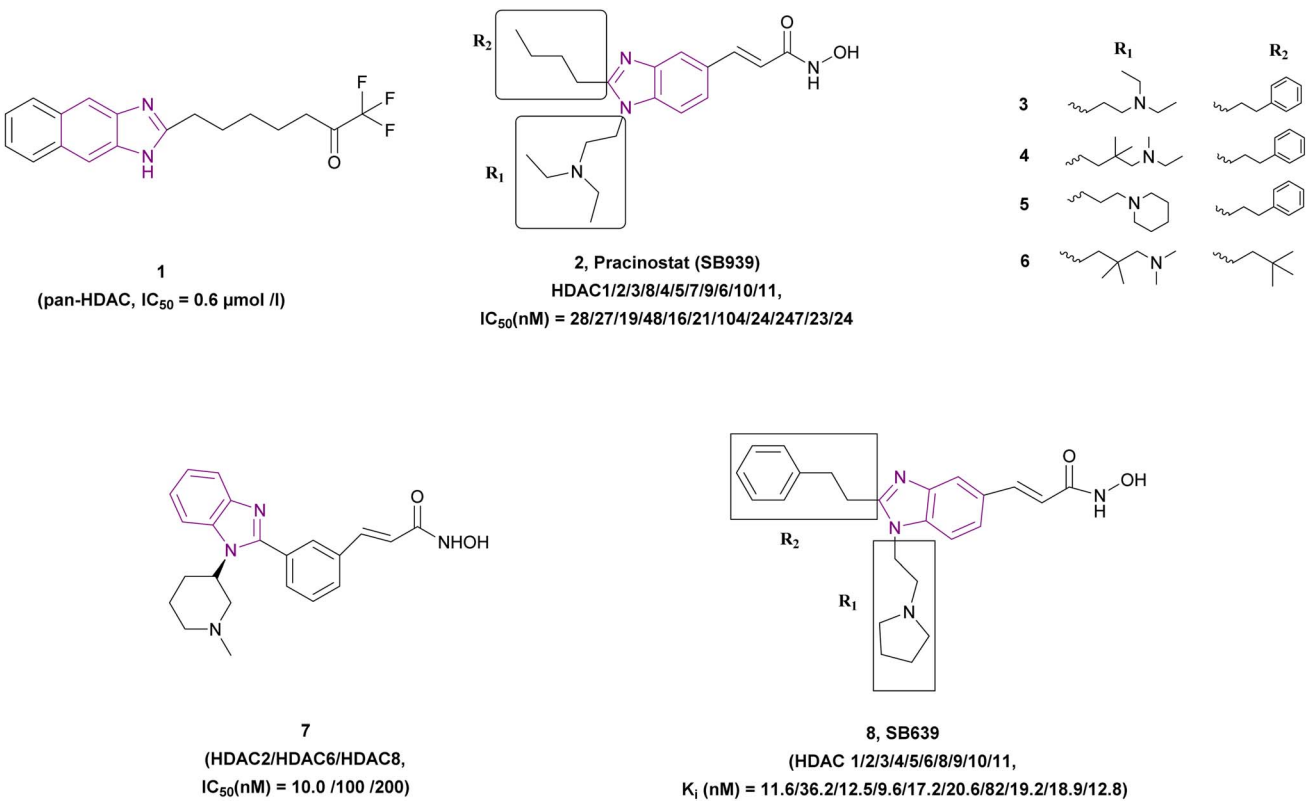


Fig. 7 Chemical structure, pharmacophore and IC_{50} of pan-HDACIs **1–8**.

(SH-EP), MYCN stably transfected cells (WAC2), and MYCN-amplified cells [BE(2)-C, Kelly] cell lines. Also, it significantly induced the expression of the cyclin-dependent kinase inhibitor 1A gene (CDKN1A/p21$^{WAF1/Cip1}$) and induced apoptosis. Moreover, it did not affect the viability of normal skin fibroblasts at 10-fold higher concentrations.[66]

Pracinostat **2**, a dialkyl benzimidazole derivative, was a result of the optimization of compounds **3–5**, which were metabolically unstable in human liver microsomal assays. With >1000-fold selectivity for HDAC Class 1 and 2 over Class 3,[67] pracinostat is a very soluble, competitive, and effective pan-HDAC inhibitor,[51] which formed essential interactions with HDAC1,

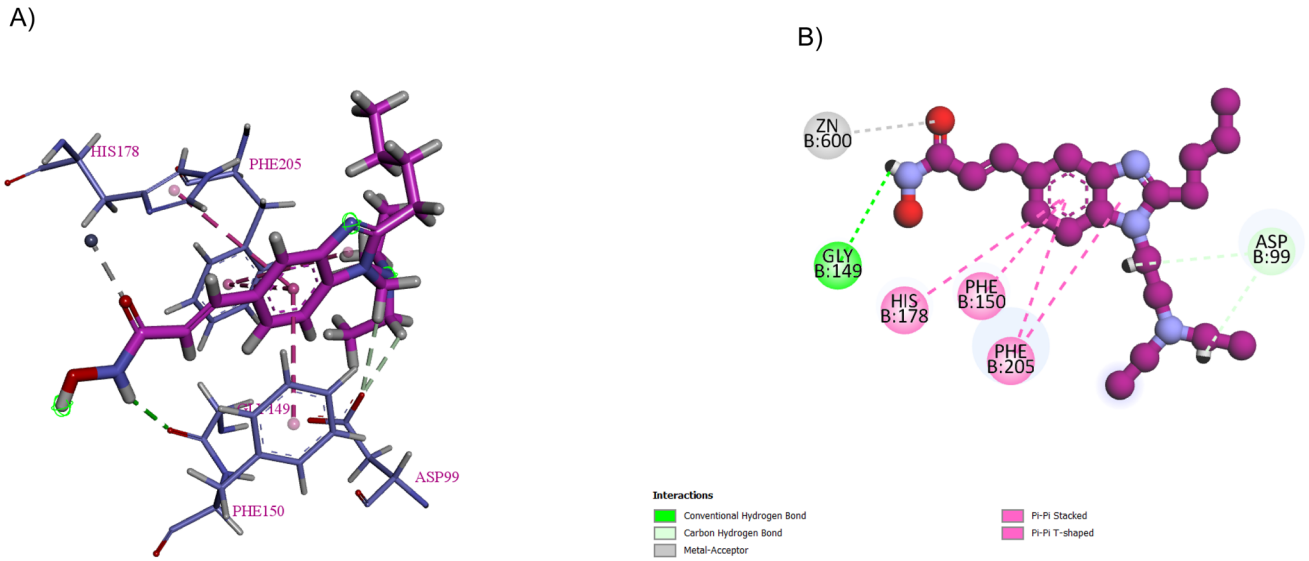


Fig. 8 Pracinostat (in purple) interacts with HDAC1 through multiple interactions. Images were generated by Discovery Studio 4.5. (A) 3D interaction of pracinostat and HDAC1 residues. (B) 2D interaction of pracinostat and HDAC1 residues (in blue). It forms four hydrophobic interactions between the PHE205, PHE150, and HIS178 residues and the benzimidazole ring, and NH of the hydroxamic group forms hydrogen bond donor interactions with GLY149, while ASP99 forms two hydrogen bond acceptors with the alkyl groups. The carbonyl oxygen forms a metal–acceptor interaction with the zinc group.

as shown in the docking structure in Fig. 8. Additionally, it has been investigated in phase I and II clinical trials for treating hematologic malignancies and solid tumours.[68] It has excellent oral bioavailability, safety, long duration of action, and good ADME. However, it has dose-dependent mild myelosuppression and gastrointestinal side effects.[69] The SAR studies be Wang *et al.* highlighted that a basic center in the R_1 group was crucial for optimal its biological activity. Additionally, the two-carbon linker of R_1 has greater potency than its three-carbon counterpart. In the case of R_2, a simple alkyl substitution at C-2 of the benzimidazole ring yields adequate potency and improved binding efficiency. Additionally, the cellular potency against COLO 205 would somewhat decrease if one of the ethyl groups from the R_1 side chain was eliminated. Moreover, when tested on a variety of cancer cell lines, including MV4-11, Ramos, PC-3, and A2780, it exhibited antiproliferative properties, indicating that it is more effective against haematological cancers than solid tumours.[68] After a pilot phase II study was carried out to investigate the efficacy and safety of a pracinostat **2** and 5-azacitidine combination in individuals with intermediate-2 or high-risk myelodysplastic syndrome (MDS), Quintás-Cardama *et al.* discovered that this combination was incredibly well tolerated in MDS patients.[70] With respect to compound **6**, the bulkiness of the *tert*-butylmethyl group at R_2 may force it to adopt a more favourable conformation for effective interaction with Asp99, making it a competitive HDAC1 inhibitor, while the presence of a bulky gemdimethyl group in the R_1 side chain increased the lipophilic interactions with HDAC1.[68]

Bressi *et al.* synthesized compound **7**, an *N*-hydroxy-3-[3-(1-substituted-1*H*-benzoimidazol-2-yl)-phenyl]-acrylamide analogue, which inhibited HDAC2 (IC_{50} of 10.0 nM), HDAC6 (IC_{50} of 100 nM), and HDAC8 (IC_{50} of 200 nM) with a good PK profile and $p21^{waf}$ induction. Its MOA was proven by H3 and H4 hyperacetylation in HL60 leukemia cells. *In vitro* studies showed its antiproliferative activity when tested in A549 lung (EC_{50} of 0.3 μM), HL60 leukemia (EC_{50} of 0.04 μM), and PC3 (EC_{50} of 0.05 μM) prostate cancer cell lines. *In vivo* studies using PANC-1 tumour-bearing mice, human HCT116 colon xenograft mouse model, and PC3 prostate model proved that **7** has anti-tumour activity.[71]

Wang *et al.* synthesized *N*-hydroxy-1,2-disubstituted-1*H*-benzimidazol-5-yl acrylamides, highlighting that compound **8** is a potent hydroxamic pan-HDAC inhibitor that inhibited HDAC1 with an IC_{50} of 0.035 ± 0.016 μM and inhibited class I, II, and IV HDAC isoenzymes in the nanomolar range, where the benzimidazole ring was a part of the cap moiety. It had antiproliferative activity when tested in HCT116, A2780, and PC3 cell lines with IC_{50} values (μM) of 0.20, 0.19, and 0.15, respectively. It is orally active and has good drug-like properties. According to SAR, hydrophobic and bulky groups were preferred for R_1 with a short, flexible linker. Both enzymatic and cellular activities were significantly improved with a monobasic amine and a two-carbon linker compared to the compounds with longer linkers. In the case of R_2, the compounds with a directly attached ring or an α-branch were not potent, whereas that with α-unsubstituted phenylalkyl chains was more potent.[72]

The optimization of compound **9**, a lysine-derived sulfamide, resulted in compound **10**, which was synthesized by Manku *et al.*, who replaced the amide group with a benzimidazole scaffold, an isosteric heterocycle, given that compound **9** was unstable due to amidic bond cleavage (Fig. 9). Compound **10** is an HDAC1 (IC_{50} of 0.20 μM) and HDAC6 (IC_{50} of 0.48 μM) inhibitor with a sulfamide group as the ZBG and benzimidazole as its cap moiety. Interestingly, the *N*-carbobenzyloxy in the

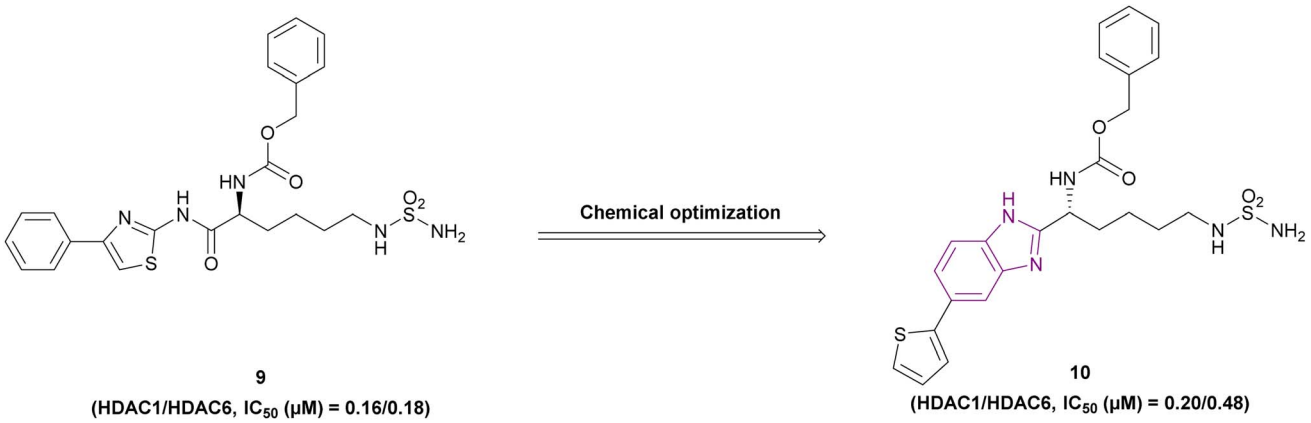


Fig. 9 Chemical structures and IC_{50} of HDAC1 and 6 inhibitors **9** and **10**.

linker was responsible for the activity against both HDAC1 and HDAC6, given that the linear linker was selective towards HDAC6. The *in vitro* studies in the 293T cell line showed anti-proliferative activity with an IC_{50} of 0.62 μM. Its PK profile showed metabolic stability and a $t_{1/2}$ of 1.2 h. However, it has poor bioavailability because of the sulfamide unit.[73]

The metabolic studies for vorinostat **11** showed its instability due to the cleavage of its benzamide bond, generating toxic secondary metabolites. A series of benzimidazole-based HDAC inhibitors was synthesized through a series of reactions, including the Suzuki coupling reaction. Among them, compound **12**, an optimized analogue of vorinostat obtained through cyclization (Fig. 10a), is considered a pan-HDAC inhibitor targeting HDAC 3, 5, 6, 9, and 10 and showed less toxic side effects than vorinostat **11** rather than exhibiting better results in *in vitro* studies. The SAR studies showed that a six-carbon linker was more potent than a five-carbon linker, and substitutions at the 5-position were more favorable than at the 4-position. Moreover, halogen substitutions (Cl, F, and Br) at the 5-position improved the cell activity more than electron-donating groups such as 5-methoxy and 5-dimethylamino, and the imidazole[4,5-*c*] pyridine system instead of the benzimidazole ring, as the cap group, eliminated the activity. Furthermore, additional groups on N-1, such as methyl, cyclopropyl, and phenyl, had deleterious effects. Compound **12** was chosen for *in vitro* studies instead of compound **13** (Fig. 10b) due to its better solubility, regardless of the enhanced cell activity of compound **13**. As a result, compound **12** was tested in the human ovarian cancer SKOV3 cell assay (IC_{50} of 0.27 μM) and other different cell lines, as well as in *in vivo* studies using a human pancreatic cancer MiaPaCa xenograft mouse model, resulting in 74% tumour growth inhibition.[74]

Rigidification of the flexible alkyl chain of vorinostat by replacing it with a benzimidazole linker enhanced its potency

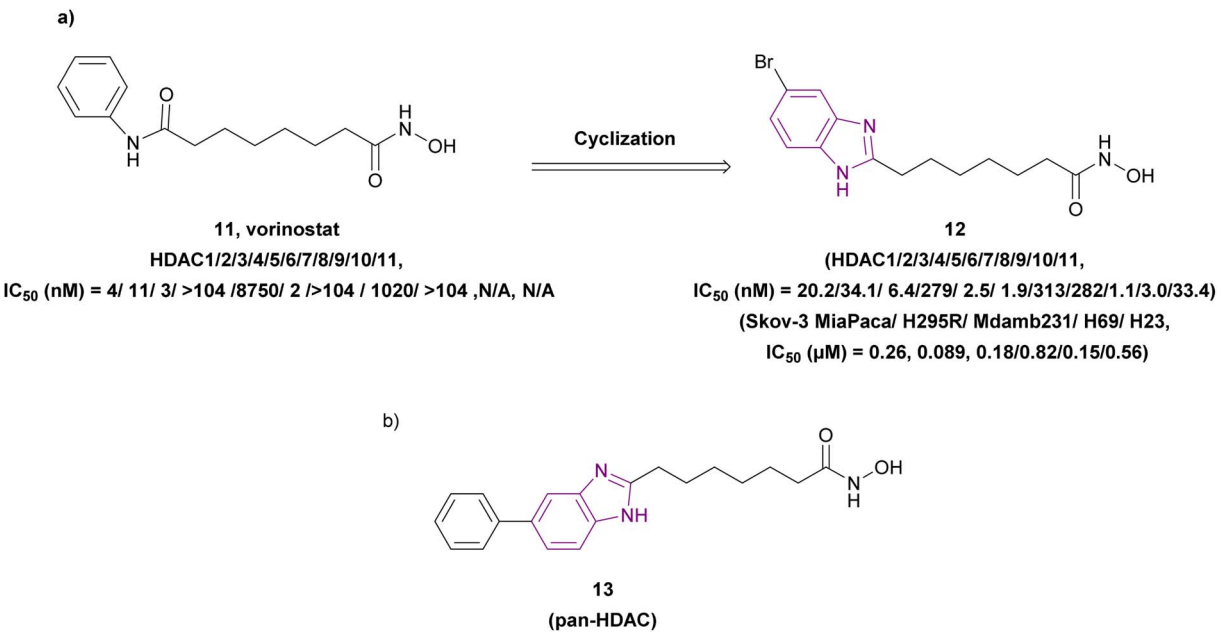


Fig. 10 Chemical structures and IC_{50} of HDAC inhibitors **11–13**.

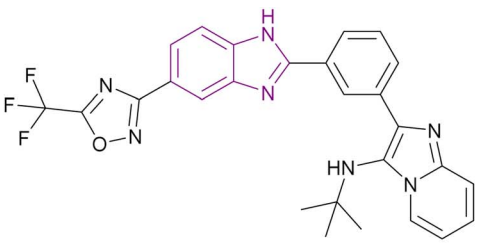
and selectivity. El-Awady *et al.* synthesized compounds **14–16**, which are imidazopyridine derivatives (Fig. 11), resulted from the post-transformation of the Groebke–Blackburn–Bienaymé [4 + 1]-cycloaddition reaction products and suggested that they could be beneficial in treating solid tumours. Compounds **14** and **15** are potent HDAC-5 inhibitors (IC_{50} = 0.31 μM and 0.30 μM, respectively) with the trifluoromethyloxadiazole group (TFMO) as a nonchelating metal-ZBG group and induce cell cycle arrest at the G1/S phase. Compound **16** showed selectivity towards HDAC-1 and HDAC-2 (IC_{50} of 0.55 and 0.4 μM, respectively), showing significant anti-tumour activity when tested in MCF7 breast cancer cells and A549 non-small cell lung cancer cells. It had no activity towards HDAC-5 because it replaces the trifluoromethyloxadiazole group with *N*-hydroxyl amidine. Also, it did not inhibit human CYP450s 2D6 and 2C9 and had moderate kinetic aqueous solubility with lower stability in human liver microsomes. These compounds reduced the expression of the oncogenic protein c-Myc, the anti-apoptotic genes BCL3 and BCL2, cyclin-dependent kinases (CDK2, CDK4, and CDK6), and cell cycle regulators (E2F1 and RB1 genes) but increased the expression of the proapoptotic proteins caspases 3 and 7. However, compounds **14** and **16** reduced transcription factor NF-kB expression, while compound **15** enhanced its expression. Moreover, compounds **14** and **16** downregulated 7 SIRT enzymes (Class III HDACs). Thus, they can be effective in treating sirtuin-associated diseases, including cancer, HIV, metabolic disorders, and neurological diseases.[75]

2.1.1.2 Benzimidazole derivatives as selective HDAC inhibitors. HDAC-selective inhibitors could reduce side effects and be more specific, given that certain HDAC isoforms have a direct correlation with certain types of illnesses.[76] Pan-HDACIs make up the majority of hydroxamic acid-based HDACIs,[77] and their hydroxamic moieties are responsible for side-effects such as nausea, thrombocytopenia, anaemia, and other metabolic abnormalities due to their strong chelation with the zinc ion. Thus, researchers are trying to find different ZBGs with weaker binding and greater selectivity.[53]

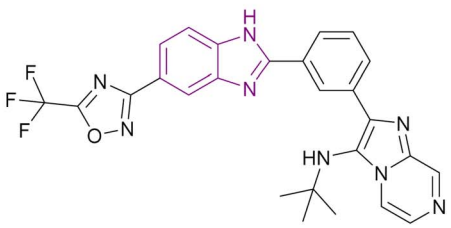
Importantly, the foot pocket (HDAC1-3), lower pocket (HDACIIa), and side pocket (HDAC8) binding groups are essential for selectivity given that they are not present in all isoforms. The homological identities and high sequence similarity of Class I HDACs are considered a challenge in the development of HDAC1/2/3-selective compounds. Currently, most synthesized selective compounds are HDAC8 inhibitors.[76]

Class I HDACs produce inflammatory cytokines, which contribute to innate immunity and possess an additional internal cavity (foot pocket) supposed to be used for water entry and acetic acid removal.[52] In this class, the HDAC channel is deeper and narrower than that of class IIb.[78] High expression levels of HDAC1, 2, and 3 have been shown to be associated with gastric and ovarian cancers.[79] HDAC1 was significantly overexpressed in various cancers, including prostate, gastric, breast, pancreatic, and colon cancers. Also, HDAC1 interacts with several non-histone protein-forming complexes. For example, HDAC1 acts as a pivotal component in its interaction with the tumour suppressor protein p53.[80]

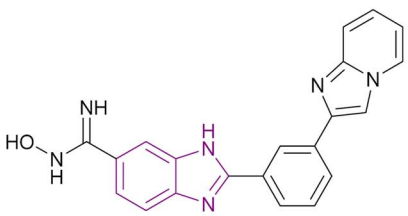
HDAC2 is overexpressed in various malignancies, including prostate, gastric, non-small lung, colon, hepatocellular, and renal cancer,[81] and is a crucial target for cancer and neurodegenerative diseases. The 14 Å foot pocket in HDAC2 can occupy benzamides well, such as chidamide and entinostat.



14
HDAC5 (IC_{50} = 0.31 μM)/ SIRT



15
HDAC5, IC_{50} = 0.30 μM



16
(HDAC1/HDAC2, IC_{50} (μM) = 0.55 /0.4)/ SIRT

Fig. 11 Chemical structures and IC_{50} of HDAC and SIRT inhibitors **14–16**.

Interestingly, the aromatic substitution of benzamide inhibitors at 5-position resulted in further entry into the foot pocket and a pronounced increase in the HDAC2 inhibitory potency.[82]

HDAC3 shares ~50% sequence homology with HDAC1 and HDAC2. The divergence is mainly in the C-terminal region, with HDAC3 containing a domain for binding to the nuclear NCoR/SMRT complexes.[83] HDAC3 inhibitors are effective for the treatment of inflammatory diseases, diabetes mellitus, cardiac diseases, HIV, different neurodegenerative disorders, rheumatoid arthritis, cancer, *etc.* The benzamide scaffold mainly as ZBG, has great selectivity towards HDAC3. However, synthesizing an effective compound against HDAC3 is still challenging.[84]

HDAC6 is the largest in size[85] and the only enzyme in the HDAC family that has two deacetylase domains and a zinc finger domain. HDAC6 catalyzes the deacetylation of acetyl groups from non-histone proteins such as α-tubulin, Hsp90, and cortactin[86] and is involved in the control of cell motility and VEGFR regulation in cancer cells.[87] It regulates cellular viral RNA sensing and promotes the angiogenesis and migration of endothelial cells.[88] Compared to the other HDACs, the rim of HDAC6 is shallower and wider. Therefore, compounds such as citarinostat, which have huge, rigid cap groups, can fit into the HDAC6 rim area easily.[46] The bulky and lipophilic cap and substituted phenyl unit as a linker are selective for HDAC6 over other isozymes more than using an alkyl chain as the linker.[86] It was reported that oxazoles attached to hydroxamate (ZBG) increased the potency and selectivity towards HDAC6.[89] The X chromosome carries the gene for HDAC8, the only known HDAC that has the ability to function as a monomer.[90] It has a larger surface opening and a wider active site pocket than HDAC1-3 (ref. 91) and is overexpressed in advanced neuroblastoma.[92] In addition, HDAC11 is overexpressed mainly in rhabdomyosarcoma.[93]

Compounds **17–23**, as shown in Fig. 12, are selective HDAC inhibitors. CID-18150975 **17** was revealed to have the most selective inhibitory effect against class I HDAC inhibitors, particularly HDAC1, due to the presence of a benzamide functional group, which is a zinc binding group.[80]

Ensulizole **18** is a selective HDAC3 inhibitor. In the HCT116 viability experiment, its IC_{50} was 13.39 ± 2.80 μM, while its efficacy was 70%.[94]

The modification of Nexturastat A by Alves Avelar *et al.* involved substituting a fragment of (1*H*-benzoimidazol-2-yl)-methylamine in place of the hydroxamic acid (a ZBG). This resulted in compound **19**, which could serve as a lead structure for the synthesis of new selective non-hydroxamic HDAC6 selective inhibitors. The fragment of (1*H*-benzoimidazol-2-yl)-methylamine was selected as a potential ZBG through a structure-based virtual screening of designed libraries, and then evaluated with molecular docking and molecular dynamics simulations. The free amino group of the methylamino group and the non-alkylated imidazolic nitrogen, both of which demonstrated interactions with the zinc ion. Given that the HDAC6 binding site is shallow and wide, it could accommodate its cap group, but class I HDACs have a tighter hydrophobic channel.[95]

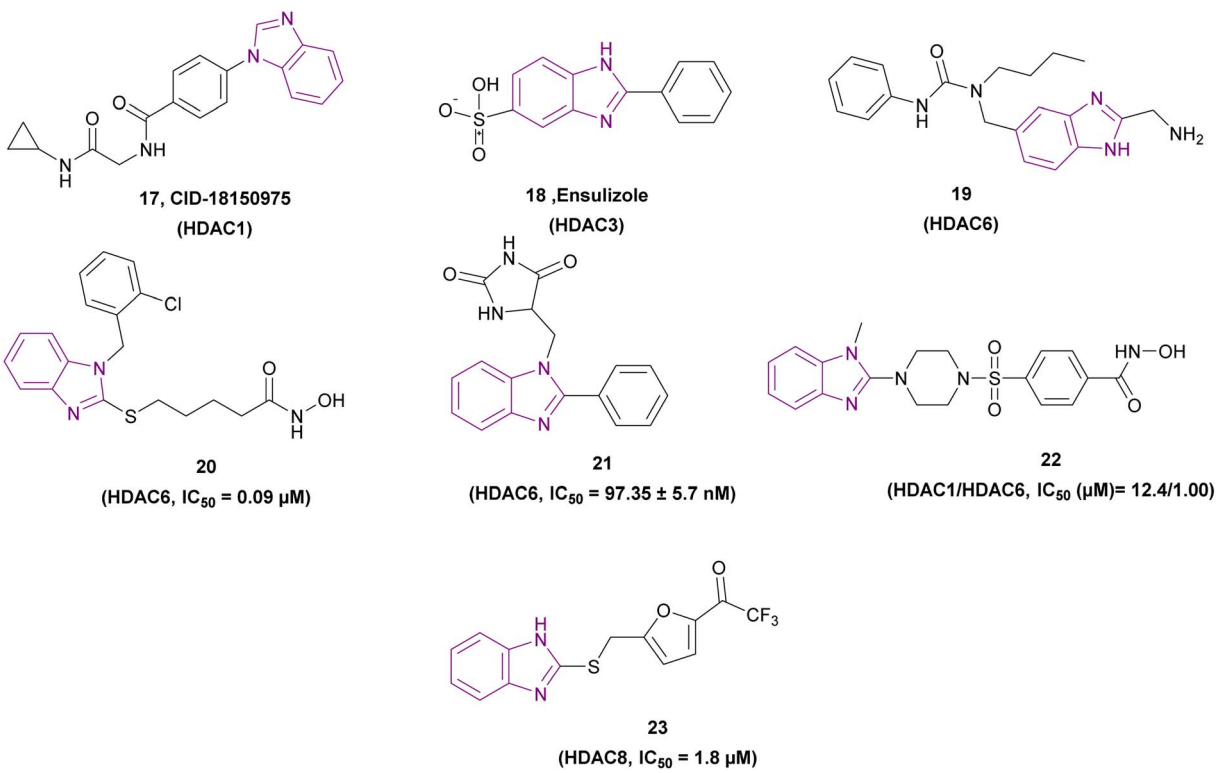


Fig. 12 Chemical structure and IC_{50} of selective HDACIs **17–23**.

Compound **20** is a selective HDAC6 inhibitor with an IC_{50} of 0.09 μM and consists of a benzimidazole cap and four carbon-chain-containing thioether linkers, providing antiproliferative effects when tested against the MCF-7 cancer cell line and causing a dose-dependent increase in the level of tubulin acetylation.[96]

Mansour *et al.* synthesized compound **21**, a Mannich base, through an aminoalkylation reaction. It is a potent HDAC6 inhibitor consisting of the benzimidazole nucleus as the capping moiety, the hydantoin moiety as the non-hydroxamate ZBG, and the CH_2 group as a linker. Both CCRF-CEM and MOLT-4 cell lines were susceptible to the cytotoxic effects of compound **21**. However, the MOLT-4 cell line was more susceptible, with an IC_{50} of 3.66 μM. The SAR studies showed that phenyl benzimidazole scaffold was more active than methyl benzimidazole, which was more active than unsubstituted benzimidazole. Also, the hydantoin derivatives were more potent than the thiohydantoin analogues.[97]

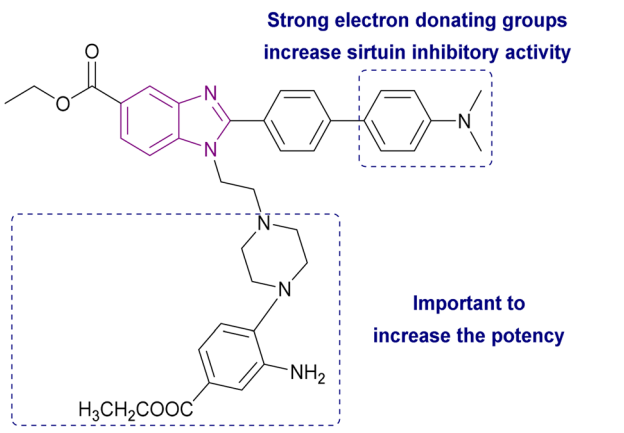
Compound **22**, a 2-benzazolyl-4-piperazin-1-ylsulfonylbenzenecarbohydroxamic acid derivative, is a selective HDAC6 inhibitor that induces cell cycle arrest in the S-phase, has antiproliferative activity with IC_{50} values of 1.49 μM and 6.1 μM in HCC4017 and HCC4018, respectively, and selectivity towards cancer cells. Its structure formed of [CO(NHOH)] as a zinc-binding group, a piperazine-sulfonylbenzene moiety as the linker, and 1-methylbenzimidazole as the cap group. The catalytic pocket surface of HDAC6 and benzimidazole fitted perfectly, indicating a greater inhibitory effect for HDAC6 over HDAC1. The compounds that were meta-substituted exhibited no biological activity. The elimination of the methyl group that was substituted on the imidazole ring led to the complete elimination of cellular activity because of the reduced permeability of the cells. Less potent than the well-known HDACIs, the compounds containing a hydroxamic acid functionality in the *para*-position of the benzene ring demonstrated good HDAC inhibitory and antiproliferative properties. However, their HDAC6 selectivity was higher.[98]

The ZBG-based pharmacophore model for HDAC8 was used to filter a virtual screening of about 200 000 chemicals. Among the 3 HITs, only compound **23** with a trifluoroacetyl group as ZBG was chosen for further research because it selectively inhibited HDAC8 over HDAC1 and HDAC4. Compound **23** exhibited anti-proliferative action when tested in the MDA-MB231 and HCT116 cell lines.[56]

2.1.1.3 Benzimidazole derivatives as sirtuins inhibitors. Class III HDACs called sirtuins use NAD^+ as a cofactor to remove acetylated groups from histones. By breaking the bonds that hold NAD^+ and niacinamide ribosomes together, sirtuins transfer the acetylated groups from proteins to ADP-ribose, and then release the deacetylated products.[99] They have been divided into seven human isoforms (SIRT1-SIRT7). Nearly all SIRT1, SIRT6, and SIRT7 are found within the nucleus. SIRT3-SIRT5 are called mitochondrial sirtuins, and SIRT2 is the main component in the cytoplasm. In the catalytic core area, SIRT2 can almost represent the structural basis of each isoform. The catalytic core of SIRT2 consists of a large and a small domain. The large domain contains an inverted Rossmann fold, which is a typical NAD^+(H) binding site. The small domain contains a helical module and a zinc finger module. The two domains are joined by a lysine channel and three large-domain polypeptide chains, all of which form a large groove. This junctional groove includes the NAD^+ binding site, which has three subpockets (A, B, and C). The ADP-ribose moiety binds to sub-pocket A. Nicotinamide-ribose is accommodated in the B sub-pocket, while during catalysis, it occupies the C sub-pocket, allowing the acetylated group to transfer from the Lys residue to the ribose portion of the substrate.[99,100] Sirtuins regulate biological pathways, such as cell cycle and apoptosis, and are associated with a variety of age-associated diseases such as type II diabetes, obesity, and Alzheimer's disease.[99,101,102]

1,2,5-Trisubstituted benzimidazole derivatives **24** and **25–27** are shown in Fig. 13 and 14, respectively, which act as inhibitors of sirtuins. Compound **24** was the most potent SIRT1, SIRT2, and SIRT3 inhibitor, followed by compound **25**, and then compound **26**. The planar benzimidazole scaffold occupied the adenosine binding site (pocket A) and ribose binding site (pocket B) of SIRT2. The *N*,*N*-dimethylbenzylamine substituent, a potent electron-donating basic terminal group, increased the sirtuin inhibitory activity. The side chain of ethyl piperazinyl benzoate was located deep inside the nicotinamide binding site, which is important for controlling the activity of the enzyme. In a comparison study, compound **27**, which has a linear octyl chain instead of ethylpiperazinyl benzoate, was less potent than compound **24**.[103]

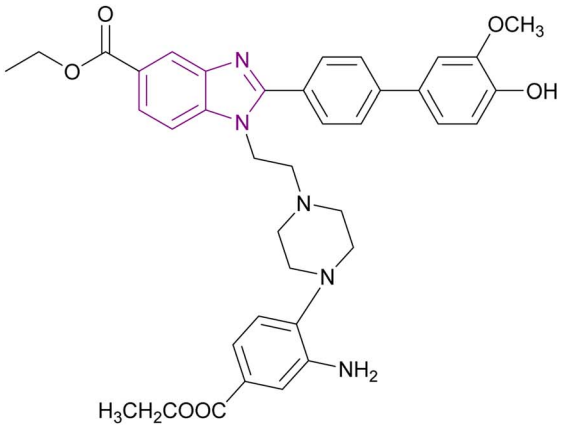
Compounds **28–31** are selective sirtuin inhibitors, as illustrated in Fig. 15. Yoon *et al.* used green chemistry to synthesize compound **28**, which is a non-toxic SIRT1 and SIRT2 inhibitor. Additionally, when it was screened against several cancer cell lines derived from HCT-116, MDA-MB-468, and CCRF-CEM cell lines, it was the most effective anti-proliferative compound. Regarding the SAR of these candidates, it was found that the dimethylamino, a potent electron-donating group, has the best sirtuin inhibitory activity, and its removal significantly reduced its activity. The substituted benzene ring of the benzimidazole



24

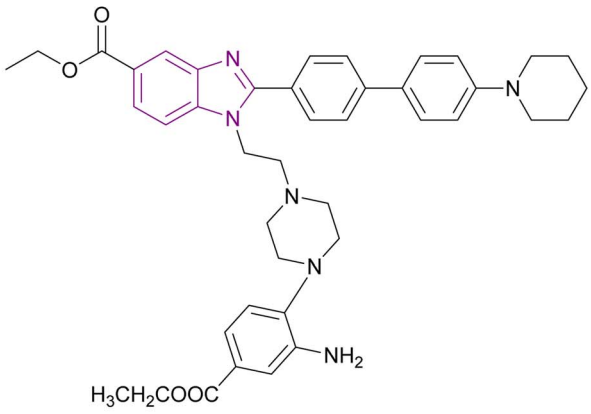
(SIRT1/2/3, IC_{50} (μM)= 10.22 /2.92/10.02)

Fig. 13 Chemical structure, SAR and IC_{50} of SIRT inhibitor **24**.



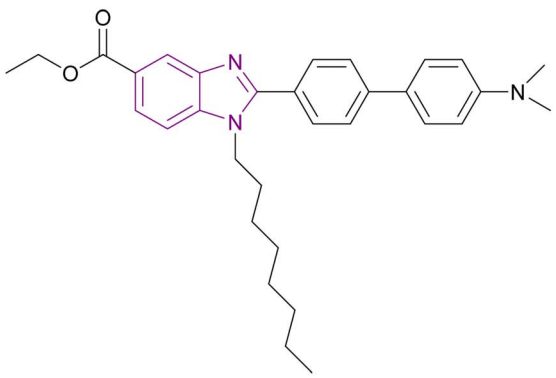
25

(SIRT1/2/3, IC_{50} (μM)= 35.07/8.61/27.91)



26

(SIRT1/2/3, IC_{50} (μM)= 36.97/13.48/29.24)



27

Fig. 14 Chemical structure and IC_{50} of SIRT inhibitors **25–27**.

core was stabilized through the π–π stacking interactions between the imidazole group of His187 and the benzene ring of Phe235. The phenyl ring of the dimethylaminobenzene substituent as well as the N from the dimethylamino group with Val233 and Phe119, respectively, formed lone-pair oxygen–π interactions.[104]

Compound **29**, an amino acid derivative of substituted benzimidazole, is an SIRT1 inhibitor with an IC_{50} of 0.77 μM and % inhibition of 89.99%, together with SIRT2 and SIRT3 inhibition of 15% and 20%, respectively. It has antiproliferative activity against human cancer cell lines (HepG2 and MCF7). The benzimidazole moiety formed π–π or π–cation interactions with the binding site. The phenyl and indole groups were important for the binding affinity and the inhibitory activity, respectively. The 4-OMe function group was necessary for the inhibitory activity of SIRT1. Also, replacing it with 2-Cl or 4-F decreased the stability of the compound.[105]

Compound **30**, a benzimidazole derivative, is an SIRT1 (IC_{50} of 58.43 μM) and SIRT2 (IC_{50} of 45.12 μM) competitive inhibitor with NAD^+ cofactor. It formed H-bond, hydrophobic, and mild polar interactions with the receptor. The H-bonds between H from the CYS324 and LYS287 residues in the pocket and N from the dimethylamino group helped to stabilize the binding complex. *In vitro* studies in breast cancer cell lines MDA-MB-468 and MCF-7 showed its good antiproliferative activity due to its potent SIRT2 inhibition. SAR studies showed that the compounds with a 2-phenylbenzimidazole moiety and a strong electron-donating group (dimethylamino) in the *para*-position have more potential antitumour activity than that with weaker electron-donating groups such as methyl, hydroxyl, and methoxyl groups. The bulkier piperidine moiety instead of the dimethylamino group was unable to fit into the cavity at the end of the pocket.[42]

BZD9L1 **31** is a potent, competitive, and reversible SIRT1/SIRT2 inhibitor with a greater affinity towards SIRT2. The strong electron-donating groups substituted at the phenyl ring, such as piperidinyl group **31** and the dimethylamino group, gave the best inhibitory activity and helped in stabilizing the

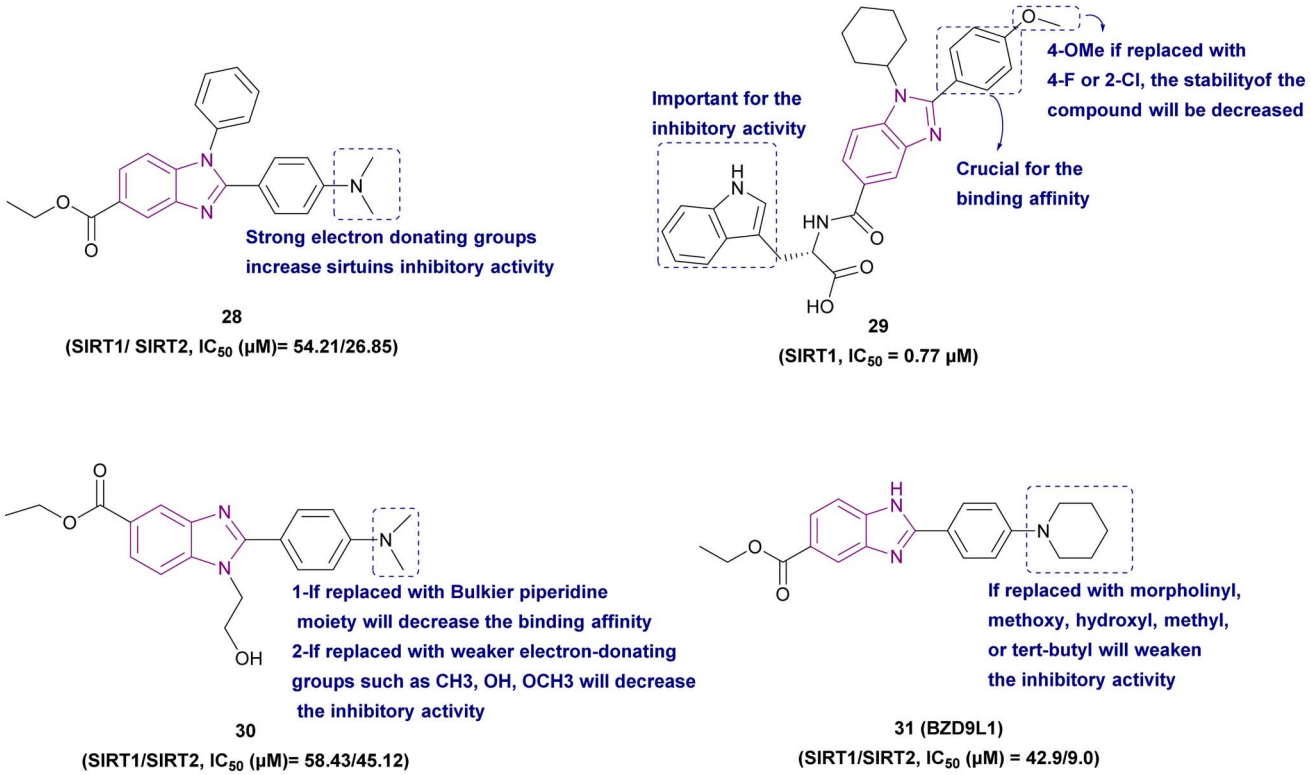


Fig. 15 Chemical structure, SAR and IC_{50} of selective SIRT inhibitors **28–31**.

benzimidazole core. In the case of replacing piperidinyl with methoxy, hydroxyl, methyl, or *tert*-butyl, it caused poor inhibition given that they shifted from the binding site with different binding orientations. Replacing the piperidinyl with the morpholinyl group weakened the inhibitory activity due to the importance of the basicity of the side chain.[106] It had strong antiproliferative effects against HCT116 and HT-29-CRC cells, with a dose-dependent reduction in cell survival and migration. It is highly selective and nongenotoxic and has no effect on the cell cycle distribution or cellular senescence. In HCT116 cells, the proapoptotic genes BAX, BCL2, and GADD45A were upregulated, while TRAF2, a prosurvival gene, was downregulated. However, it had no effect on TRAF2 gene expression in the HT-29 cell line. At lower concentrations, cleaved PARP, a biological indicator of apoptosis, was seen in HCT-116 cells and HT-29. Only in HCT-116 cells, the p21 expression was reduced, while the PUMA protein was activated, given that both of them were not expressed in the p53 mutant cell line HT-29.[107]

2.1.1.4 Benzimidazole derivatives as dual-targeting HDAC inhibitors. Nowadays, multitarget-directed ligands (MTDLs) have marked importance, especially for complex multifactorial diseases such as diabetes, neurodegenerative syndromes, cardiovascular diseases, and cancer. They may be a new way to reduce drug resistance. Merged-pharmacophore mode, non-cleavable linked-pharmacophore mode, fused-pharmacophore mode, and cleavable linked-pharmacophore mode are the most common strategies for the synthesis of MTDLs.[108] MTDLs are compounds consisting of more than one pharmacophore, each having a different target, which can be beneficial for cancer treatment.[109] Multidrug targets are an alternative approach to combination therapy with the advantage of overcoming the problems associated with combination therapy, such as patient compliance, simplified dosing schedules, more predictable pharmacokinetics and pharmacodynamics, solubility/bioavailability issues, drug–drug interactions, and decreasing toxicity.[110,111] For HDAC inhibitors to be a part of MTDLs, only their cap group could be replaced without impairing their potency due to the crucial effect of ZBG in the HDAC inhibitory activity, which could be useful in solid tumour treatment.[57,78] This is because they resist treatment through some mechanisms such as signaling proteins, thioredoxin expression, cell cycle proteins, NF-kB expression, and apoptosis-related proteins.[112] However, there are some challenges associated with dual inhibitors including poor pharmacokinetics due to their large molecular weight given that they are synthesized through either a pharmacophore-linked or -fused design technique. The second difficulty is in optimizing the synergistic therapeutic efficacy of the drug *in vivo* and maximizing its safety profile. Moreover, most of the dual epigenetic inhibitors are alkyl hydroximic acid-based HDAC inhibitors. However, the alkyl hydroximic acid scaffold has pharmacological liabilities, often resulting in short pharmacokinetic half-lives, poor oral bioavailability and toxicity.[113]

Huang *et al.* linked vorinostat **11** (an HDAC inhibitor) and abemaciclib **32** (a CDK inhibitor), resulting in compound **33**, which is a CDK/HDAC dual-targeting hybrid inhibitor, and also targets JAKs (Fig. 16). To achieve HDAC inhibition, hydroxamic acid is essential for good zinc binding and activity, as in

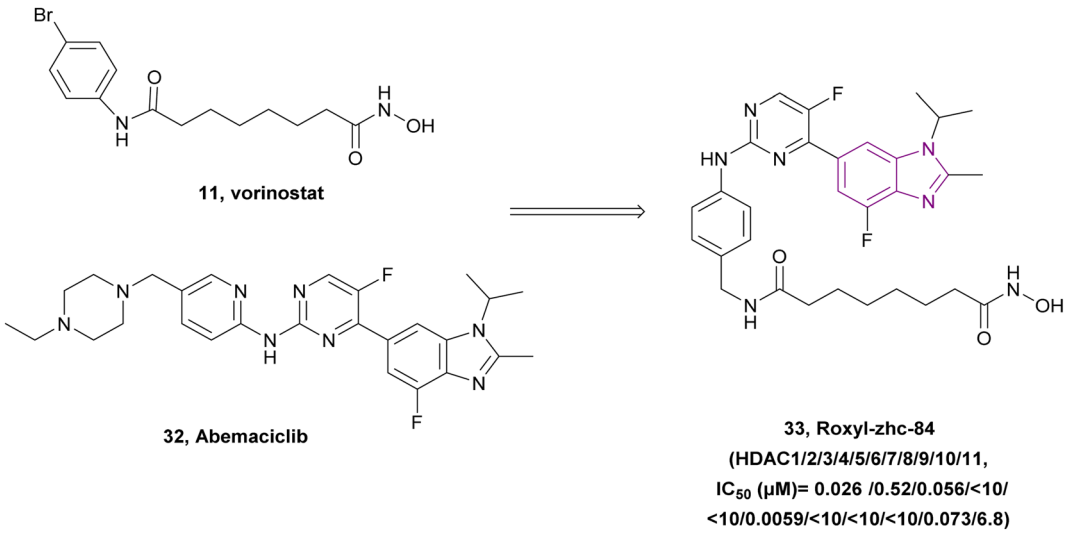


Fig. 16 Combination between compound **11** and **32** resulted in the CDK/HDAC dual inhibitor roxyl-zhc-84 **33**.

vorinostat, and the CH_2–NH–(C═O)– moiety acts as a linker. Moreover, the 2-aminopyrimidine moiety is responsible for CDK inhibition, as in abemaciclib **32**. Compared to the pan-HDAC inhibitor vorinostat, compound **33** inhibited HDAC classes I, II, and IV with significantly greater selectivity. Moreover, it showed anti-tumour activity when evaluated in MDA-MB-231, MDA-MB-468, OVCAR-5, and SK-OV-3 cell lines, and considerably outperformed vorinostat or abemaciclib in the *in vivo* tests using both the 4T1 and MDA-MB-468 mouse models. Also, it downregulated the antiapoptotic protein BCL2, upregulated p21 expression, induced caspase-3 cleavage, and caused cell cycle arrest at the G1 phase. Interestingly, compound **33** was sensitive to solid tumours given that it inhibited the phosphorylation of JAK1 in the MDA-MB-231 and MDA-MB-468 (TNBC) cell lines, which activated STAT3-stimulated anti-apoptotic signaling and limited the effect of HDAC inhibitors on solid tumours. Additionally, it had good kinase selectivity and a favourable pharmacokinetic profile.[114]

2-Dimethylamino-4,5,6,7-tetrabromo-benzimidazole (DMAT) scaffold **34** and hydroxamic acid (ZBG), which inhibited CK2 and HDAC, were linked, forming compounds **35** and **36** as selective dual HDAC/CK2 inhibitors, respectively (Fig. 17). Various cell lines such as Jurkat, HCT-116, MCF-7, HL-60/vinc, HL-60/adr, and HL-60 were used in the *in vitro* studies and demonstrated that both compounds inhibited HDAC-1, HDAC-6, and CK2. However, compound **36** showed the highest cytotoxic activity, proapoptotic capability, mitochondria-targeting, and multidrug-circumventing properties. Thus, compound **36** was employed in further *in vivo* studies. SAR demonstrated that the addition of the triazole ring in the linker lowered the cytotoxic and pro-apoptotic activities, while having no effect on the inhibitory action, and the 4-carbon linker was less active in both enzymes. Comparing compounds **35** and **36** to derivatives with aromatic linkers, the former had greater action against several types of cancer cells. The cytotoxic action of inhibitors **35** and **36** was influenced by their aliphatic chain length. Specifically, among the dual inhibitors that were evaluated, compound **36** was the most active.[41]

Due to the critical role played by HDAC in DNA synthesis and repair in cancer cells, DNA/HDAC dual-targeting compounds have been synthesized to overcome the resistance of cancer cells to genotoxic treatments.[115] Mehrling *et al.* fused vorinostat **11** and bendamustine **37**, resulting in tinostamustine **38**, which has DNA alkylating and HDAC inhibitory activity, as shown in

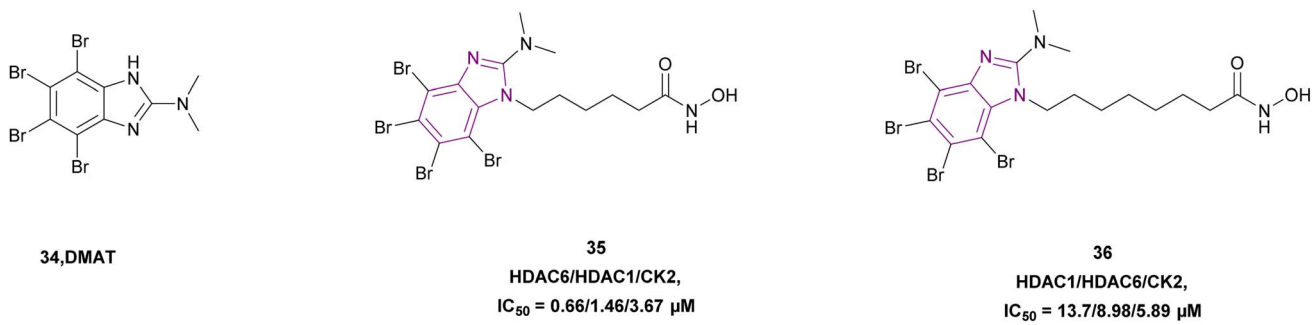


Fig. 17 Chemical structure of CK2 inhibitor **34** and HDAC/CK2 inhibitors **35** and **36** with their IC_{50}.

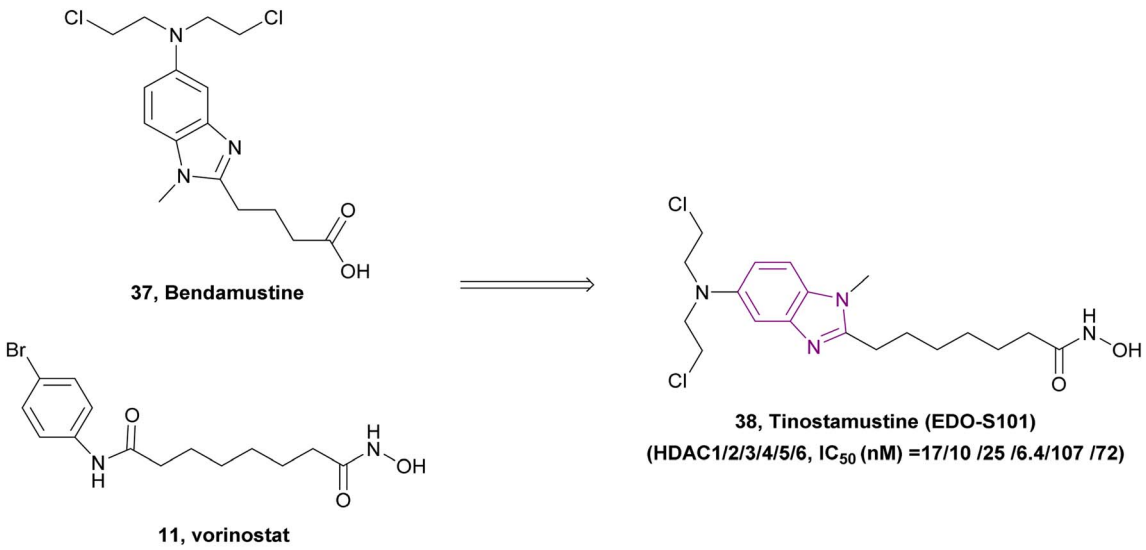


Fig. 18 Combination of compounds **37** and **11** resulted in DNA/HDAC inhibitor **38**.

Fig. 18. Tinostamustine inhibited HDAC1, 2, 3, 8, 6, and 10 with IC_{50} values of 9, 9, 25, 107, 6, and 72 nM, respectively, and formed essential interactions with HDAC1, as shown in the docking structure of Fig. 19. The inhibition of HDACs in living cells was assessed in PBMC from fifteen female rats, proving that HDAC inhibition of tinostamustine was at the maximum activity. It caused DNA cross-linking at lower concentrations than bendamustine and melphalan and had antitumour activity when evaluated in HL60 and Daudi cells. In the Daudi xenograft model, it triggered apoptosis,[116] reduced the expression of genes that control DNA repair, and showed selectivity towards cancer cells.[115] An open-label phase I/II study (NCT03345485) was

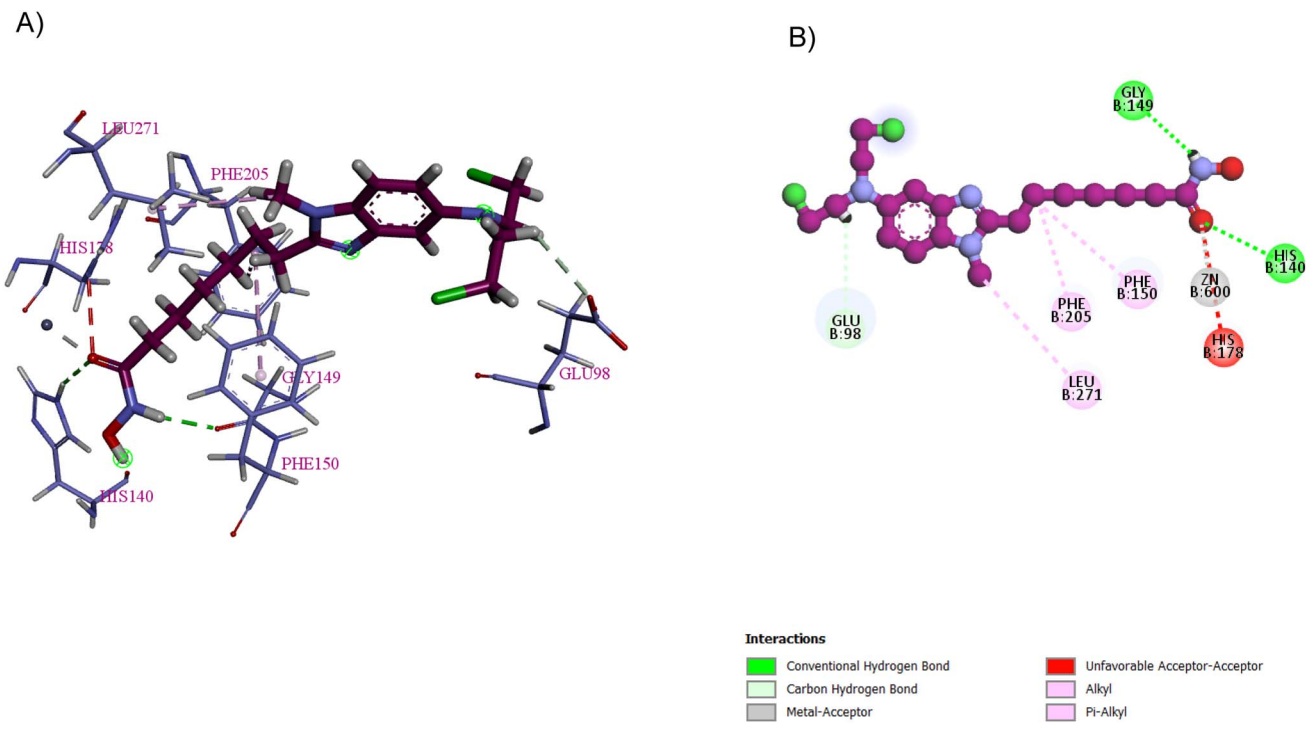


Fig. 19 Tinostamustine (in purple) interacts with HDAC1 through multiple interactions. Images were generated by Discovery Studio 4.5. (A) 3D interaction of tinostamustine and HDAC1 residues. (B) 2D interaction of tinostamustine and HDAC1 residues (in blue). The carbonyl oxygen, amidic H, and CH_2 of the alkyl group form three hydrogen bond donor interactions with the HIS140, GLY149, and GLU98 residues, respectively. The CH_3 group forms hydrophobic interactions with LEU271 and the second CH_2 of the linker forms two hydrophobic interactions with the PHE205 and PHE150 residues. Moreover, the carbonyl oxygen forms metal–acceptor interactions with the zinc group.

conducted to investigate the safety, pharmacokinetics, and efficacy of tinostamustine in treating patients with advanced solid tumours, which indicated that it was well tolerated.[117] Also, the cohort-expansion stage of a phase I study in relapsed/refractory (R/R) Hodgkin lymphoma (HL) patients suggested that it was well tolerated with no unexpected adverse effects.[118] The same results were obtained in another R/R HL expansion cohort of a phase I study (NCT02576496). The main TEAEs were haematological and thrombocytopenia, leading to treatment discontinuation.[119]

2.1.2 Histone acetylated readers

2.1.2.1 Bromodomain. Bromodomains (BRDs) are acetylated histone readers. The human genome encodes 61 bromodomains in 46 proteins with different cellular functions.[120,121] BRDs are comprised of the bromodomain and extra-terminal domain (BET) family and non-BET bromodomain family. The BET family members are bromodomain-containing protein 2 (BRD2), BRD3, BRD4, and BRDT localized in the nucleus and consist of two N-terminal bromodomains (BD1 and BD2), which are responsible for the process of locating and binding to Kac.[122–125] BRD2, BRD3, and BRD4 are expressed in different cells, but BRDT is only expressed in tissues such as pachytene spermatocytes, diplotene spermatocytes and round spermatids.[121]

The bromodomain inhibitors bind to the acetyl-binding sites of bromodomains, which are deep hydrophobic binding pockets and highly selective and competitive inhibitors of Kac.[126] BRD4 reads and binds to acetylated lysine in histones H3 and H4 through BD1 and BD2. Inhibition of BRD4 has been shown to decrease the expression of MYC, an oncogene,[127] and Bcl-2, an antiapoptotic.[121] Also, BRD4 was proven to be overexpressed in non-small cell lung cancer, promoting cancer progression.[128] BRD2 is supposed to be a promising target to prevent KSVH transmission. BIC1 is a BRD2 bromodomain inhibitor given that it inhibits its binding to acetylated H4K12.[122]

2.1.2.1.1 Benzimidazole derivatives as BET family inhibitors. Compounds **39–43**, as shown in Fig. 20, are BET family inhibitors. 3,5-Dimethylisoxazole aryl-benzimidazole derivative **39** is a BRD4 inhibitor that has anticancer activity against colorectal cancer (RKO) and selectivity towards BET bromodomains with sufficient hepatic stability. It competitively inhibited binding to BD1 (K_i of 7.5 nM) and BD2 (K_i of 3.1 nM) of BRD4. The SAR demonstrated that the cyclopropyl benzimidazole enhanced both its biochemical and cellular potency, and the two methyl groups of the isoxazole ring were crucial for its maintaining potency. When compound **39** was used to treat MM.1S tumour-bearing mice and SU-DHL-10 tumour-bearing animals, it was well tolerated and inhibited the tumour growth.[127]

Xing J., *et al.* synthesized BRD4 inhibitors **40** and **41**, 5-(1*H*-imidazol-1-ylmethyl)-8-quinolinol analogues, and proved that they could treat acute monocytic leukaemia and basal-type triple-negative breast cancer, respectively, with little risk of hERG toxicity. 5-(1*H*-Imidazol-1-ylmethyl)-8-quinolinol was optimized to increase its cellular membrane permeability. As a result, the imidazole group in 5-(1*H*-imidazol-1-ylmethyl)-8-

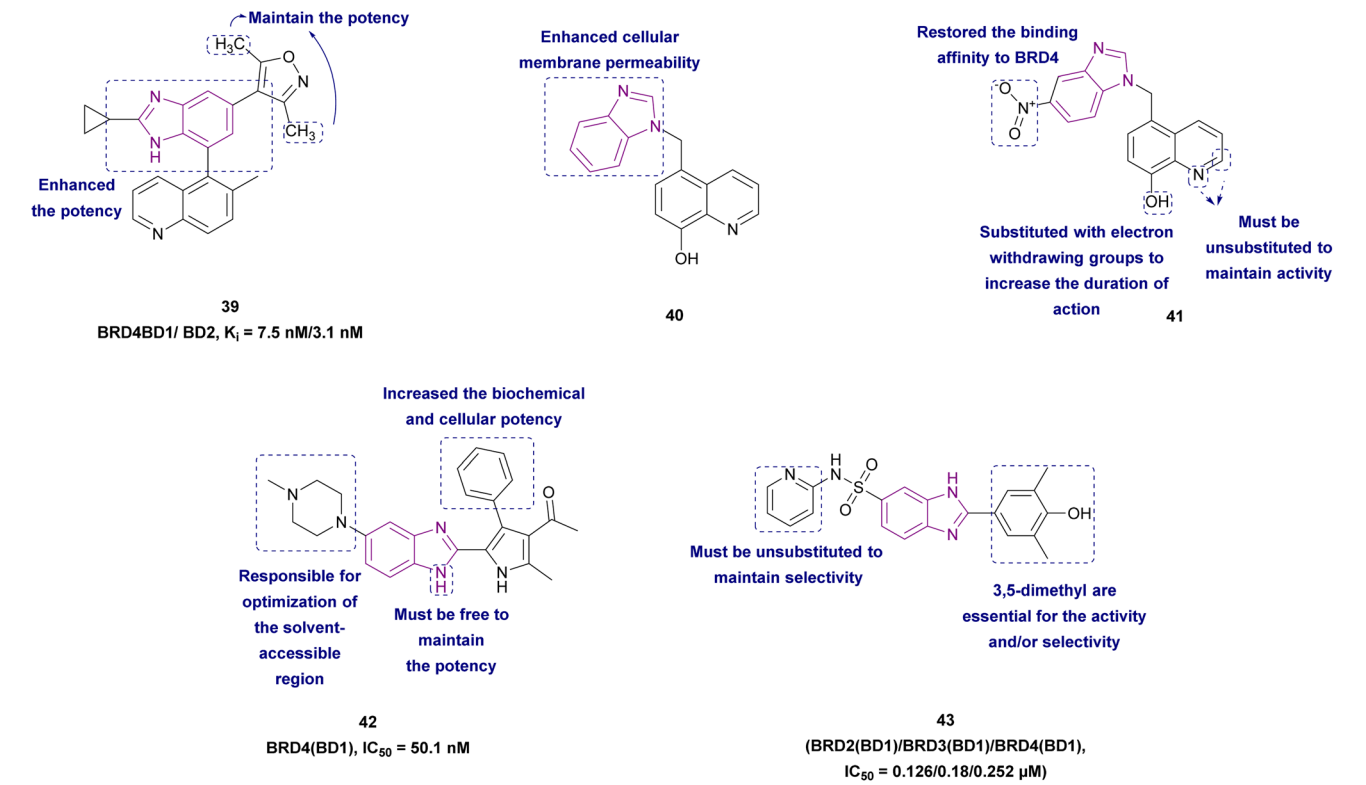


Fig. 20 Chemical structure, SAR and IC_{50} of BET family inhibitors **39–43**.

quinolinol was changed to 1*H*-benzoimidazole in **40**, which made it very potent but decreased the binding affinity to BRD4. To restore the activity at both the molecular and cellular levels, a nitro group was added to the benzene ring in **40** to produce **41**. According to SAR, the non-substituted nitrogen in the quinoline ring was required for activity, and the methylene linker directed the benzimidazole group to the WPF shelf of the pocket. For a longer duration effect, stronger electron-withdrawing groups may be added to the 8-hydroxyl group to reduce its phase II metabolism. Position 2 of quinoline must be unoccupied given that replacements would make the molecule less likely to act as a BRD4 inhibitor. In contrast to **41**, which was sensitive to MDA-MB-231 (IC_{50} 1.1 μM), **40** was sensitive to the MV4-11 cell line. In the MV4-11 cell line, **40** and **41** downregulated the c-Myc levels.[123]

Compound **42** resulted from the optimization of (1-(2,4-dimethyl-5-(thiophen-3-yl)-1*H*-pyrrol-3-yl)ethan-1-one). Kong *et al.* synthesized **42** *via* acetylation, bromination, Suzuki cross-coupling, and cyclization reactions. It is a well-tolerated BRD4 inhibitor with good metabolic stability and good oral bioavailability. Significantly, it may be a potential candidate for the treatment of AML and some solid tumours. It induced cell cycle arrest at the G0/G1 phase through the upregulation of p21 and apoptotic marker-cleaved PARP and downregulation of c-Myc. The introduction of *N*-methylpiperazine (a hydrophilic function group) at the 5-position of benzimidazole optimized the binding interactions towards the solvent-accessible region. The rigid phenyl substituent at the 4-position of the pyrrole increased the biochemical and cellular potency given that it was surrounded by a hydrophobic pocket. Substituting the *ortho*-, *meta*-, and *para*-position of the phenyl with –Cl or $–OCH_3$ or the *para*-/*meta*-position with methyl, methylthio, ethoxyl, dimethylamino, 3-fluoro-4-methoxyl, 3,4-dimethoxyl, benzo[*d*][1,3]dioxol-5-yl and 2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl had a negative influence on the potency.[129]

Compound **43**, a benzoimidazole-6-sulfonamide derivative, exhibited a better selectivity profile for all BD1 BETs with features of solubility, cell permeability, and stability. It caused a decrease in the expression of c-Myc without significant cytotoxicity. The benzimidazole scaffold was responsible for acceptable cellular permeability and was used as a bioisostere for the azobenzene scaffold. SAR studies revealed that the removal of the pyridine decreased both the affinity and selectivity. The separation of the pyridine ring from the sulfonamide function by a CH_2 group caused complete loss of activity. The activity shifted to non-BET proteins when the pyridine moiety was replaced with an aliphatic ring; however, when the pyridine moiety was replaced with a cyclopropyl group, there was significant off-target binding and a partial loss of affinity to BETs, with only binding to the BD2 of BRDT remaining. Its activity was attributed to the substitution of 3,5-dimethyl-4-phenol; removal of the 5-methyl or shifting the position of dimethyl to the 2,6 positions resulted in the loss of the inhibitory activity. Moreover, the activity of the unsubstituted phenol derivative was significantly less selective.[124]

Three HITs were chosen among 224 205 compounds using the 3D-QSAR pharmacophore model and docking as BRD4 inhibitors. Subsequently, the total binding free energy was determined using the MM/PBSA method, which showed that only the **44**/BRD4 complex had the best binding affinity towards BRD4 (Fig. 21) with the lowest binding free energy. The ADMET results showed that it has low aqueous solubility, good absorption, non-hepatotoxic and does not inhibit CYP2D6. In compound **44**, the benzimidazole moiety formed an H-bond. Besides, the 2*H*-chromen-2-one moiety formed π–π T-shaped interaction and π–anion interaction. Additionally, it formed van der Waals interactions and hydrophobic interactions.[121] Compound **45** is a BRD2 bromodomain inhibitor given that it inhibits its binding to acetylated H4K12.[122]

2.1.2.1.2 Benzimidazole derivatives as CBP/p300 bromodomain inhibitors. CBP and its paralog p300 are members of the lysine acetyltransferase superfamily. CBP and p300 share highly similar sequences and structural organizations, with overlapping but distinct functions, and thus they are typically referred to as CBP/p300.[130] CBP and p300 are multidomain proteins containing HAT and BRD domains. The CBP/p300 bromodomain has been associated with malignancies and is considered a therapeutic agent.[131] Moreover, CBP/p300 coactivates important oncogenes such as MYC and IRF4, which are significant in many types of haematological cancers.[132]

Compounds **46–50** are CBP/p300 bromodomain inhibitors (Fig. 22). CBP30 **46** is a potent and selective CBP/p300 bromodomain inhibitor (K_d = 0.021 and 0.032 μM, respectively) with a pIC_{50} of 7.1 ± 0.049. Its dimethylisoxazole mimics the key Kac binding interactions of the CBP BRD.[133] Z. Chen *et al.* optimized CBP30 **46** utilizing bioisosterism and conformational restriction strategies, which resulted in compound **47**, which is a potent p300 bromodomain inhibitor with a benzimidazole scaffold and a propiolactam linker, showing better

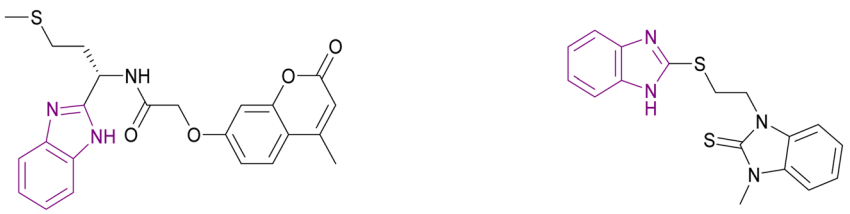


Fig. 21 Chemical structure of BET family inhibitors **44–45**.

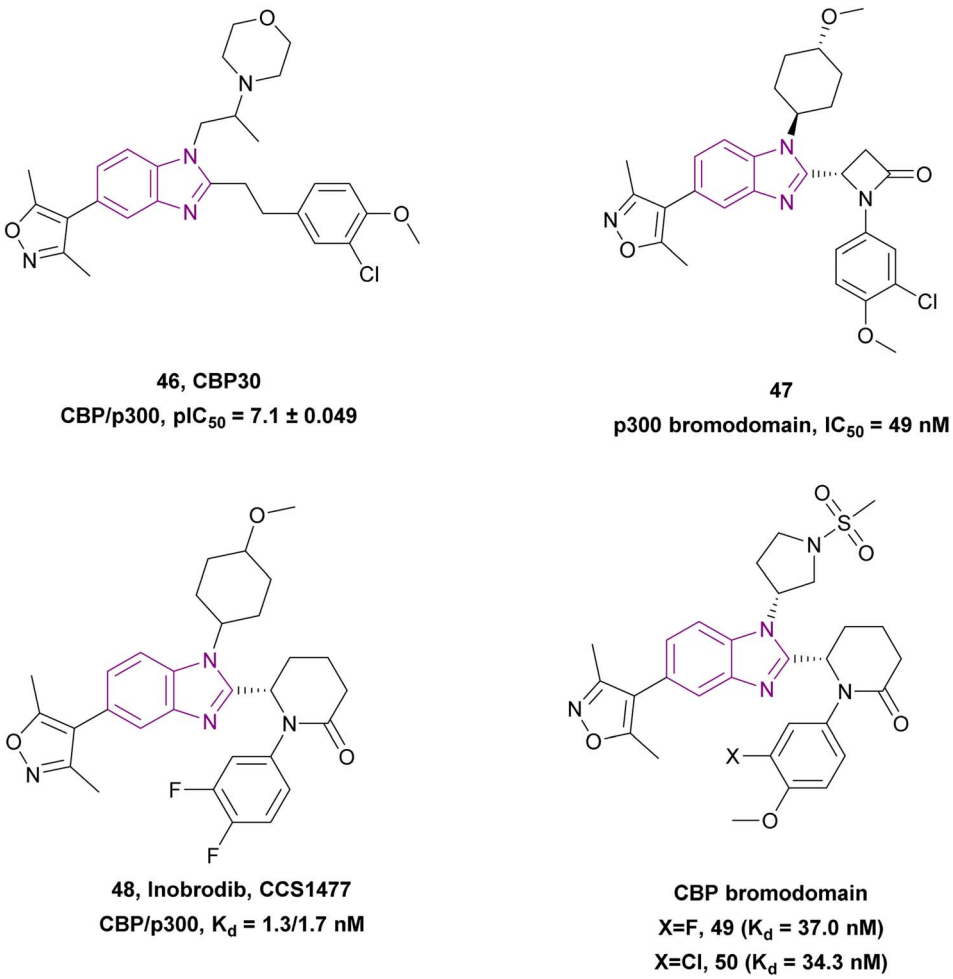


Fig. 22 Chemical structure, pIC_{50}, IC_{50} and K_d of CBP/p300 bromodomain inhibitors **46–50**.

antiproliferative activity against the OPM-2, 22RV1, MCF-7, and SCC-9 cell lines compared to CBP30 **46**. Moreover, it down-regulated c-Myc expression and induced apoptosis and cell cycle arrest in the G1/G0 phase.[134]

CCS1477 **48** is currently in phase I/II clinical trials for the treatment of AML, non-Hodgkin lymphoma, and MM (NCT04068597).[132] It is a potent, selective, and orally active small molecule inhibitor of the p300/CBP bromodomain with high affinity (K_d = 1.3/1.7 nM) and selectivity. It has anti-proliferative activity against prostate cell lines (22Rv1 and VCaP) with IC_{50} values of 96 and 49 nM, respectively. In 22Rv1 cells, p300/CBP inhibition resulted in the complete inhibition of plasma PSA. Moreover, the 22Rv1 xenograft model treated with it showed complete tumour growth inhibition.[135] It is promising for the treatment of advanced prostate cancer given that it exhibited antitumour activity, regulating AR and c-Myc signalling in androgen receptor (AR) splice variant (SV)-driven models, and could regulate CRPC biopsy biomarker expression and modulate the KLK3 blood levels.[136] Further studies showed that CCS1477 was effective in haematological malignancies, including MM and AML, as monotherapy or in combination with standard care agents. It arrested the cell cycle and inhibited differentiation through the upregulation of selected differentiation markers (*e.g.*, CD11b and CD86).[137] Moreover, it could be used in combination with azacitidine and/or venetoclax in AML and B-cell lymphomas. In an OPM-2 xenograft model, it had additive or synergistic inhibitory activity when combined with each of bortezomib, lenalidomide and vorinostat, suggesting its clinical utility in the treatment of MM as monotherapy and/or in combination with standard care agents.[138]

Compounds **49** and **50** (analogues of CCS1477 **48**) are CBP bromodomain inhibitors. Compound **49** showed better selectivity than CCS1477 **48**. Compound **49** displayed a similar binding affinity to that of CCS1477, with a K_d value of 37 nM.[120]

Compound **51**, (*R*)-5-methylpyrrolidin-2-one derivative, is a structurally modified compound of CCS1477 **48**. Compound **51** is a p300 bromodomain inhibitor with antiproliferative activity against different cell lines. With its high cell permeability, it could overcome the defect of CCS1477 **48** of high efflux rate. Moreover, it is metabolically stable and has no hERG risk, indicating its safety for the heart. Replacing the five-membered ring (pyrrolidine) with a four-membered (azetidine) or six-membered ring (piperidine) weakened its inhibitory activity. The methoxy substituted on *trans*-cyclohexyl group was important for its binding affinity. However, the methoxy group was

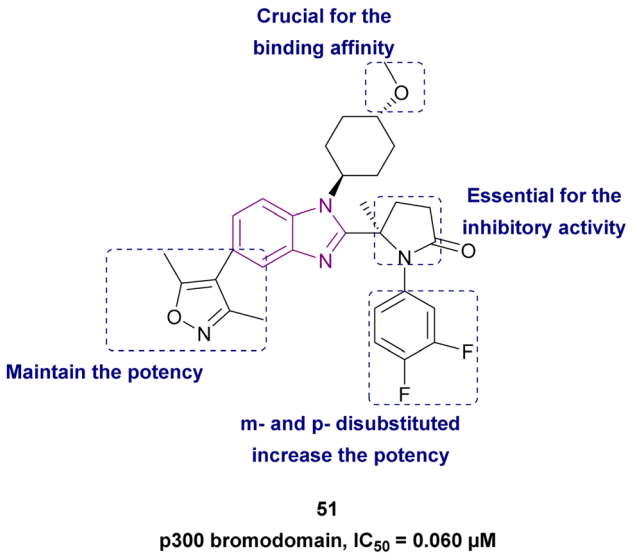


Fig. 23 Chemical structure, SAR and IC_{50} of CBP/p300 bromodomain inhibitor **51**.

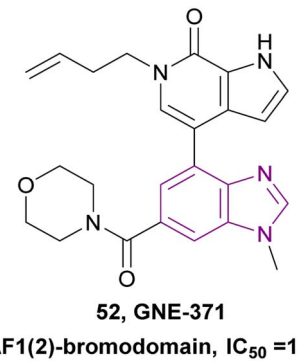


Fig. 24 Chemical structure and IC_{50} of TAF1 bromodomain inhibitor **52**.

a potential metabolizable site. Additionally, its potency was diminished when alternative heterocycles were used in place of 3,5-dimethylisoxazole. Generally, the *meta*- and *para*-disubstituted phenyl-containing compounds were more potent than the *para*- or *meta*-monosubstituted compounds (Fig. 23).[139]

2.1.2.1.3 Benzimidazole derivatives as TAF1 bromodomain inhibitors. TAF1 is a non-BET bromodomain protein that contains a histone acetyltransferase (HAT) domain and a tandem bromodomain module (TAF1(1) and TAF1(2)). TAF1(2) could recognize acetyl lysine as well as butyryl and crotonyl lysine. Moreover, TAF1(2) synergized with BRD4 in controlling cancer cell proliferation. Compound **52** is a TAF1(2)-bromodomain inhibitor with an IC_{50} of 10 nM and excellent selectivity over other bromodomain-family members, showing that the benzimidazole ring is essential for improving the potency and selectivity (Fig. 24). It exhibited antiproliferative synergy with the BET inhibitor JQ1.[140]

2.1.2.1.4 Benzimidazole derivatives as BRPF bromodomain inhibitors. The BRPF family (bromodomain and PHD finger-containing family of BRPF1, BRPF2/BRD1, and BRPF3) is non-BET bromodomain-containing proteins (1). BRPF1 contains multiple epigenetic reader modules, including a unique double PHD, zinc finger assembly (PZP), a bromodomain, and a chromo/Tudor-related Pro-Tyr-Tyr-Pro (PWWP) domain (2). Demont *et al.* showed that (5,6)-disubstituted benzimidazolones has greater selectivity for BRPF1 over BRD4. They synthesized compound **53** given that the amide at the 5-position interacted with a BRPF1 ZA loop and 2-methoxy phenyl is more potent than 3-methoxy phenyl carboxamide and unsubstituted phenyl.[141] Bamborough *et al.* optimized compound **53** (pIC_{50} = 7.1) by replacing piperidine with piperazine, where the extra basic nitrogen formed an additional hydrogen bond and improved the solubility, resulting in compound **54**, which was a potent and selective BRPF1 bromodomain inhibitor (pIC_{50} = 8.1) with greater solubility, potency (300 pM), and cell activity (20 nM) (Fig. 25). However, changes at the 3- and 5- positions of the benzimidazolone had detrimental effects.[142]

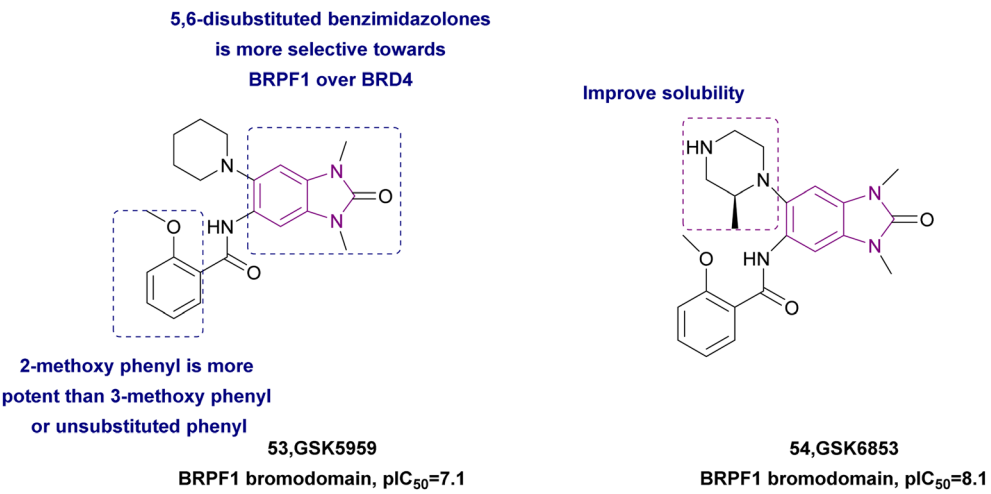


Fig. 25 Chemical structure, SAR, and pIC_{50} of TAF1 bromodomain inhibitors **53** and **54**.

2.1.2.1.5 Benzimidazole derivatives as BAZ2A bromodomain inhibitors. BAZ2A is a non-BET bromodomain-containing protein. Acetylated Lys16 on histone H4 is recognized by the bromodomain and PHD zinc finger domain of BAZ2A, which then bind HDAC1 to create heterochromatin. The metastatic potential of prostate cancer cells has been revealed to be impacted by BAZ2A downregulation, whereas overexpression of the protein has been linked to the aggressiveness of the disease. Among 54 794 molecules from the Maybridge Screening Collection and Enamine Golden Fragment libraries docked into the crystal structure of the BAZ2A bromodomain, only compound **55** inhibited the BAZ2A bromodomain with an IC_{50} of 28 μM, forming 3 H-bonds by the benzimidazole nitrogen and the N2 and N3 nitrogen atoms of the triazole ring. Moreover, compounds **56**, **57**, and **58** from the docked compounds showed an increase in binding affinity. The potency of compound **58** could be further improved by substituting the methyl group on the triazole ring with a longer aliphatic tail (Fig. 26).[143]

2.1.2.1.6 Benzimidazole derivatives as dual inhibitors. Further structure optimization of compound **59** resulted in compound **60** (Fig. 27), which is a BRD4, phosphatidylinositol 3-kinase (PI3K) (IC_{50} = 13.12 nM) and aurora A (IC_{50} = 10.19 nM) multi-targeted inhibitor, providing a lower dose, better safety, enhanced efficacy, and delayed drug resistance. In compound **60**, SAR studies revealed that replacing its benzyl moiety with an aromatic heterocyclic group or increasing the length of the alkyl side chain attached to its amide group may decrease its anti-cancer activity. It was tested in drug-resistant cell lines (A549 and H1975 cells) and non-drug-resistant cell lines (HCC827 cells), showing antiproliferation activity with IC_{50} values of 0.83 μM, 1.02 μM, and 0.26 μM, respectively. The pro-apoptotic

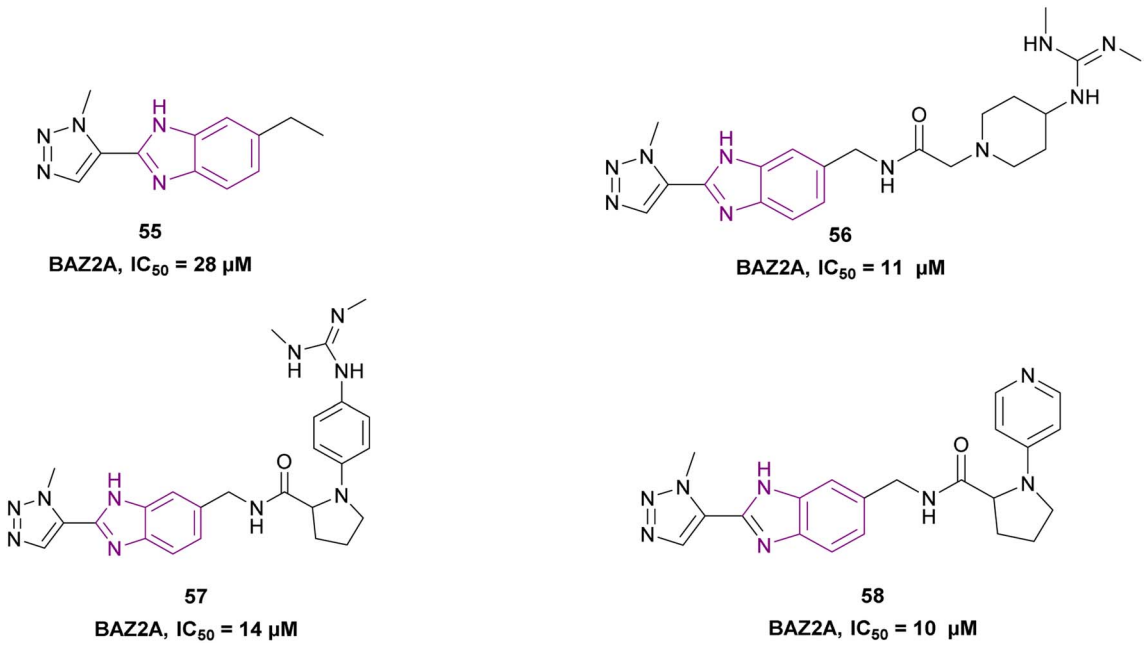


Fig. 26 Chemical structure and IC_{50} of BAZ2A bromodomain inhibitors **55**–**58**.

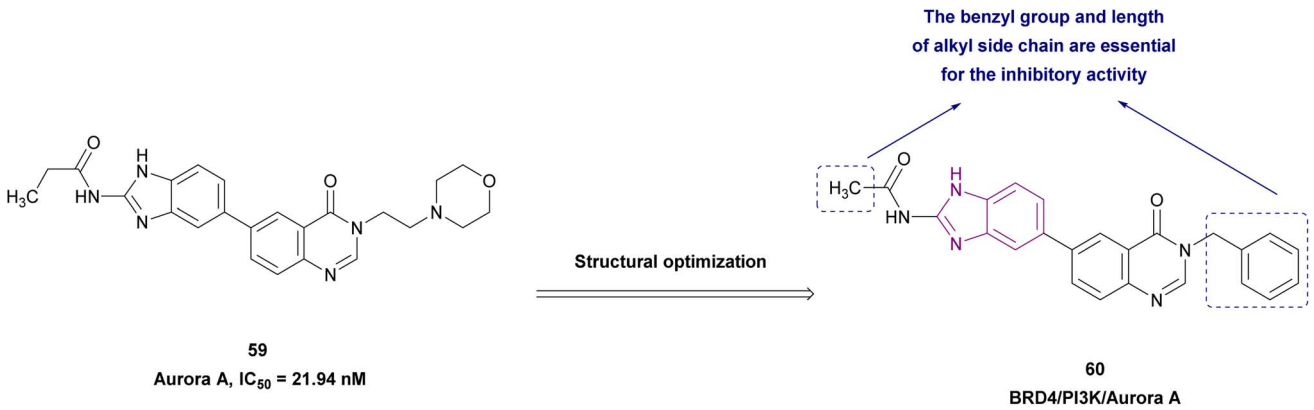


Fig. 27 Chemical structure, IC_{50}, and SAR of **59** and **60**.

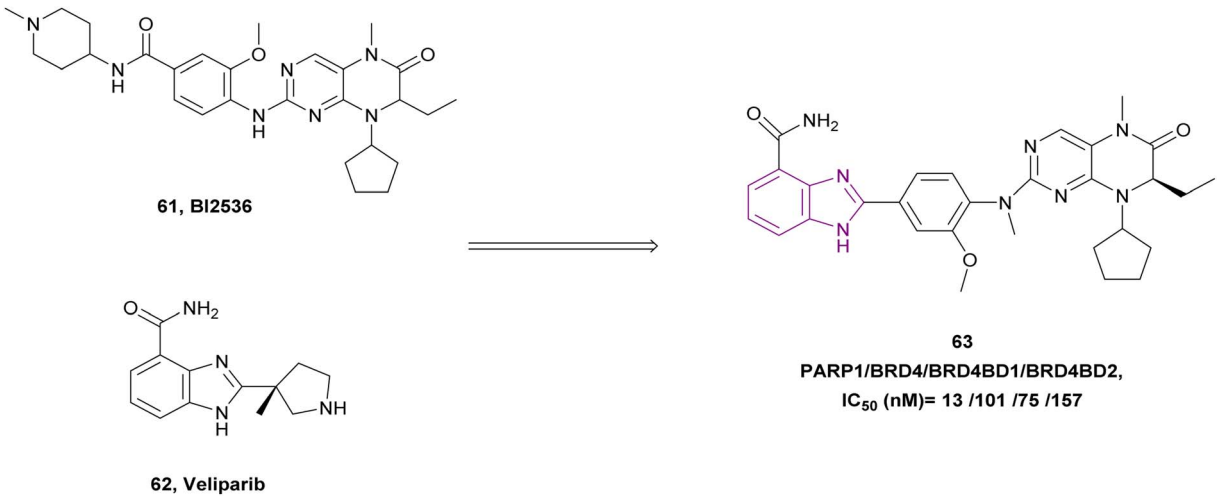


Fig. 28 Combination of compound **61** and **62** resulted in PARP1/BRD4 inhibitor **63**.

proteins cleaved-PARP and cleaved-caspase 9 were upregulated by it, while the anti-apoptotic protein Bcl-2 was downregulated. Also, it showed superior selectivity towards cancer cells to normal cells and induced cell cycle arrest in the G2/M phase.[128]

BI2536 **61**, a BRD4 inhibitor, and veliparib **62**, a PARP1/2 inhibitor, were combined to create compound **63**, a dual PARP/BRD4 inhibitor (Fig. 28). Compound **63** inhibited PARP1, BRD4, BRD4 BD1, and BRD4 BD2 with IC_{50} of 13 ± 2.5 nM, 101 ± 11 nM, 75 ± 10 nM, and 157 ± 15 nM, respectively. It exhibited anti-tumour action in pancreatic ductal adenocarcinoma (PDAC) SW1990 cells and breast cancer MDA-MB-231 cells with IC_{50} of 0.98 μM and 0.34 μM, respectively. Moreover, it could reverse olaparib-induced resistance by promoting cell cycle arrest, DNA damage, and autophagy-related cell death.[144]

Inhibition of BRD4 elevates homologous recombination (HR), the most effective repair pathway, and sensitizes cancer cells to PARP1/2 inhibitors. Huang, Cao *et al.* linked the 1*H*-benzimidazole-4-carboxamide moiety of veliparib acting as a PARP1 inhibitor with compound **64** acting as a BRD4 inhibitor to synthesize compound **65** (Fig. 29). Compound **65** is a dual PARP1/BRD4 inhibitor that arrests the cell cycle at G0/G1 and G2/M phases, increases DNA damage, and reverses olaparib-induced adaptive resistance. It was more effective than JQ-1 and olaparib in inhibiting pancreatic cancer cell (CFPAC-1, Capan-1, and SW1990) growth and had an antiproliferative effect on BRCA-proficient triple-negative breast cancer (MDAMB-231 and MDA-MB-468), BRCA2-mutated colonic cancer (HCT116), and leukaemic cancer (THP-11) cell lines. The inhibitory effects of compound **65** at a dose of 30 mg kg^{-1} on SW1990 xenograft tumour growth were more significant than that of JQ1, olaparib, and their combination.[145]

2.1.2.2 Benzimidazole derivatives as YEATS family inhibitors. YEATS is named after the 5 protein members containing this

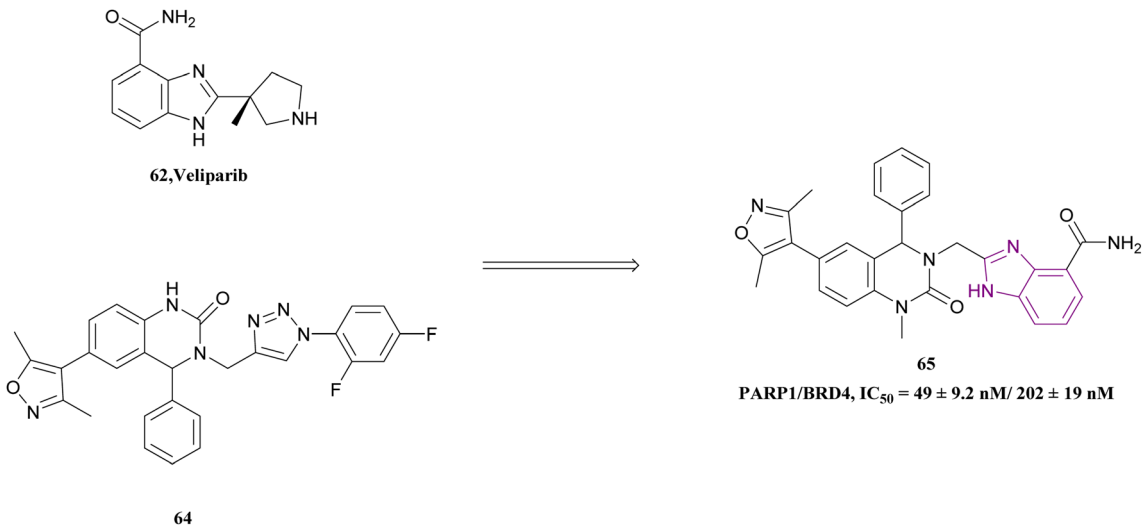


Fig. 29 Combination of compound **62** and **64** resulted in PARP1/BRD4 inhibitor **65**.

conserved domain (Yaf9, ENL, AF9, Taf14, and Sas5).[146] The YEATS domain is an acetylated and crotonylated lysine reader.[147] Humans have four proteins that contain the YEATS domain, GAS41 (YEATS4), AF9, YEATS2, and ENL.[148,149] Native ENL is necessary for leukaemia development, and thus the YEAST domain of ENL is considered as target for leukemogenesis.[146,148]

Compounds **66–68** inhibit ENL, as shown in Fig. 30. SGC-iMLLT **67** inhibited the YEATS domain (YD) of ENL (IC_{50} = 0.26 μM) and AF9. It resisted metabolism with the primary process for metabolism by *N*-demethylation, upregulated the expression of CD86, and downregulated the expression of MYC and DDN. Moustakim *et al.* optimized compound **66** due to its labile methyl ester, revealing SGC-iMLLT **67**, which has more potent binding affinity. Replacement of the amide bond with sulfonamide eliminated the inhibitory activity. Incubation of cells with SGC-iMLLT in the presence of vorinostat significantly decreased $t_{1/2}$ for MLLT1 and MLLT3 (MLLT1: $P < 0.0001$, MLLT3: $P = 0.0185$).[150]

To improve the anti-leukemic cell activities of SGC-iMLLT **67**, Guo, Jia, *et al.* replaced the indazole ring with a larger aromatic system, such as methyl phenanthridinone in compound **68**. This increased the π–π stacking interaction with residue histidine 56 (H56), which is located at the substrate entrance through which the H3Kac27 peptide entered. Compound **68**, an ENL inhibitor with a K_d of 17.0 nM, is a therapeutic target in leukemogenesis, which was the most powerful SGC-iMLLT analogue because of its exceptional target engagement in the cellular context. It downregulated the expression of the target gene MYC alone or in combination with JQ-1. It had inhibitory activities towards MLL-rearranged cell lines (MOLM-13 and MV4-11) and showed the highest thermal stability for the ENL domain but did not affect the GAS41 YEATS domain. The *in vivo* pharmacokinetic properties of **68** showed a $t_{1/2}$ of 5.2 h and C_{max} of 33.6 ng mL^{-1} at 0.5 h with better oral exposure. The mean resident time after oral administration was 5.5 times higher than that of SGC-iMLLT. However, it needs further optimization due to its low oral bioavailability.[148]

2.2 Histone methylation

2.2.1 Histone methyltransferases enzymes. Histone methylation is a reversible reaction through histone methyltransferases and demethylases. Methylation occurs primarily at the lysine and arginine residues in the side chains (N-terminal) of histones.[151] Depending on which residue of the histone tail is modified, histone methylation may promote transcription repression or activation.[152] Also, it is a key in epigenetic modification that regulates many nuclear processes, including transcription, DNA replication, and DNA repair.[153] Histone lysine methylation is mediated by two enzyme families, including Su(var)3–9, an enhancer-of-zeste and trithorax (SET) domain-containing proteins, and DOT1L. Alternatively, histone arginine methylation is mediated by the protein arginine *N*-methyltransferase (PRMT) family.[154]

2.2.1.1 Histone lysine methyltransferases (HKMTs). All known lysine histone methyltransferases contain a conserved methyltransferase domain termed a SET domain. It consists of 4 families including SUV39, SET1, SET2, and RIZ.[155] HKMTs catalyze the transfer of a methyl group from *S*-adenosylmethionine (SAM) to the ε-amino group of lysine.[156] H3K4, 9, 27, 36, 79 and H4K20 are the commonly recognized lysine methylation sites.[151] The six lysine methylation sites have 24 distinct HKMTs including SETD1A, SETD1B, MLL1, MLL2, MLL3, MLL4, SETD7, and PRDM9 for H3K4 methylation, EZH1 and EZH2 for H3K27 methylation, SETD2, NSD1, NSD2, NSD3, and

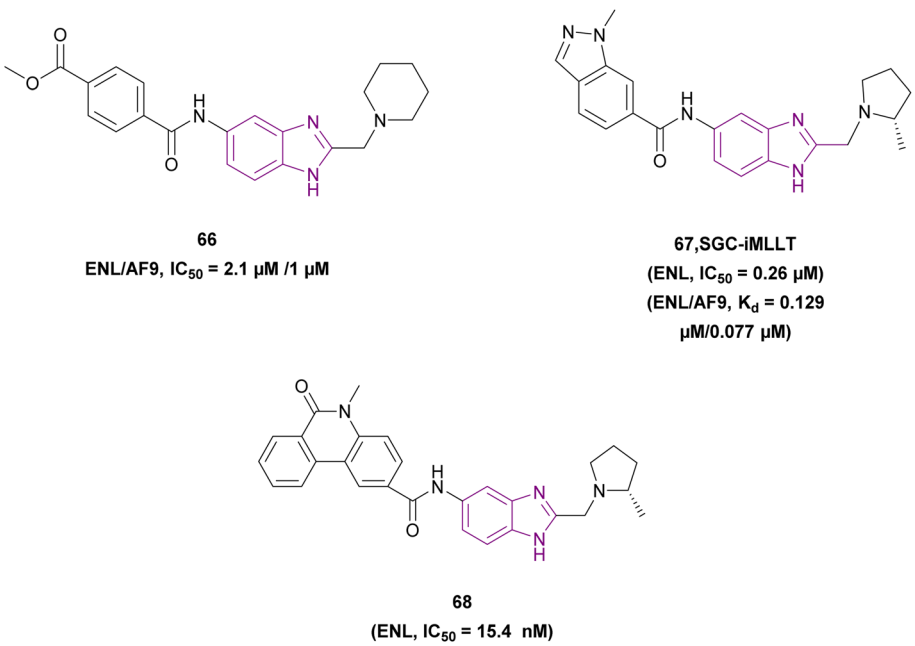


Fig. 30 Chemical structure and IC_{50} of ENL inhibitors **66–68**.

ASH1L for H3K36 methylation, SUV39H1, SUV39H2, SETDB1, G9A/EHMT2, and GLP/EHMT1 for H3K39 methylation, DOT1L for H3K79 methylation, and SETD8, SUV420H1 and SUV420H2 for H4K20 methylation.[157] Here, we discuss some benzimidazole derivatives that target lysine histone methyltransferases, MLL1 and EZH2, which belong to the SET1 family, G9a, belonging to the SUV39 family, and DOT1L, as shown in Fig. 31.

2.2.1.1.1 Benzimidazole derivatives as DOT1L inhibitors. DOT1L is a histone methyltransferase enzyme that causes mono-, di-, or trimethylation of H3K79. The hypermethylation of H3K79 has been associated with the development of acute leukemias characterized by MLL (mixed-lineage leukemia) rearrangements (MLLr cells). Therefore, the growth of MLLr cells is inhibited by the suppression of H3K79 methylation.[158] Optimization of EPZ004777 **69** using the structure-guided design resulted in EPZ-5676 **70** (Fig. 32), which is a potent and selective aminonucleoside DOT1L inhibitor. It has recently entered clinical evaluation as a therapeutic agent for MLL-rearranged leukemia because it decreased HOXA9 and MEIS1 mRNA. By occupying the SAM binding pocket, EPZ-5676 **70** altered the conformation of DOT1L, which allowed additional interactions to be formed. It had an antiproliferative effect on MV4-11 cells with an IC_{50} of 3 nM. Also, *in vivo* studies using MV-411 xenograft-bearing rats showed that continuous IV infusion for 14 days or longer gave optimal activity.[159,160]

2.2.1.1.2 Benzimidazole derivatives as mixed lineage leukemia 1(MLL1) inhibitors. The mixed lineage leukemia 1 (MLL1) protein belongs to the SET1 family and catalyzes H3K4 methylation in all three states (mono-, di-, and tri-). Also, it is required for the maintenance of normal hematopoiesis. Dysregulation of the function of MLL1 is responsible for acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). The MLL1 core complex consists of MLL1, WDR5, Ash2L, RBBP5, and DPY30. Hence, disturbing the MLL1-WDR5 interaction is an attractive approach for MLL-rearranged leukemias to downregulate the methylation level of H3K4 and

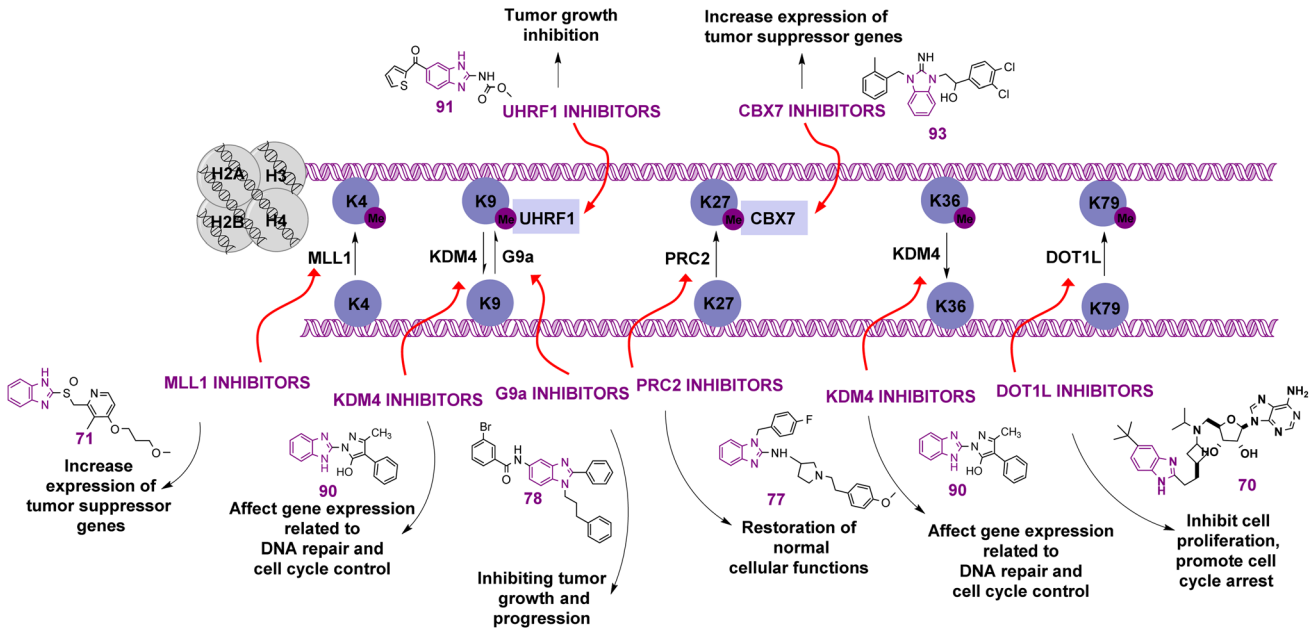


Fig. 31 Effects of benzimidazole derivatives as histone lysine methyl transferase inhibitors, histone demethylase inhibitors, and histone methylated readers inhibitors on cancer cell growth and gene expression.

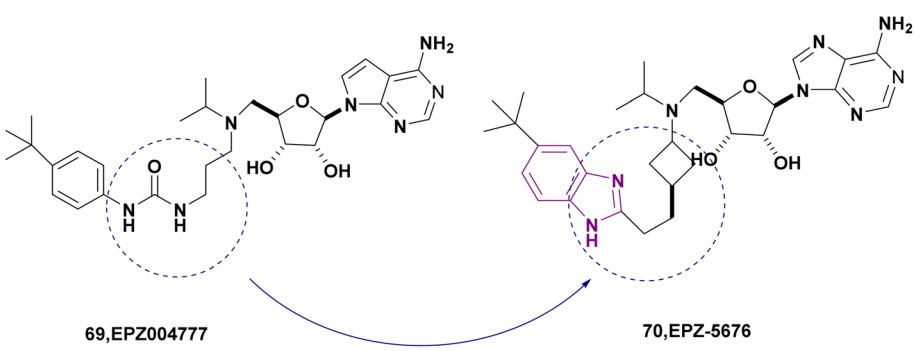
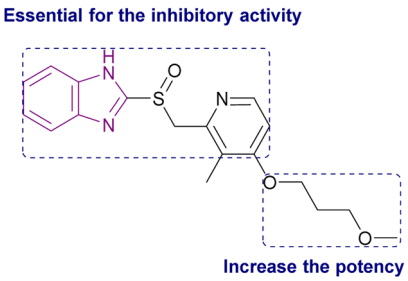


Fig. 32 Chemical structure of DOT1L inhibitors **69**–**70**.



71,Rabeprazole
(MLL1-WDR5, IC_{50} = 0.49 ± 0.1 μM)
(MV4-11/Molm-13, GI_{50} = 14.1 μM /17.5 μM)

Fig. 33 Chemical structure, IC_{50}, SAR, and *in vitro* potency of MLL1 inhibitor **71**.

the overexpression of Hox genes, which are known to induce leukemia.[161]

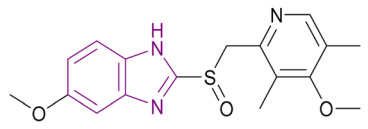
The outcome of cell-based screening on 1200 compounds from an in-house approved drug database was rabeprazole **71** (Fig. 33), which inhibited the MLL1-WDR5 interaction competitively. It displayed antiproliferation activity selective for cancer cell lines such as MLL-fusion leukemia cell lines MV4-11 (MLL-AF4) and MOLM-13 (MLL-AF9). However, it had no inhibitory effect on K562 (wild-type MLL1). Moreover, it down-regulated the expression of the Hoxa9 and Meis1 genes.

The MLL1-WDR5 complex was competitively inhibited by further proton pump inhibitors such as omeprazole **72**, esomeprazole **73**, lansoprazole **74**, and *R*-lansoprazole **75**, showing anti-proliferation effects against MV4-11 (MLL-AF4) and Molm-13 (MLL-AF9) (Fig. 34). The SAR of omeprazole **72** showed that removing $–OCH_3$ from its benzimidazole ring did not affect its biochemical potency. Also, its biochemical potency was unaffected by the addition of a difluoromethoxy moiety to C-5 on the benzimidazole ring, but the MLL1-WDR5 inhibition activity decreased by the removal of the pyridine ring substituents or their replacement with a phenyl ring. In addition, the benzimidazole group was inserted into the central channel of WDR5, and the sulfoxide chain and benzimidazole ring were necessary for the inhibitory action.

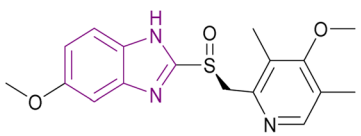
In the fluorescence polarization (FP) assay, lansoprazole **74** and (*R*)-lansoprazole **75** had similar activity, suggesting that the sulfoxide-induced conformational isomerism did not significantly impact inhibitory activity. Rabeprazole **71**, carrying a hydrophobic chain, showed the most potent activity in disturbing the MLL1-WDR5 interaction. More modifications are needed to repurpose the scaffold of substituted 2-pyridyl methyl/sulfinyl benzimidazole to eliminate its proton pump inhibitory effects in the future.[161]

2.2.1.1.3 Benzimidazole derivatives as polycomb repressive complex 2 (PRC2) inhibitors. PRC2 is a methyltransferase that responsible for the mono/di/trimethylation of histone H3k27 and is comprised of various catalytic subunits, including EZH1/2, EED, and SUZ12.[8] Heterozygous mutations in EZH2 cause hyper-trimethylation in H3K27, causing GC B-cell type diffuse large B-cell lymphoma (GCB-DLBCL) and follicular lymphoma. Importantly, the interaction between the EZH2 and EED subunits of PRC2 is crucial for the trimethylation of H3K27. Thus, destabilizing of the PRC2 complex by inhibiting the interaction of EZH2-EED subunits can be a good target in the treatment of cancer.[162]

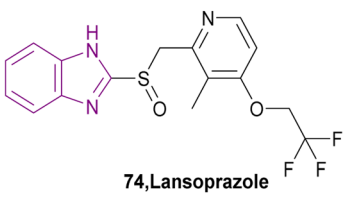
Virtual screening of 1000 compounds revealed that the FDA-approved astemizole **76** drug could displace the EZH2 peptide with an IC_{50} of 93.80 μM by binding to EED. Thus, it competed with EZH2, causing dissociation of the PRC2 complex as the benzimidazole ring bound to the hydrophobic groove of EED. This study proved the invalidity of previous studies, which explained that the antiproliferative effect of astemizole was due to the blockage of the EAG1 ion channel. The combination of



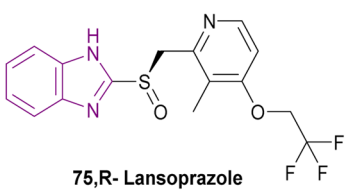
72,Omeprazole
(MLL1-WDR5, IC_{50} = 1.7 ± 0.1 μM)
(MV4-11/Molm-13, GI_{50} =37.3 μM /34.1 μM)



73,Esomeprazole
(MLL1-WDR5, IC_{50} = 1.8 ± 0.2 μM)
(MV4-11/Molm-13, GI_{50} =47.6 μM /32.0 μM)



74,Lansoprazole
(MLL1-WDR5, IC_{50} = 5.9 ± 0.9 μM)
(MV4-11/Molm-13, GI_{50} = 21.1 μM /30.1 μM)



75,R- Lansoprazole
(MLL1-WDR5, IC_{50} = 5.7 ± 1.1 μM)
(MV4-11/Molm-13, GI_{50} = 25.1 μM /27.2 μM)

Fig. 34 Chemical structure, IC_{50}, and *in vitro* potency of MLL1 inhibitors **72–75**.

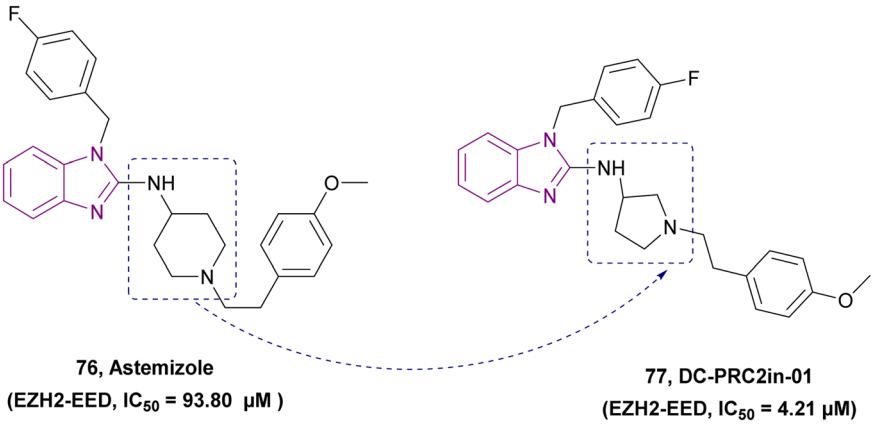


Fig. 35 Chemical structure and IC_{50} of PRC2 inhibitors **76–77**.

astemizole and EPZ (an EZH2 inhibitor) had a synergistic effect on DB and Toledo cells.[162] Given that the distance between the two nitrogen atoms was essential for the formation of hydrogen bonds, the optimization of astemizole led to DC-PRC2in-01 **77** by substituting the 4-aminopiperidine of astemizole with 3-aminopyrrolidine (Fig. 35). The methoxy group of astemizole and DC-PRC2in-01 maintained potency due to the generation of more hydrophobic contacts. DC-PRC2in-01 **77** inhibited the interaction of EZH2-EED by binding to EED and arrested the cell cycle at the G0/G1 phase. The Pfeiffer cell line was the most sensitive cell line to DC-PRC2in-01 **77**, according to studies using the SU-DHL-4, Karpsa-422, DB, and Pfeiffer cell lines to demonstrate its antiproliferative activity.[163]

2.2.1.1.4 Benzimidazole derivatives as G9a inhibitors. G9a (also known as KMT1C or EHMT2) is a histone methyltransferase of histone H3 (H3K9me1 and H3K9me2). G9a is overexpressed in various human cancers, such as prostate cancer, leukemia, hepatocellular carcinoma, pancreatic cancer, lung cancer, and breast cancer.[15,164]

The chemical structure of compounds **78–81**, which are G9a inhibitors, is provided in Fig. 36. Virtual screening for compounds from the ChemBridge CORE library followed by *in silico* studies revealed 10 hits with a benzoxazole moiety in 4 of them. The benzoxazole moiety was subjected to further optimization by replacing it with a benzimidazole moiety and introducing phenylpropyl, a strong hydrophobic group, to the nitrogen atom of benzimidazole. Compound **78** was the most selective and potent inhibitor for G9a with antiproliferative activity when tested in MCF7 cells. The MTT assay showed that MCF-7 was the most sensitive cell line to it among five different cancer cell lines (MCF-7, T-47D, MDA-MB-231, MDA-MB-468, and MDA-MB-435 cells). It could induce autophagy *via* AMPK in MCF7 cells. In addition, high concentrations of **78** could induce apoptosis through p21-Bim signalling cascades in MCF7 cells. Thus, it activated the tumour suppressor p53 by inhibiting G9a, which regulated p21 activation.[164]

Compound **80**, an analogue of BIX-01338 **79**, was found to inhibit G9a more effectively in cells than the latter. **80** is a SAM mimetic prodrug, which is converted to **81** (the active form) in cells. Compound **81** was a more potent biochemical G9a inhibitor than **80**, but it had no activity in cell-based assays due to its impaired cell permeability. Thus, it must be in the methyl-ester form (**80**). Compound **81** inhibited G9a and PRC2 activity,

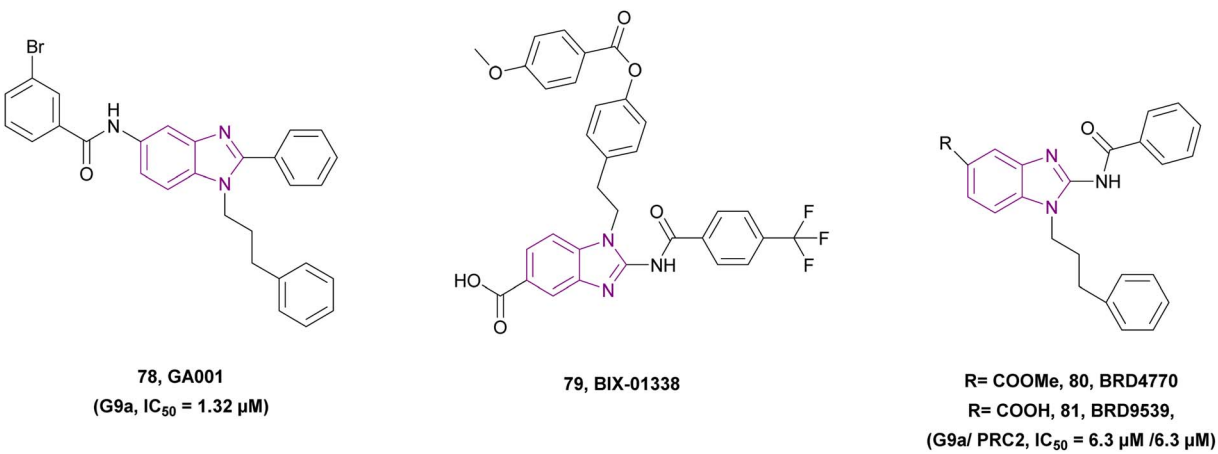


Fig. 36 Chemical structure and IC_{50} of G9a inhibitors **78–81**.

but there was no evidence for PRC2 inhibition by **80**. Compound **80** was nontoxic and induced a senescent phenotype in a pancreatic cancer cell line, which inhibited both anchorage-independent and anchorage-dependent, and caused cell-cycle arrest at the G2/M phase. In addition, compound **80** activated the ATM pathway without inducing DNA damage, while the ATR pathway was not affected.[165]

2.2.1.2 Benzimidazole derivatives as arginine methyltransferases inhibitors. The post-translational methylation of arginine residues is catalyzed by protein arginine methyltransferases (PRMTs) type I–III; type I PRMT enzymes (PRMT1, -2, -3, -4, -6, and -8), type II enzymes (PRMT5 and PRMT9), and type III enzymes (PRMT7).[166] These enzymes carry the methyl group from *S*-adenosylmethionine (AdoMet) to a terminal guanidino nitrogen of arginine, which resulted in S-adenosylhomocysteine (AdoHcy) and methylarginine.[167] Importantly, PRMT inhibitors can reverse drug resistance and sensitize cancer cells to anti-cancer therapies, especially PRMT5.[168]

The increased PRMT5 levels in lymphomas, breast cancer, lung cancer, colorectal cancer, and glioblastoma were validated as a target in mantle cell lymphoma and glioblastoma. The optimization of compound **82**, a PRMT5 inhibitor, and its SAR studies led to the development of compound **83**, which selectively inhibited PRMT5 with IC_{50} of 0.33 μM and K_d of 0.987 μM. Compound **83** is a SAM competitive inhibitor and a peptide noncompetitive inhibitor (Fig. 37 and 38). The benzimidazole ring and the phenyl ring were crucial for maintaining activity. Substitutions at C-4 of the benzimidazole caused a loss of activity, but methoxy substitution at C-5 improved the potency. The methoxycarbonyl on the phenyl ring must be retained as the oxygen of the carbonyl forms a hydrogen bond with the L315 residues. It was sensitive only to MV4-11 cells, with an EC_{50} of 6.53 μM.[166]

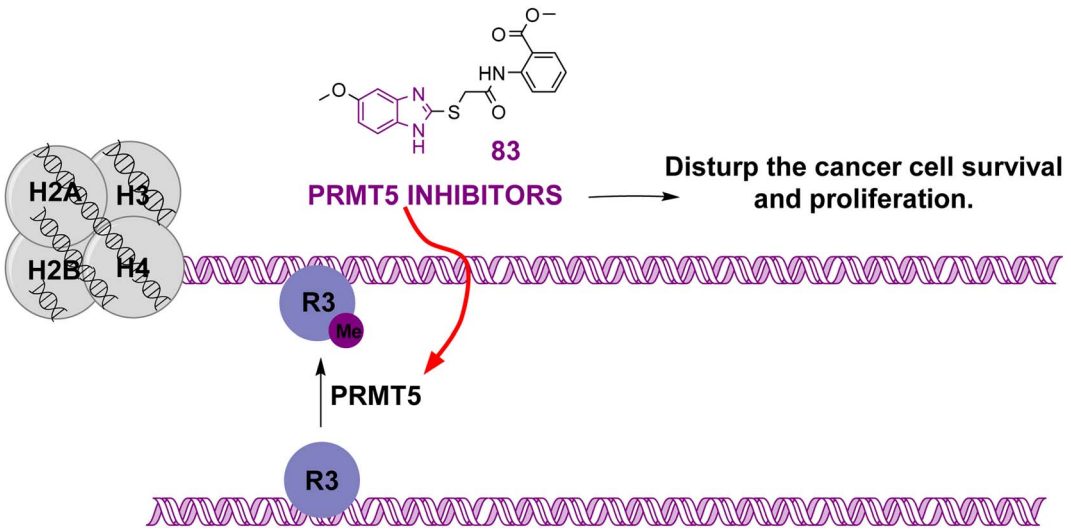


Fig. 37 Effects of benzimidazole derivatives act as arginine methylation inhibitors on cancer cell growth and gene expression.

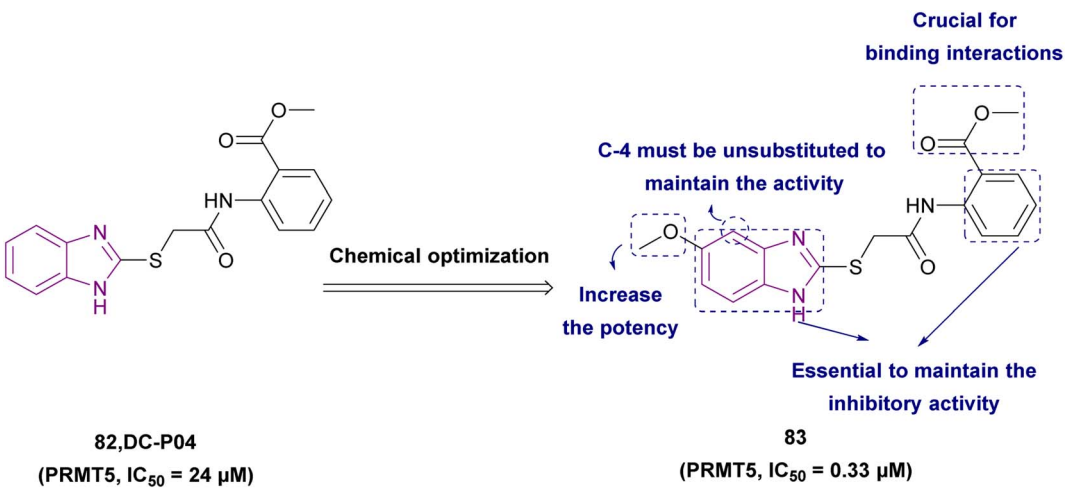


Fig. 38 Chemical structure and IC_{50} of PRMT5 inhibitors **82**–**83**.

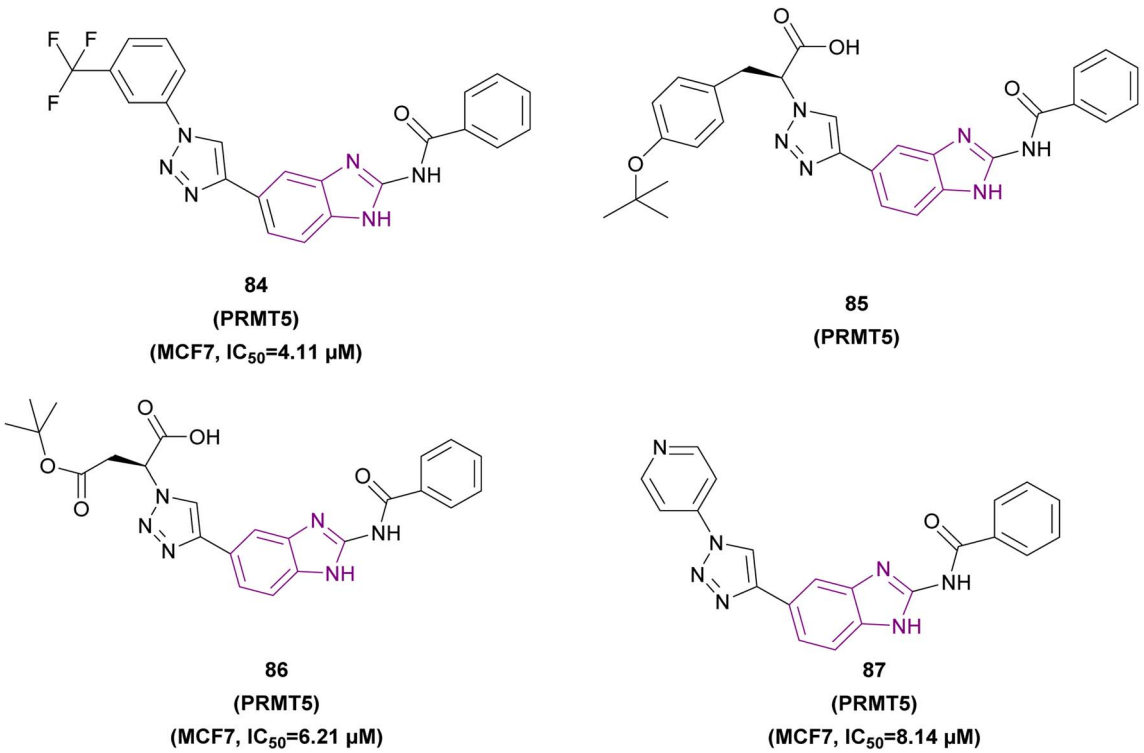


Fig. 39 Chemical structure, IC_{50}, and *in vitro* potency of PRMT5 inhibitors **84–87**.

A new triazole-benzimidazole derivative, **84**, is a PRMT5 inhibitor with anti-tumour activity against the MCF7, DU145, PC3, and HepG2 cell lines. The benzimidazole ring formed π–π interactions and H-bonds with the active site residue. It was synthesized through a series of reactions, including Sonogashira coupling, HATU-promoted amide coupling, and copper-catalyzed cycloaddition reaction to form **84**. Also, compounds **85**, **86**, and **87** had inhibitory activity against cancer cells and PRMT5 inhibition, but to a lesser extent than **84** (Fig. 39).[169]

2.2.2 Histone demethylases enzymes. There are two families of lysine demethylases (KDMs), KDM1/LSD1 [flavin adenine dinucleotide (FAD)-dependent KDM] and KDM2–7 [Fe(II)– and 2-oxopentadiene acid (2-OG)-dependent KDM],[170] which are involved in different stages of gene transcription and play a role in diseases, notably cancer.[171]

2.2.2.1 Benzimidazole derivatives as jumonji domain-containing protein (JMJD) inhibitors. Jumonji domain-containing proteins (JMJDs) are a class of histone lysine demethylases.[172] The KDM4 subfamily consists of KDM4A-E (JMJD2A-E). KDM4 demethylates di- and tri-methylated lysine 9 (KDM4A-E) and lysine 36 (KDM4A-C) of histone H3 and requires Fe^{2+} and α-ketoglutarate (α-KG) as cosubstrates.[173,174]

JMJD2A is a promoter of colon, lung, and breast cancer cell proliferation. An abnormality in the expression of JMJD2A can lead to colon cancer, lung cancer, and breast cancer. Due to the low cell permeability of 5-carboxy-8-hydroxyquinoline, it was optimized using structure-based design, leading to compound **88** (Fig. 40), which was synthesized through a series of reactions, including the Doebner–Miller reaction. It selectively

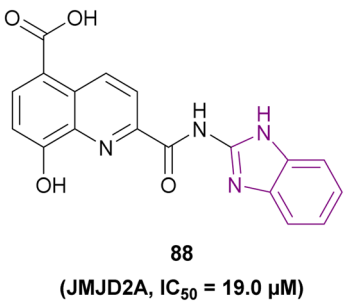


Fig. 40 Chemical structure and IC_{50} of JMJD inhibitor **88**.

inhibited JMJD2A with an IC_{50} of 19.0 μM and had anti-proliferative activity against HCT116, MCF-7, and A549 cell lines with good aqueous solubility and permeability.[172]

JMJD inhibitors **89** and **90** are illustrated in Fig. 41. A library of 32 032 compounds was screened and filtered by fluorescence-based and FDH-mediated coupled enzyme assays. CBN-209350 **89** was revealed after choosing PAINS compounds and excluding false-positive compounds. CBN-209350 had a benzimidazole pyrazolone scaffold with KDM4A-E selectivity. Also, it inhibited KDM2A and PHF-8. Higher concentrations of CBN-209350 had cytostatic effects on LnCaP, DU145, and PC-3 cell lines given that it was less permeable to the cell membrane because of its large polar surface area and low clogP value. Its safety for non-cancerous cells was established, and it produced unique biphasic inhibition curves in both the fluorescence assay and ELISA.[174]

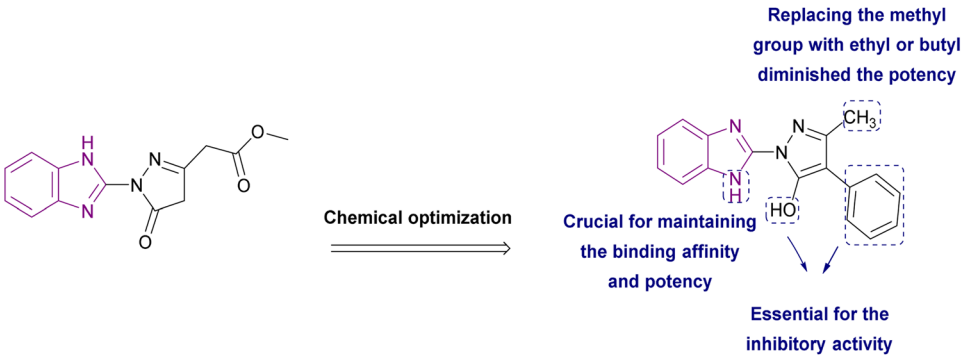


Fig. 41 Chemical structure and IC_{50} of JMJD inhibitors **89** and **90**.

Carter *et al.* optimized CBN-209350 **89**, resulting in compound **90**, given that CBN-209350 **89** lacks the pyrazole-substituted sidechains that mimic the terminal ethyl carboxylate present in the α-KG and are necessary for interacting with the active site of the enzyme. Compound **90** had an uncompetitive and non-competitive mechanism of inhibition with the α-KG cofactor and peptide substrate, respectively. It downregulated AR-dependent PSA gene expression in LnCaP cells and inhibited the growth of all the PCa cell lines tested, including LnCaP cells, DU145 cells, PC3 cells, and a non-disease control cell line (HuPrEC). In compound **90**, the phenyl group (nonpolar, electron-rich sidechain) and hydroxyl moiety were crucial, given that their replacement would reduce the inhibitory activity. The bidentate heterocyclic nitrogen atoms were essential for binding affinity, given that replacing the benzimidazole NH moiety with a sulfur atom or methylating the NH moiety reduced the potency. Furthermore, the potency was diminished when the methyl group was replaced with ethyl or butyl. Replacing the benzimidazole moiety with pyridine or 3-chloropyridazine decreased the potency.[173]

2.2.3 Histone methylated readers

2.2.3.1 Benzimidazole derivatives as UHRF1 inhibitors. UHRF1 is a histone-methylated reader. UHRF1 upregulation resulted in silencing of tumour suppressor genes. Nocodazole **91** is a UHRF1 inhibitor (Fig. 42) that causes downregulation of UHRF1. It acquired an L-shaped conformation in the binding cavity, with the core benzimidazole ring surrounded by Leu462, Gly464, Gly465, and Tyr466, according to molecular docking studies conducted within the SRA domain of UHRF1.[175]

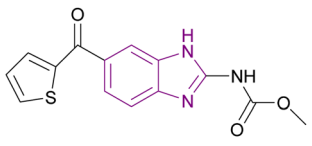


Fig. 42 Chemical structure of UHRF1 inhibitor **91**.

2.2.3.2 Benzimidazole derivatives as CBX7 inhibitors. CBX7, a subunit of polycomb repressive complex 1 (PRC1), with its chromodomain (CBX7ChD) is a reader of H3K27me3 (ref. 176) and is responsible for decreasing the transcription of p16INK4a, a tumour suppressor gene, in prostate cancer cells. Hence, CBX7ChD is an important target in cancer treatment. *In silico* chemical screening of approximately 100 000 drug-like compounds resulted in MS452 **92**, which had poor cell permeability. Consequently, a ligand development strategy and cell-based SAR study discovered MS351 **93**, which is a chromodomain antagonist that inhibited H3K27me3 binding to CBX7ChD and selectively bound to CBX7ChD (Fig. 43). The crystal structure of the CBX7ChD/**93** complex revealed that the iminobenzimidazole core interacted with the aromatic cage residues Phe11, Trp32, and Trp35, which normally house the methyl-lysine of the bound H3K27me3 peptide. It upregulated the expression of p16INK4a in human prostate cancer PC3 cells. Further chemical optimization of **93** was suggested, such as a conformationally constrained linker connecting the iminobenzimidazole and dichlorobenzene and certain substituent groups on the iminobenzimidazole might optimize its interactions with the cage residues.[177]

2.3 Benzimidazole derivatives inhibit other epigenetic enzymes

2.3.1 Benzimidazole derivatives as deubiquitinating enzymes inhibitors. USP5 is a deubiquitinating enzyme that prevents the degradation of the MYCN protein, a key driver gene in neuroblastoma, and is overexpressed in several cancers, including glioblastoma, hepatocellular carcinoma, melanoma, and pancreatic cancer. Compound **94** is a pyrimido[1,2-*a*] benzimidazole derivative and is bound to the USP5 protein with a K_d of 0.47 μM (Fig. 44), stopping the formation of the USP5-

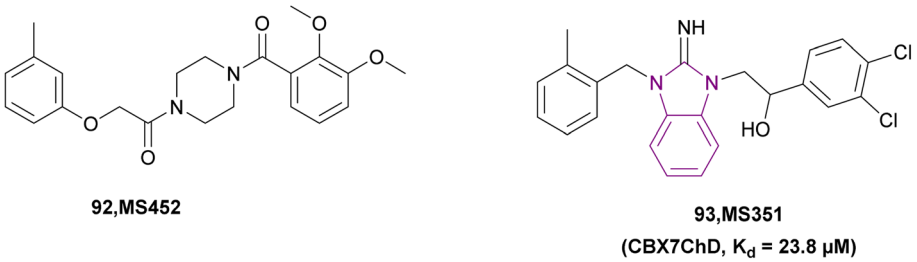


Fig. 43 Chemical structure and K_d of CBX7 inhibitors **92** and **93**.

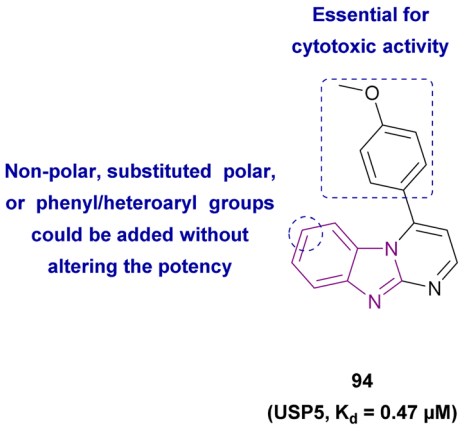


Fig. 44 Chemical structure and K_d of USP5 inhibitor **94**.

MYCN complex. It preferred USP5 over other MYCN deubiquitinating enzymes (USP3, USP7, and USP28), with selectivity towards cancer cell lines. It had a synergistic effect when combined with vorinostat and panobinostat. It had cytotoxicity against MYCN-amplified and MYCN-non-amplified neuroblastoma cells. The 4-methoxyphenyl group at the C-4 position was crucial, given that shifting it from C-4 to C-2 resulted in a loss of activity, and substituting it with H, F, Cl, or Br atoms led to diminishing cytotoxicity. Even when the OMe group was replaced by trifluoromethoxy, the cytotoxicity was lost. There were slight reductions in cytotoxicity when the methoxy group was substituted in the *meta* or *ortho* positions. The potency of **94** remained unaltered by adding non-polar, substituted polar, or phenyl/heteroaryl groups in place of the *H* atom at the C-7 position.[178]

2.3.2 Benzimidazole derivatives as citrullination enzymes inhibitors. Citrullination causes proteins to gain 0.98 mass units and lose one positive charge per deamination. Peptidyl arginine deiminases (PADs) are calcium-dependent enzymes that mediate citrullination. There are five peptidyl arginine deiminase (PAD) isozymes (PADs 1–4 and 6), which are uniquely distributed in various tissues.[179]

GSK-484 **95** is a PAD4 inhibitor, where replacing its the *N*-cyclopropylmethyl group on the indole ring with *N*-benzyl resulted in a more potent PAD4 inhibitor, compound **96** (Fig. 45). Compound **96** is a non-covalent PAD4 inhibitor, which was synthesized through the copper-catalyzed C–H arylation of

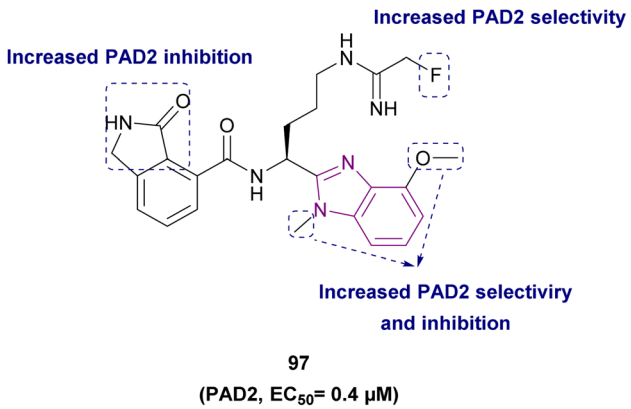


Fig. 46 Chemical structure and EC_{50} of PAD inhibitor **97**.

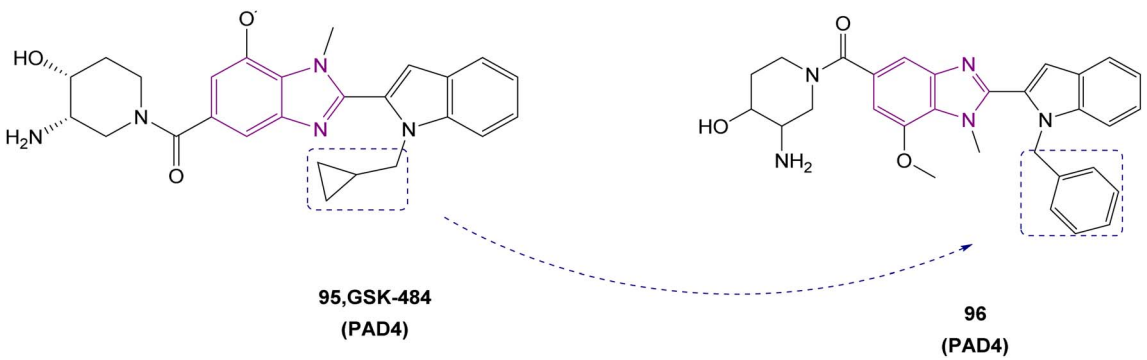


Fig. 45 Chemical structure and EC_{50} of PAD inhibitors **95** and **96**.

benzimidazoles. The indole ring was important for the activity, given that replacing it with benzofuran, naphthalene, and pyridine diminished the activity. *In vitro* studies of **96** in 4T1 cells caused a marked decrease in viability, and the MTT assay showed potent activity.[180]

PAD2 is overexpressed in luminal breast cancers. Compound **97** is a potent PAD2 inhibitor with selectivity towards PAD2 over other PADs with a large therapeutic window (Fig. 46).[181]

3 Conclusions and future perspectives

The benzimidazole is a promising scaffold. It has many biological activities given that it mimics the purine ring. It this review, it was highlighted that the benzimidazole ring has been implicated in the structure of many epigenetic inhibitors, especially in HDACIs, and targeting different epigenetic enzymes, which plays a critical role in cancer therapy. HDAC inhibitors containing a benzimidazole ring could act as pan-inhibitors, dual inhibitors, or selective-isoforms inhibitors, where benzimidazole acts as a cap, linker, or zinc-binding group, respectively. These compounds had antiproliferative activity against many cell lines in the nanomolar range. Further research and investigations can be beneficial in synthesizing less resistant and selective HDAC inhibitors and dual acting benzimidazole derivatives, given the promising future of treating cancer with epigenetics, and especially because the dual-targeting of HDAC as a single target did not show great efficiency in cancer therapy. Alternatively, further efforts should focus on improving their pharmacokinetics and optimizing the synergistic therapeutic efficacy of the drug *in vivo* and maximizing its safety profile. The benzimidazole moiety plays a crucial role in inhibiting other epigenetic targets through crucial interactions with the essential residues of the target pocket. However, this area of research was not fully and precisely covered and needs further research to make the benzimidazole scaffold well established in the field of epigenetic target inhibitors. Also, benzimidazole derivatives that act as PRMT5 inhibitors are a great step in overcoming the anticancer drug resistance. However, their exact mechanism is not known yet. Synthesizing non-hydroxamic ZBG as in compound **19** and **21** was beneficial given that the hydroxamic moieties are responsible for side-effects such as nausea, thrombocytopenia, anaemia, and other metabolic abnormalities due to their strong chelation with the zinc ion. Thus, researchers are trying to find different ZBGs with weaker binding and greater selectivity. The most promising benzimidazole derivatives were pracinostat **2**, a pan-HDAc inhibitor that entered phase I and phase II clinical trials for treating hematologic malignancies and solid tumours, tinostamustine **38**, a DNA alkylating and HDAC inhibitor, which entered phase I/II studies in advanced solid tumours and phase I study in relapsed/refractory (R/R) Hodgkin lymphoma (HL), exhibiting haematological and thrombocytopenia adverse effects, and Inobrodib (CCS1477) 48, which inhibited the p300/CBP bromodomain in phase I/II clinical trials in non-Hodgkin lymphoma (including B-cell lymphoma), AML, and multiple myeloma. This area of research seems to be promising and requires more effort from researchers. Accordingly, this review article can help researchers design better, less resistant, and less toxic benzimidazole derivative anticancer agents.

Abbreviations

22RV1	Human prostate carcinoma epithelial cell line
A2780	Ovarian cancer cell line
A549	Human lung carcinoma cell line
AML	Acute myeloid leukaemia
Ash2L	Absent, small or homeotic 2-like protein
BAX	Bcl-2-associated X protein
BCL3	B-cell chronic lymphatic leukemia protein 3
BCL2	Anti-apoptotic B cell lymphoma 2
BRD	Bromodomain
BRDT	Bromodomain testis-specific protein
BRPF	Bromodomain and PHD finger-containing family
CBP	cAMP-regulated-enhancer (CRE)-binding protein (CREB)-binding protein
CBX7	Chromobox protein homolog 7
CCRF-CEM	Blood leukemia
CD86	Cluster of differentiation 86
CDK	Cyclin-dependent kinase
CDKN1A	Cyclin-dependent kinase inhibitor 1A
CK2	Casein kinase 2
CREB	Cyclic-AMP response element binding
CRPC	Castration-resistant prostate cancer
DNMT	DNA methyltransferases
DOT1L	Disruptor of telomeric silencing 1 (Dot1)-like proteins
DU145	Human prostate cancer cell line
E2F1	E2 promoter binding factor 1
EED	Embryonic ectoderm development
ENL	Eleven-nineteen-leukemia protein
EZH2	Enhancer of zeste homolog 2
G9a	Histone H3 Lys-9 methyltransferase
GADD45A	Growth arrest and DNA damage-inducible 45
GNAT	Gen-5-related *N*-acetyltransferase
H1975	Anlotinib-resistant lung cancer cell line
HATs	Histone acetyltransferases
HCC827	Lung adenocarcinoma cell line
HCC4017	Lung large cell carcinoma
HCC4018	Small cell lung cancer
HCT-116	Human colorectal carcinoma cell line
HDACs	Histone deacetylases
HepG2	Human liver cancer cell line
hERG	Human ether-a-go-go-related gene
HKMTs	Histone lysine methyltransferases
HL-60	Human Caucasian promyelocytic leukemia cell line
HL-60/adr	Adult acute myeloid leukemia cell line
HL-60/vinc	Adult acute myeloid leukemia cell line
HOXA9	Homeobox A9
Hsp90	Heat shock protein 90
HT-29	Human colorectal adenocarcinoma cell line
IRF4	Interferon regulatory factor 4
JAK1	Janus kinase 1

JmjC	Jumonji C domain-containing demethylases
JNK	c-Jun N-terminal kinase
Kac	Acetylated lysine
KDMs	Lysine demethylase enzymes
KLK3	Kallikrein related peptidase 3
KMTs	Lysine methyltransferase
LSD1	Lysine-specific demethylase 1
LSD2	Lysine-specific demethylase 2
KSP	Kinesin spindle protein
KSVH	Kaposi's sarcoma-associated herpesvirus
LnCaP	Lymph node carcinoma of the prostate cell line
lncRNA	Long non-coding RNAs
MBD	Methyl-CpG binding domain family
MCF7	Human breast cancer cell line
MDA-MB-231	Triple-negative breast cancer(TNBC) cell line
MDA-MB-435	Amelanotic melanoma cell line
MDA-MB-468	Triple-negative breast cancer(TNBC) cell line
MEIS1	Myeloid ecotropic viral integration site 1
MLL1	Mixed lineage leukemia 1
MM	Multiple myeloma
MOLM-13	Human leukemia cell line
MOLT-4	T lymphoblast cell line
MTDLs	Multitarget-directed ligands
MTT	(3-(4,5-Dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay
MV4-11	Acute monocytic leukemia cell line
MYC	Master regulator of cell cycle entry and proliferative metabolism
MYCN protein	Avian myelocytomatosis viral oncogene neuroblastoma derived homolog
MYST	MOZ/YBF2/SAS2/TIP60
NAD^+	Nicotinamide adenine dinucleotide
ncRNA	Non-coding RNA
NF-κB	Nuclear factor kappa B
OPM-2	Human myeloma cell line
OVCAR-5	High-grade serous ovarian adenocarcinoma cell line
p16INK4a	Cyclin-dependent kinase inhibitor 2A
$p21^{WAF1/Cip1}$	Wildtype activating factor-1/cyclin-dependent kinase inhibitory protein-1
p53	Tumour protein P53
PAD	Peptidyl arginine deiminase
PARP	Poly-ADP ribose polymerase
PBMC	Peripheral blood mononuclear cell
PC-3	Human prostate cancer cell line
PHDs	Plant homeodomains
piRNA	Piwi-interacting RNA
PRC2	Polycomb repressive complex 2
PRMTs	Arginine methyltransferase
PUMA	p53 upregulated modulator of apoptosis
RAMOS	B lymphocyte cell line
RB1	Retinoblastoma 1
RBBP5	Retinoblastoma binding protein 5
SAM	*S*-adenosyl methionine
SCC-9	Squamous carcinoma cell line
SET	Su(var)3–9, enhancer-of-zeste and trithorax
siRNA	Short interfering RNA
SK-OV-3	Ovarian cancer cell line
snoRNAs	Small nucleolar RNAs
SRA	SET- and ring finger-associated domain family
STAT3	Signal transducer and activator of transcription 3
T-47D	Human breast cancer cell line
TAF1	TATA-box binding protein associated factor 1
TEAEs	Treatment-emergent adverse events
TET	Ten-to-eleven translocation
THP-11	Leukaemic cancer cell lines
TNBC	Triple-negative breast cancer
TRAF2	Tumour necrosis factor (TNF) receptor associated factor-2
UHRF1	Ubiquitin-like with containing PHD and RING finger domains 1
USP5	Ubiquitin-specific protease 5
VCaP	Prostate cancer cell line
WDR	WD40 repeat domains
ZBD	Zinc-binding domain

Data availability

No primary research results, software or code has been included and no new data were generated or analyzed as part of this review.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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