Polycomb repressive complex 2 inhibitors: emerging epigenetic modulators

Danishuddin[1], Naidu Subbarao[1], Mohammad Faheem[1] and Shahper Nazeer Khan[2]

[1] School of Computational and Integrative Sciences, Jawaharlal Nehru University, New Delhi, India
[2] Interdisciplinary Nanotechnology Centre, Aligarh Muslim University, Aligarh, India

Polycomb repressive complex 2 (PRC2) plays a significant part in histone methylation – trimethylating K27 at H3, an epigenetic hallmark of gene silencing. Inhibition of PRC2 has been reported as a promising strategy for the treatment of various cancers. Significant efforts have been made toward the development of PRC2 inhibitors and some of them have progressed to clinical trials. The binding mode of these inhibitors is well understood. Here, we summarize the advances in drug discovery and development for PRC2 component inhibitors by focusing on their chemotypes, activity, selectivity and binding modes. We believe that such analysis will provide new avenues for the design and development of next-generation PRC2 inhibitors through establishment of a structure-based drug design platform.

Introduction

Polycomb repressive complex 2 (PRC2)-mediated histone methylation plays an important part in aberrant gene silencing [1–3]. Heterochromatization by PRC2 is a key marker in various cancer types. PRC2 transfers a methyl group from the cofactor S-adenosyl-L-methionine (SAM) to the ε-amino group of H3K27 which results in H3K27Me3. High levels of H3K27 trimethylation (H3K27me3) in the coding region generally correlate with transcription repression [4,5]. These severe chromatin marks add to cancer-associated *de novo* DNA methylation and gene silencing, which play a part in normal cellular differentiation and function (Fig. 1). The H3K27me3 catalyzed reaction is thought to recruit other factors such as PRC1 resulting in the silencing of genes involved in cellular process like cell differentiation, cell fate decision and cell cycle regulation. The catalytic activity of PRC2 is related to the arrangement of a core complex structure.

Dysregulation of PRC2 is involved in several cancer types, making it a promising therapeutic target for cancer therapy, attracting interest in pharmaceutical and academic settings. However, significant changes in PRC2 activity with its inhibitors and their modes of interaction will need to be carefully considered in any therapeutic context [6]. Herein, we focus on summarizing the PRC2 components and advances in drug discovery and development of its inhibitors.

Components of PRC2

The human PRC2 complex includes four core components: enhancer of Zeste homolog 2 (EZH2), embryonic ectoderm development (EED), suppressor of Zeste 12 (SUZ12) and RbAp48 [1,2]. Careful consideration of favorable interactions of these components is essential to confer the methyltranferase activity.

EZH2

EZH2 is overexpressed mostly in metastatic prostate and breast cancer and has been linked with breast malignancy aggressiveness [7]. The ~750 amino acid EZH2 protein is characterized by the various structural and functional domains dispersed on the N and C terminus regions [8]. The N terminus region has domains such as EED-binding domain (EBD), SANT1 and SET activation loop (SAL), among others, composed of an extended structural scaffold for interaction with EED and SUZ12 [7]. The activation loop (SAL) plays a significant part in bringing about certain conformation changes in the SET domain. A pull-down assay suggests that the

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Corresponding authors: Subbarao, N. (nsrao@mail.jnu.ac.in), Khan, S.N. (shahper01@gmail.com)

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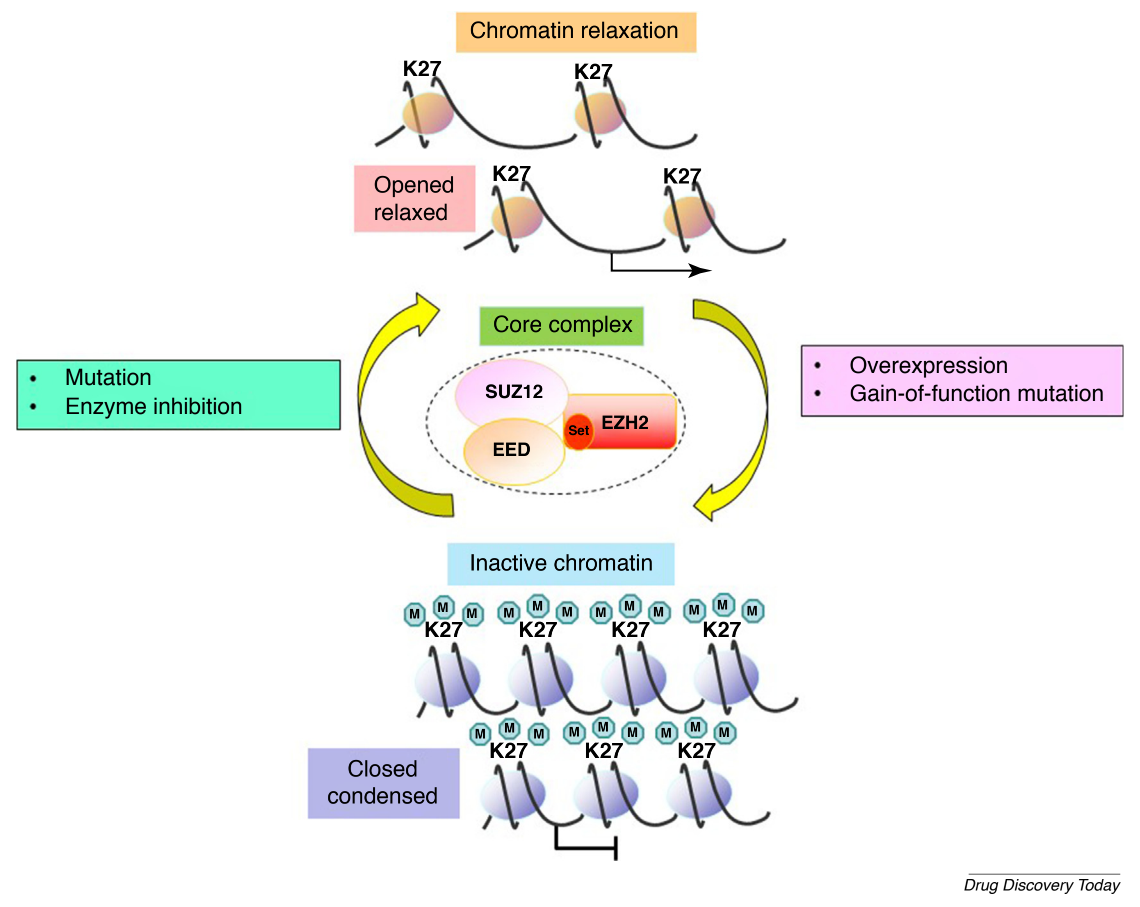

FIGURE 1

Systemic diagram of polycomb repressive complex 2 (PRC2) complex and its function.

N-terminal fragment of EZH2 forms a stable complex with EED [9]. The catalytic activity of PRC2 is conferred by the C terminus SET domain of EZH2. Mutations in either protein lead to EZH2 destabilization and deficient methylation of H3K27 [10,11]; whereas, the C-terminal domain did not exhibit any sign of interaction with EED [9,12]. A series of EZH2 constructs of varying lengths suggested that amino acids 39–68 interact with EED, and this region was the EBD [9]. Structure analysis of the EZH2 catalytic domain (AA520–746) revealed that the isolated catalytic domain is inactive and sheds some light on how this inactive conformation is maintained [13,14]. Conformational changes in the pre-SET and post-SET domains take place for cofactor- and substrate-binding activities [15]. These changes are significant for EED and SUZ12 binding and catalysis activity. In human PRC2, the tight turn under the cofactor-binding site has the sequence GWG (623–625), with the tryptophan side-chain oriented away from the SET domain into a hydrophobic pocket composed of residues V107 to M110 from the SAL(EZH2), F566 to S568 from SUZ12 and L616 to F618 from N-SET and F686 from I-SET. However, in the isolated SET domain structure, these interactions are absent. Two amino acids G643 and G681 are consistent among SET domains of lysine methyltransferases and can serve as the flexible 'hinge' about which the I-SET could 'swing' between its different conformational states. The overall differences between these two states were found to be 1.6 Å. Another significant α-helical domain of EZH2 (AA40–68) that involves EED established the basis for designing hydrocarbon-stapled derivatives to disrupt the specific protein interaction [16].

EED

EED contains seven WD-40 repeats at its C terminus (AA81–441) followed by an N-terminal domain (AA1–80). The WD-40 repeat domain is a structural motif of ~40 residues which forms a four-stranded antiparallel β-sheet [17], capable of recognizing a diverse group of ligands including proteins [18]. PRC2 association with repressive chromatin marked by H3K27me3 is mediated through the β-propeller WD-40 domain of EED. Margueron *et al.* [19] showed that the C-terminal domain of EED interacts with histone tails having trimethyl-lysine residues associated with repressive chromatin marks leading to the allosteric activation of the methyltransferase activity of PRC2. Mutations in EED prevent it from recognizing repressive trimethyl-lysine marks, thus preventing the activation of PRC2 [19]. EED anchored to the N-terminal helix of EZH2 was achieved through Kme3 (trimethyl-lysine) binding into the aromatic pocket formed by the seven WD-40 repeats [13,19]. Binding analyses showed that EED binds to H1K26me3, H3K27me3, H3K9me3 and H4K20me3 peptides with dissociation constant K_d of 10–45 μM. However, this binding was found to reduce fourfold with the loss of the methyl group from the methylated lysine [19]. In EED-H3K27me3 the lysine residue was inserted into the aromatic cage and was stabilized by van der Waals and cation-Pi interactions with the trimethylated ammonium group. Another aromatic chain (W364) interacts with the aliphatic moiety of the lysine side-chain by a hydrophobic interaction [20]. In addition, other interactions such as H-bonds between W364 and H3R26 and the backbone of H3A25 and side-chain of R414 were also involved in stabilizing the EED-H3K27me peptide complex. An aromatic cage in the binding site is a conserved feature of methyl-lysine-binding motifs [21] and is also present in other classical trimethylated histone-binding proteins, such as HP1 and polycomb [20,22,23]. This has been proved by Xu *et al*. [24] and showed that the WD-40 domains of EED form a histone methyl-lysine-binding motif that preferentially recognizes the ARKS-motif-containing trimethylated H3K27, H3K9 and H1K26 peptides. The significant part played by the methyl-lysine-binding residues in EED was verified by various mutagenesis experiments. Fluorescence polarization measurements showed that substitution of F97, Y148, W364 and Y365 to alanine completely abolished or reduced the binding of EED to the H3K27me3 peptide [19,20]. Structural and mutagenesis studies indicate that this peptide-binding groove mainly lined with hydrophobic residues can be important to the function of the PRC2 complex. Thus, EED is a potential cancer drug target that novel inhibitors to prevent the binding of EZH2 to EED could be designed to inhibit.

SUZ12

SUZ12 was also characterized as an essential component of PRC2 for its HMTase enzymatic activity. This protein contains two evolutionary conserved domains: C-terminal VEFS (AA ~140) and a zinc finger domain. SUZ12 contacts with EED and EZH2 to confer an unusual split active SET domain for catalysis**.** Removal of the entire VEFS domain abolishes the PRC2 assembly formation [25]. Crystal structures of an active PRC2 complex from the yeast *Chaetomium thermophilum* and humans [26] showed the VEFS domain in SUZ12 interacts with EED and SET regions of EZH2. It also plays a significant part in ordering the activation loop of EZH2. Additionally, the helical contents of VEFS form a 10-helix bundle with the MCSS and SANT2L. The importance of this domain can be highlighted with mutation studies that showed that substitution in the conserved VEFS domain reduced its activity in PRC2 formation [26–29]. Conserved amino acid W584 at the junction of the helical and loop contents of SUZ12 (VEFS) is known to involve a hydrophobic interaction with Y231 and Y232 of EED, and mutation of these tryptophan residues leads to a reduced enzyme activity of *Drosophila* PRC2. A C2–H2 zinc finger (ZnF) motif outside the VEFS domain of SUZ12 facilitates binding of PRC2 to the genomic target [26]. Currently, as far as we are aware, no inhibitors are available for SUZ12. Thus, considering the significant role of this protein in PRC2 complex formation, we believe that it could be a crucial drug target for the development of novel PRC2-based inhibitors.

EZH2 inhibitors

Given the evidence that EHZ2 is a significant component of PRC2 and a cancer driver, development of specific inhibitors against it has been an active area of investigation. Various scientific organizations and pharmaceutical companies have engaged in development of potential compounds. Promising preclinical results for some inhibitors for EZH2 have been obtained and human clinical trials are now underway, with early results suggesting their potential activity (Fig. 2). All inhibitors characterized to date target EZH2 via competition with the methyl-donating cofactor SAM for binding to the SET domain.

DZNeP

Via library-based drug screening, Tan and co-workers identified a SAM-dependent methyltransferase inhibitor (SAH-hydrolase): DZNeP [30]. This compound targets the degradation of EZH2, preferentially inducing apoptosis in various hematological malignancies. It was reported that DZNeP diminishes the expression levels of the PRC2 components EZH2, SUZ12 and EED with concomitant loss of H3K27me3, inducing efficient apoptotic cell death in cancer cells. However, this compound was not efficient for inhibiting H3K9 methylation [30]. DZNeP also inhibits proliferation and promotes the apoptosis in different types of cancer cells [31,32]. Treatment of DZNeP along with other inhibitors was found to be a potential epigenetic therapy for various cancers [33,34]. In 2014, Girard *et al*. demonstrated that targeting the EZH2 with DZNeP in a chondrosarcoma cancer cell line inhibits its expression and reduces H3K27me3 [35].

EPZ005687

EPZ005687 has the potency to inhibit wild-type and EZH2 mutants (Y641 and A677) and showed great selectivity (>500-fold) over human protein methyltransferases [36]. Structural recognition elements of the indazole series do not differ between wild-type and Y641 mutants and have similar potency. The A677G mutant has greater affinity (5.4-fold; $P < 0.05$). This increased potency for the A677G mutant suggests that EPZ005687 can engage additional interactions as a result of this mutation. In 2016, Shen *et al*. studied the effect of EPZ005687 on synovial sarcoma cell proliferation [37]. This compound also showed an acceptable inhibition activity at the micromolar level in Aska-SS ($IC_{50} = 0.72\ \mu M$), Fuji ($IC_{50} = 1.5\ \mu M$), SYO ($IC_{50} = 2.1\ \mu M$) and Yamato-SS ($IC_{50} = 3.5\ \mu M$) cell lines [37]. A docking study of EPZ005687 carried out by Kyle *et al*. showed that the morpholinomethyl moiety was solvent exposed and does not interact with EZH2, whereas other moieties form H-bonds and hydrophobic interactions with the SAM-binding site [38].

In 2012, Verma *et al*. identified highly potent, SAM-competitive and selective inhibitors for EZH2 [39]. These compounds are highly selective (>1000-fold) for EZH2 over most other methyltransferases. SAR studies showed that the regioisomeric piperazinylpyridine group at the 6-position in GSK926 significantly increase the potency with $K_i = 7.9 \pm 3$. Addition of an *n*-propyl group at the 4-position of the pyridone increased activity at least tenfold ($K_{iapp} = 0.60 \pm 0.05$) against EZH2. Molecular docking analyses using Genetic Optimisation for Ligand Docking (GOLD) showed that these compounds could dock into the binding pocket with same binding mode as those made by the adenine moiety of SAM (Fig. 3). GSK126 inhibits EZH2 with high potency ($IC_{50} = 9.9$ nM) compared with EZH1 ($IC_{50} = 680$ nM) and it was highly selective (>1000-fold) over other methyltransferases [40]. This compound decreases total H3K27me3 levels and reactivates silenced PRC2 target genes. GSK126 was found to be effective in the treatment of non-Hodgkin's lymphoma (NHL) and other solid

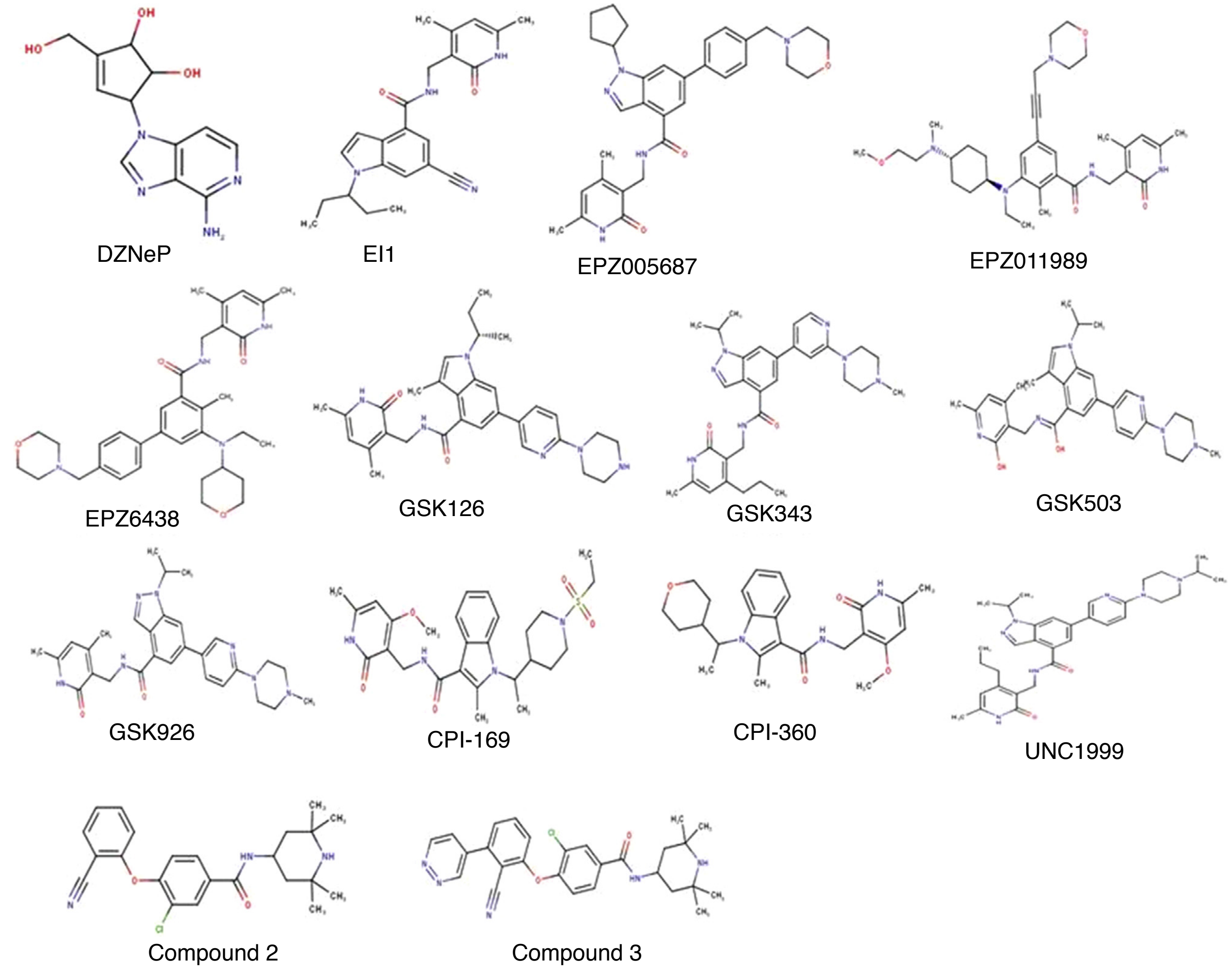

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FIGURE 2

Chemical structures of the selected enhancer of Zeste homolog 2 (EZH2) inhibitors.

tumors. A docking study revealed the SAM-binding pocket as the most plausible binding site for GSK126, making extensive contacts with the post-SET domain which forms one side of the SAM-binding pocket. Two cell lines: MGC803 and A549, treated with GSK126 demonstrated inhibited cell migration and angiogenesis through downregulation of vascular endothelial growth factor (VEGF)-1 expression [41]. This compound also has a significantly longer residence time of inhibition on the activated form of EZH2/PRC2 as compared with inactivated EZH2/PRC2, occurring in wild-type and mutants [42]. Another compound, GSK503, similar to GSK126 and GSK343, known to significantly reduce H3K27me3 levels and inhibiting tumor growth, prevents the methyltransferase activity of wild-type and mutant EZH2 with similar potency (K_i = 3–27 nM) [43,44]. This compound has >200-fold selectivity over EZH1 and is >4000-fold selective over other histone methyltransferases (HMTs).

EI1

A potent and selective small-molecule inhibitor, EI1 inhibits the enzymatic activity of the wild-type (IC_{50} = 15 ± 2 nM) and Y641N mutant (IC_{50} = 13 ± 3 nM) of EZH2. EI1 directly binds to the enzyme and competes with the methyl group donor SAM [45]. It is highly selective over EZH2 and closely homologous with other HMTs [45,46]. EI1 was found to be effective in treatment of NHL and other solid tumors. Treatment of cells with EI1 exhibited genome-wide reduction of H3K27 methylation and activation of PRC2 target genes. Molecular modeling suggested that the residues I626 and C663 around the EI1-binding pocket might contribute to the selectivity.

UNC1999

UNC1999 is the first orally bioavailable highly potent and selective inhibitor for EZH2 and EZH1 [47]. This compound has potency for the wild-type (IC_{50} < 10 nM) and Y641N mutant (EC_{50} of 633 nM) EZH2. UNC1999 potently reduced H3K27me3 activity and selectively killed the DB cells in a diffuse large B cell lymphoma (DLBCL) cell line harboring the Y641N mutant EZH2. The binding mode of UNC1999 within the active site of EZH2 showed that the secondary amide and pyridone form H-bonds with N688 and H689 residues, whereas indazole is buried in a hydrophobic pocket. Two

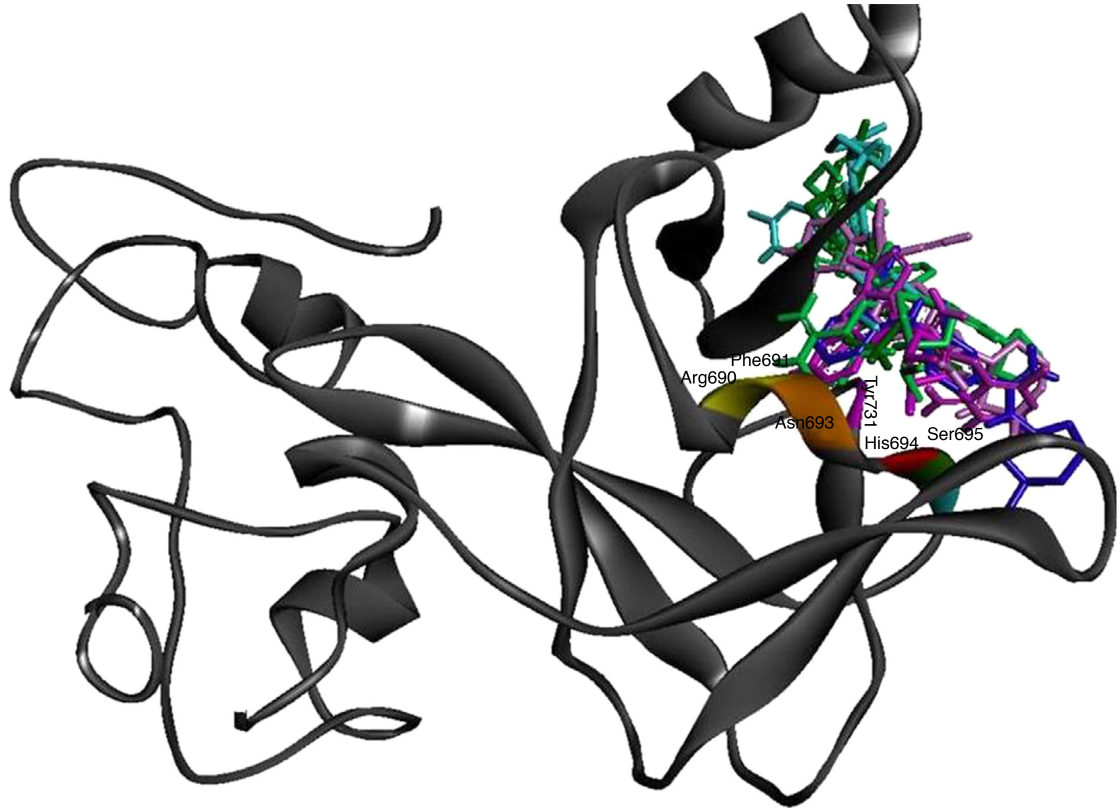

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FIGURE 3

Predicted binding mode of compounds in complex with SET domain of enhancer of Zeste homolog 2 (EZH2). Compounds were docked within the active site of the SET domain [38]. Important and common amino acids were labeled. Figure was prepared using BIOVIA Discovery Studio 4.5 (Accelrys).

methyl groups at R1 and R2 were found to be significant in potency of UNC1999. Xu *et al*. studied the effects of UNC1999 and its inactive analog UNC2400 among MLL-rearranged leukemia and found that UNC1999 suppressed H3K27me3/2 activity [48].

EPZ-6438

EPZ-6438 has been obtained by substitution of EPZ005687 which exhibited excellent potency, selectivity and pharmacokinetic properties over EPZ005687 [36,49]. This compound showed similar potency for wild-type and the EZH2 mutants (Y646F, A682G, etc.) ($K_i = 2.5 \pm 0.5$ nM). Treatment of EZH2-mutant NHL-xenograft-bearing mice with EPZ-6438 causes dose-dependent tumor growth inhibition, including complete and sustained tumor regression with correlative diminution of H3K27Me3 levels in tumors and selected normal tissues. Selective inhibition of EZH2 by EPZ-6438 leads to potent antitumor activity in EZH2 mutant NHL. It inhibits EZH2 in a manner competitive with the substrate SAM, displayed a 35-fold selectivity versus EZH1 and >4500-fold selectivity relative to 14 other HMTs tested. EPZ-6438 is used for the treatment of lymphoma and is currently in a Phase I clinical trial.

EPZ011989

Campbell *et al*. discovered EPZ011989, a potent, selective and orally bioavailable compound for EZH2 having significant potency against tumor growth in a mouse xenograft model of human B cell lymphoma [50]. This compound was discovered with the modification of the pyran substituent in EPZ-6438. It appears that, a trans-*N*,*N*-dimethylcyclohexylamine substituent appears to maintain biochemical activity. A series of compounds were developed to attenuate the pK_a of the amine components and improvement in potency was achieved through the addition of a methoxyethyl group to the cyclohexylamine (p$K_a = 9.8$) combined with the substitution of the second benzene ring in EPZ-6438 and an acetylene linker to modify the adjacent morphine (p$K_a = 5.7$). These modifications led to EPZ011989 having equal potency against the wild-type and mutant EZH2 with an inhibition constant (K_i) of <3 nM (Table 1). It reduces the cellular H3K27 methylation in the Y641F-mutant-bearing human lymphoma cell line (WSU-DLCL2) with an IC_{50} <100 nM. It is a specific EZH2 inhibitor with a >15-fold selectivity over EZH1 and >3000-fold selectivity over other HMTs tested.

Benzamide inhibitors

In 2013, Garapaty-Rao *et al*. reported the discovery of the series of highly selective SAM-competitive, small benzamide inhibitors of EZH2 and EZH1 [51]. These compounds were shown to exhibit significant potency for wild-type and mutants of EZH2, suppressing H3K27 methylation [52]. Importantly, these compounds represent a structurally distinct EZH2 inhibitor chemotype for the exploration of the role of PRC2-mediated H3K27 methylation in various biological contexts. Severally structurally distinct

TABLE 1

Overview of EZH2 inhibitors

Compound	Smile	IC_{50}/K_i		Refs
		Wild	*Mt#*	
EPZ6438	CCN(C1CCOCC1)C2=CC(=CC(=C2C)C(=O)NCC3=C(C=C(NC3=O)C)C)C4=CC=C(C=C4)CN5CCOCC5	2.5 nM (K_i)	Na	[36,49]
DZNeP	C1=CN=C(C2=C1N(C=N2)C3C=C(C(C3O)O)CO)N	K_i = 50 pM	Na	[30]
UNC1999	CCCC1=C(C(=O)NC(=C1)C)CNC(=O)C2=C3C=NN(C3=CC(=C2)C4=CN=C(C=C4)N5CCN(CC5)C(C)C)C(C)C	2 nM	633 nM (EC_{50})	[47]
EI1	CCC(CC)N1C=CC2=C(C=C(C=C21)C#N)C(=O)NCC3=C(C=C(NC3=O)C)C	15 nM	13 nM	[45]
GSK343	CCCC1=C(C(NC(C)=C1)=O)CNC(C2=C3C=NN(C3=CC(C4=CC=NC(N5CCN(CC5)C)=C4)=C2)C(C)C)=O	4 nM	Na	[39]
GSK126	CC1=CN([C@@H(C)CC)C2=CC(C(C=C3)=CN=C3N4CCNCC4)=CC(C(NCC5=C(C)C=C(C)NC5=O)=O)=C21	9.9 nM	3.2–8.0 μM	[39]
EPZ005687	CC1=CC(=C(C(=O)N1)CNC(=O)C2=C3C=NN(C3=CC(=C2)C4=CC=C(C=C4)CN5CCOCC5)C6CCCC6)C	~24 nM (K_i)	Na	[36]
CPI-169	CCS(=O(=O)N1CCC(CC1)C(C)N2C(=C(C3=CC=CC=C32)C(=O)NCC4=C(C=C(NC4=O)C)OC)C	0.24 nM	0.5 nM	Na
CPI-360	CC1 CC(=C(C(=O)N1)CNC(=O)C2=C(N(C3=CC=CC=C32)C(C)C4CCOCC4)C)OC	0.5 nM	2.5 nM	[54]
EPZ011989	CN(CCOC)[C@@H]1CC[C@@H (N(C2=CC (C#CCN3CCOCC3)=CC(C(NCC4=C(C)C=C(C)NC4=O)=O)=C2C)CC)CC1	<3 nM	<3 nM	[50]
Compound 2	CC1(C)CC(CC(C(C)N1)NC(=O)c2ccc(Oc3ccccc3C#N)c(Cl)c2	0.45 μM	Na	[51]
Compound 3	CC1(C)CC(CC(C(C)N1)NC(=O)c2ccc(Oc3cccc(c4ccnnc4)c3C#N)c(Cl)c2	0.032 μM	197 nM	[51]
GSK926	CC1=CC(=C(C(=O)N1)CNC(=O)C2=C3C=NN(C3=CC(=C2)C4=CN=C(C=C4)N5CCN(CC5)C)C(C)C)C	2.5 μM	Na	[39]
GSK503	CC(N(C1=CC(C2=CN=C(N3CCN(CC3)C)C=C2)=CC(/C(O)=N/CC4=C(O)N=C(C=C4C)C)=C51)C=C5C)C	3 μM	Na	[39]
Compound 44	ClC=1C=C(C(=O)NC2CC(NC(C2(C)C(C)C)C=CC1OC1=C(C(=CC=C1)C1=CN=NC=C1)C#N	0.032 μM	Na	[51]

Abbreviation: Na, not available.

compounds (288 confirmed hits) have been identified from a compound library of ~150 000 compounds. Compound **2** inhibited wild-type EZH2 (IC_{50} = 0.45 μM) more potently compared with the Y641N mutant of EZH2. Further substitution to a pyridazine at the 3-position provided a significant improvement in potency. Compound **3** exhibited significant potency for EZH2 wild-type (IC_{50} = 32 nM) and the EZH2 Y641N mutant (IC_{50} = 197 nM) and EZH1 (IC_{50} = 213 nM). Furthermore, analogs were developed for compound **6** that were found to be selective and have moderate potency (EZH2 wild-type IC_{50} = 51 μM) in a biochemical assay. Several modifications in compound **6** led to the development of a SAM-competitive compound **44**, having >1000-fold more potentcy against EZH2 (Table 1) [53]. Further exploration revealed a 15-fold increment in potency with the incorporation of a 2-cyano substituent, suggesting the cyano nitrogen was making a favorable interaction with the binding site in EZH2. The 2-cyano substituent was utilized as the preferred substituent for further SAR development. Substitution of a pyrazole alpha to the 2-cyano group led to a modest improvement, additionally a 6-membered heterocycle enabled sub 100 nM potency against the wild-type enzyme. Further, the nitrogen aryl substitution from 4-pyridyl to pyridazine then pyrimidine led to improvement in affinity and efficiency. Taking advantage of these compounds, Bradley *et al.* [54] generated a hybrid compound series with significantly improved potency which led to the identification of compound CPI-360. This compound showed the *in vivo* efficacy in an EZH2-containing GCB-DLBCL xenograft model. This potency was lost upon compound removal suggesting that EZH2-inhibitor-induced effects are reversible. Moreover, this compound inhibited the EZH2 catalytic activity with similar potency to GSK126 and EPZ-6438 [35,36]. The remarkable similarity of the induced gene expression changes within a given lymphoma model by these three compounds provided the evidence of their on-target activity.

EED inhibitors

There is continuing interest in developing novel inhibitors against PRC2 components with more-acceptable pharmacological properties. Inhibition of H3K27me3 binding to EED would be an alternative approach to disrupt the function of the PRC2 complex. Significant efforts from academia and the pharmaceutical industry have been provided to discover some inhibitors of EED, indicating that targeting the H3K27me3 pocket in EED is a promising approach for treating cancers that are dependent on PRC2 activity (Figs 4 and 5).

A-395

A-395 is a potent, selective and cellularly active PPI inhibitor of the EED–histone interface [55]. A-395 potently binds EED in the H3K27me3-binding site and ultimately inhibits H3K27 methylation *in vitro* via inhibition of PRC2 activation. A-395 is highly selective against other methyltransferase enzymes or other methyl-lysine-binding proteins. Structural data demonstrate the bind-

TABLE 2

Overview of EED inhibitors

Compound	Smile	IC$_{50}$/K_i	PDBID	Refs
A-395	[F]c5cccc4CC[C@]([H])(N3C[C@]([H])(c1ccc(cc1)N2CCN(CC2)[S](=O)(C)=O)[C@]([H])(C3)N(C)C)c45	7 nM	5K0M	[55]
Compound 1	CN1C=C(c2ccccc12)[C@@]3([H])CN(C[C@]3([H])N(C)C)Cc4ccccc4OC	K_i = 600 nM	5U69	[56]
Compound 35	Cc1cccc([F])c1CN4C[C@]([H])(c2ccc(cc2)C=3C=NN(C=3)CC(N)=O)[C@]([H])(C4)N(C)C	K_i = 2.6 nM	5U6D	[56]
EED666	C/C1=C/C(C)=NN1c2cc(ccc2N(=O)=O)N4Cc3ccccc3CC4	8.76 μM	5H14	[57]
EED162	N#C/C4=C(\NCc1ccco1)N2C=NN=C2C=3CN(CCC=34)Cc5ccccc5	0.32 μM	5H19	[57]
EED210	COc3cccc2C[C@]1([H])N(C)C[C@]([H])(C[C@@]1([H])Cc23)Cc4cc(ccc4)OC	0.8 μM	5H17	[57]
EED226	C[S](=O)(=O)c1ccc(cc1)C3=C/N=C(/NCc2ccco2)N4C=NN=C34	23.4 nM	5GSA	[57]
EED709	CN1C=C(c2ccccc12)[C@@]3([H])CN(C[C@]3([H])N(C)C)Cc4ccccc4OC	1.57 μM	5H15	[57]
EED396	COc1cc(ccc1OC(C)(C)[H])C5=CC3N(CCc4cc2OCOc2cc34)C(N)=C5C#N	11.58 μM	5H13	[57]
Compound 11	[F]c1ccccc1/C3=C/c2ccccc2C4=NCCN34	6.04 μM	5H25	[58]
Compound 8	N#CC2C=CC1=NN=CN1/C2=N/Cc3occc3	1 μM	5H24	[58]
Peptidomimetic	Peptide	1.14 ± 0.14 μM	5TTW	[60]
Compound 14	NC(N)([H])N1C[C@]([H]) CCC1)Cc2cccc3OCOc23	3.9 μM	5U5T	[61]
Compound 16	N/C2=N/C=C1CC[C@]([H])(CN12)Cc4cccc3OCOc34	1.3 μM	5U62	[61]

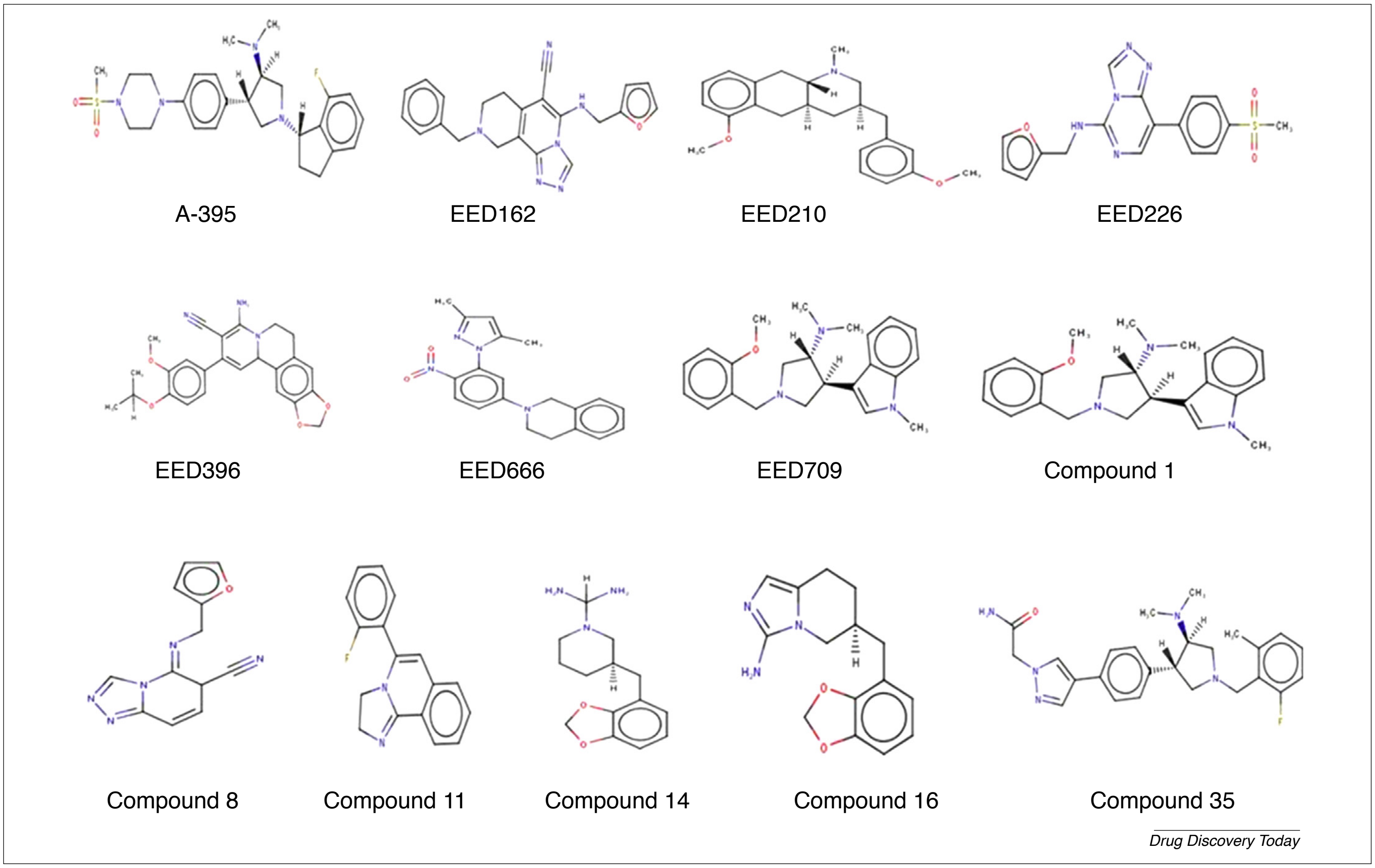

FIGURE 4

Chemical structures of the selected embryonic ectoderm development (EED) inhibitors.

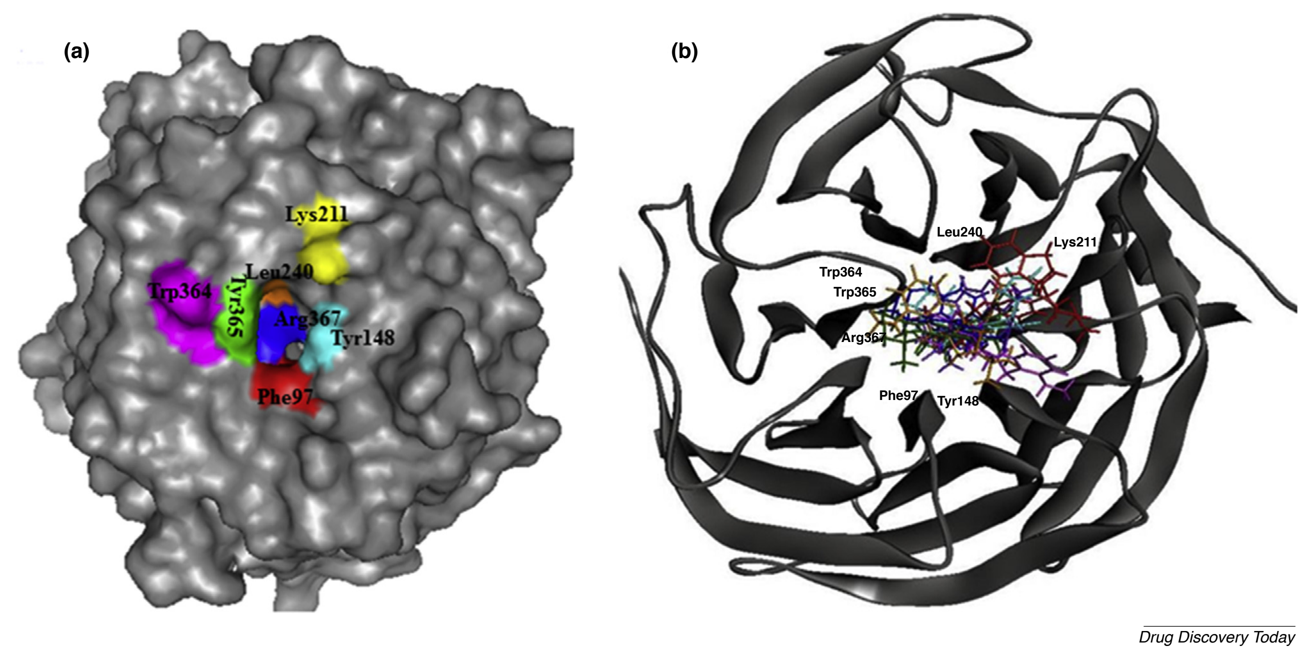

FIGURE 5

Mode of interaction of available embryonic ectoderm development (EED) inhibitors in the active site. Figure was prepared using BIOVIA Discovery Studio 4.5 (Accelrys). (a) Important catalytic residues. (b) Binding mode of inhibitors in the active site.

ing of A-395 to the H3K27me3-binding site on the β-propeller WD-40 domain of EED, thereby hampering the allosteric activation of the catalytic activity of PRC2. This compound was capable of competing for H3K27me3 peptide binding to EED ($IC_{50} = 7$ nM) as confirmed by an AlphaScreen® binding assay. The crystal structure of EED in complex with A-395 (PDBID: 5K0 M) demonstrates that A-395 occupies the H3K27me3-binding site. Binding of A-395 brings rotamer changes in W364, Y365 and R367 that disrupt the aromatic cage, which ordinarily accommodates the trimethyl-lysine head group to expose a larger druggable binding pocket [55]. This alternate rotamer orientation forms a new aromatic cage that consists of the original three residues (F97, Y148 and Y365) and accommodates the pyrrolidine core of A-395 (Fig. 5). The indane of A-395 occupies the space previously held by Y365 and R367 and forms a cation–π interaction. Moreover, this compound also stabilizes the complex key interactions such as H-bonds from the dimethylamine to a highly coordinated water, as well as hydrophobic and van der Waals interactions with catalytic amino acids in the active site. A SAR study by Curtin *et al*. [56] on this compound demonstrated that modification of the indole and benzyl moieties substantially improved binding and cellular activities. The SAR work began with a thorough survey of *N*-benzyl substituents and revealed a strong preference for 2,6-disubstitution. The 2-bromo-6-fluorobenzyl showed the improved binding. A-395 was found to inhibit the growth of DLBCL xenografts and reduced H3K27me3 levels similar to EZH2 inhibitors.

MAK683

MAK683 is an allosteric inhibitor of PRC2, with potential antineoplastic activity (NCI Thesaurus Code: C133819). It selectively binds to the domain of EED that interacts with trimethylated H3K27me3, which leads to a conformational change in the EED-H3K27me3-binding pocket thus preventing the interaction of EED with the histone methyltransferase EZH2. This binding disrupts the EED–EZH2 interaction and reduced the PRC2 activity. Currently, this compound is in clinical trials. MAK683 was tested in patients with DLBCL, nasopharyngeal carcinoma and potentially other solid tumors.

Peptidomimetic ligands

Characterization of the binding of trimethylated Jarid2 (116Kme3) to EED at low micromolar potency ($K_d = 3$ μM) provided a rational starting point for peptide optimization to target the reader interface of EED [57]. Recently, Barnash *et al*. [58] identified the smaller, more-potent peptidomimetic ligands for the EED aromatic cage using the combinatorial and structure-based approaches. These ligands exhibited improved potency (~tenfold) and physicochemical properties compared with Jarid2-K116me3 (114–118). The crystal structure of this peptide has been determined in complex with EED (PDBID: 5TTW). Binding mode analyses shows that Kme3 and phenylalanine residues adopt identical conformation to Jarid2-K116me3. However, the C terminus binds in a unique orientation that positions the hydrophobic leucine side-group toward the EED surface. The I363 side-chain and the carbon chain of Q382 form van der Waals contacts with the side-chain of leucine. Overall, these peptidomimetic ligands disrupt the reader function of EED, leading to allosteric inhibition of PRC2 catalytic activity by preventing the stabilization and orientation of the stimulation response motif (SRM) helix of EZH2. This alteration in the SRM helix cannot in turn stabilize the SET-I helix, which contributes many of the residues that interact with the substrate peptide and is required for more-efficient catalytic activity. The disruption of PRC2 catalytic activity by these peptide ligands presents a unique opportunity to probe the allosteric regulation of H3K27me3.

Structure-guided EED binders

Lingel *et al*. introduced the structure-guided compounds with submicromolar inhibition in functional assays and cellular activity [59]. A stepwise optimization was performed on an earlier identified compound that showed noncompetitive and competitive inhibition with the substrates SAM and H3K27Me0 and H3K27Me3. The charged guanidinium group of compound **14** interacts with EED directly with the side-chains of N194 and E238 and via structured water with the second oxygen of E238 [59]. The cocrystal structure of compound **14** with EED (PDBID: 5U5T) illustrated that the benzodioxole moiety is placed on the R367 side-chain, whereas the position of the phenyl ring is completely retained. An interaction between the benzodioxole moiety and R367 explained its potency. Another well-developed compound in this series is compound **16**. It was made by replacing the guanidinium group in compound **14** with a 2-aminoimidazole. This modification improved the permeability (pK_a of 9.2) and led to water-mediated interaction with N194 and E238. Further, analogs have been prepared with the fluorine at the ortho-position. Ortho-fluoro meta-methoxy substituted in compound **19** showed biochemical potency at 1 μM and an IC_{50} of 2.9 μM in the G-401 ELISA assay. The co-crystal structure of compound **19** with EED reveals that the methoxyphenyl group is retained, whereas the fluorine atom faces toward the N194 and makes a positive interaction with the side-chain amide with a distance of 3.6 Å [59]. In contrast to SAM-competitive EZH2 inhibitors, the mode-of-inhibition of these molecules is allosteric in nature. Inspired by the allosteric inhibition of PRC2 by inhibitor EED226, which binds to the H3K27me3 pocket of EED, Ling *et al*. identified the diverse set of small compounds (EED666, EED162, EED210 and EED709) targeting EED [60]. These compounds were highly selective and showed no binding activity to other HMT enzymes. The X-ray crystal structures of these compounds have been solved (Table 2). Their binding brings significant conformation changes for W364 and Y365 and minor changes for residues F97 and Y148, which form a much deeper pocket compared with the original cage found when H3K27me3 peptide is bound. The tetrahydroisoquinoline moiety of EED666 led to rearrangement of Y365, which enabled the nitrophenyl moiety to interact with Y148 and Y365 through the phi–phi interactions. The triazolo-pyridine core and amide linker of EED162 made H-bonds with the side-chain of N194 and K211. The amino group of the octahydrobenzo-quinoline moiety of EED210 forms cation-Pi stacking with Y148 and Y365. An enzyme kinetics study suggests that EED226 inhibits the PRC2 with an IC_{50} of 23.4 nM along with H3K27me0 peptide (used as substrate) and an IC_{50} of 53.5 nM when the mononucleosome was used as the substrate. Upon binding, the side-chain of W364 and Y365 induced conformational changes that allow them to move toward the solvent and create a deeper pocket. This allows EED226 to insert into this rearrangement with the furan group on the inside and the methylsulfone group toward the solvent. The triazolopyrimidine core was sandwiched between Y148 and Y365, forming Pi–Pi interactions with F97, Y148 and Y365. These interactions were found to be significant in maintaining the potency of the inhibitors [61]. EED226 inhibits the growth of DLBCL and reduced the H3K27me3 levels of EZH2 inhibitor.

Concluding remarks and future perspectives

The role of PRC2 in various cancers is becoming increasingly evident. Drug discovery against the components of PRC2 has gained new momentum as demonstrated by the development of new therapeutics. Promising preclinical results have been obtained and human Phase I/II trials are now underway, with early results for EZH2 inhibitors suggesting their potential clinical activity. Recent encouraging results for EED inhibitors in epigenetic regulation hold great potential but still need to be validated in the clinic. Hence, in the present review, we have analyzed the interaction pattern of the PRC2 components and their inhibitors from the available literature. We believe that these molecules can serve as a simplified entry point for lead optimization and might be helpful in development of an alternative strategy for selective targeting of PRC2 methyltransferase activity by disrupting the interaction of its components.

Conflicts of interest

The authors have no conflicts of interest to declare.

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References

1 Kondo, Y. (2014) Targeting histone methyltransferase EZH2 as cancer treatment. *J. Biochem.* 156, 249–257

2 von Schimmelmann, M. *et al.* (2016) Polycomb repressive complex 2 (PRC2) silences genes responsible for neurodegeneration. *Nat. Neurosci.* 19, 1321–1330

3 Yang, C.Y. and Wang, S. (2017) Allosteric inactivation of polycomb repressive complex 2 (PRC2) by inhibiting its adapter protein: embryonic ectodomain development (EED). *J. Med. Chem.* 60, 2212–2214

4 Nekrasov, M. *et al.* (2007) Pcl-PRC2 is needed to generate high levels of H3-K27 trimethylation at Polycomb target genes. *EMBO J.* 26, 4078–4088

5 Sarma, K. *et al.* (2008) Ezh2 requires PHF1 to efficiently catalyze H3 lysine 27 trimethylation *in vivo*. *Mol. Cell. Biol.* 28, 2718–2731

6 Stanley, C.W. *et al.* (2015) Polycomb repressive complex 2 component Suz12 is required for hematopoietic stem cell function and lymphopoiesis. *Blood* 26, 167–175

7 Justin, N. (2016) Structural basis of oncogenic histone H3K27M inhibition of human polycomb repressive complex 2. *Nat. Commun.* 7, 11316

8 Li, L. (2014) EZH2: novel therapeutic target for human cancer. *BioMedicine* 4, 1

9 Han, Z. (2007) Structural basis of EZH2 recognition by EED. *Structure* 15, 1306–1315

10 Montgomery, N.D. *et al.* (2005) The murine polycomb group protein Eed is required for global histone H3 lysine-27 methylation. *Curr. Biol.* 15, 942–947

11 Cao, R. and Zhang, Y. (2004) SUZ12 is required for both the histone methyltransferase activity and the silencing function of the EED-EZH2 complex. *Mol. Cell.* 15, 57–67

12 Lindsay, E. *et al.* (2017) Structure, mechanism, and regulation of polycomb repressive complex 2. *J. Biol. Chem.* 14 R117.800367

13 Antonysamy, S. *et al.* (2013) Structural context of disease-associated mutations and putative mechanism of auto-inhibition revealed by X-ray crystallographic analysis of the EZH2-SET domain. *PLoS One* 8, e84147

14 Wu, H. *et al.* (2013) Structure of the catalytic domain of EZH2 reveals conformational plasticity in cofactor and substrate binding sites and explains oncogenic mutations. *PLoS One* 8, e83737

15 Graham, S.E. *et al.* (2016) Dynamic behavior of the post-SET loop region of NSD1: implications for histone binding and drug development. *Protein Sci.* 25, 1021–1029

16 Kim, K.H. *et al.* (2016) Targeting EZH2 in cancer. *Nat. Med.* 22, 128–134

17 Smith, T.F. *et al.* (1999) The WD repeat: a common architecture for diverse functions. *Trends Biochem. Sci.* 24, 181–185

18 Xu, C. and Min, J. (2011) Structure and function of WD40 domain proteins. *Protein Cell* 2, 202–214

19 Margueron, R. *et al.* (2009) Role of the polycomb protein EED in the propagation of repressive histone marks. *Nature* 461, 762–767

20 Yun, M. *et al.* (2011) Readers of histone modifications. *Cell Res.* 21, 564–578

21 Adams-Cioaba, M.A. and Min, J. (2009) Structure and function of histone methylation binding proteins. *Biochem. Cell Biol.* 87, 93–105

[22 Jacobs, S.A. and Khorasanizadeh, S. (2002) Structure of HP1 chromodomain bound to a lysine 9-methylated histone H3 tail. *Science* 295, 2080–2083

23 Fischle, W. *et al.* (2003) Molecular basis for the discrimination of repressive methyl-lysine marks in histone H3 by polycomb and HP1 chromodomains. *Genes Dev.* 17, 1870–1881

24 Xu, C. *et al.* (2010) Binding of different histone marks differentially regulates the activity and specificity of polycomb repressive complex 2 (PRC2). *Proc. Natl. Acad. Sci. U. S. A.* 107, 19266–19271

25 Ketel, C.S. *et al.* (2005) Subunit contributions to histone methyltransferase activities of fly and worm polycomb group complexes. *Mol. Cell Biol.* 25, 6857–6868

26 Rai, A.N. *et al.* (2013) Elements of the polycomb repressor SU(Z)12 needed for histone H3-K27 methylation, the interface with E(Z), and *in vivo* function. *Mol. Cell. Biol.* 33, 4844–4856

27 Brooun, A. *et al.* (2016) Polycomb repressive complex 2 structure with inhibitor reveals a mechanism of activation and drug resistance. *Nat. Commun.* 7, 11384

28 Score, J. *et al.* (2011) Inactivation of polycomb repressive complex 2 components in myeloproliferative and myelodysplastic/myeloproliferative neoplasms. *Blood* 119, 1208–1213

29 Veneti, Z. (2017) Polycomb repressor complex 2 in genomic instability and cancer. *Int. J. Mol. Sci.* 18, 1657

30 Tan, J. *et al.* (2007) Pharmacologic disruption of polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells. *Genes Dev.* 21, 1050–1063

31 Hayden, A. *et al.* (2011) S-adenosylhomocysteine hydrolase inhibition by 3-deazane-planocin A analogues induces anti-cancer effects in breast cancer cell lines and synergy with both histone deacetylase and HER2 inhibition. *Breast Cancer Res. Treat.* 127, 109–119

32 Miranda, T.B. *et al.* (2009) DZNep is a global histone methylation inhibitor that reactivates developmental genes not silenced by DNA methylation. *Mol. Cancer Ther.* 8, 1579–1588

33 Takashina, T. *et al.* (2016) Combined inhibition of EZH2 and histone deacetylases as a potential epigenetic therapy for no small-cell lung cancer cells. *Cancer Sci.* 107, 955–962

34 Avan, A. *et al.* (2012) Molecular mechanisms involved in the synergistic interaction of the EZH2 inhibitor 3-deazaneplanocin A (DZNeP) with gemcitabine in pancreatic cancer cells. *Mol. Cancer Ther.* 11, 1735–1746

35 Girard, N. *et al.* (2014) 3-Deazaneplanocin A (DZNep), an inhibitor of the histone methyltransferase EZH2, induces apoptosis and reduces cell migration in chondrosarcoma cells. *PLoS One* 9, e98176

36 Knutson, S. *et al.* (2012) A selective inhibitor of EZH2 blocks H3K27 methylation and kills mutant lymphoma cells. *Nat. Chem. Biol.* 8, 890–896

37 Shen, J.K. *et al.* (2016) Targeting EZH2-mediated methylation of H3K27 inhibits proliferation and migration of synovial sarcoma *in vitro*. *Sci. Rep.* 6, 25239

38 Kyle, D.K. *et al.* (2013) An orally bioavailable chemical probe of the lysine methyltransferases EZH2 and EZH1. *ACS Chem. Biol.* 8, 1324–1334

39 Verma, S.K. *et al.* (2012) Identification of potent, selective, cell-active inhibitors of the histone lysine methyltransferase EZH2. *ACS Med. Chem. Lett.* 3, 1091–1096

40 McCabe, M.T. *et al.* (2012) EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2. *Nature* 492, 108–112

41 Chen, Y.T. (2016) The novel EZH2 inhibitor, GSK126, suppresses cell migration and angiogenesis via down-regulating VEGF-A. *Cancer Chemother. Pharmacol.* 77, 757–765

42 Van Aller, G.S. *et al.* (2014) Long residence time inhibition of EZH2 in activated polycomb repressive complex 2. *ACS Chem. Biol.* 9, 622–629

43 Béguelin, W. *et al.* (2013) EZH2 is required for germinal center formation and somatic EZH2 mutations promote lymphoid transformation. *Cancer Cell* 23, 677–692

44 Zingg, D. *et al.* (2015) The epigenetic modifier EZH2 controls melanoma growth and metastasis through silencing of distinct tumour suppressors. *Nat. Commun.* 6, 6051

45 Wei, Q.I. (2012) Selective inhibition of Ezh2 by a small molecule inhibitor blocks tumor cells proliferation. *Proc. Natl. Acad. Sci. U. S. A.* 109, 21360–21365

46 Bitler, B.G. *et al.* (2015) Synthetic lethality by targeting EZH2 methyltransferase activity in *ARID1A*-mutated cancer. *Nat. Med.* 21, 231–238

47 Konze, K.D. *et al.* (2013) An orally bioavailable chemical probe of the lysine methyltransferases EZH2 and EZH1. *ACS Chem. Biol.* 8, 1324–1334

48 Xu, B. *et al.* (2015) Selective inhibition of EZH2 and EZH1 enzymatic activity by a small molecule suppresses MLL-rearranged leukemia. *Blood* 125, 346–357

49 Knutson, S.K. *et al.* (2014) Selective inhibition of EZH2 by EPZ-6438 leads to potent antitumor activity in EZH2-mutant non-Hodgkin lymphoma. *Mol. Cancer Ther.* 13, 842–854

50 Campbell, J.E. *et al.* (2015) EPZ011989, a potent, orally-available EZH2 inhibitor with robust *in vivo* activity. *ACS Med. Chem. Lett.* 6, 491–495

51 Garapaty-Rao, S. *et al.* (2013) Identification of EZH2 and EZH1 small molecule inhibitors with selective impact on diffuse large B cell lymphoma cell growth. *Chem. Biol.* 20, 1329–1339

52 Dhanak, D. and Jackson, P. (2014) Development and classes of epigenetic drugs for cancer. *Biochem. Biophys. Res. Commun.* 455, 58–69

53 Nasveschuk, C.G. *et al.* (2014) Discovery and optimization of tetramethylpiperidinyl benzamides as inhibitors of EZH2. *ACS Med. Chem. Lett.* 5, 378–383

54 Bradley, W.D. *et al.* (2014) EZH2 inhibitor efficacy in non-Hodgkin's lymphoma does not require suppression of H3K27 monomethylation. *Chem. Biol.* 21, 1463–1475

55 He, Y. *et al.* (2017) The EED protein–protein interaction inhibitor A-395 inactivates the PRC2 complex. *Nat. Chem. Biol.* 13, 389–395

56 Curtin, M.L. *et al.* (2017) SAR of amino pyrrolidines as potent and novel protein–protein interaction inhibitors of the PRC2 complex through EED binding. *Bioorg. Med. Chem. Lett.* 7, 1576–1583

57 Sanulli, S. *et al.* (2015) Jarid2 methylation via the PRC2 complex regulates H3K27me3 deposition during cell differentiation. *Mol. Cell* 57, 769–783

58 Barnash, K.D. *et al.* (2017) Discovery of peptidomimetic ligands of EED as allosteric inhibitors of prc2. *ACS Comb. Sci.* 19, 161–172

59 Lingel, A. (2017) Structure-guided design of EED binders allosterically inhibiting the epigenetic polycomb repressive complex 2 (PRC2) methyltransferase. *J. Med. Chem.* 60, 415–427

60 Ling, L. *et al.* (2017) Discovery and molecular basis of a diverse set of polycomb repressive complex 2 inhibitors recognition by EED. *PLoS One* 12, e0169855

61 Huang, Y. *et al.* (2017) Discovery of first-in-class, potent, and orally bioavailable embryonic ectoderm development (EED) inhibitor with robust anticancer efficacy. *J. Med. Chem.* 60, 2215–2226