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Identification of novel EZH2 inhibitors through pharmacophore-based virtual screening and biological assays

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ABSTRACT

Polycomb repressive complex 2 (PRC2) acts as a primary writer for di- and tri-methylation of histone H3 at lysine 27. This protein plays an essential role in silencing gene expression. Enhancer of zeste 2 (EZH2), the catalytic subunit of PRC2, is considered as a promising therapeutic target for cancer. GSK126, a specific inhibitor of EZH2, is undergoing phase I trials for hypermethylation-related cancers. In addition, many derivatives of GSK126 are also commonly used in laboratory investigations. However, studies on the mechanism and drug development of EZH2 are limited by the absence of structural diversity of these inhibitors because they share similar SAM-like scaffolds. In this study, we generated a pharmacophore model based on reported EZH2 inhibitors and performed in silico screenings. Experimental validations led to the identification of two novel EZH2 inhibitors, DCE_42 and DCE_254, with IC_{50} values of 23 and 11 μM, respectively. They also displayed significant anti-proliferation activity against lymphoma cell lines. Thus, we discovered potent EZH2 inhibitors with novel scaffold using combined in silico screening and experimental study. Results from this study can also guide further development of novel specific EZH2 inhibitors.

Epigenetic modifications, such as DNA methylation, histone methylation, and histone acetylation, have been demonstrated as promising pathways for oncotherapy.[1] Polycomb group (PcG) was first discovered in *Drosophila*.[2] Mutation of this group of proteins results in deviant comb-like body segmentation. Subsequent study has confirmed that deletion of PcG leads to embryonic lethality.[3] Among PcGs, polycomb repressive complex 2 (PRC2) is a set of polycomb repressive complexes composed of embryonic ectoderm development, enhancer of zeste 2 (EZH2), suppressor of zeste 12 (SUZ12), retinoblastoma-associated protein 46/48 (RBAP46/48), adipocyte enhancer binding protein 2 (AEBP2), polycomb-like 2 (PCL2), and Jumonji AT rich interactive domain 2 (JARID2).[4] EZH2 is the catalytic subunit of PRC2 with a SET domain, which catalyzes the transfer process of methyl groups from the cofactor SAM to histone H3 at lysine27.[2] The SET domain is highly conservative within different methyltransferases.[5–7] Moreover, the gain of function mutation of SET domain in EZH2 is closely associated with tumorigenesis.[8,9] Several effective drugs, such as 5-azacytidine, 5-aza-2′-deoxycytidine, and GSK126, are currently clinically used as new anti-tumor drugs that target epigenetic machinery.[10] The significance of histone methylation, which is a highly reversible and dynamic process, is related to the occurrence and development of multiple tumors.[11] GSK126, an identified histone methylation inhibitor, has been successful in treating diffuse large B-cell lymphoma (DLBCL) and transformed follicular lymphoma. Thus, an increasing number of studies have focused on the exploration of inhibitors, and EZH2 has been considered as a promising target to treat hypermethylation-related cancers.[12]

Almost all EZH2 inhibitors, such as GSK126,[12] EPZ005687,[13] EI1[14] and UNC1999,[15] (Table 1) were found through high throughput screening (HTS) or inadvertently. GSK126 and EPZ005687 are

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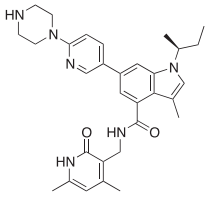
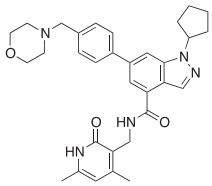
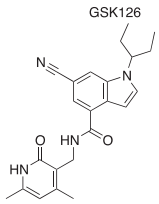
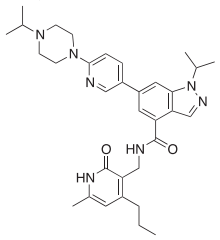
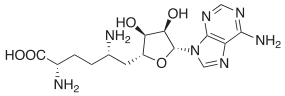
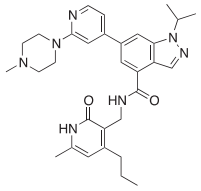
Table 1
Some current known EZH2 inhibitors and their characters

Name	Structure	Selectivity	Clinical advances
GSK126		GSK126 was highly potent and selective for EZH2 and EZH1	Phase 1 Clinical
EPZ005687		Structure–activity relationship is not fully revealed	Preclinical
EI1		EI1 inhibits H3K27 di- and tri-methylation without affecting H3K4, H3K9 and H3K79	Preclinical
UNC1999		UNC1999 exhibits an excellent selectivity, even to EZH1 over a broad range of epigenetic and non-epigenetic targets	Preclinical
Sinefungin		Sinefungin can inhibit SAM-mediated methyl transfer process without selectivity as a SAM analog	Preclinical
GSK343		GSK343 is a highly selective EZH2 inhibitors with selectivity over EZH1	Preclinical

SAM competitive inhibitors of EZH2, which are widely used in evaluating models, mechanism studies, and EZH2 inhibitor development.[14] However, almost all these inhibitor compounds share similar SAM-like scaffolds, and the lack of structural diversity seriously restricts the mechanism study, as well as drug development. Moreover, EZH2 only possesses enzymatic activity when incorporated into PRC2.[16–18] Thus, PRC2 complex, instead of isolated EZH2, is used for HTS, which further increases the complexity of assay development and cost.

Computer-aided drug design is now an emerging subject in medicinal chemistry. Rational drug design based on ligand and structure is attracting increasing attention.[19] All rational design methods have facilitated the discovery of novel bioactive hits from large compound databases through a deep understanding of their physical chemistry or structural features. Ligand-based pharmacophore model is a shared solution when the crystal structure of the target protein is unknown. Compared with docking method, ligand-based pharmacophore model can also provide results without understanding the relationship between the protein and ligands. This characteristic is very applicable to the case in which the receptor crystal structure cannot be obtained.[20] Pharmacophore model is highly accepted in ligand-based drug design, which leads to the discovery of novel hit compounds using 3D chemical information, such as hydrogen-bond donor and receptor, electrostatic force interaction, aromatic ring, hydrophobic and hydrophilic sites, and geometry conformation volume.[21,22] Numerical studies have proved that this method is simple and convenient to apply to epigenetics,[23,24] among other studies.

Antonysamy et al. obtained the crystal structure of EZH2 SET, which resolved the overall catalytic core structure of EZH2 (PDB code: 4MI5).[25] However, the catalytic pocket presents as an inactive state, so the SAM or other designing inhibitors could not access the catalytic pocket. Thus, the catalytic mechanism, is difficult to elucidate, and the discovery and design of novel inhibitors using this crystal structure is hindered.[10] The absence of active EZH2 structure hampers the discovery of new scaffold inhibitors.[26,27] However, the molecular shape and structure-based pharmacophore techniques can still be used to identify novel hit