

Introduction

Enhancer of Zeste Homolog 2 (EZH2) is the catalytic subunit of the Polycomb Repressive Complex 2 (PRC2) that regulate downstream target genes expression, and then promotes tumor cell proliferation, metastasis and drug resistance. EZH2 also performs some functions in a PRC2-independent manner. Most of reported EZH2 inhibitors are S-adenosyle-methionine (SAM)-competitive inhibitor, and are less selective for EZH2 close homolog EZH1, which resulted in safety concerns and insufficient efficacy. To obtain irreversible EZH2 inhibitor, a novel covalent inhibitor was developed and characterized.

Methods

SKLB-03220 and its derivatives were designed, synthesized and confirmed as EZH2 covalent inhibitor by us. The mechanism of covalent binding of SKLB-03220 was confirmed by SAM competition experiments, mass spectrometry, and washing-out assays. Furthermore, the anti-tumor activities of SKLB-03220 in ovarian cancer were investigated by MTT assay, colony formation assay, flow cytometry, western blot assay, and xenograft model. The reversible analogue of SKLB-0322 (SKLB-03224) was used as negative control.

Results

1. SKLB-03220 were covalently bound with EZH2 protein

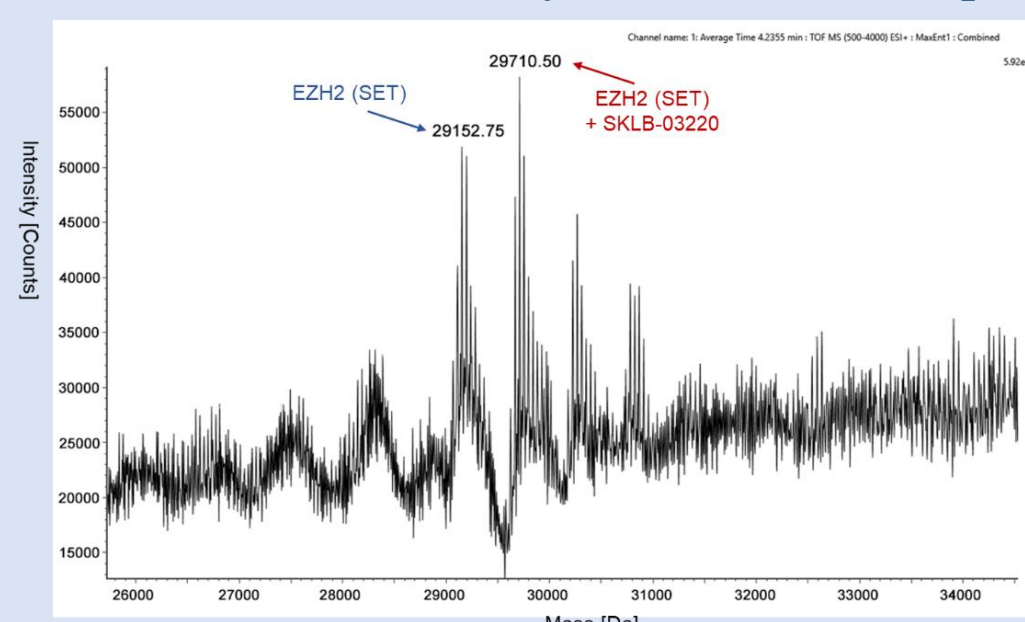


Fig. 1 Mass spectra of EZH2 protein (SET) preincubated with SKLB-03220.

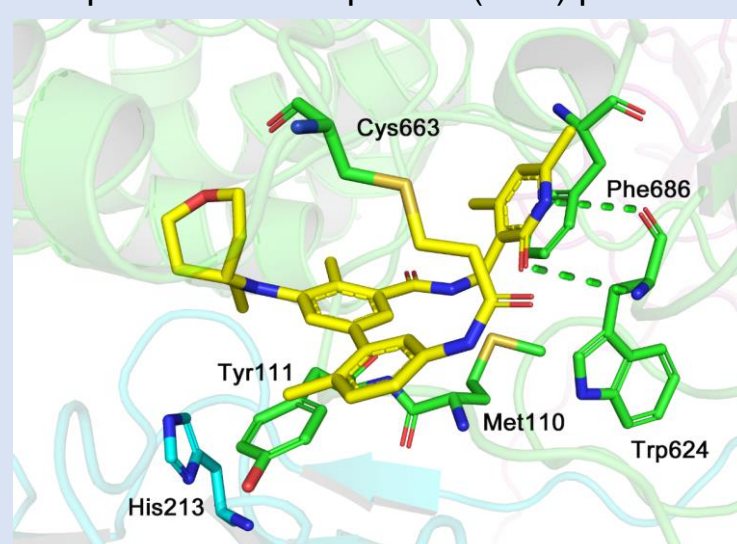


Fig. 2 Predicted binding mode of SKLB-03220 (yellow stick) with EZH2

Results

2. Inhibitory activity of SKLB-03220 on EZH2^{WT} and EZH2^{MUT}

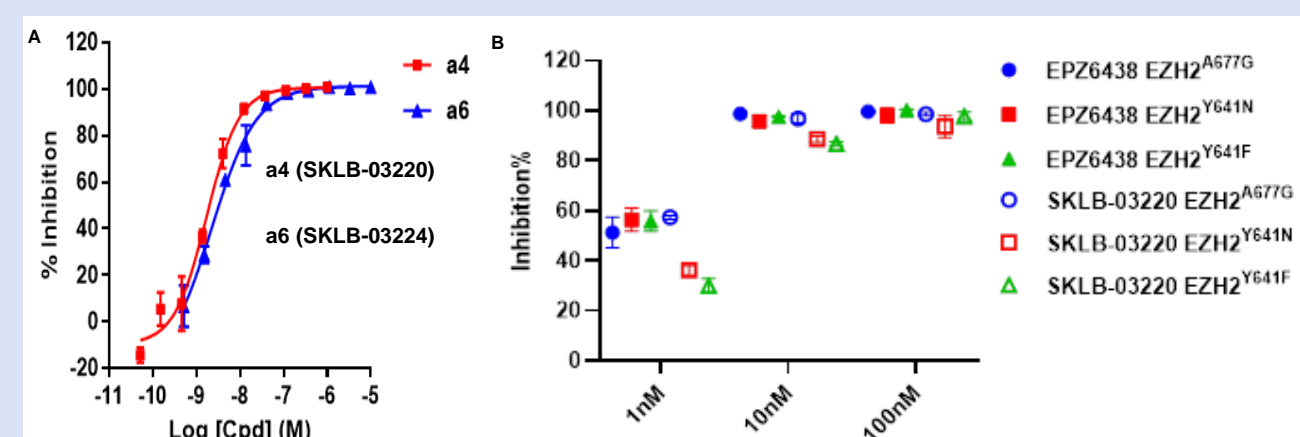


Fig. 3 Potency of SKLB-03220 and reversible analogs SKLB-03224 against EZH2^{WT}(A) and EZH2^{MUT}(B).

3. SKLB-03220 inhibited the cell proliferation in ovarian cancer cells

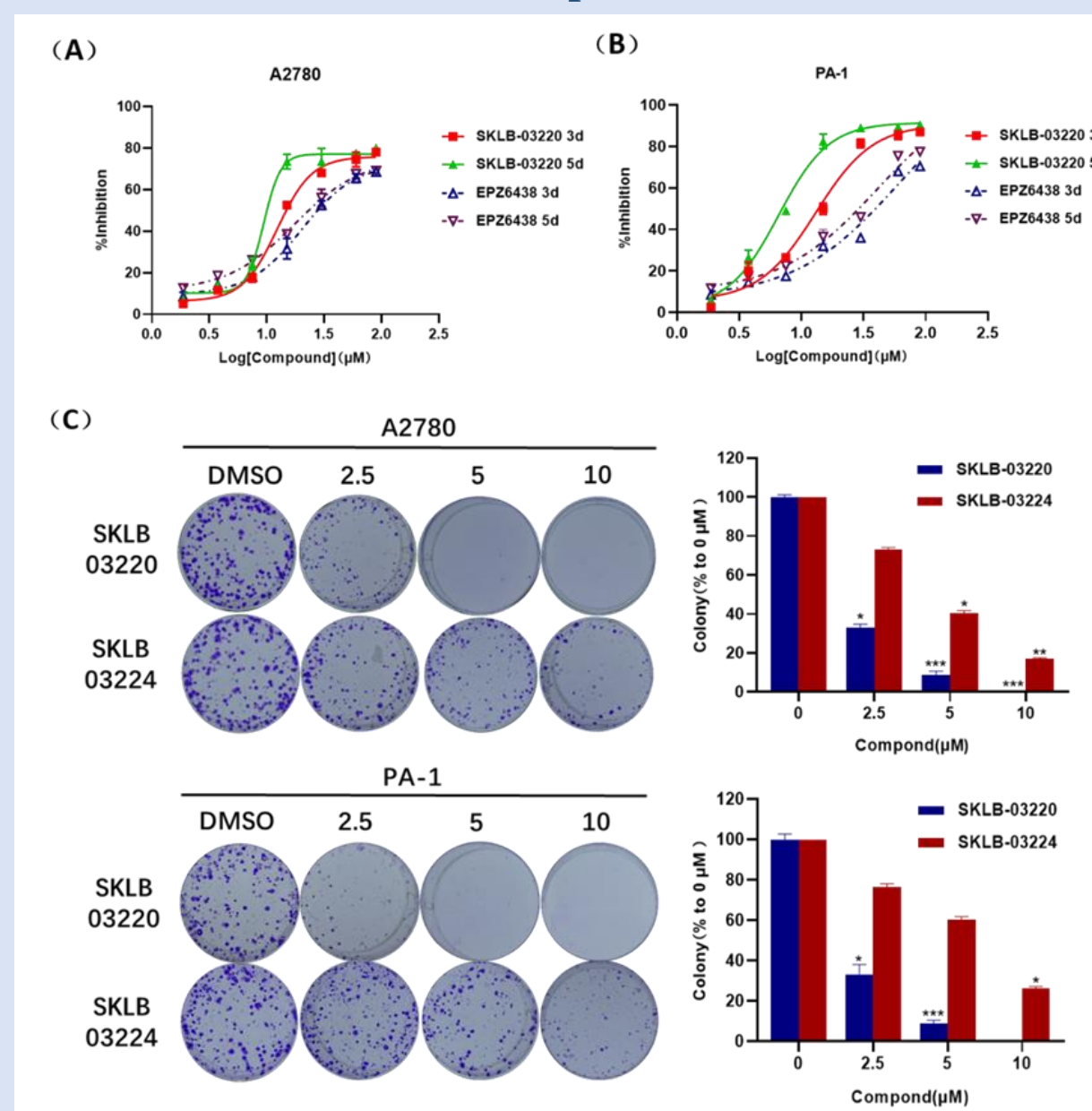


Fig. 4 Proliferation inhibitory effect of SKLB-03220 and reversible analogs SKLB-03224 against Ovarian cancer cell lines A2780 and PA-1.

4. SKLB-03220 induced apoptosis of ovarian cancer cells

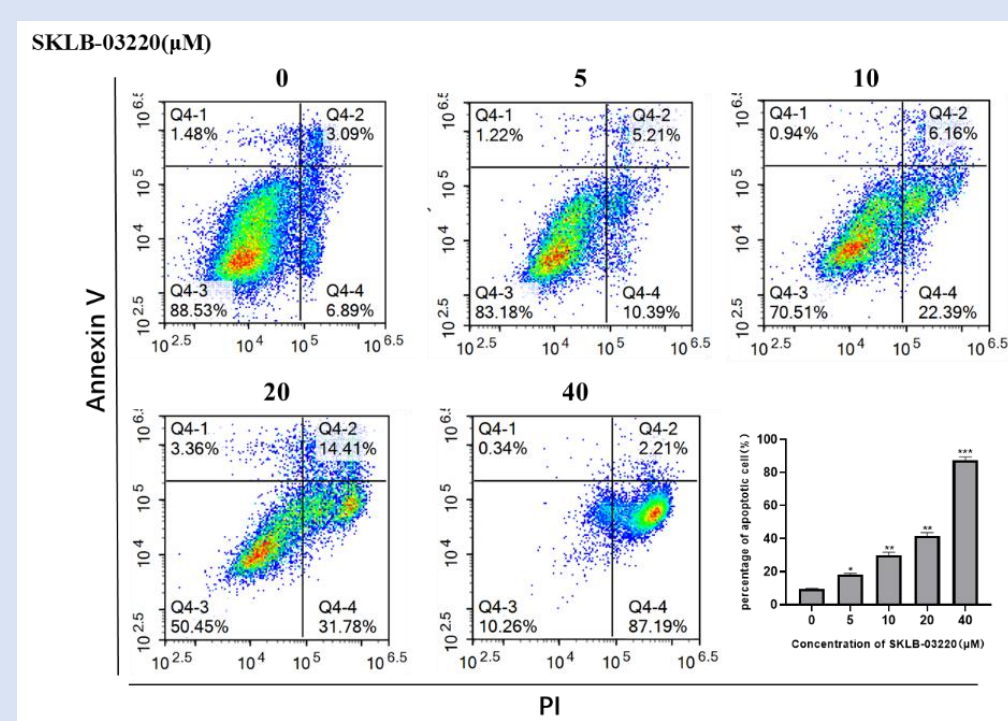


Fig. 5 SKLB-03220 induced apoptosis of PA-1

Results

5. SKLB-03220 inhibited the expression of H3K27me3

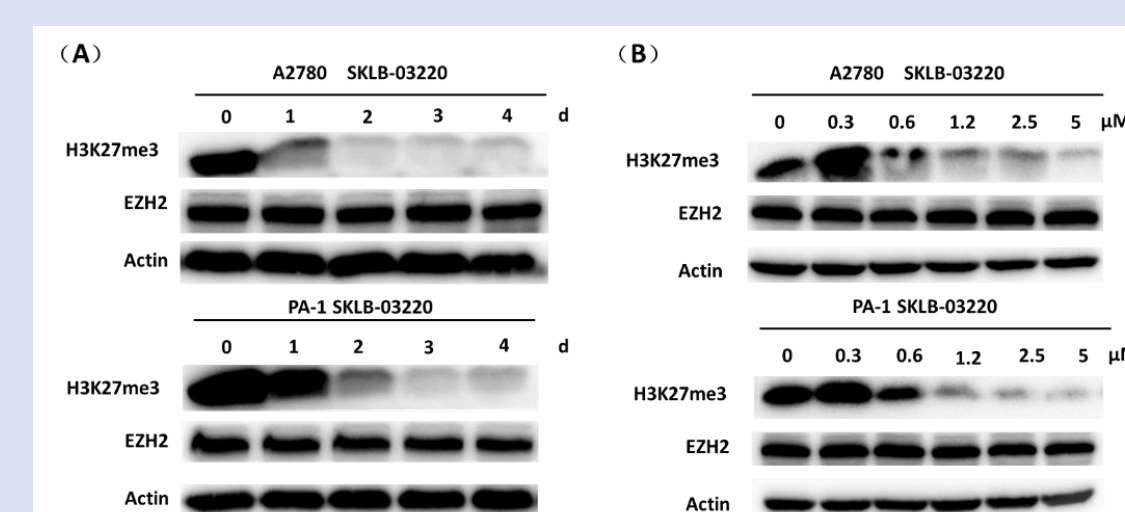


Fig.6 SKLB-03220 inhibited the expression of H3K27me3 in A2780 and PA-1.

6. Washout experiments confirmed SKLB-03220 is covalent inhibitor

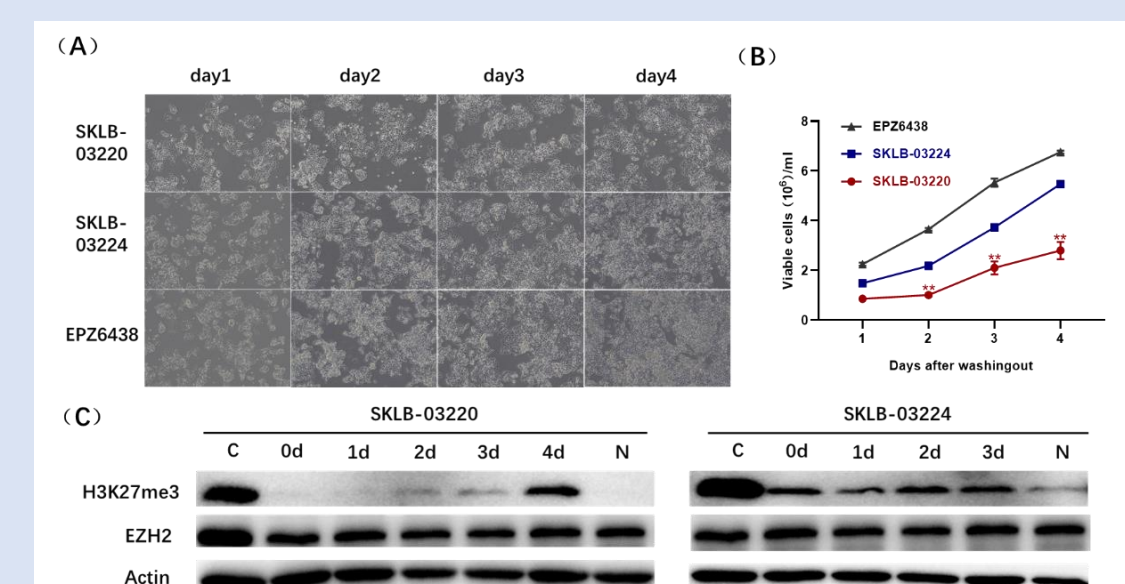


Fig.7 Cellular washout experiments confirmed SKLB-03220 is covalent inhibitor

7. SKLB-03220 inhibited tumor growth in PA-1 xenograft animal model

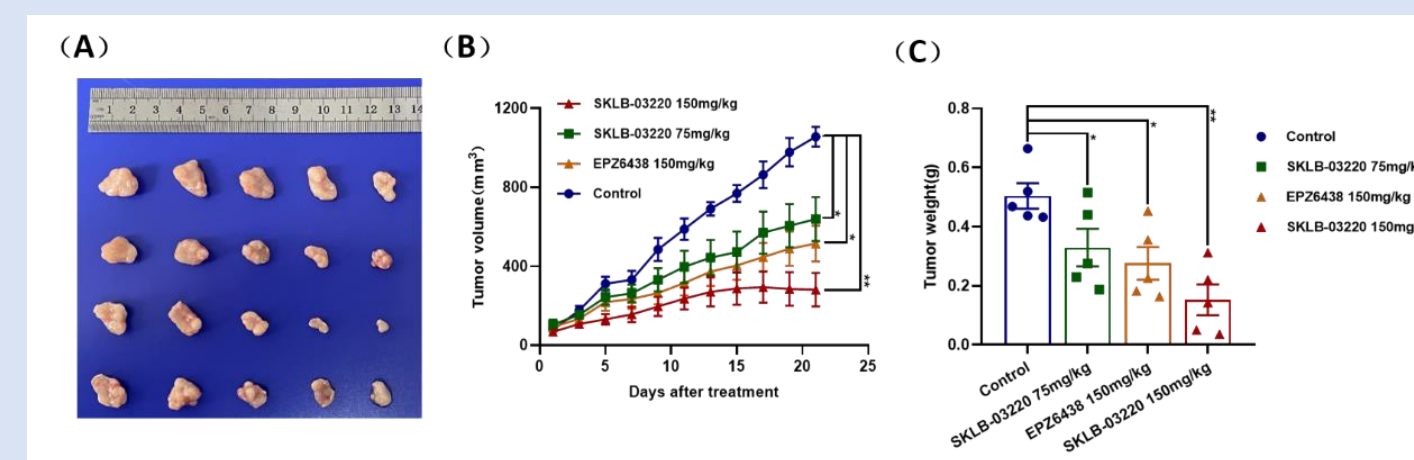


Fig.8 SKLB-03220 was more effective in reducing the growth of PA-1 xenograft animal model than EPZ6438

Conclusion

Taken together, SKLB-03220 is a potent, selective EZH2 covalent inhibitor with noteworthy potency against ovarian cancer both *in vitro* and *in vivo*.