Dual targeting, a new strategy for novel PARP inhibitor discovery

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SUMMARY   As a hallmark for cancer treatment, PARP inhibitors can effectively kill tumor cells with a mechanism termed as synthetic lethality, and are used to treat various cancers including ovarian, breast, prostate, pancreatic and others with DNA repair defects. However, along with the clinical trials progressing, the limitations of PARP-1 inhibitors became apparent such as limited activity and indications. Studies have shown that a molecule that is able to simultaneously restrict two or more targets involving in tumors is more effective in preventing and treating cancers due to the enhancing synergies. In order to make up for the shortcomings of PARP inhibitors, reduce the development cost and overcome the pharmacokinetic defects, multiple works were carried out to construct dual targeting PARP inhibitors for cancer therapy. Herein, they were summarized briefly.

Keywords   PARP, BRCA, dual targeting, inhibitor, antitumor

1. Introduction

Poly (ADP-ribose) polymerase (PARP), a family of at least 17 members, catalyzes the transfer of ADP-ribose from nicotinamide adenine dinucleotide (NAD^+) to itself and substrate proteins (*1*) and plays a role in recruiting DNA repair proteins to the site of DNA injury and triggering the DNA damage repair *via* homologous recombination (HR) pathway (*2*). PARP is also involved in chromatin remodeling, gene transcription, signal transduction, cell cycle regulation, cell death control and aging (*3*). Abnormal expression of PARP is associated with many human disorders including cancers, oxidative stress, metabolic diseases and inflammation (*4*). Since its discovery, PARP has become an important drug targets.

Among all PARPs, PARP-1 is the founding member and the most widely investigated one. As a 116-kDa protein, PARP-1 is one of the most abundant proteins in the nucleus, and plays a role of more than 90% in DNA damage repair (*5*). PARP-1 can be activated up to 500-fold by DNA strand breaks (*6*). PARP-1 contains three functional domains: The N-terminal DNA-binding domain contains two zinc fingers which are important for the PARP-1 binding to single-strand break (SSB) and double-strand break (DSB). The central auto-modification domain, specific glutamate and lysine residues serve as acceptors of ADP-ribose moieties, thereby allowing the enzyme to poly(ADP-ribosyl)ate (PARylate) itself. Finally, the C-terminal catalytic domain transfers ADP-ribose subunits from NAD^+ to protein acceptors, and forming poly (ADP-ribose) polymer. C-terminal catalytic domain is also the binding site of PARP inhibitors (*7*).

PARP can directly participate in the base resection repair pathway and repair single-stranded DNA gaps by activating ataxia telangiectasia mutated (ATM) to promote homologous recombination (HR) (*8*). When DNA damage occurs, PARP binds to DNA gap as a dimer through DNA binding domain which causes a conformational change that activates PARP-1 to cleave NAD^+ into nicotinamide and an ADP-ribose moiety. ADP ribose is covalently transferred to PARP-1 itself or other receptor proteins and forms poly ADP ribose (PAR) chains, whose high negative charge alters the function of the nuclear receptor protein. The high steric hindrance generated causes chromosome relaxation and sends signal to recruit DNA repair proteins and guide them to bind to the gap and repair the damaged site (*9*).

1.1. Mechanism of action of PARP inhibitors

Studies have shown that targeting more than two DNA repair pathways in tumor cells can induce "synthetic lethality" mechanism and PARP inhibitors are the first anticancer drugs approved for clinical use under the concept of synthetic lethality (*10*). BRCA1 and BRCA2 are two tumor suppressors responsible for DSB repair by HR and humans with mutations in these two genes are inclined to suffer from breast and other cancers (*11*). Inhibition of PARP-1 bring about the blockage of base excision repair and the persistent SSB of DNA chain,

finally resulting in DSBs. In normal cells, these DSBs can be repaired through HR but not in BRCA1- and/or BRCA2-deficient tumor cells (*12,13*). Accumulation of DSBs becomes highly toxic to tumor cells and results in synthetic lethality. So, cancer patients with disabled BRCA1/2 are susceptible to the treatment with PARP inhibitors, and due to this feature, the efficacy of PARP inhibitors is expected to be extended to cancers with the same DNA repair dysfunction, such as breast cancer, ovarian cancer (*14*), prostate cancer (*15*), pancreatic cancer (*16*) and lung cancer (*17*). Besides the synthetic lethality mechanism, PARP inhibitors also exert cytotoxic effects through trapping PARP-DNA complexes, thereby preventing DNA replication and transcription (*18*).

The development of PARP inhibitors based on synthetic lethality is a hallmark for cancer therapy. Accordingly, the research and development of PARP inhibitors has become a hot topic in the anticancer field.

1.2. The binding mode of PARP inhibitors

The NAD^+ binding domain of PARP-1 is divided into three subdomains, including the nicotinamide-ribose binding domain, the phosphate binding domain and the adenine-ribose binding domain. Most of the reported PARP inhibitors were designed to mimic the nicotinamide structure and bind competitively with NAD^+ at the nicotinamide-ribose binding domain (*19*).

So far, the most important pharmacophore in PARP inhibitors is a amide group free or in an cycle system which is capable of competing with the natural substrate NAD^+ at the catalytic site of PARP (*20*). The amide groups can form two critical hydrogen bonds (HBs) with Ser904 and Gly863 residues. Apart from the amide group, the aromatic ring to which the amide group attaches can form a π-π stacking with Tyr907 residue, which is another key interaction between inhibitors and PARP (*21*). The other part of the PARP inhibitors go through another two domains. Some conserved water molecules can form extra HBs and contribute to the design of PARP-1 selective inhibitors.

After the failure of iniparib, currently, a total of six PARP inhibitors are approved on the market, including olaparib (1), rucaparib (2), niraparib (3), talazoparib (4), fluzoparib (5), and pamiparib (6) (*22*) as showed in Figure 1 below.

1.3. Existing problems of PARP inhibitors

As clinical trials were published, the limitations of PARP inhibitors became apparent. On the one hand, PARP inhibitors as monotherapy are only effective against BRCA1/2 defective cancers (*23*). To cancers with normal expression of BRAC, the effectiveness of PARP inhibitors is pale. On the other hand, long-term use of PARP inhibitors also faces drug resistance problems

induced by different mechanisms (*24*).

The pathogenesis of cancer is complex, and a single antitumor drug usually cannot provide effective and lasting inhibition. There are several strategies to address this problem (*25*), one of which is the design of dual targeting PARP inhibitors which can inhibit other cancer related targets other than PARPs.

2. Dual targeting PARP inhibitors

2.1. PARP/HDAC dual targeting inhibitor

Acetylation as the best studied epigenetic modification plays important roles in the regulation of a host of normal cellular processes such as cell differentiation, proliferation, angiogenesis, and apoptosis. Dysregulation of acetylation is implicated in diverse cellular events in pathologies of cancer.

The level of acetylation of histones and non-histone proteins is governed by two antagonistic families of enzymes: histone deacetylases (HDACs) and histone acetyl transferases (HATs) (*26*). HDACs are a family of ubiquitous enzymes capable of removing acetyl groups from the ε-amino groups of lysine residues present within core histones and many non-histone substrates (*27*). Consequently, the positive charge on the N-terminal of core histones increases and strengthens interactions with negatively charged DNA while blocking access of transcriptional machinery to the DNA template, leading to gene silencing.

There are 18 known members in HDAC family which is further subdivided into four classes based on their sequence homology: Class I HDACs include HDAC1, 2, 3, and 8; Class II HDACs include Classes IIa (HDAC4, 5, 7, and 9) and IIb (HDAC6 and 10); Class III HDACs, known as sirtuins (sirt1-7); and Class IV HDAC (HDAC11) (*28*). Silencing or
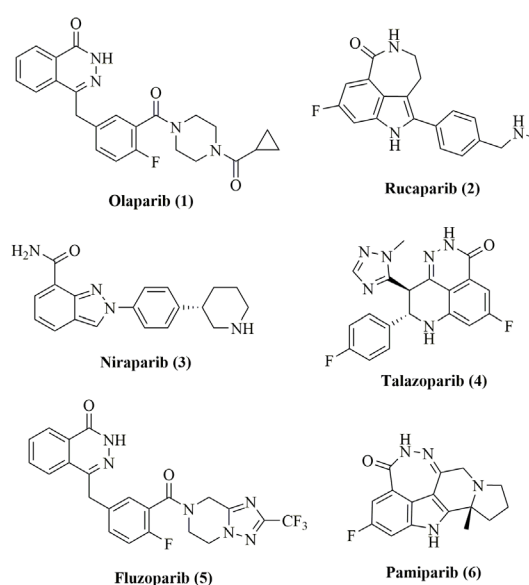

Figure 1. Six approved PARP inhibitors.

inhibiting HDACs can impair cell cycle, cell growth, chromatin decondensation, and angiogenesis and induce cell differentiation, apoptosis in several cancer cell types. HDACs have therefore emerged as important therapeutic targets for cancers. Accompanied by the extensive elucidation of mechanisms and functions of HDAC in tumorigenesis, the development of HDAC inhibitors represents a powerful weapon fighting against for cancers.

In 2006, the FDA approved SAHA used in treating the rare cutaneous T-cell lymphoma (*29*). Following SAHA, romidepsin, belinostat and panobinostat were approved by the FDA for treatment of cancers including cutaneous T-cell lymphoma, peripheral T-cell lymphoma (PTCL), and multiple myeloma. The benzamide-based Class I HDAC-selective inhibitor chidamide was approved by NMPA for the treatment of relapsed or refractory PTCL. Apart from these five drugs, several HDAC inhibitors are undergoing different stages of clinical trials against human disorders.

The canonical pharmacophore of the HDAC inhibitors is composed of three parts: a cap structure that can interact with the rim at the entrance of the active pocket of HDACs; a zinc ion (Zn^{2+}) binding group (ZBG); and a linker responsible for the connection of cap and ZBG and for interaction with the hydrophobic tunnel of the active site (*30*). Of these three constitutive parts of the HDAC inhibitor, the cap can accept extensive structural derivatization which make it possible to design huge amounts of HDAC inhibitors.

It was reported that acetylation blocks DNA damage-induced chromatin ADP-ribosylation (*31*) and HDAC inhibitors can decrease expression of proteins involved in DNA repair (*32,33*), which support the combinatorial application of PARP and HDAC inhibitors for the treatment of PARP-dependent cancers. As a fact, many works have proved that HDAC inhibitors can synergize with PARP inhibitors in treating cancers (*34,35*), which validate the design of dual PARP/HDAC inhibitors.

Yuan *et al.* firstly designed four hydroxamic acid containing derivatives of compound 1 as dual PARP and HDAC inhibitors to induce cancer cell death (*36*). All four compounds displayed potent inhibitory activities against PARP-1/2 and HDAC1/6. Compounds 7 and 8 (Figure 2) showed the best HDAC6 inhibitory activities with the IC_{50} values of 8.21 and 10.18 nM. These two hybrids also potently inhibited the activity of PARP-2 with IC_{50} value of 5.02 and 2.53 nM.

In vitro, 7 and 8 possessed excellent antiproliferative activities, comparable to compound SAHA and much better than 1, suggesting that the HDAC inhibitory activities of 7, 8 should play a predominant role in tumor cell response. The significant antiproliferative activities of 7 and 8 were maintained even to the Raji and HCC1937 tumor cell lines that have been reported to be resistant to SAHA treatment. The inhibitory activities of 7 (IC_{50} = 1.29 μM) and 8 (IC_{50} = 0.81 μM) against Raji were 7- and 12-fold more potent than that of SAHA, respectively, while the IC_{50} value of 1 was more than 50 μM. In HCC1937 tumor cells, both 7 (IC_{50} = 2.02 μM) and 8 (IC_{50} = 0.45 μM) exhibited more potent antiproliferative activities than 1 (IC_{50} = 8.65 μM) and SAHA (IC_{50} = 4.23 μM). So, compounds 7 and 8 may represent especially bona fide leads for further optimization in the development of novel antitumor agents against both PARP and HDAC.

Besides hydroxamic acid, *o*-amine aniline is another frequently used ZBG. Reported by Liao, *et al.*, a series of PARP/HDAC inhibitors was synthesized still with 1 as the core skeleton and *o*-amine aniline as the ZBG (*37*). Compound 9 (Figure 2) came up as the most promising candidate possessing balanced
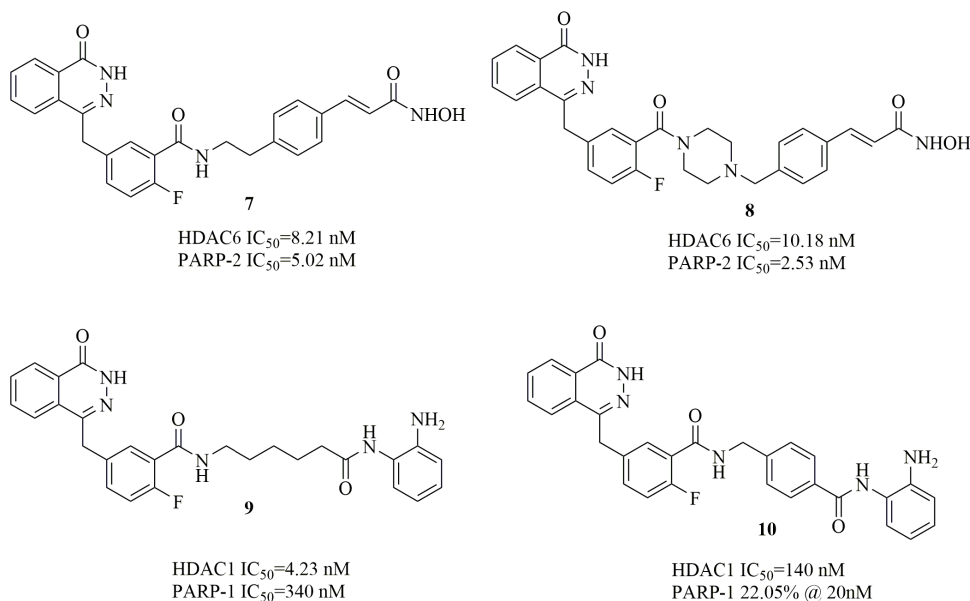

HDAC6 IC_{50}=8.21 nM
PARP-2 IC_{50}=5.02 nM

HDAC6 IC_{50}=10.18 nM
PARP-2 IC_{50}=2.53 nM

HDAC1 IC_{50}=4.23 nM
PARP-1 IC_{50}=340 nM

HDAC1 IC_{50}=140 nM
PARP-1 22.05% @ 20nM

Figure 2. PARP/HDAC dual targeting inhibitors.

inhibitory activities toward PARP-1 and HDAC1 with the IC_{50} values of 4.23 and 340 nM, respectively. 9 showed potent inhibition against growth of K562 and MDA-MB-231 cells with GI_{50} values of 5.6 and 4.3 μM, respectively. However, in this series, compound 10 (Figure 2) with benzyl as linker showed the most potent antiproliferative activity against K562 and MDA-MB-231 cells with GI_{50} values of 0.4 and 1.9 μM, respectively, due to its strong inhibitory activity to HDAC1 (IC_{50} = 140 nM), however, the PARP-1 inhibition activity was much weaker (inhibition rate = 22.06 % at 20 nM) than 9.

2.2. PARP/PI3K dual target inhibitor

Phosphatidylinositol 3-kinases (PI3Ks) are a family of intracellular signal transducer enzymes possessing regulatory roles in critical cellular processes including cell growth, proliferation, differentiation, motility, and intracellular trafficking (38). These lipid kinases specifically phosphorylate the 3-position hydroxyl group upon the inositol ring of phosphatidylinositol. The generation PIP3 resulting from PI3K activation activates Akt which is also known as protein kinase B through phosphorylation, Akt targets many downstream proteins that results in many cancer-related consequences such as tumor cell survival, cell cycle progression, cell proliferation and growth (39-41).

PI3K-Akt-mTOR is one of the most frequently activated signaling pathways controlling a number of essential cellular processes including cell survival, proliferation, motility, and differentiation in tumors, and mediated nearly 50% of malignant tumors. This pathway has been one of the most extensive studied pathways for cancer therapeutics and the development of its inhibitor is attractive. The first antitumor drugs targeting the PI3K/mTOR signaling pathway are the rapamycin derivatives temsirolimus and everolimus which are inhibitors of mTORC1. The former is used for the treatment of advanced renal cell carcinoma (42), and the latter is used for the treatment of advanced breast cancer (43), renal cell carcinoma, and pancreatic neuroendocrine tumors (44). Besides that, more than 40 PI3K inhibitors with different isoform selectivity have advanced to clinical trials, four of those, idelalisib, copanlisib, duvelisib, and alpelisib have been approved by the FDA (40).

It has been proved that the PI3K signaling pathway maintains the stability of HR repair pathway and controls the repair process of DNA double-strand damage (45). Inhibition of the PI3K signaling pathway activates ERK, enhances ETS1 activity, and thus inhibits the expression of BRCA1/2, resulting in HR defects that sensitize tumor cells to PARP inhibitors (46). Inhibition of the PI3K related pathway has displayed synergistic effects with PARP inhibitors for the treatment of cancers (47).

Triple negative breast cancer (TNBC) as an invasive

breast cancer with poor prognosis and high recurrence rate, currently, the only treatment option for it is still highly toxic and incurable chemotherapy, so more effective and safety therapeutics are urgently needed. Ibrahim and Juvekar both found that PI3K inhibition attenuate BRCA1/2 expression which making TNBC cells much more sensitive to PARP inhibitors (46,48). In another work, Yang, *et al.* reported that combinatory treatment with PI3K inhibitor BKM120 and PARP inhibitor 1 is effective in inhibiting the gastric cancer cells with ARID1A deficiency (49). The combination of BKM120 and 1 also showed promising efficacy for the treatment of ovarian cancer due to the low expression of BRCA (50). Considering the synergistic effect of dual inhibition of PI3K and PARP, some works have been reported for the PI3K and PARP dual targeting inhibitors.

In the work of Wu *et al.*, the authors discovered highly effective dual PARP/PI3K inhibitors through pharmacophores combination and scaffold hopping strategy, demonstrating the practicability of targeting PARP and PI3K together with a single chemical entity (51) . Compound 1 was firstly selected as the starting point to design the hybrid inhibitors, and the imperative structure of a PI3K inhibitor GDC-0980 was merged. In this serial, compounds 11 (Figure 3) exhibited excellent inhibitory activities against PARP-1 (IC_{50} = 1.57 nM) and PI3Kα (IC_{50} = 2.0 nM). In another serial, the benzofuran carboxamide structure was utilized to design the hybrid inhibitors in place of compound 1. Compound 12 (Figure 3) exhibited balanced and more potent inhibitory activities against two targets (PARP-1: IC_{50} = 0.91 nM, PI3Kα: IC_{50} = 1.5 nM). Compound 11 and 12 showed promising antiproliferative activities against both BRCA-deficient (HCT-116, HCC-1937) and BRCA-proficient (SW620, MDA-MB-231/468) tumor cells with IC_{50} values in μM or sub-μM ranges. 11 and 12 also exhibited considerable *in vivo* antitumor efficacy in an MDA-MB-468 xenograft mouse model, with tumor growth inhibition values of 56.39% and 48.77%, respectively. Excitingly, 12 possessed promising profiles including high kinase selectivity and low cardiotoxicity. Overall, this work indicates two compounds 11 and 12 might be potential PARP/PI3K dual inhibitors for cancer therapy and deserve further research.

In another work, Wang, *et al.* also reported the design and synthesis of novel PARP/PI3K dual inhibitors (52). By taking the enzyme inhibitory activity, solubility and pharmacokinetic parameters all into account, compound 13 (Figure 3) was obtained whose structure encompassed a benzofuran carboxamide moiety for PARP inhibition and 1,3,5-triazine scaffold for PI3K inhibition. 13 potently inhibited the activities of PARP-1 and PI3Kα with IC_{50} values of 13.8 and 64 nM, respectively. 12 also showed excellent antiproliferative activity against MDA-MB-468 cells with the IC_{50} value 1.40 μM, much stronger than compound 1 (IC_{50} = 13.72
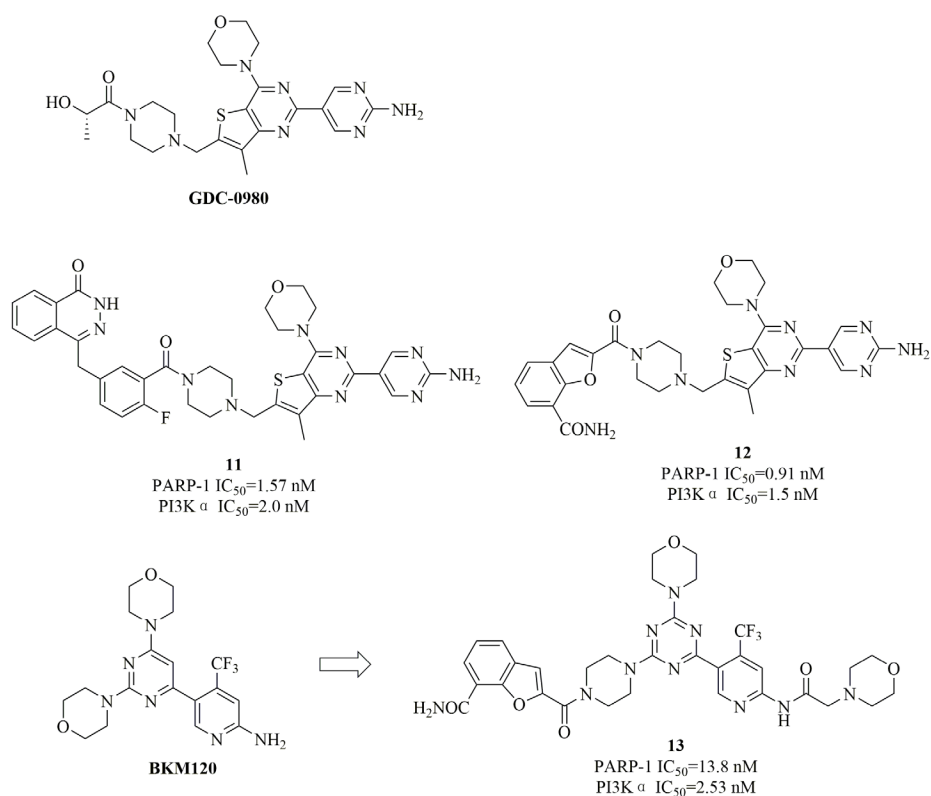
Figure 3. PARP/PI3K dual targeting inhibitors.

μM). Compound 13 displayed a stronger capability to downregulate the expression of BRCA1/2 at the mRNA level than 1 and BKM120, a PI3K inhibitor, suggesting that compound 13 was likely to induce HR deficiency through the downregulation of BRCA1/2. In MDA-MB-468 cell derived xenograft model, compound 13 displayed excellent antitumor efficacy at a dose of 50 mg/kg, more efficacious than the single administration of 1 or BKM120.

2.3. PARP/topoisomerase dual targeting inhibitors

Topoisomerase, including type I and II (Topo I/ II), are well-studied enzymes that participate in the cleavage and religation of DNA. They can dissolve topological problems caused by supercoiling of DNA and play important roles in cell replication and gene transcription. Topo I transiently unknits a single strand of DNA, while Topo II can cleave double strands of DNA powered by ATP. Both of these two processes can change the topological structure of DNA and relax it, ultimately facilitating the process of DNA replication during cell division (*53*).

Topo I/II also plays key roles in cancer cell proliferation. Since cancer cells divide much more rapidly than normal cells, cancer cells can be killed disproportionately by inhibition of Topo inhibition, which makes Topo I/II important targets for anticancer drug development. Compounds targeting Topo can be divided into poisons and catalytic inhibitors, with the majority of Topo I/II inhibitors belonging to the former class that firmly bind to cleaved DNA-Topo complex to prevent DNA relinking and lead to tumor cell death (*54*).

The overexpression of PARP-1 and other various DNA damage repair proteins may contribute to the repair of DNA lesions induced by Topo I/II inhibitors, and then enable tumor cells to resist to Topo I/II inhibitors treatment. While, the combination of a Topo inhibitor and PARP inhibitor is considered to be an option to obtain a synergistic effect against tumors. Topo I poisons such as camptothecin (CPT) stabilize the complex in the broken conformation leading to persistent SSB. While PARP-1 plays a major role in the sensing and repair of DNA SSB and contributes to the restart of stalled replication forks during HRR. PARP-1 is activated by CPT-induced DNA breaks and promotes the separation of Topo I from the DNA and the subsequent DNA repair (*55*). So, PARP reduces CPT-induced replication fork reversal and limits DNA strand breakage. While, inhibition of PARP-1 will sensitize cells to Topo I poisons and PARP inhibitors could enhance the cytotoxicity of Topo I poisons by the inhibition of DNA repair (*56*). It was also reported that inhibition of PARP was able to potentiate the antitumor activity of Topo II inhibitors (*57,58*). So, it is reasonable to design Topo/PARP dual targeting inhibitors.

Acridine and its derivatives which possess tricyclic planar structures have been widely explored as Topo I or II inhibitors (*59,60*). In a work accomplished by Yuan, *et al.*, an acridine derivative 14 (Figure 4) was

selected as the skeleton to design Topo I/PARP dual targeting inhibitors by retaining the key pharmacophore of veliparib, a potent PARP inhibitor under clinical trials (61). A serial of fourteen compounds was finally obtained. Out of them, compound 15 (Figure 4) displayed the most potent PARP-1 inhibition activity with IC_{50} value of 90 nM. While another compound 16 (Figure 4) with moderate PARP-1 inhibitory activity (IC_{50} = 450 nM) possessed the highest antiproliferative activity toward MCF-7 cells with GI_{50} value of 2.14 μM. On the other hand, 16 exhibited comparable Topo I inhibitory potency with that of reference compound CPT at the concentration of 100 μM which proved that 16 was a Topo I/PARP dual targeting compounds. As the candidate, *in vivo* antitumor activity was also assessed with 16 against the xenografts tumor models of MCF-7. At two dose, 20 and 40 mg/kg, 16 significantly reduce the tumor growth compared to the blank control group.

2.4. PARP/EZH2 dual targeting inhibitors

Like acetylation mentioned above, lysine methylation is another important protein covalent modification in the field of epigenetics. Many enzymes function lonely or as a subunit in a complex act as lysine methyltransferase such as DOT1L (62), SMYD (63), G9a (64) and enhancer of zeste homolog 2 (EZH2). EZH2 an enzymatic subunit of PRC2 complex, catalyzes histone H3 lysine 27 trimethylation, which results in multiple gene silencing (65). EZH2 is frequently overexpressed or mutated in many kinds of cancers (66). Many studies have shown that EZH2 promotes cancer cell proliferation, tumor growth, cancer stem cell (CSC) expansion and metastasis (67).

Thus, EZH2 is considered as a promising anticancer drug target. Tazemetostat as an EZH2 inhibitor was approved in 2020 for treating epithelioid sarcoma (68). Following the HDAC inhibitors, one more drug in epigenetics was approved.

EZH2 is subjected to multiple posttranslational modifications including phosphorylation (69), ubiquitination (70) and O-GlcNAcylation (71), all of which participate in the regulation of EZH2 activity. Yamaguchi, *et al.* demonstrated that PARylation of EZH2 mediated by PARP-1 negatively regulates EZH2 activity, leads to its dissociation from the PRC2 complex and subsequent degradation. Conversely, PARP inhibitor could induce EZH2 activity and increased cancer stem cell population which could attenuate the therapeutic efficacy of the PARP inhibitor. Inhibition of EZH2 could sensitize BRCA-mutant cancers to PARP inhibition. So, concurrent inhibition EZH2 and PAPR-1 should be a promising therapeutic strategy for BRCA-mutated breast and ovarian cancers (72). Accordingly, it is reasonable to design dual EZH2/PARP inhibitors.

In the work reported by Wang, *et al.*, they used compound 1 and tazemetostat as the starting point to design the dual PARP and EZH2 inhibitors (73). Analysis of the complex of tazemetostat-EZH2 revealed that the benzylmorpholine moiety oriented to the solvent and can tolerate structural modification. Thus, the authors replace the benzylmorpholine with a linker in order to incorporate the key pharmacophore of compound 1. In addition, different substitution groups were installed on the benzene ring of the tazemetostat to investigate their influence on the activity towards two targets. Finally, compound 17 (Figure 5) was selected as the candidate. 17 potently inhibited the PARP-1 and EZH2 with IC_{50} values of 6.87 and 36.51
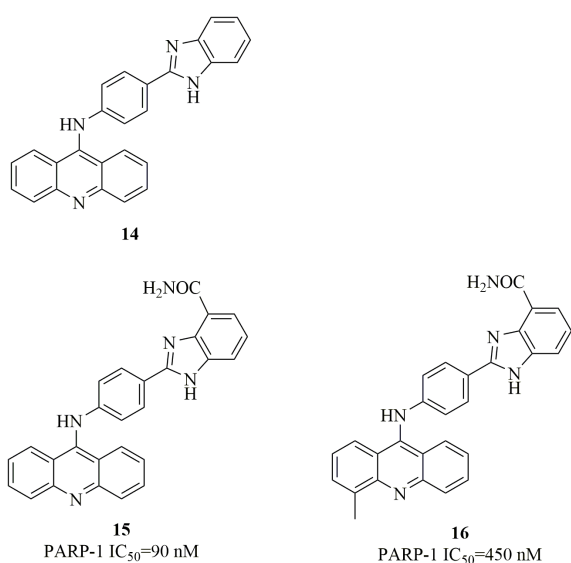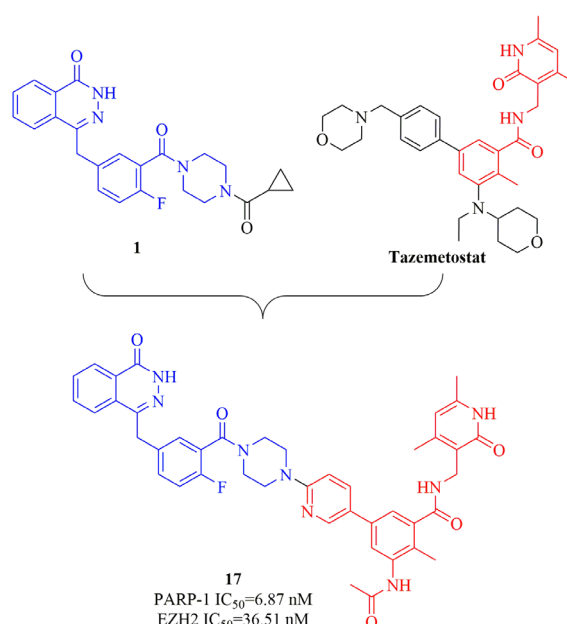

Figure 4. PARP/Topo I dual targeting inhibitors.

14

15
PARP-1 IC_{50}=90 nM

16
PARP-1 IC_{50}=450 nM

Figure 5. PARP/EZH2 dual targeting inhibitors.

1

Tazemetostat

17
PARP-1 IC_{50}=6.87 nM
EZH2 IC_{50}=36.51 nM

nM, respectively, comparable to two positive controls (IC$_{50}$ of compound 1 against PARP-1 = 7.09 nM, IC$_{50}$ of tazemetostat against EZH2 = 13.05 nM) In cellular assay, 17 could suppress the proliferation of MDA-MB-231 and MDA-MB-468 breast tumor cells with IC$_{50}$ values of 2.63 and 0.41 μM, respectively, much more potent than 1 (36.92 and 35.57 μM) and tazemetostat (44.45 and 46.76 μM). 17 could induce autophagy death of tumor cells and cause less damage to normal cells. Therefore, compound 17 as a first-in-class dual PARP and EZH2 inhibitor should be a potential anticancer drug candidate for breast cancer treatment.

2.5. PROTAC for PARP

Targeted protein degradation (TPD) which can eliminate a protein of interest (POI) have been drawing immense attentions and holds great promise for the development of novel drugs for human diseases (*74*). One focus of TPD is the development of hetero-bifunctional small-molecule degraders, such as Proteolysis Targeting Chimera (PROTAC). A PROTAC molecule is composed of two different ligands, one is for binding to corresponding POI and another binding to an E3 ligase. These two ligands are tethered *via* a linker. PROTACs are able to tag the POI with ubiquitination and then hijack the ubiquitin-proteasome system (UPS) to bring about the degradation of the POI (*75*).

Comparing to the traditional small molecular inhibitors (SMIs) of POI, PROTAC possess several advantages. PROTACs can function in a low concentration, just like a catalysis in the field of organic chemistry, which enable it to degrade multiple POIs with single PROTAC molecule (*76*). Accordingly, PROTAC can confine the toxicities induced by SMIs. Another advantage of PROTAC over SMIs is that PROTAC can degrade undruggable proteins such as transcription factors (TFs), of which no suitable binding pocket exist on the surface, such as STAT3 and Ras. Additionally, PROTACs can overcome the drug resistance resulted from the mutations of one or more amino acids.

So far, the most frequently used in literature including CRBN with immunomodulatory imide drugs (IMiD) as the ligand (*e.g.* thalidomide, lenalidomide and pomalidomide), VHL with the peptoid as the ligand, MDM2 with nutlin-3 derivatives as ligand and IAPs with bestatin, a CD13 inhibitor, as the ligand.

Following the upsurge of the PROTAC technology and considering the promising therapeutic value of harnessing PARP-1, Zhao, *et al.* was encouraged to develop potential PROTAC for PARP-1 degradation (*77*). Niraparib (3) was selected as the PARP-1 ligand. The analysis of the crystal structure of 3 in the complex with PARP-1 (PDB# 4R6E, Figure 6) suggested that the piperidine ring of 3 experiences the opening of the ligand binding pocket and thus may represent a suitable site for tethering the linker and E3 ligase ligand. Five molecules were synthesized coupling with the ligands of three different E3 ligases: MDM2, CRBN and VHL. Finally, compound 18 (Figure 6) equipped with nutlin-3, a MDM2 ligase, could effectively induce PARP-1 degradation in a concentration dependent manner, but not 3, nutlin-3 or their combination. In cellular level, 18 could induce the apoptosis of MDA-MB-231 cells and suppress the proliferation of MDA-MB-231 cells with the IC$_{50}$ of 8.45 μM and 6.12 μM for 24 h and 48 h treatment, respectively. In contrast, only marginal or no inhibitory effects were observed upon treatment with 3, nutlin-3 or in their combination. All these results demonstrated that compound 18 was
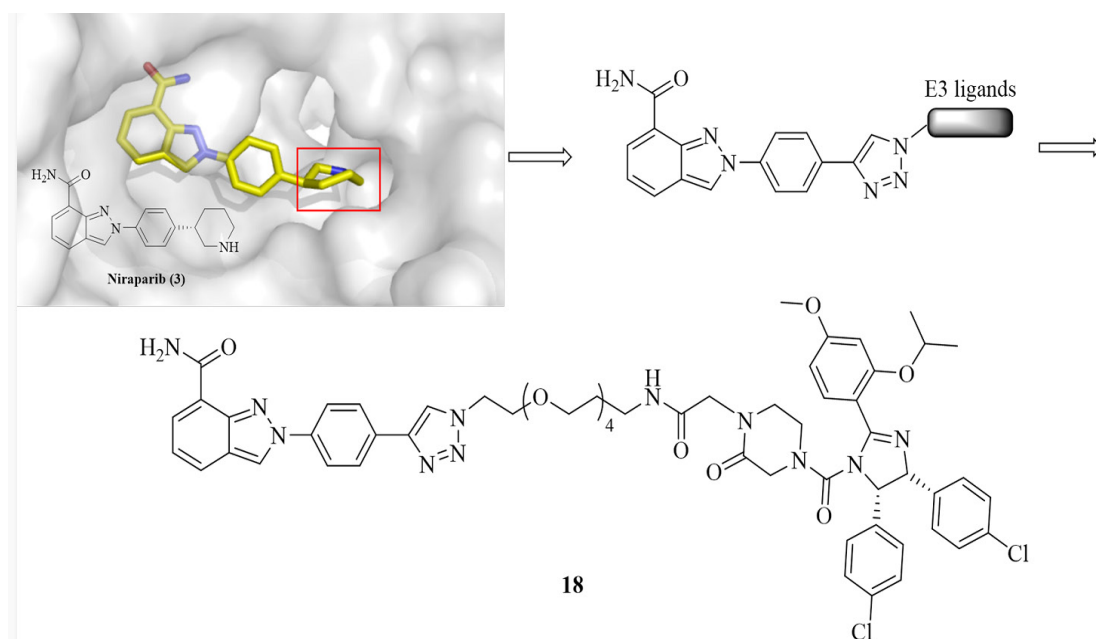

Figure 6. The PROTAC for PARP degradation.

a promising PROTAC for PARP-1 of value for further biological activity tests. In our opinion, this work also shed light on the work for design of more PARP PROTACs.

3. Conclusion

Since 2010, many important advances in the field of PARP inhibitors for cancer therapy have been achieved. As the most successful tumor targeting agents in the field of synthetic lethality, six PARP inhibitors have been approved for clinical use mainly for breast and ovarian cancers, which verified the theory that PARP inhibition can block the single-chain repair pathway and kill tumor cells with BRCA mutations. Although the use of PARP inhibitors is a hallmark event, some bottlenecks are still present. PARP inhibitors have limited indications in cancer therapy. And it is apparent that patients with or without BRCA-mutant cancers will eventually become resistant to PARP inhibitors. Therefore, PARP inhibitors are usually used in combination with other anticancer drugs in order to extend the therapeutic spectrum, enhance the efficacy and conquer the drug resistance. But drug combinations meet some drawbacks including unpredictable metabolism, complex drug-drug interaction that induces undesirable side effects and poor patients' compliance. The design of multi targeting drugs that are capable of repressing more than one pathway can promisingly address these problem including the PARP inhibitors. But till now, only a few of dual targeting PARP inhibitors are reported. There is still a large space for the development of dual targeting PARP inhibitors. In theory, inhibition of drug targets that are associated with PARP or involved in DNA breaks repair have the potential to cooperate with PARP inhibitors and design dual targeting PARP inhibitors. The key point is to pinpoint the structural moiety that can tolerate modification without effecting the inhibitory activities towards two targets. There are many PARP inhibitors with abundant structure features available which make great opportunity to design more dual targeting PARP inhibitors possessing druggable characteristics.

But, the design of dual targeting PARP inhibitors has its dark side which is that the toxicity maybe increased along with the inhibition of two targets which should be paid attention. More works need to be accomplished to assess the toxicity of the dual targeting PARP inhibitors.

Moreover, PROTACs as a sophisticated technology for drug discovery have drawing more and more attentions. But to our best knowledge, only one work reported the PROTACs for PARP-1. Along with the increasing number of PARP inhibitors and E3 ligase ligands, more PROTACs for PARP can be designed. Overall, design of dual targeting PARP inhibitor is newly emerging filed and hold great promise for novel anticancer drug discovery, so, more attentions should be paid.

References

1. Wang YQ, Wang PY, Wang YT, Yang GF, Zhang A, Miao ZH. An update on poly(ADP-ribose)polymerase-1 (PARP-1) inhibitors: opportunities and challenges in cancer therapy. J Med Chem. 2016; 59:9575-9598.
2. Houtgraaf JH, Versmissen J, van der Giessen WJ. A concise review of DNA damage checkpoints and repair in mammalian cells. Cardiovasc Revasc Med. 2006; 7:165-172.
3. Curtin NJ, Szabo C. Poly(ADP-ribose) polymerase inhibition: past, present and future. Nat Rev Drug Discov. 2020; 19:711-736.
4. Peralta-Leal A, Rodriguez-Vargas JM, Aguilar-Quesada R, Rodriguez MI, Linares JL, de Almodovar MR, Oliver FJ. PARP inhibitors: new partners in the therapy of cancer and inflammatory diseases. Free Radic Biol Med. 2009; 47:13-26.
5. Shen H, Ge Y, Wang J, Li H, Xu Y, Zhu Q. Design, synthesis and biological evaluation of novel molecules as potent PARP-1 inhibitors. Bioorg Med Chem Lett. 2021; 47:128169.
6. D'Amours D, Desnoyers S, D'Silva I, Poirier GG. Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. Biochem J. 1999; 342 (Pt 2):249-268.
7. Boraei ATA, Singh PK, Sechi M, Satta S. Discovery of novel functionalized 1,2,4-triazoles as PARP-1 inhibitors in breast cancer: Design, synthesis and antitumor activity evaluation. Eur J Med Chem. 2019; 182:111621.
8. Haince JF, Kozlov S, Dawson VL, Dawson TM, Hendzel MJ, Lavin MF, Poirier GG. Ataxia telangiectasia mutated (ATM) signaling network is modulated by a novel poly(ADP-ribose)-dependent pathway in the early response to DNA-damaging agents. J Biol Chem. 2007; 282:16441-16453.
9. Zhao Y, Zhang LX, Jiang T, Long J, Ma ZY, Lu AP, Cheng Y, Cao DS. The ups and downs of Poly(ADP-ribose) Polymerase-1 inhibitors in cancer therapy-Current progress and future direction. Eur J Med Chem. 2020; 203:112570.
10. Zhang B, Tang C, Yao Y, Chen X, Zhou C, Wei Z, Xing F, Chen L, Cai X, Zhang Z, Sun S, Liu Q. The tumor therapy landscape of synthetic lethality. Nat Commun. 2021; 12:1275.
11. Boyd J. BRCA: the breast, ovarian, and other cancer genes. Gynecol Oncol. 2001; 80:337-340.
12. Jain PG, Patel BD. Medicinal chemistry approaches of poly ADP-Ribose polymerase 1 (PARP1) inhibitors as anticancer agents - A recent update. Eur J Med Chem. 2019; 165:198-215.
13. Chen Y, Du H. The promising PARP inhibitors in ovarian cancer therapy: From Olaparib to others. Biomed Pharmacother. 2018; 99:552-560.

14. Mercier-Vogel L, Bodmer A, Castiglione M. PARP inhibitors: new therapeutic agents in breast and ovarian cancer. Rev Med Suisse. 2011; 7:1137-1140.

15. Lavery A, Gilson C, Chowdhury S. PARP inhibitors and stratified treatment of prostate cancer. Expert Rev Anticancer Ther. 2016; 16:1213-1215.

16. Pant S, Maitra A, Yap TA. PARP inhibition - opportunities in pancreatic cancer. Nat Rev Clin Oncol. 2019; 16:595-596.

17. Ji W, Weng X, Xu D, Cai S, Lou H, Ding L. Non-small cell lung cancer cells with deficiencies in homologous recombination genes are sensitive to PARP inhibitors. Biochem Biophys Res Commun. 2020; 522:121-126.

18. Alvarez Secord A, O'Malley DM, Sood AK, Westin SN, Liu JF. Rationale for combination PARP inhibitor and antiangiogenic treatment in advanced epithelial ovarian cancer: A review. Gynecol Oncol. 2021; 162:482-495.

19. Ferraris DV. Evolution of poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors. From concept to clinic. J Med Chem. 2010; 53:4561-4584.

20. Penning TD. Small-molecule PARP modulators – current status and future therapeutic potential. Curr Opin Drug Discov Devel. 2010; 13:577-586.

21. Yu J, Luo L, Hu T, Cui Y, Sun X, Gou W, Hou W, Li Y, Sun T. Structure-based design, synthesis, and evaluation of inhibitors with high selectivity for PARP-1 over PARP-2. Eur J Med Chem. 2021; 227:113898.

22. Markham A. Pamiparib: first approval. Drugs. 2021; 81:1343-1348.

23. Kamel D, Gray C, Walia JS, Kumar V. PARP inhibitor drugs in the treatment of breast, ovarian, prostate and pancreatic cancers: An update of clinical trials. Curr Drug Targets. 2018; 19:21-37.

24. Noordermeer SM, van Attikum H. PARP inhibitor resistance: A tug-of-war in BRCA-mutated cells. Trends Cell Biol. 2019; 29:820-834.

25. Petrelli A, Valabrega G. Multitarget drugs: the present and the future of cancer therapy. Expert Opin Pharmacother. 2009; 10:589-600.

26. Ho TCS, Chan AHY, Ganesan A. Thirty years of HDAC inhibitors: 2020 insight and hindsight. J Med Chem. 2020; 63:12460-12484.

27. Luan Y, Li J, Bernatchez JA, Li R. Kinase and histone deacetylase hybrid inhibitors for cancer therapy. J Med Chem. 2019; 62:3171-3183.

28. Peng X, Sun Z, Kuang P, Chen J. Recent progress on HDAC inhibitors with dual targeting capabilities for cancer treatment. Eur J Med Chem. 2020; 208:112831.

29. Paris M, Porcelloni M, Binaschi M, Fattori D. Histone deacetylase inhibitors: from bench to clinic. J Med Chem. 2008; 51:1505-1529.

30. De Vreese R, D'Hooghe M. Synthesis and applications of benzohydroxamic acid-based histone deacetylase inhibitors. Eur J Med Chem. 2017; 135:174-195.

31. Liszczak G, Diehl KL, Dann GP, Muir TW. Acetylation blocks DNA damage-induced chromatin ADP-ribosylation. Nat Chem Biol. 2018; 14:837-840.

32. Munshi A, Kurland JF, Nishikawa T, Tanaka T, Hobbs ML, Tucker SL, Ismail S, Stevens C, Meyn RE. Histone deacetylase inhibitors radiosensitize human melanoma cells by suppressing DNA repair activity. Clin Cancer Res. 2005; 11:4912-4922.

33. Marijon H, Lee DH, Ding L, Sun H, Gery S, de Gramont A, Koeffler HP. Co-targeting poly(ADP-ribose) polymerase (PARP) and histone deacetylase (HDAC) in triple-negative

breast cancer: Higher synergism in BRCA mutated cells. Biomed Pharmacother. 2018; 99:543-551.

34. Min A, Im SA, Kim DK, Song SH, Kim HJ, Lee KH, Kim TY, Han SW, Oh DY, Kim TY, O'Connor MJ, Bang YJ. Histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA), enhances anti-tumor effects of the poly (ADP-ribose) polymerase (PARP) inhibitor olaparib in triple-negative breast cancer cells. Breast Cancer Res. 2015; 17:33.

35. Yin L, Liu Y, Peng Y, Peng Y, Yu X, Gao Y, Yuan B, Zhu Q, Cao T, He L, Gong Z, Sun L, Fan X, Li X. PARP inhibitor veliparib and HDAC inhibitor SAHA synergistically co-target the UHRF1/BRCA1 DNA damage repair complex in prostate cancer cells. J Exp Clin Cancer Res. 2018; 37:153.

36. Yuan Z, Chen S, Sun Q, Wang N, Li D, Miao S, Gao C, Chen Y, Tan C, Jiang Y. Olaparib hydroxamic acid derivatives as dual PARP and HDAC inhibitors for cancer therapy. Bioorg Med Chem. 2017; 25:4100-4109.

37. Tian Y, Xie Z, Liao C. Design, synthesis and anticancer activities of novel dual poly(ADP-ribose) polymerase-1/ histone deacetylase-1 inhibitors. Bioorg Med Chem Lett. 2020; 30:127036.

38. Garces AE, Stocks MJ. Class 1 PI3K clinical candidates and recent inhibitor design strategies: A medicinal chemistry perspective. J Med Chem. 2019; 62:4815-4850.

39. Saurat T, Buron F, Rodrigues N, *et al.* Design, synthesis, and biological activity of pyridopyrimidine scaffolds as novel PI3K/mTOR dual inhibitors. J Med Chem. 2014; 57:613-631.

40. Zhang K, Ji M, Lin S, Peng S, Zhang Z, Zhang M, Zhang J, Zhang Y, Wu D, Tian H, Chen X, Xu H. Design, synthesis, and biological evaluation of a novel photocaged PI3K inhibitor toward precise cancer treatment. J Med Chem. 2021; 64:7331-7340.

41. Andrs M, Korabecny J, Jun D, Hodny Z, Bartek J, Kuca K. Phosphatidylinositol 3-kinase (PI3K) and phosphatidylinositol 3-kinase-related kinase (PIKK) inhibitors: importance of the morpholine ring. J Med Chem. 2015; 58:41-71.

42. Ravaud A, Bernhard JC, Gross-Goupil M, Digue L, Ferriere JM. mTOR inhibitors: temsirolimus and everolimus in the treatment of renal cell carcinoma. Bull Cancer. 2010; 97:45-51.

43. Tartarone A, Lerose R, Aieta M. Everolimus in HR-positive advanced breast cancer. N Engl J Med. 2012; 366:1739; author reply 1739-1740.

44. Yao JC, Phan AT, Jehl V, Shah G, Meric-Bernstam F. Everolimus in advanced pancreatic neuroendocrine tumors: the clinical experience. Cancer Res. 2013; 73:1449-1453.

45. Alemi F, Raei Sadigh A, Malakoti F, Elhaei Y, Ghaffari SH, Maleki M, Asemi Z, Yousefi B, Targhazeh N, Majidinia M. Molecular mechanisms involved in DNA repair in human cancers: An overview of PI3k/Akt signaling and PIKKs crosstalk. J Cell Physiol. 2021.

46. Ibrahim YH, Garcia-Garcia C, Serra V, *et al.* PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient triple-negative breast cancer to PARP inhibition. Cancer Discov. 2012; 2:1036-1047.

47. Alderton GK. Therapeutics: PI3K-PARP combination. Nat Rev Cancer. 2012; 12:658.

48. Juvekar A, Burga LN, Hu H, *et al.* Combining a PI3K inhibitor with a PARP inhibitor provides an effective therapy for BRCA1-related breast cancer. Cancer Discov.

2012; 2:1048-1063.

49. Yang L, Yang G, Ding Y, Huang Y, Liu S, Zhou L, Wei W, Wang J, Hu G. Combined treatment with PI3K inhibitor BKM120 and PARP inhibitor olaparib is effective in inhibiting the gastric cancer cells with ARID1A deficiency. Oncol Rep. 2018; 40:479-487.

50. Wang D, Wang M, Jiang N, Zhang Y, Bian X, Wang X, Roberts TM, Zhao JJ, Liu P, Cheng H. Effective use of PI3K inhibitor BKM120 and PARP inhibitor Olaparib to treat PIK3CA mutant ovarian cancer. Oncotarget. 2016; 7:13153-13166.

51. Wu Z, Bai Y, Jin J, Jiang T, Shen H, Ju Q, Zhu Q, Xu Y. Discovery of novel and potent PARP/PI3K dual inhibitors for the treatment of cancer. Eur J Med Chem. 2021; 217:113357.

52. Wang J, He G, Li H, Ge Y, Wang S, Xu Y, Zhu Q. Discovery of novel PARP/PI3K dual inhibitors with high efficiency against BRCA-proficient triple negative breast cancer. Eur J Med Chem. 2021; 213:113054.

53. Salerno S, Da Settimo F, Taliani S, Simorini F, La Motta C, Fornaciari G, Marini AM. Recent advances in the development of dual topoisomerase I and II inhibitors as anticancer drugs. Curr Med Chem. 2010; 17:4270-4290.

54. Capranico G, Binaschi M. DNA sequence selectivity of topoisomerases and topoisomerase poisons. Biochim Biophys Acta. 1998; 1400:185-194.

55. Mouly L, Mamouni K, Gence R, Cristini A, Cherier J, Castinel A, Legrand M, Favre G, Sordet O, Monferran S. PARP-1-dependent RND1 transcription induced by topoisomerase I cleavage complexes confers cellular resistance to camptothecin. Cell Death Dis. 2018; 9:931.

56. Matsuno Y, Hyodo M, Fujimori H, Shimizu A, Yoshioka KI. Sensitization of cancer cells to radiation and topoisomerase I inhibitor camptothecin using inhibitors of PARP and other signaling molecules. Cancers (Basel). 2018; 10:364.

57. Sabisz M, Wesierska-Gadek J, Skladanowski A. Increased cytotoxicity of an unusual DNA topoisomerase II inhibitor compound C-1305 toward HeLa cells with downregulated PARP-1 activity results from re-activation of the p53 pathway and modulation of mitotic checkpoints. Biochem Pharmacol. 2010; 79:1387-1397.

58. Wesierska-Gadek J, Zulehner N, Ferk F, Skladanowski A, Komina O, Maurer M. PARP inhibition potentiates the cytotoxic activity of C-1305, a selective inhibitor of topoisomerase II, in human BRCA1-positive breast cancer cells. Biochem Pharmacol. 2012; 84:1318-1331.

59. Haider MR, Ahmad K, Siddiqui N, Ali Z, Akhtar MJ, Fuloria N, Fuloria S, Ravichandran M, Yar MS. Novel 9-(2-(1-arylethylidene)hydrazinyl)acridine derivatives: Target topoisomerase 1 and growth inhibition of HeLa cancer cells. Bioorg Chem. 2019; 88:102962.

60. Kozurkova M. Acridine derivatives as inhibitors/poisons of topoisomerase II. J Appl Toxicol. 2021.

61. Yuan Z, Chen S, Chen C, Chen J, Chen C, Dai Q, Gao C, Jiang Y. Design, synthesis and biological evaluation of 4-amidobenzimidazole acridine derivatives as dual PARP and Topo inhibitors for cancer therapy. Eur J Med Chem. 2017; 138:1135-1146.

62. Sarno F, Nebbioso A, Altucci L. DOT1L: a key target in normal chromatin remodelling and in mixed-lineage leukaemia treatment. Epigenetics. 2020; 15:439-453.

63. Yi X, Jiang XJ, Fang ZM. Histone methyltransferase SMYD2: ubiquitous regulator of disease. Clin Epigenetics. 2019; 11:112.

64. Chen WL, Sun HP, Li DD, Wang ZH, You QD, Guo XK. G9a - An appealing antineoplastic target. Curr Cancer Drug Targets. 2017; 17:555-568.

65. Kim KH, Roberts CW. Targeting EZH2 in cancer. Nat Med. 2016; 22:128-134.

66. Eich ML, Athar M, Ferguson JE, 3rd, Varambally S. EZH2-targeted therapies in cancer: hype or a reality. Cancer Res. 2020; 80:5449-5458.

67. Duan R, Du W, Guo W. EZH2: a novel target for cancer treatment. J Hematol Oncol. 2020; 13:104.

68. Hoy SM. Tazemetostat: first approval. Drugs. 2020; 80:513-521.

69. Chen S, Bohrer LR, Rai AN, Pan Y, Gan L, Zhou X, Bagchi A, Simon JA, Huang H. Cyclin-dependent kinases regulate epigenetic gene silencing through phosphorylation of EZH2. Nat Cell Biol. 2010; 12:1108-1114.

70. Wang X, Cao W, Zhang J, *et al.* A covalently bound inhibitor triggers EZH2 degradation through CHIP-mediated ubiquitination. EMBO J. 2017; 36:1243-1260.

71. Chu CS, Lo PW, Yeh YH, Hsu PH, Peng SH, Teng YC, Kang ML, Wong CH, Juan LJ. O-GlcNAcylation regulates EZH2 protein stability and function. Proc Natl Acad Sci U S A. 2014; 111:1355-1360.

72. Yamaguchi H, Du Y, Nakai K, Ding M, Chang SS, Hsu JL, Yao J, Wei Y, Nie L, Jiao S, Chang WC, Chen CH, Yu Y, Hortobagyi GN, Hung MC. EZH2 contributes to the response to PARP inhibitors through its PARP-mediated poly-ADP ribosylation in breast cancer. Oncogene. 2018; 37:208-217.

73. Wang C, Qu L, Li S, Yin F, Ji L, Peng W, Luo H, Lu D, Liu X, Chen X, Kong L, Wang X. Discovery of first-in-class dual PARP and EZH2 inhibitors for triple-negative breast cancer with wild-type BRCA. J Med Chem. 2021; 64:12630-12650.

74. Lai AC, Crews CM. Induced protein degradation: an emerging drug discovery paradigm. Nat Rev Drug Discov. 2017; 16:101-114.

75. Schapira M, Calabrese MF, Bullock AN, Crews CM. Targeted protein degradation: expanding the toolbox. Nat Rev Drug Discov. 2019; 18:949-963.

76. Khan S, Zhang X, Lv D, *et al.* A selective BCL-XL PROTAC degrader achieves safe and potent antitumor activity. Nat Med. 2019; 25:1938-1947.

77. Zhao Q, Lan T, Su S, Rao Y. Induction of apoptosis in MDA-MB-231 breast cancer cells by a PARP1-targeting PROTAC small molecule. Chem Commun (Camb). 2019; 55:369-372.

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