Inhibitors targeting the SUMOylation pathway: A patent review 2012-2015 (Review)

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Abstract. Small ubiquitin‑related modifier (SUMO) proteins bind to the lysine residue of target proteins to produce functionally mature proteins. The abnormal SUMOylation of certain target proteins is associated with diseases including cancer, heart disease, diabetes, arthritis, degenerative diseases and brain ischemia/stroke. Thus, there has been growing appreciation for the potential importance of the SUMO conjugation pathway as a target for treating these diseases. This review introduces the important steps in the reversible SUMOylation pathway. The SUMO inhibitors disclosed in the patents between 2012 and 2015 are divided into different categories according to their mechanisms of action. Certain compounds disclosed in this review have also been reported in other articles for their inhibition of the SUMOylation pathway following screening in cell lines. Although there are few studies using animal models or clinical trials that have used these compounds, the application of bortezomin, a ubiquitylation inhibitor, for treating cancer indicates that SUMO inhibitors may be clinically successful.

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1. Introduction
Precursor proteins are not active, and thus, need further processing to become functional mature proteins. Post-translational protein modification is the chemical modification of proteins prior to or following protein biosynthesis, and includes phosphorylation, ubiquitylation, methylation, acetylation, and modifications by ubiquitin‑like modifiers (Ubls).
Ubiquitin (Ub) is a globular protein consisting of 76 amino acid residues, and the UPS (ubiquitin‑proteasome system) is responsible for degrading 80‑90% of intracellular proteins. Ub binds the lysine residues of target proteins by a series of enzymes including the ubiquitin‑activating enzyme E1, ubiquitin‑conjugating enzyme E2 and the ubiquitin E3 ligases. Subsequently, these ubiquitylated proteins are recognized and degraded by the 26S proteasome. Modifications by Ubls are similar to ubiquitylation and they compete for the lysine residues of certain substrates (1).

Small ubiquitin‑related modifier (SUMO) proteins are one type of Ubls. Although they only have ~18% sequence identity with ubiquitin, they share a common three-dimensional structure; namely, a globular β-grasp fold and a characteristic C-terminal diglycine (Gly-Gly) motif that is exposed following maturation (2). Mammalian cells express four SUMO isoforms: SUMO‑1, SUMO‑2, SUMO‑3 and SUMO‑4. SUMO1‑3 is ubiquitously expressed in human tissues, whereas SUMO4 is only expressed in the kidney, lymph nodes and spleen (3). SUMO‑2 and 3 are 95% identical to each other and only 50% identical to SUMO‑1 (4); it is not yet clear whether these three isoforms are functionally distinct (6). Unlike SUMO‑1, SUMO‑2 and 3 have internal SUMO modification sites at their N‑termini (K11), which can form poly‑SUMO chains (5). The expression level and activity of SUMO proteins affect their conjugation to substrates, although it remains unknown if SUMO‑4 can be conjugated to cellular proteins. Similar to the ubiquitylation pathway,
SUMO conjugation is reversible and catalyzed by a three-step enzymatic reaction that includes activation, conjugation, and ligation (Fig. 1), which does not result in protein decomposition, but rather, in the regulation of normal cell functions such as protein-protein interactions, subcellular localization, DNA repair, and cell cycle and transcription factor regulation (6).

In SUMOylation, initially, sentrin-specific proteases (SENP)s cleave the C-terminus of SUMO using their endopeptidase activities to expose the Gly-Gly motif required for conjugation, which is also termed the SUMO-interacting motif (SIM) (7-9). SENPs have six isoforms (SENP1, 2, 3, 5, 6 and 7) in mammals, which are divided into three subfamilies based on their sequence homology, substrate specificity, and subcellular localization (10). The catalytic cysteine protease domain at the C-terminus is ~250 amino acid residues in length, and controls the specificity and function of SENP isoforms (11). The catalytic domain crystal structures of SENP1, SENP2 and SENP7, which are comprised of the typical catalytic triad (Cys-His-Asp), are very similar (10). SENP1 and SENP2 have SUMO maturation and deSUMOylation abilities, whereas the other SENPs are unable to induce maturation and prefer SUMO-2/3 over SUMO-1 for deSUMOylation (10,12).

In the second step, SUMO-activating enzyme (SAE) activates the C-terminus of SUMO in a two-step reaction that involves ATP hydrolysis. There is only one heterodimeric E1 enzyme [SAE1/SAE2, also termed activator of SUMO1 (Aos1)/ubiquitin like modifier activating enzyme 2 (Uba2)] involved in the SUMOylation pathway. SAE1 (Aos1) promotes adenylation of the SUMO C-terminus to form the SUMO-AMP intermediate, followed by transfer of SUMO to SAE2 (Uba2) and thioester bond formation (13).

The third step is conjugation. Specifically, SUMO is transferred from SAE to the catalytic Cys173 residue on SAE2 (Uba2) and thioester bond formation (13). The third step is conjugation. Specifically, SUMO is transferred from SAE to the catalytic Cys173 residue on SAE2 (Uba2) and thioester bond formation (13). The third step is conjugation. Specifically, SUMO is transferred from SAE to the catalytic Cys173 residue on SAE2 (Uba2) and thioester bond formation (13).

The fourth step is ligation. Ubc9 catalyzes formation of an isopeptide bond between the C-terminal glycine of SUMO and a lysine residue in the substrate, usually together with a specific SUMO E3 ligase, which increases the efficiency of
this reaction by associating with both the substrate protein and Ubc9 (2,15). Specific E3 ligases in mammals include the protein inhibitor of activated STAT-1 (PIAS) protein family (PIAS1, PIAS3, PIASxα, PIASxβ and PIASy), the nucleoporin Ran binding protein 2 and the human polycomb protein Pc2 (2). The tripartite motif family was recently described as a fourth group of SUMO E3 ligases (16).

In the last step, SUMO is removed from the lysine residues of target proteins by SENPs using their isopeptidase activities (10). Ubiquitin-specific protease-like 1 was identified as a new SUMO isopeptidase, with roles in Cajal body functions (17). DeSUMOylating isopeptidase 1 is another SUMO isopeptidase, which recognizes different sets of substrates to SENPs (18).

All SUMOylation proteins are associated with cancer, as elevated levels of SAE, Ubc9, SUMO E3s and SENPs have been observed in various types of cancer (12,14-16). As target proteins of SUMOylation, the Ras-related nuclear protein GTPase-activating protein (RanGAP1), the nuclear factor-κB regulatory inhibitor-α, the tumor suppressor gene p53, the androgen receptor, the progesterone receptor and certain other substrates have been reported (19). The abnormal SUMOylation of these target proteins is also associated with heart disease, diabetes, arthritis, degenerative diseases, and brain ischemia/stroke (6). Thus, there has been growing appreciation for the potential importance of the SUMO conjugation pathway as a target for treating these diseases (20-25).

This review discusses the compounds that inhibit the SUMOylation pathway referred to in patents written in English or Chinese (Table I). These compounds can be divided into four categories according to their mechanisms of action. Category 1 compounds are SUMO mimics that can affect the entire pathway. Category 2 comprises SAE and Ubc9 inhibitors, because all SUMO isoforms are activated and conjugated by SAE and Ubc9. Category 3 compounds are ligand inhibitors because of the specificity of SUMO E3 ligases to target proteins. Category 4 compounds are SENP inhibitors that may inhibit maturation and deSUMOylation.

### 2. Category 1: SUMO mimics

**Multivalent poly-Ubi chain inhibitors (US0302815) (26)**

**Structure.** Multivalent poly-SUMO chain inhibitors comprise gold nanoparticle (AuNP)-ligand conjugates and include at least two components: AuNPs and modified SIM mimics (26). The basic structures (formula 1 and 2) of SIM mimics and potential compound 1 are shown in Fig. 2 (26). The thiol tail \[R_3\text{-SH or SH-(CH}_2\text{)2-CO-NH-}\] allows the modified SIM mimic to be conjugated to an AuNP by disulfide bonds (26).

**Mechanism.** The SIM mimic may recapitulate the action of SUMO in protein modification to inhibit the SUMOylation pathway by: i) Forming thioester conjugates with SAE; ii) being transferred from SAE to Ubc9; and iii) being further transferred to the SUMOylation target protein. Once the mimics are conjugated to SAE and Ubc9, they block full-length SUMO from entering the cascade composed of SAE and Ubc9. Thus, SUMO mimics can function as mechanism-based inhibitors of the protein SUMOylation reaction (27).

### Table I. Summary of compounds included in the patents reviewed.

<table>
<thead>
<tr>
<th>No.</th>
<th>Patent ID</th>
<th>Invention</th>
<th>Mechanism</th>
</tr>
</thead>
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<tr>
<td>2.1</td>
<td>US0302815</td>
<td>AuNP-ligand conjugates</td>
<td>SIM mimic</td>
</tr>
<tr>
<td>3.1</td>
<td>EP2402334</td>
<td>MLN4924</td>
<td>AMP mimic</td>
</tr>
<tr>
<td>3.2</td>
<td>WO002994</td>
<td>Heteroaryl compounds</td>
<td>SAE inhibitor</td>
</tr>
<tr>
<td>3.3</td>
<td>US0317101</td>
<td>MLS-0437113 etc.</td>
<td>Singleton SAE/Ubc9 inhibitor</td>
</tr>
<tr>
<td>3.4</td>
<td>WO064897</td>
<td>MLS-0207587 etc.</td>
<td>Tricyclic SAE/Ubc9 inhibitor</td>
</tr>
<tr>
<td>3.5</td>
<td>EP2545935</td>
<td>HLS5 polypeptide</td>
<td>Degenerating Ubc9/PIAS1</td>
</tr>
<tr>
<td>4.1</td>
<td>WO064877</td>
<td>NSC5068 etc.</td>
<td>SENP1, 2 and 7 inhibitor</td>
</tr>
<tr>
<td>4.2</td>
<td>CN104436196</td>
<td>shRNA</td>
<td>SENP1 inhibitor</td>
</tr>
<tr>
<td>4.3</td>
<td>CN103961348</td>
<td>Agents for treating prostate cancer</td>
<td>SENP1 inhibitor</td>
</tr>
<tr>
<td>4.4</td>
<td>CN103877078</td>
<td>Agents for treating breast cancer</td>
<td>SENP2 inhibitor</td>
</tr>
</tbody>
</table>

AuNP, gold nanoparticle; SIM, SUMO-interacting motif; AMP, adenosine monophosphate; SAE, SUMO-activating enzyme; Ubc9, ubiquitin conjugating enzyme E2; HLS5, hemopoietic lineage switch 5; PIAS1, protein inhibitor of activated STAT-1; shRNA, short hairpin RNA; SENP, sentrin-specific protease.
absorb more X-ray energy than soft tissues, thus augmenting the effects of ionizing radiation when delivered to cells (28,29).

**Application.** Multivalent poly-SUMO chain inhibitors can kill cancer cells by increasing radiation sensitivity, while sparing normal cells (26). Thus, these inhibitors provide a viable approach for treating cancer by enhancing the effects of ionizing radiation therapy, which is commonly used in cancer treatment. Combining the properties of nanomaterials with nanoparticles as platforms for multivalent interactions creates important potential therapeutic applications (30).

### 3. Category 2: SUMO enzyme inhibitors

**Activating E1 inhibitors (EP2402334)** (31)

**Structure.** There are 86 compounds disclosed in EP2402334, of which most are 4-substituted [(1S,2S,4R)-2-hydroxy-4-{7H-pyrrolo(2,3-d)pyrimidin-7-yl}cyclopentyl]methyl sulfamates (formula 3; Fig. 3) (31).

**Mechanism.** The E1 inhibitors described herein are selective and preferably target human NEDD8-activating enzyme (NAE), SAE, or ubiquitin-activating enzyme, with the most preference for NAE (31). The disclosed SAE inhibitors can also be referred to as AMP mimics; they directly bind to SAE1 and inhibit formation of the SAE-SUMO intermediate. MLS 4924 (compound 3; Fig. 3), an AMP mimic, is a selective and highly potent inhibitor of NAE, and also inhibits SAE with a half maximal inhibitory concentration (IC50) value of 8.2 (32).

**Application.** SAE inhibitors are useful for treating disorders, particularly cell proliferation disorders, including cancer (not limited to solid tumors and hematological tumors), inflammatory and neurodegenerative disorders, and inflammation associated with infection and cachexia (31).

**Heteroaryl compounds (WO002994)** (33)

**Structure.** The basic structure (formula 4) of heteroaryl compounds is shown in Fig. 4. The -Y- is often replaced by -O-, -X1- is often replaced by -N-, -X2- is often replaced by -N- or -CH-, Ra can be -OH or -F or -H, and Rc is often replaced by -H. I-1 (compound 4; Fig. 4) and I-5 (compound 5; Fig. 4) are two examples of these heteroaryl compounds (33).

**Mechanism.** These heteroaryl compounds may be useful as SAE inhibitors (33).

**Application.** Heteroaryl compounds can be used for the treatment of proliferative, inflammatory, cardiovascular and neurodegenerative disorders (33). The altered expression of proteins in the SAE pathway have been noted in a variety of cancer types including multiple myeloma (MM) (34) and breast cancer (21). In addition, preclinical studies have indicated that Myc-driven cancers may be especially sensitive to SAE inhibition (35).

**Singleton inhibitors [US0317101 (36) and WO064898 (37)]**

**Structure.** The structure (formula 5) of singleton inhibitors is presented in Fig. 5 (36,37).

**Mechanism.** Singleton inhibitors inhibit the function of SAE and/or Ubc9. MLS-0437113 (compound 5; Fig. 4) is the lead and most potent compound in this series, and strongly inhibits SUMO-RanGap1 and Ubc9 conjugation (36,37). MLS-0417120 (compound 6; Fig. 4) is another potential inhibitor (36,37). There are 16 SAE-specific inhibitors in these two patents. MLS-0437317 (compound 7; Fig. 5) is a representative SAE-specific inhibitor that can bind to SAE with high affinity and specificity, and inhibits global SUMOylation in a dose-dependent manner (36,37).

**Application.** Singleton inhibitors have toxicity in cancer and other diseases by sensitizing cells to genotoxic treatments. Chemoradiotherapy (CRT) is frequently used as a preoperative treatment for colorectal cancer to facilitate surgical
intervention and improve long-term survival (38,39). Novel SAE inhibitors can enhance the effects of CRT and/or impair tumor viability, improving treatment outcomes, preserving quality of life and reducing healthcare costs (37). Such inhibitors may be similarly useful in other cancer types (16), diseases, and conditions associated with the overexpression of SAE or Ubc9 (36,37).

**Bicyclic and tricyclic inhibitors [WO064897 (40), US0245032 (41) and US9045483 (42)]**

**Structure.** The basic structure in these three patents is formula 6 (Fig. 6). Most tricyclic inhibitors disclosed herein are derived from formulas 7, 8 and 9 (Fig. 6). There are 13 tricyclic SAE inhibitors disclosed in these patents. Compound 8 (Fig. 5) is similar to MLS-0207587 (compound 9; Fig. 6), which also showed an IC50 of 0.5 µM (40-42).

**Mechanism.** The tricyclic inhibitors competitively inhibit ATP binding, sensitizing cells to genotoxic stress and inhibiting HIV infection. Some compounds also inhibit Ubc9 (40-42).

**Application.** These inhibitors can be used to treat cancer, degenerative diseases, and viral infections (e.g. HIV). The characterized tricyclic SAE inhibitors may also induce significant sensitivity to radiation in various cancer cell lines (40-42).

**Hemopoietic lineage switch 5 polypeptide [EP2545935 (43) and US0330738 (44)]**

**Structure.** Hemopoietic lineage switch 5 (HLS5) is a member of the RING finger B-box coiled-coil protein family. The gene is located on chromosome 8p21, a region implicated in numerous leukemias and solid tumors (45). The overexpression of HLS5 in HeLa cells inhibited cell growth, clonogenicity, and tumorigenicity; thus, it is conceivable that HLS5 is a tumor suppressor protein (46).

**Mechanism.** HLS5 binds SUMO-1 and is SUMOylated in vivo. It also binds Ubc9 and PIAS1, and has a global effect on SUMOylation by causing the degradation of PIAS1 and Ubc9 through its coiled-coil domain (43,44). In addition, HLS5 also increases the SUMOylation of some proteins. It is possible that the effects of HLS5 are concentration-dependent, in that it may promote greater cell death at higher concentrations (47).

Application. Ligation inhibitors can be used to treat diseases such as Parkinson's disease, diabetes, Huntington's disease, familial neuronal intranuclear inclusion disease, Alzheimer's disease, neuronal intranuclear inclusion disease, cancer (48‑52), polyglutamine disease and human immunodeficiency virus (HIV) infection (43,44).

4. Category 3: SENP inhibitors

Inhibitors of deSUMOylation enzymes [WO064887 (53) and US0302525 (54)]

Structure. Compounds 10-11 in Fig. 7 are derived from formula 10 and compounds 12-13 are derived from formula 11 (53,54). They inhibit SENPs and also inhibit HIV infection (55).

Mechanism. SENPs share a conserved catalytic domain that is a cysteine protease of ~225 amino acids (11). Nuclear magnetic resonance and enzyme kinetics data indicate that SENP inhibitors bind in the vicinity of the catalytic center and inhibit the enzyme by a mixed inhibitory mechanism (56). The catalytic domain crystal structures of SENP1, SENP2 and SENP7 are very similar (10). As a result, the inhibitors disclosed herein mostly inhibit SENP1, SENP2 and SENP7.

Application. The SENP inhibitors may sensitize target cells/cancer cells to DNA‑damaging therapy, and then minimize or eliminate harm to healthy cells at reduced doses. Inhibitors of deSUMOylation significantly reduce viral infectivity, and limit maintenance of the latent HIV reservoir, which may be valuable to future HIV eradication strategies (57). The SENP inhibitors described herein may also be used to treat cardiovascular disease, neurodegenerative diseases and diabetes in a preventative manner (53,54).

An SENP1 inhibitor: small hairpin RNA [shRNA; CN 104436196 (58)]

Structure. The sequence of the shRNA described herein is 5'-CCGGCGCCAGAUUGAAGAACAGACUCGAGUUCGUGUCUUAUCGGCUUUUU-3' (SEQ ID no:1) (58).

Mechanism. The SENP1 inhibitor described herein is an shRNA, including the shRNA recombinant vector and host cells containing the recombinant vector (58). As a precursor of small interfering RNA, shRNA can inhibit expression of the SENP1 protein via the RNA interference mechanism, leading to inhibition of cell proliferation and induction of apoptosis of malignant tumor cells (58).
Application. The SENP1 inhibitors described herein can be used for the preparation of certain special agents that can prevent or treat malignant hematological tumors, and those that inhibit the proliferation of malignant hematological tumor cells and/or promote apoptosis in malignant hematological tumor cells (58).

SENP1 inhibitors [CN 103961348 (59)]

Structure. These SENP1 inhibitors were derived from formula 12 and 13 in Fig. 8 (59), of which the representatives are 2-(4-chlorophenyl)-2-oxoethyl 4-benzamidobenzoate derivatives (60).

Mechanism. The compounds in this patent inhibited the activity of SENP1 in vitro and certain cloned prostate cancer strains (59). Compounds 14 and 15 (Fig. 8) have been reported to have IC50 values of 2.38 and 1.08 µM, respectively (10).

Application. SENP1 affects the function of hypoxia-inducible factor 1α in tumor angiogenesis, thus promoting the development of various tumors (61). The SENP1 inhibitors described herein can inhibit the growth of prostate cancer cells stimulated by androgen (62-64). Thus, these inhibitors may be useful candidate anticancer drugs.

SENP 2 inhibitors [CN 103877078 (65)]
Structure. These inhibitors comprise SENP2 inhibitors containing formula 14 (Fig. 9) as an active ingredient, including its pharmacological derivatives or acceptable salts. This patent has 864 compounds derived from formula 14, differing in Z, Y, R, R1 and R2, compounds 16 and 17 are used here as two examples (65).

Mechanism. The SENP2 inhibitors described have high efficiency and low toxicity, and significantly inhibit SENP2 activity in vitro (65).

Application. The inhibitors of SENP2 described herein can be used to treat breast cancer. By removing the SUMO modification of Pc2 in the protein regulator of cytokinesis 1 protein, the regulation of polycomb group target gene activity is achieved. SENP2 is involved in the regulation of cell differentiation and development, and is highly expressed in a large number of patients with breast cancer (65). Thus, SENP2 inhibitors may be novel drug candidates for anticancer therapy.

5. Discussion

The proteasome inhibitors, bortezomib and carfizomib, which target the ubiquitin pathway, have been used for the treatment of MM and hematologic malignancies (66). The second-generation proteasome inhibitor, carfizomib, induces responses in a minority of patients with MM that relapsed from or were refractory to bortezomib treatment, and experienced dose-limiting peripheral neuropathy. However, carfizomib still results in some adverse reactions that are commonly shared with other antineoplastic agents. To identify more effective agents with less toxicity, inhibitors targeting the SUMOylation pathway have been investigated.

This review summarizes compounds inhibiting the SUMOylation pathway that were described in patents written in English or Chinese between 2012 and 2015. Among these compounds, SAE and SENP1/2 inhibitors appear to have the greatest therapeutic potential, as once protein maturation or activation is blocked, downstream biological events will be inhibited. However, SAE, Ubc9 and SENP1/2 inhibitors do not have target selectivity, whereas SUMO E3 ligases inhibitors may be more selective with fewer side effects.

Research studies should not just focus on SUMO inhibitors, but should also take SUMO activators, such as cysteine protease polypeptides, into consideration (67-70). These proteins function as SENPs, with the ability to cleave SUMO from a target protein and/or cleave the precursor form of SUMO to release its active form. These SENP analogs (cysteine protease polypeptides) may be useful in the treatment of various cancers and some other diseases related to cysteine family members (71). PIAS1 is a breast cancer suppressor protein (72). An artificial SUMO ligase reportedly promotes the process of ligation (73).

The application of SUMO inhibitors for treating cancer is of utmost importance. Thus, additional studies should focus on identifying the roles of SUMO enzymes in different cancer
types. In addition, an understanding of the basic biology and specificity/selectivity of SUMO inhibitors is required before they can be identified and developed for use in the clinic. Although, to date, no SUMO inhibitors have been tested in humans, and further experiments are required in cells and animals, compounds targeting the SUMOylation pathway may represent a new direction for the treatment of cancer and other diseases.

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