

REVIEW

SCR7, a potent cancer therapeutic agent and a biochemical inhibitor of nonhomologous DNA end-joining

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Abstract

Background: DNA double-strand breaks (DSBs) are harmful to the cell as it could lead to genomic instability and cell death when left unrepaired. Homologous recombination and nonhomologous end-joining (NHEJ) are two major DSB repair pathways, responsible for ensuring genome integrity in mammals. There have been multiple efforts using small molecule inhibitors to target these DNA repair pathways in cancers. SCR7 is a very well-studied anticancer molecule that blocks NHEJ by targeting one of the critical enzymes, Ligase IV.

Recent findings: In this review, we have highlighted the anticancer effects of SCR7 as a single agent and in combination with other chemotherapeutic agents and radiation. SCR7 blocked NHEJ effectively both in vitro and ex vivo. SCR7 has been used for biochemical studies like chromosomal territory resetting and in understanding the role of repair proteins in cell cycle phases. Various forms of SCR7 and its derivatives are discussed. SCR7 is also used as a potent biochemical inhibitor of NHEJ, which has found its application in improving genome editing using a CRISPR-Cas system.

Conclusion: SCR7 is a potent NHEJ inhibitor with unique properties and wide applications as an anticancer agent. Most importantly, SCR7 has become a handy aid for improving genome editing across different model systems.

KEYWORDS

chemotherapy, DNA double-strand break, DNA repair inhibitors, genome editing, homologous recombination, nonhomologous DNA end-joining

1 | INTRODUCTION

DNA repair pathways play a significant role in maintaining genome integrity. Defects in the DNA repair pathways inside cells can predispose them to cancer.^{1,2} DNA repair proteins, when over-expressed in cancers, promote their growth and provide resistance to therapy.^{3,4} One of the cancer therapeutic modalities involves targeting the repair of DNA double-strand breaks (DSBs), resulting in the inhibition of the repair machinery. This results in the

accumulation of DSBs and cell death.^{5,6} Among different DNA damages, DSBs are considered as most deleterious as a failure of their repair results in cell death.

Three different DNA repair pathways homologous recombination (HR), nonhomologous end-joining (NHEJ), and alternative NHEJ, are responsible for the repair of DNA DSBs.⁷⁻⁹ HR is known to operate during the S-phase of the cell cycle, whereas NHEJ is functional throughout the cell cycle. HR utilizes the information from a template to mend the breaks and is precise without errors.¹⁰⁻¹² NHEJ is an

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error-prone pathway involving insertion or deletion at the site of repair as it involves a template-independent mode of joining. When there is a DSB, the KU proteins (KU70/KU80) localize to the site of the break to protect them from degradation. KU70/80 then recruit other proteins like Artemis and DNA-PKcs, which are endonucleases and bring about end resection.¹²⁻¹⁵ PAXX, another protein recently been known to stabilize the repair machinery.¹⁶ Pol μ and Pol λ , belonging to the Pol X family, fill the gap, thereby carrying out the polymerization at the repair site. The final step of NHEJ being ligation involves DNA ligase IV/XRCC4/XLF complex to seal the ends and repair the broken DNA^{11,17} (Figure 1). Another pathway known as alternative NHEJ (A-NHEJ), which is a back-up pathway to classical NHEJ (c-NHEJ) is present in cells and use a short terminal microhomology region for sealing the DNA break.^{9,12,14,18}

Several small-molecule inhibitors targeting DSB repair pathways have been developed.^{2,19} One such inhibitor is SCR7, which interferes with the sealing of the nick, which is the last step of the NHEJ.^{6,12,20} Ligase IV, the enzyme dedicated to the final ligation of broken ends, is one of the most critical proteins associated with NHEJ. SCR7 binds to the DNA binding domain of Ligase IV to block NHEJ and leave the ends unrepaired, resulting in the accumulation of DNA breaks and thus leading to activation of cell death pathways.²⁰ The activity of SCR7 has been demonstrated both in vitro in several cancer cell lines and in vivo in multiple animal tumor models.²⁰

SCR7 was first reported in 2012 and has made an impact across different fields.^{12,19} SCR7 has been used in biochemical studies and, most importantly, to improve genome editing using CRISPR-Cas

technology.^{6,12} The recent advances in genome engineering technology are due in part to the use of small-molecule inhibitors like SCR7, which has enhanced the efficiency of genome editing. However, in this short review, we discuss the evolution of SCR7 as a cancer therapeutic agent and its other applications.

2 | THERAPEUTIC ROLE OF SCR7

Cancer cells alter DNA repair protein expression to gain a survival advantage.²¹ Harnessing the defects in DNA repair genes and utilizing differential expression of repair proteins in cancer cells compared to normal cells can be a great strategy for inhibiting the progression of cancer.^{22,23} One such example that has given successful results is the FDA-approved PARP inhibitor Olaparib that can bring about synthetic lethality in breast and ovarian cancers that are deficient in BRCA1/2.^{24,25} ATM inhibitor KU-55933 has shown promising results in Fanconi anemia deficient pancreatic cancer cell line.²⁶

NHEJ is a rapid and quick fixing DNA repair process that takes care of 70% DSBs within the cells particularly operating when homology is absent.^{8,11} When NHEJ is inhibited at the initial steps of damage recognition or end resection, there is a chance of them being rerouted to the back-up alternative NHEJ pathway.^{9,18,27} Cancer cells escape cell death signals as they can shift from one repair pathway to the other efficiently. Therefore, blocking a protein like Ligase IV at the final step of the repair process does not allow for shift easily and lead to efficient cell death signaling.²

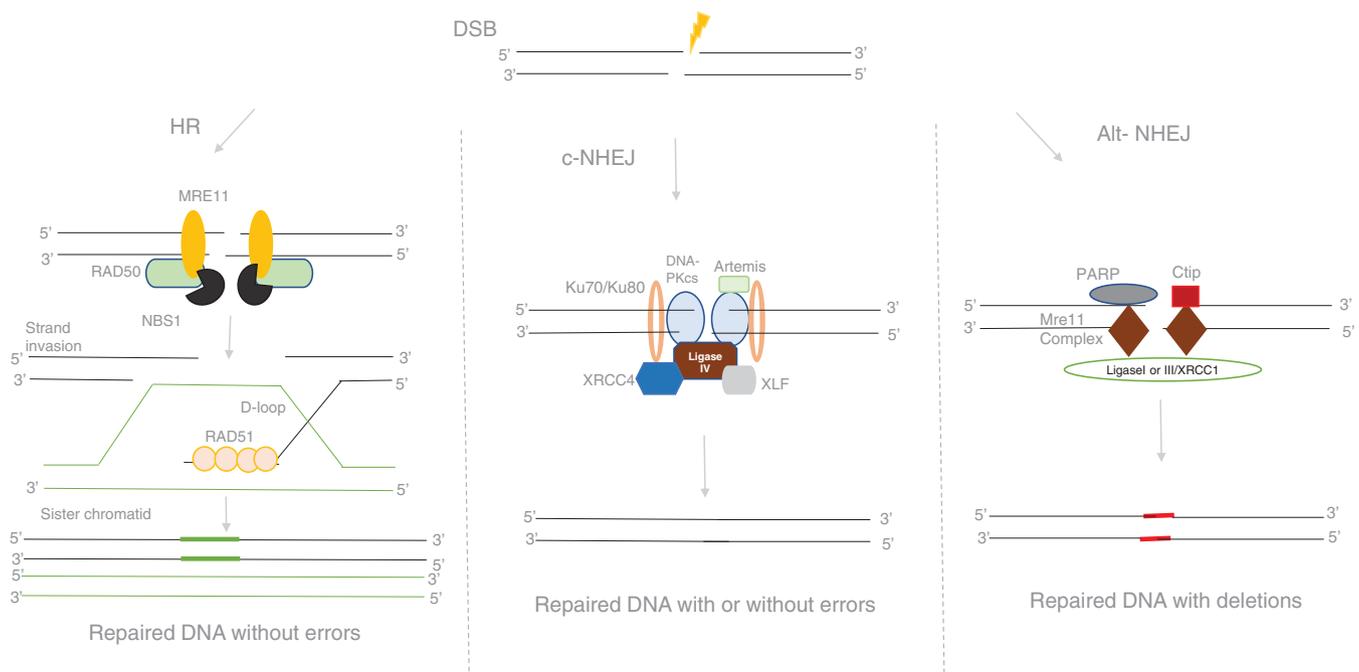


FIGURE 1 Schematic representation of DSB repair pathways that operates in a mammalian cell. There are mainly three pathways to take care of the repair of DSBs in mammalian cells; homologous recombination (HR), classical nonhomologous end-joining (c-NHEJ), and alternative NHEJ (A-NHEJ). As shown in the figure, each pathway has its own repertoire of proteins, which aids in repairing the broken DNA. HR repairs DNA breaks based on the homology and occurs at the replicative phase of the cell cycle and is error-free. c-NHEJ occurs throughout the cell cycle and is error-prone. Ligation of broken ends is mediated by Ligase IV/XRCC4/XLF complex. Alternative NHEJ is also error-prone, leading to extensive deletions, and repair occurs based on the microhomology regions

Chen et al developed L189, a pan-ligase inhibitor, which inhibits all three ligases, Ligase IV, III, and I. However, this molecule was not extensively used as anticancer therapeutics.²⁸

The most effective and widely studied DNA Ligase IV/XRCC4 inhibitor is SCR7.¹² Chemically, SCR7 is 5,6-bis(benzylidene amino)-2-mercapto-pyrimidin-4-ol that binds to the DNA-binding domain of Ligase IV and exerts its action. In the cell-free repair assays, SCR7 showed concentration-dependent inhibition of NHEJ.²⁰ The sensitivity of cancer cell lines to SCR7 correlated with the expression levels of Ligase IV. Knockdown studies elucidated the specificity of SCR7.²⁰ Western blotting analysis of MCF7 cells treated with SCR7 showed a decrease in anti-apoptotic proteins that coincided with an increase in pro-apoptotic proteins. Induction of the intrinsic pathway of apoptosis in mouse tumors was evident in the form of DNA fragmentation using TUNEL assay. In three different mouse models including xenografts, significant tumor regression was observed even leading to an increase in lifespan²⁰ (Figure 2).

The significant effect of SCR7 that is of additional interest is that it sensitizes the cancer cells to chemo and radiotherapeutic agents when given in combination.²⁰ Studies by Gkatzamanidou et al²⁹ revealed that in patients with multiple myeloma, combination therapy involving DNA repair (SCR7, NU7026, and RS-1) inhibitors along with an alkylating agent, melphalan showed better cytotoxicity.²⁹ Another chemotherapeutic drug, doxorubicin, when administered along with SCR7, showed a 15% to 50% higher cytotoxicity in cervical cancer.³⁰ Gopalakrishnan et al³¹ showed that SCR7 enhances ionizing radiation's effects in one of the most common types of non-Hodgkin lymphoma, diffuse large B cell lymphoma.³¹ Further, inhibition of the NHEJ pathway by SCR7 overcomes HSP110 conferred resistance to oxaliplatin in the SW480 colorectal adenocarcinoma cell line.³²

3 | DIFFERENT FORMS OF SCR7

Vartak et al showed that parental SCR7 get autocyclized to cyclized-SCR7 that possess the same molecular weight (334.09), formula ($C_{18}H_{14}N_4OS$), and melting temperature ($221^{\circ}C-225^{\circ}C$).³³ Although parental SCR7 and cyclized version are the same in molecular formula and other properties, SCR7-pyrazine is an entirely different molecule with a molecular weight of 332.07, formula $C_{18}H_{12}N_4OS$, and melting point $194^{\circ}C$ to $196^{\circ}C$. Studies have shown that SCR7-cyclized and SCR7-pyrazine exhibits a Ligase IV-dependent inhibition of NHEJ; however, the latter shows less specificity inside cells (Figure 3). The nanopolymer-encapsulated version of SCR7, E-SCR7, induced not only 5-fold higher cytotoxicity in the cells but also increased its bio-availability.^{34,35} SCR7 and its pyrazine form were further developed as water-soluble versions that regressed tumors in mice.³⁶⁻³⁸ However, water-soluble SCR7 was Ligase IV specific, whereas water-soluble SCR7-pyrazine had pan-ligase activity.^{6,36,37}

One of the drawbacks of SCR7 is its high IC_{50} in cancer cells. Recently, SCR130, a spiro derivative of SCR7, was shown to induce cytotoxicity at $2 \mu M$ in Nalm6 cells, 20 times higher than that of SCR7 ($40 \mu M$) and exerts its effect in Ligase IV dependent manner.³⁹ It induces cytotoxicity by activating both the intrinsic and extrinsic apoptotic pathways. Like SCR7, SCR130 also sensitizes cancer cells to γ -radiation when used in combination.³⁹

4 | SCR7 IS A BIOCHEMICAL INHIBITOR OF NHEJ

Apart from being an excellent anticancer agent, SCR7 has also been known for its biochemical role. From previous studies, it is evident

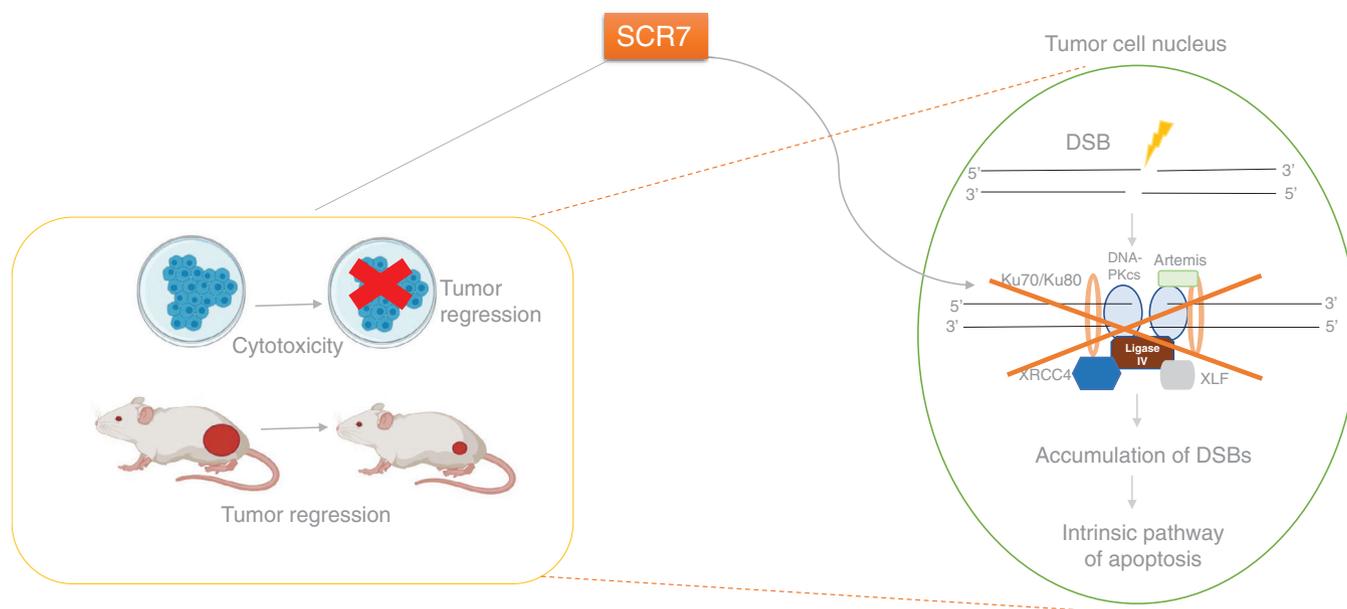


FIGURE 2 Mechanism of SCR7-mediated cell death during tumor regression. SCR7, an inhibitor of NHEJ, is known to induce cytotoxicity in cancer cells leading to tumor regression in mice. Once administered, SCR7 is diffused inside the nucleus and binds to the DNA binding domain of Ligase IV. This prevents the recruitment of Ligase IV to DSBs, resulting in inhibition of NHEJ, thus, culminating in the accumulation of DSBs inside the cells. Finally, activation of the intrinsic apoptotic pathway led to cancer cell death and thus tumor regression

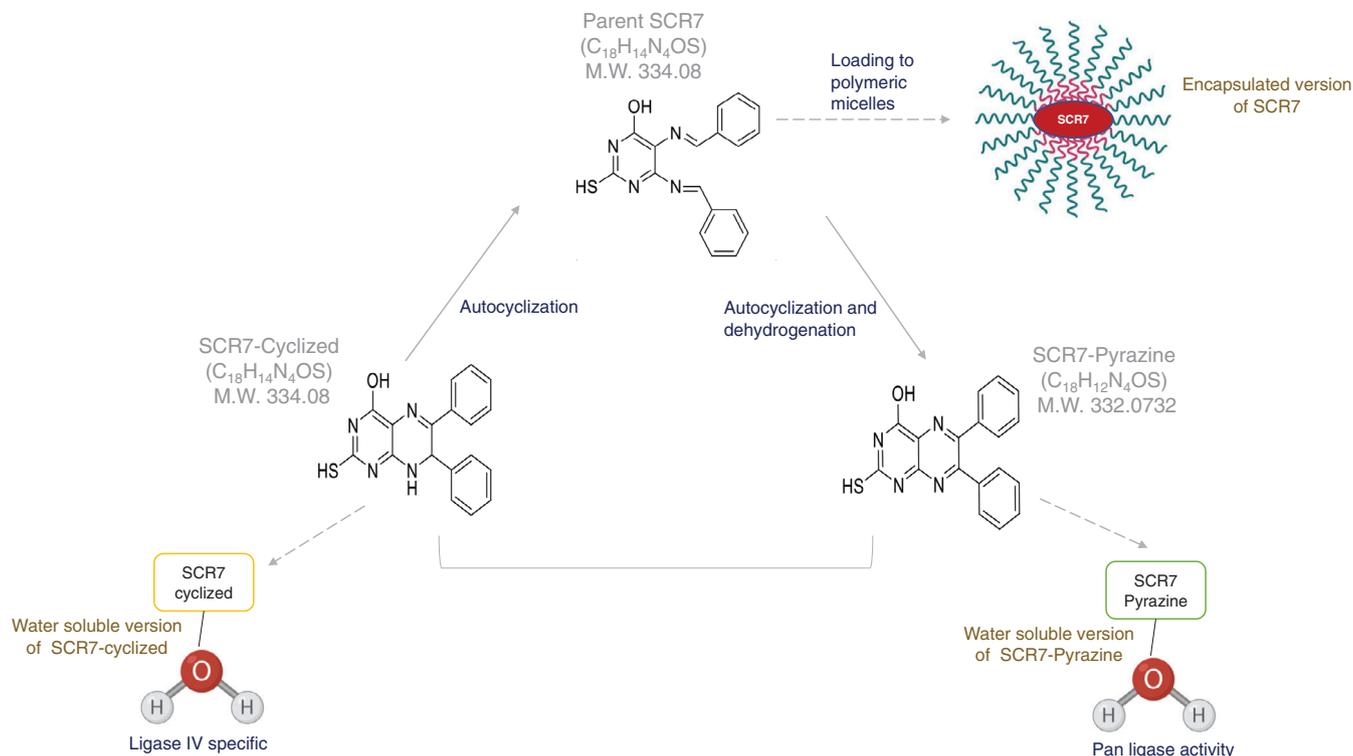


FIGURE 3 Different forms of SCR7. Parental SCR7 gets autocyclized to form a cyclized version of SCR7 having the same molecular weight, formula, and melting temperature as that of parental SCR7. SCR7-pyrazine is the oxidized version of parental SCR7. It is a different entity compared to parental SCR7 with a molecular weight of 332.07 and melting point 194°C–196°C. When parent SCR7 loaded onto polymeric micelles, called encapsulated SCR7, it showed a better bioavailability. The water-soluble version of SCR had ligase IV activity, whereas SCR7-pyrazine had pan ligase activity. Part of the figures made using biorender.com

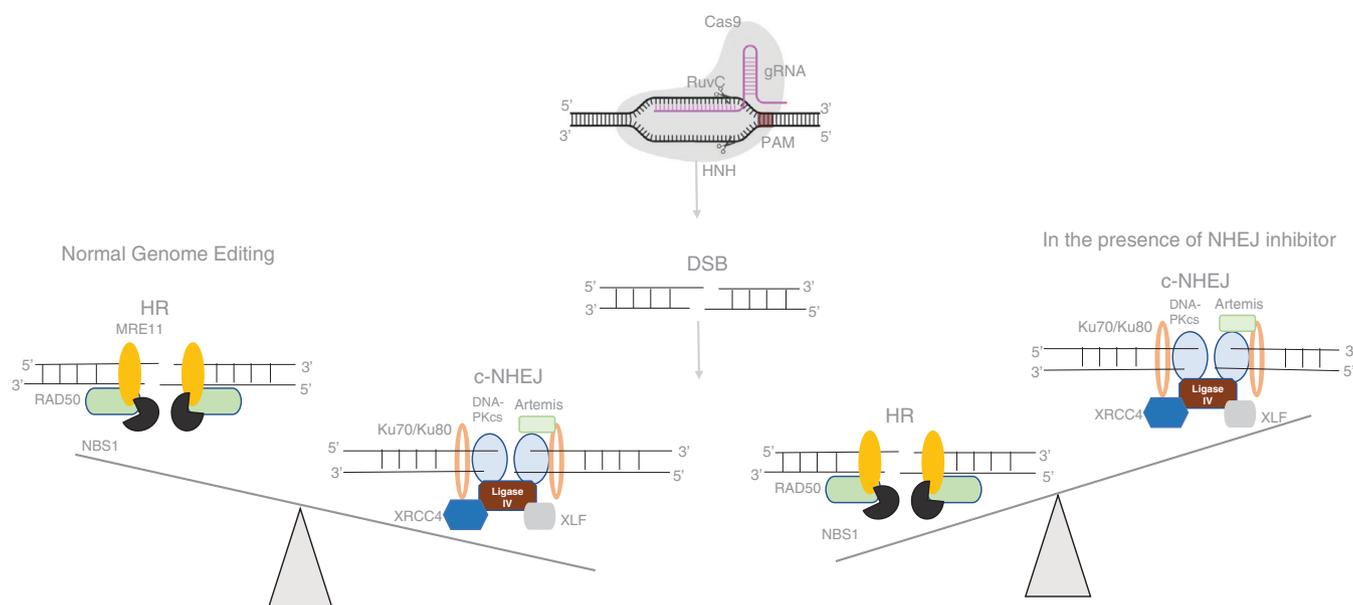


FIGURE 4 SCR7 tilts the balance toward HR during genome editing. Incubation of SCR7 and CRISPR/Cas9 mixture with mammalian cells leads to inhibition of nonhomologous end-joining and thus, the DSB repair occurs primarily only through HR. In the absence of c-NHEJ, the cell is dependent on HDR for mending the broken ends that result in an error-free repair of the target site

that SCR7 can block the ligation of DSBs with either compatible or noncompatible ends.²⁰ SCR7 can inhibit the amalgamation of breaks present in both plasmid and oligomers^{6,12,19,20} [in press]. These

results have given a new dimension to SCR7, focussing on roles other than anticancer activity. To capture the dynamics of NHEJ in the cell, single-molecule FRET along with super-resolution

localization microscopy was used. In this study, 100 μ M of SCR7 was used as a control to elucidate the mechanism and dynamics of NHEJ.^{40,41} Tripathi et al⁴² used SCR7 as a biochemical inhibitor to understand the DNA repair protein's role at different phases of the cell cycle.⁴² SCR7 has also been used to understand the resetting of chromosome territory upon DNA damage.⁴³ The Zebrafish as a model system has been developed to screen for inhibitors of NHEJ, owing to the high frequency of NHEJ in early embryonic development. SCR7 alleviated SV40 fragment-induced embryonic lethality. SCR7-induced disruption in NHEJ established Zebrafish as a model system for screening targets against NHEJ, which might be useful for the development of novel anticancer drugs.⁴⁴

5 | ROLE OF SCR7 IN GENOME EDITING

Another vital role of SCR7 is its use in genome editing.^{6,12,38} In CRISPR/Cas9 mediated genome editing, after the induction of DSBs by Cas9, endogenous DNA repair pathways, HR or NHEJ are activated. NHEJ being error-prone in its action results in gene disruption by generating indels at the target; on the other hand, HR can integrate the donor DNA into the target site.⁴⁵ Efforts have been put in the direction of shifting the balance toward HR during the editing process.⁶ Various studies had shown that the addition of SCR7 showed a 2-19-fold enhancement in the efficiency of genome editing in mammalian cells and mice embryos. Inhibition of NHEJ by SCR7 shifts the mode of repair to HR⁴⁶⁻⁴⁸ (Figure 4). The efficiency of CRISPR/Cas mediated gene editing increased by 2 to 3-folds in porcine fetal fibroblast and cancer cells in the presence of SCR7.^{49,50} Apart from mammalian systems, SCR7 also increased HDR by 40% when transfection studies were performed after cloning genes of interest from *Saccharomyces cerevisiae*.^{51,52} One of the gene inactivation methods involves the integration of markers (resistance cassette) for productive editing.⁵³ showed that SCR7 increased the efficacy of productive (integration) editing. Similarly, enhanced knock-in efficiency using CRISPR/Cas in the presence of SCR7-pyrazine in *Xenopus* oocyte has been reported,⁵⁴ suggesting the use of SCR7 in inhibiting NHEJ independent of the model system and gene editing mechanisms using CRISPR/Cas.

6 | CONCLUSION

It has become evident that inhibiting DNA DSB repair pathways has a broad range of applications. Among that, NHEJ inhibitor SCR7 and its novel derivative SCR130 are the most potent to date as they block NHEJ efficiently both in vitro and ex vivo. Not only as a monotherapy, in combination with radiation but SCR7 has also shown promising results. Both autocyclized and oxidized forms of SCR7 inhibit NHEJ efficiently, although the oxidized pyrazine version is less efficient inside the cells. The broad spectrum of SCR7 utility is evident in the form of cancer therapeutics to its use as a biochemical inhibitor. Besides, SCR7 is also an attractive

tool for improving genome editing using CRISPR/Cas across model systems.

7 | FUTURE DIRECTIONS

The use of DNA repair inhibitors, combined with DNA-damaging drugs, has revealed its therapeutic importance both in laboratories and clinical trials. Olaparib is one of the successful examples of DNA repair, which is currently used in clinics. NHEJ inhibitor, SCR7 in combination with chemotherapeutic agents and radiation, has been tested in preclinical settings; however, more studies are required to assess its clinical relevance. Although SCR130 showed improved IC₅₀ and NHEJ inhibition, its relevance in vivo tumor regression is yet to be seen. More potent derivatives of SCR7 need to be explored in combination with chemotherapy to achieve efficient tumor regression at lower doses. Besides, to classify cancers that would respond to Ligase IV inhibitors based on the expression of Ligase IV is essential for its use as therapeutics. Patients with low levels of ligase are generally sensitive to radiation. Cataloging of patients based on polymorphisms and Ligase IV expression can be used as a standard in deciding the therapeutic regimen using NHEJ inhibitors. Also, it would be interesting to see how new SCR7 derivatives contribute toward biochemical inhibition of NHEJ and their impact on improving CRISPR/Cas mediated gene editing, the future of cancer therapeutics as well as other genetic diseases.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS CONTRIBUTION

All authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Conceptualization*, B.C., S.C.R.; *Writing - Original Draft*, M.M.; *Writing - Review & Editing*, B.C., S.C.R.; *Supervision*, B.C., S.C.R.

ETHICAL STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

Data sharing does not apply to this article as no new data were created or analyzed in this study.

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