Human DNA Ligases: A Comprehensive New Look for Cancer Therapy

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Published online 19 August 2013 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/med.21298

Abstract: Living organisms belonging to all three domains of life, viz., eubacteria, archaeabacteria, and eukaryotes encode one or more DNA ligases. DNA ligases are indispensable in various DNA repair and replication processes and a deficiency or an inhibition of their activity can lead to accumulation of DNA damage and strand breaks. DNA damage, specially strand breaks at unsustainable levels can lead to replication block and/or cell death. DNA ligases as potential anticancer targets have been realized only recently. There is enough rationale to suggest that ligases have a tremendous potential for novel therapeutics including anticancer and antibacterial therapy, specially when the world is facing acute problems of drug resistance and chemotherapy failure, with an immediate need for new therapeutic targets. Here, we review the current state of the art in the development of human ligase inhibitors, their structures, molecular mechanisms, physiological effects, and their potential in future cancer therapy. Citing examples, we focus on strategies for improving the activity and specificity of existing and novel inhibitors by using structure-based rational approaches. In the end, we describe potential new sites on the ligase I protein that can be targeted for the development of novel inhibitors. This is the first comprehensive review to compile all known human ligase inhibitors and to provide a rationale for the further development of ligase inhibitors for cancer therapy. Med. Res. Rev., 34, No. 3, 567–595, 2014

Key words: DNA ligase; ligase inhibitor; cancer target; cancer drug; ligase; inhibitor

1. INTRODUCTION

DNA ligases were discovered in 1967 by the Gellert, Lehman, Richardson, and Hurwitz laboratories.[1] DNA ligases join adjacent 3′-hydroxyl and 5′-phosphoryl termini to form a phosphodiester bond in duplex DNA and thus have an indispensable role in DNA replication,

Contract grant sponsor: Government of India; *Contract grant numbers*: SB/FT/LS-163/2012, BT/PR6421/GBD/27/436/2012), BSC0106.

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recombination, and repair. DNA ligases are grouped into two families, ATP-dependent ligases and NAD^+-dependent ligases, according to the cofactor required for ligase–adenylate formation. The bacterial NAD^+-dependent DNA ligases belong to a highly conserved phylogenetic cluster of enzymes and have been described only in eubacteria. In contrast, the ATP-dependent DNA ligases are found in all three domains of life, viz., eubacteria, archaeabacteria, and eukaryotes and form a somewhat loosely defined cluster of enzymes.[2,3] In this review, we will restrict ourselves to human ATP-dependent DNA ligases only and unless otherwise specified, the word ligase will stand for human DNA ligase. The reaction mechanism for ATP-dependent ligases involves the formation of a covalent enzyme–AMP intermediate from the cleavage of ATP to AMP and pyrophosphate. The adenylate group from AMP is then transferred to the 5′-phosphate of the nicked DNA molecule. Finally, the DNA ligase seals the gap by phosphodiester bond formation, via the displacement of the AMP residue with the 3′-hydroxyl group from the adjacent DNA strand.

DNA ligases have an indispensable role in replication. During replication, the two antiparallel strands of DNA are copied distinctively. One strand is copied in a continuous manner (and called the leading strand) whereas the other strand, due to its opposite orientation, is copied discontinuously in small stretches called Okazaki fragments, after its discoverer Reiji Okazaki. These fragments then have to be sealed together to make a continuous strand of DNA called the lagging strand. This important function during replication is performed by DNA ligase I (hLigI). Other important DNA repair processes such as nucleotide excision repair (NER) (by hLigI and hLigIII),[4] base excision repair (BER) (by hLigI and hLigIII),[5,6] and double-strand break repair (DSBR) pathways such as nonhomologous end joining (NHEJ) (by hLigIV)[7] and microhomology-mediated end joining (by hLigI and hLigIII)[8] are also dependent on ligases. A deficiency in DNA ligation leads to severe cell lethality, increased genomic instability, and hypersensitivity to DNA damage. It has been reported that an individual with an inherited mutation in the hLigI gene exhibited retarded growth, development, and immunodeficiency.[9] LigIV deficiency (known as LIG4 syndrome) is characterized by microcephaly and immunodeficiency and is common to severe combined immunodeficiency with microcephaly, growth retardation, and sensitivity to ionizing radiation due to NHEJ1 deficiency (NHEJ1 syndrome).[10] No patients with ligase III deficiency have been identified till date.

Inhibiting the function of ligases can have potentially serious consequences for DNA replication and repair events, processes that are most often targeted in cancer therapy. Several inhibitors against human DNA ligases have been discovered or synthesized, but not a single inhibitor is presently used in therapy. In the sections below, we begin with a brief introduction to the working of ligases and then undertake a comprehensive review of all classes of human DNA ligase inhibitors that are known till now. The inhibitors have been reviewed for their mode of inhibition and target specificity and their potential for use in future cancer therapy. In the end we present potential new sites on the human ligase I protein that could be targeted for development of specific inhibitors.

2. A DESCRIPTION OF HUMAN DNA LIGASES

A. Human DNA Ligase I (hLigI)

The human gene encoding DNA ligase I is located at chromosome 19q13.2-13.3. The gene covers 53 kb and contains 28 exons.[11] DNA ligase I is required for the ligation of Okazaki fragments during lagging strand DNA synthesis as well as several DNA repair pathways. Elevated levels of DNA ligase I have been found in several cancer cells such as breast, lung, and ovarian cancer cells.[12–14] In 2001, Sun et al.[14] showed that there are higher levels of DNA

ligase I in human tumors than in benign normal tissues and normal peripheral lymphocytes, suggesting that DNA ligase I plays more of a role in proliferating cells than in resting cells. Unexpectedly for a protein involved in replication, an individual with an inherited defect in DNA ligase I has been identified.[15] The individual exhibited severe immunodeficiency, stunted growth, and sun sensitivity and died at a young age from an abdominal lymphoma with an associated acute infection suggesting immunodeficiency.[16] A fibroblast cell strain, 46BR was derived from the individual and showed deleterious mutations in both alleles of the DNA ligase I gene. One allele carries a mutation that inactivates the active-site region, whereas the other has a mutation in a conserved part of the carboxy-terminal region that allows a small amount of residual DNA ligase activity. The leakiness of the latter point mutation suggests that even low levels of DNA replication enzymes may be sufficient for cellular viability.[17]

An available SV40-transformed 46BR.lG1 subline carries mutations in either one allele (hemizygous mutation) or both alleles (homozygous mutations) of hLigI, but in either case a leaky expression of hLigI is enough to keep the cells viable. 46BR cells show inefficient joining of Okazaki fragments and anomalous gap filling during excision repair.[17] However, no problems with V(D)J joining is observed in 46BR cells.[18] The 46BR patient was sun sensitive, and since the late steps of lagging-strand DNA replication and NER share many factors,[19] it seems plausible that DNA ligase I is responsible for the final DNA-joining step in NER. hLigI is also involved in UV damaged proliferating cells having UV photolesions at late G1 and S phase of cell cycle,[4] long patch BER,[5,6] and microhomology-mediated end-joining pathway (DSBR pathway).[8] Thus, inhibition of ligase I activity can potentially block replication as well as introduce mutations due to failure in DNA repair pathways. The high levels of hLigIp in several cancer cells raises the probability that it may serve as a good target for cancer therapy. Accumulation of single-strand (ss) breaks followed by double-strand (ds) breaks in subsequent cycles of replication is the hallmark of cells deficient in hLigI protein. Several inhibitors against hLigI have been reported but there is a need for the development of more potent-specific inhibitors. These are discussed in the later sections of this review.

B. DNA Ligase III (hLigIII)

The human gene encoding DNA ligase III (hLigIII) is located at chromosome 17q11.2-q12. DNA ligase III encodes multiple forms of DNA ligase. Nuclear and mitochondrial forms of human DNA ligase IIIα are generated by alternative translations of the same gene.[20] Additionally, a germ cell specific form of human DNA ligase IIIβ is generated by alternative splicing.[9] Cell lines with reduced activity for one of the DNA ligases show different phenotypes suggesting that these enzymes have distinct cellular functions.[15,21–23] The nuclear form of DNA ligase IIIα is partnered with another protein called XRCC1 and is unstable in its absence.[24] In the germ cell specific form of DNA ligase IIIβ, a small positively charged peptide sequence replaces the C-terminal BRCT domain of DNA ligase IIIα.[9] hLigIII is restricted to higher eukaryotes and has been associated with short patch BER and NER pathway in quiescent as well as proliferating cells at all phases of cell cycle.[17–19] DNA ds breaks pose a great danger to the genome and cell survival, and must be repaired. In higher eukaryotes, such damage is repaired efficiently by NHEJ in which hLigIV has been implicated. The alternative NHEJ pathway is implicated in the generation of large deletions and chromosomal translocations that are frequently observed in cancer cells. A recent study has shown that in DNA ligase IV deficient cells, the MRN complex (hMRE11/hRAD50/Nbs1) interacts with LigIIIα/XRCC1 upon DNA damage, and stimulates intermolecular ligation via a reaction that mimics alt-NHEJ.[25] Thus, LigIIIα may be a target for therapeutic use in hLigIV-deficient cancers.

Another recent study has shown functional redundancy between DNA ligase I and III in vertebrate cells.[26] Using conditional knockdown of hLigIII along with inactivation of hLigI

and hLigIV genes, they demonstrated in DT40 (white leghorn chicken bursal lymphoma) cells that hLigIII can support semiconservative DNA replication. These observations suggest an alternative pathway for Okazaki fragment processing. To us this observation suggests that an inhibitor with activity against both hLigI and hLigIII will be more effective than an inhibitor with activity against hLigI alone. However, such a molecule will have far greater side effects for normal cells as compared to specific inhibitors for individual ligases. Interestingly, another recent study suggests that in cells with dysfunctional hLigI, hLigIII can contribute to the ligation of the replication intermediates but not to the prevention of telomeric instability.[27] Therefore, specific inhibitors against hLigI might still be effective in blocking cellular proliferation in cells with functional hLigIII. hLigIII is majorly responsible for NER and BER (SSBR) pathways, and in certain situations for alternative NHEJ and MHEJ (microhomology-mediated end joining) DSBR pathways.[8,17,19,20,26,28,29] Thus, the inhibition of hLigIII will almost certainly have an adverse affect on DNA repair pathways as well as replication. Inhibitors against hLigIII are discussed in detail later. However, specific inhibitors against hLigIII are yet to be reported.

C. DNA Ligase IV (hLigIV)

hLigIV is the most recently discovered human DNA ligase. It is a 911 amino acid polypeptide that plays a role in the repair of ds DNA breaks. hLigIV is exclusively nuclear and functions in DNA NHEJ processes,[30–33] and may also be responsible for the V(D)J recombination in lymphoid cells.[22] Similar to the nuclear form of LigIIIα, hLigIV is unstable without its partner protein called XRCC4. Late embryonic lethality and impaired V(D)J recombination was reported in mice lacking DNA ligase IV.[34] In yeast, DNA ligase IV was reported to mediate nonhomologous DNA end joining.[7] According to another report by Grawunder et al.,[35] the function of the XRCC4–DNA ligase IV complex in mammals may be to carry out the final steps of V(D)J recombination and joining of DNA ends. In 1998, Deborah E. Barnes et al.[36] has reported that the targeted disruption of the gene encoding DNA ligase IV leads to lethality in embryonic mice. Microcephaly and immunodeficiency are common to DNA ligase IV deficiency (LIG4 syndrome) and severe combined immunodeficiency with microcephaly, growth retardation, and sensitivity to ionizing radiation due to NHEJ1 deficiency (NHEJ1 syndrome).[10] Tobin et al.[37] showed that relative to nontumorigenic breast epithelial MCF10A cells, estrogen receptor positive (ER+)-MCF7 breast cancer cells and progesterone receptor positive (PR+)-MCF7 breast cancer cells have reduced steady-state levels of DNA ligase IV, whereas the steady-state levels of LigIIIα are higher. The main proteins involved in NHEJ in eukaryotes are DNA ligase IV, XRCC4, the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs), Ku proteins, and possibly Artemis. Deficiencies in any one of these proteins result in hypersensitivity to DNA DSB-inducing agents such as ionizing radiation. Once the blunt ends are in place, the XRCC4/DNA ligase IV ligation complex is recruited to join the DNA ends together. DNA ligase IV carries out the ligation step, but it requires the binding of XRCC4 to do so. XRCC4 functions as a regulatory element to stabilize DNA ligase IV, stimulate ligase activity, and direct the ligase to the site of DNA breaks via its recognition helix and DNA-binding capacity. Thus, it is clear from the above discussion that hLigIV is a crucial enzyme for classical NHEJ and if we inhibit hLigIV, the cellular machinery will not be capable of repairing ds breaks by NHEJ. This will predictably lead to triggering of cell death and/or apoptotic pathways.

From the above it can be safely concluded that inhibition of all three ligases at the same time will most definitely kill a proliferating cancer cell. However, the side effects for normal cells might be too great to consider such a scenario for therapy. Hence, specific inhibitor for individual ligases will be more desirable and inhibitors for hLigI will perhaps be the first ones

Table I. List of Major DNA Repair and Replication Pathways Targeted by Human DNA Ligases and Abnormalities Caused by Their Deficiencies

DNA ligases	Functions	Adverse effect due to deficiency of human DNA ligases	References
DNA ligase I	• Ligation of Okazaki fragments. • Long patch BER • NER in proliferating cells at G1, S phase during UV photolesion. • MHEJ.	Abnormalities in hematopoiesis and lymphopoiesis, severe immunodeficiency, stunted growth, and sun sensitivity.	5–7,9
DNA ligase III	• Ligation of Okazaki fragments in ligase I deficient cells. • Short patch BER. • NER in all types of cells at each phase of cell cycle. • MHEJ. • Alt NHEJ.	Defects in BER, defects in mitochondrial DNA metabolism.	6–9,25,26
DNA ligase IV	• NHEJ. • VDJ recombination.	Microcephaly, immune deficiency, growth retardation, and sensitivity to ionizing radiation.	9,30–33,37

to be inducted for therapy given its direct role in replication. See Table I for a brief functional description of human DNA ligases.

D. A Comparative Domain Analysis of the Three Human Ligases

The crystal structure of human DNA ligase I bound to nicked DNA has been recently solved.[38] It shows that hLigI protein has three domains:

1. An N-terminal DNA-binding domain (DBD; amino acid residues 262–535).
2. An adenylation domain (AdD; comprising amino acid residues 536–748), where Lys 568 covalently interacts with AMP. The AdD contains two metal-binding sites. One interacts with Glu720 to stabilize the negative charge on the 5′-phosphate of AMP leaving group in the transition step for the third step in ligation. The other one that interacts with Glu621 is closer to the nick and helps in ligation by activating the nucleophilic 3′-OH to attack the 5′-phosphate.
3. An OB (oligonucleotide-binding) fold domain (OBD; amino acid residues 749–919). These three domains completely encircle the DNA substrate and form extensive interactions with the DNA.[38]

A comparison of the domain mapping of hLigI with the other two human ligases shows the presence of some conserved domains such as DBD, AdD, and OB fold domains (Fig. 1). According to available literature, the DBD and the AdD domains have most often been targeted for inhibition.[39,40] In the AdD, the metal-binding site and the AMP-binding site have been targeted most for designing inhibitors.[40,41] Not surprisingly, inhibitors targeting the domains common to the three ligases also show activity (inhibition) against all three ligases.[42] Specific inhibitors would perhaps target domains that are nonconserved or target nonconserved residues inside conserved domains. Potential new sites on the ligase I protein that can be targeted

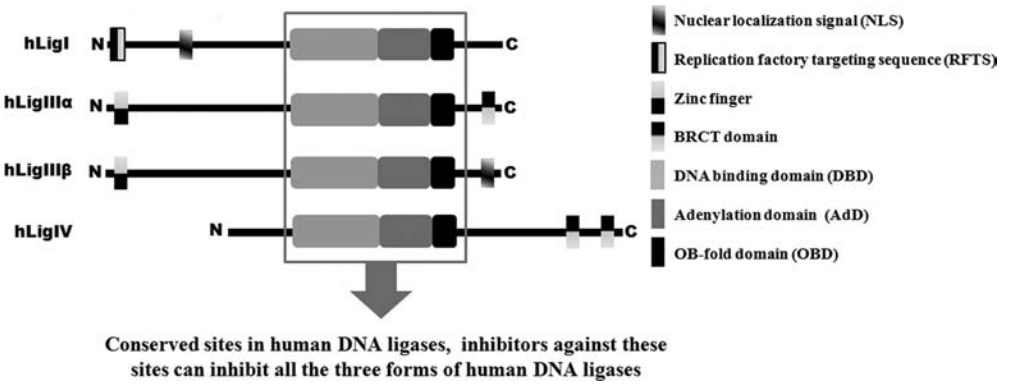


Figure 1. A comparative domain analysis of the human DNA ligases showing the conserved and unconserved domains.

for designing specific hLigI inhibitor/s have been discussed in the later part of this review (Section 5,C).

3. STRATEGIES FOR TARGETING DNA LIGASES FOR INHIBITION

DNA ligase activity can be inhibited either by targeting the enzyme or the DNA to which ligases bind.

A. Approach I: Inhibition of Ligation by Targeting the DNA Ligase Enzyme

1. DBD as a Target for Human DNA Ligase Inhibitors

The DBD of human ligases plays two important roles in ligase activity. First, it interacts with the minor groove of DNA, contacting the DNA upstream and downstream of the nick. Second, it directly interacts with the AdD and the OBD of hLigI and stimulates the DNA end-joining activity of the catalytic core.[38] Thus, if we target the DBD with specific inhibitors, we would achieve ligation inhibition. Some small molecule inhibitors have been recently reported that act on the DBD and inhibit ligation competitively. For example, small molecule inhibitors such as L67 and L189 compete with DNA for binding to the DBD whereas L82 inhibits ligation by stabilizing a reaction intermediate.[39,42] According to reports in Zhong et al.[39] and our own docking results, it is seen that (Fig. 2A) L67 binds to the DBD of hLigI, hence leading to inhibition of DNA binding and ligation. These inhibitors are discussed in more detail in Section 4,I (ligase inhibitors from structure-based drug designing). A comparison of the DBD and catalytic domains of human ligases is discussed in Section 3,A.4 to stress upon the specificity issue.

2. Metal-Binding Sites as Targets for Human DNA Ligase Inhibitors

Mg^{2+} plays an important role in ligation activity by human DNA ligases. There are two potential metal-binding sites present in hLigI. The first is coordinated by Glu720, which stabilizes the negative charge on the 5′-phosphate of the AMP-leaving group in the ligation reaction. The other metal-binding site identified in the hLigI-DNA complex is adjacent to the 2′-OH of AMP and is coordinated by the side chain of Glu621, which is close to the 3′-OH of nicked DNA, where it could participate in ligation activity.[38] Thus, molecules that can compete with Mg^{2+} for the metal-binding site will be good inhibitors of ligases. For example, $ZnCl_2$ and $CdCl_2$ have

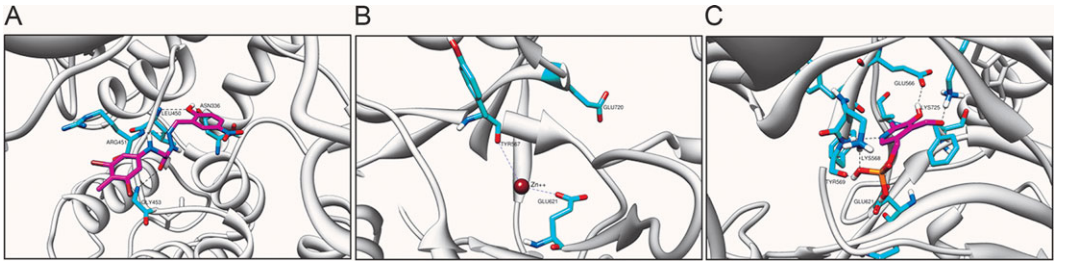


Figure 2. Docking of inhibitors with ligase I to highlight residues important for interaction. (A) Docking of compound L67 (magenta) within DNA-binding domain of LigI reveals a conformation showing H-bond interactions of L67 with the neighboring residues Asn336, Leu450, Arg451, and Gly453. Binding of L67 occupies the receptor site for DNA, hence inhibiting ligation. (B) At the metal-binding site, Zn^{2+} forms a coordination bond with Tyr567 and Glu621. This leads to occupation of Mg^{2+}-binding site and inhibition of ligation. (C) Conformation of the compound PLP (magenta) within adenylation domain of human DNA ligase I shows interactions with neighboring residues (Glu566, Lys568, Tyr569, and Glu621) through H-bonds. Binding of PLP blocks the AMP-binding site, hence inhibiting ligation.

both shown inhibition of ligation activity.[41] A docking study done by us shows that one of the residues that is occupied by Mg^{2+} (viz., Glu621) can also be occupied by Zn^{2+}, hence leading to inhibition of ligation (Fig. 2B).

3. AMP-Binding Site as a Target for Human DNA Ligase Inhibitor

In the first step of ligation reaction, the ε-amino group of Lys 568 and an α-phosphorus of ATP make a covalent enzyme–AMP adduct, which is necessary for ligation. This step of the ligation reaction can be inhibited in two ways, either by mutating the AMP-binding site so that it is not available for adenylation, or by using a nucleotide analogue instead of ATP so that ATP is not able to bind to ligase and hence not be able to transfer AMP from ligase to the 5′-end of nicked DNA. For example, pyridoxal phosphate(PLP)[40] and 9-β-D-arabinofuranosyl-2-fluoroadenine (F-ara-A)[43] are some such compounds reported in the literature. A docking study done by us shows that the PLP molecule can bind to the AMP-binding site (Lys 568), hence leading to inhibition of ligation (Fig. 2C).

4. Rational Inhibitor Design Based on Comparative Domain Analysis

Among all domains, DBD is the most potent and specific site for development of ligase inhibitors since it is unique to human ligases, conserved among the three human ligases, and can potentially inhibit all human ligases. An example of a compound that targets the DBD and inhibits all the three human DNA ligases is L189.[42] However, such a molecule may not be the best choice as an anticancer molecule due to issues of side effects to normal cells. Specific inhibitors for hLigI, hLigIII, and hLigIV that can be used together when necessary may be more desirable for use in combination therapy with other drugs. Figure 3A shows the normal process of ligation that leads to nick sealing. Figure 3B shows the various sites of the ligase I enzyme that can or have been targeted for development of inhibitors. In Figure 3B1, the ATP-binding site is shown to be targeted by PLP/F-ara-A; in Figure 3B2, the metal-binding site is shown to be targeted by $ZnCl_2$ and $CdCl_2$; and in Figure 3B3, the DBD is shown to be targeted by the small molecule inhibitors L67, L189, and L82.

De Ioannes et al.[44] have compared the sequences and structures of the DBD domains of the human ligases. They found that although the DBDs share a low sequence identity (only 14% for LigI vs. LigIII-DBD and 13% for LigI vs. LigIV-DBD) (Fig. 4A), their structures are quite similar (Fig. 4C). Another study done by Srivastava et al.[45] shows that the motifs involved in interaction of LigI with nicked DNA (RLRLG and ELGVED of the DBD of LigI; PDB

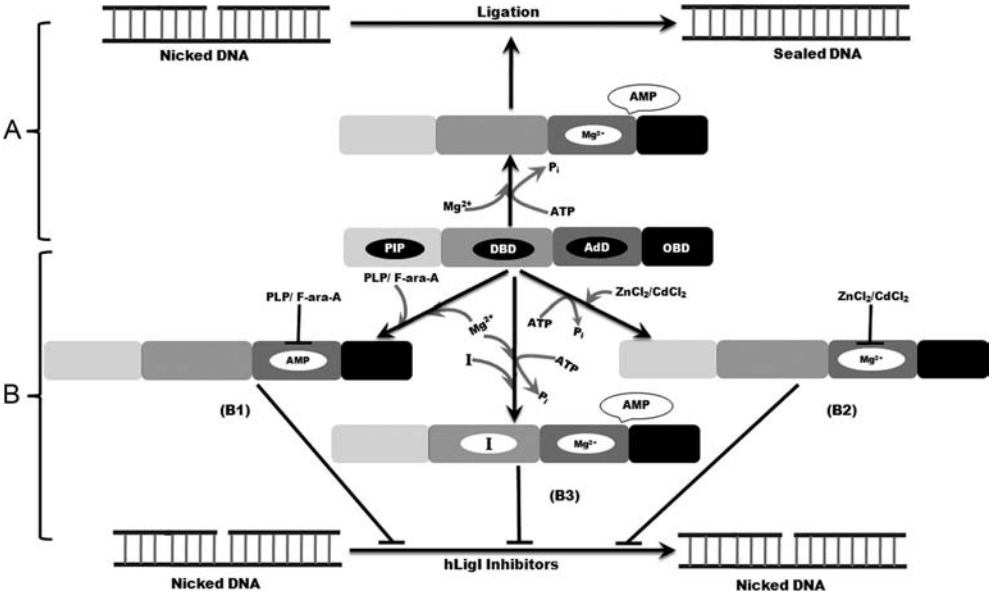


Figure 3. Depiction of various sites on the ligase enzyme that have been targeted by different inhibitors. (A) The normal process of ligation that leads to nick sealing. In (B), the various sites of the human ligase I enzyme are shown that are known to be targeted by inhibitors. In (B1), ATP-binding site is shown to be targeted by PLP and F-ara-A; in (B2), metal-binding site is targeted by $ZnCl_2$ and $CdCl_2$; and in (B3), the DBD is targeted by the small molecule inhibitors L67, L189, and L82 (denoted by I).

ID-1X9N) are structurally similar to the DBD of LigIII (DLKMN and VYNLND; PDB ID-3L2P) and to LigIV (DLKLG and AYGIKE; PDB ID-3W1G) (Fig. 4C). Interestingly, in spite of this similarity, they were able to design an inhibitor with activity against LigIV by modifying a small molecule inhibitor (L189) already known to inhibit all the three human ligases (by targeting the DBD).[42] This suggests that it may be possible to modify existing inhibitors in a manner that they show specific activity against one ligase only.

Alignment of the catalytic domains also shows low sequence identity among the human ligases (Fig. 4B). However when compared structurally, the catalytic domains look very similar to each other[46] (Fig. 4D). Nevertheless, from the experience of Srivastava et al., it may be extrapolated that given the sequence dissimilarity, existing or new inhibitors that target the catalytic domains of the three ligases nonspecifically could be optimized to act against a specific ligase only, provided that they are designed to target residues that are specific to each individual ligase.

B. Approach II: Inhibition of Ligation by Targeting the DNA

Not many inhibitors in this category have been reported in the literature. Some DNA intercalators such as anthracyclines (Table II) and distamycin A (Fig. 7D) have been reported that inhibit ligation by interacting with DNA. Two natural product compounds known to inhibit ligation by intercalation are nitidine chloride and fagaronine chloride (Table IV). One of the major problems with these intercalating agents is their nonspecific mechanism of action because of which the activities of several DNA-directed enzymes are affected.[47] However, the best among these are some anthracycline class of molecules, bearing a free amino group on the 3′-position of the sugar (e.g., esorubicin, 4′-deoxydaunorubicin, 4′-deoxy-4-demethoxydoxorubicin)

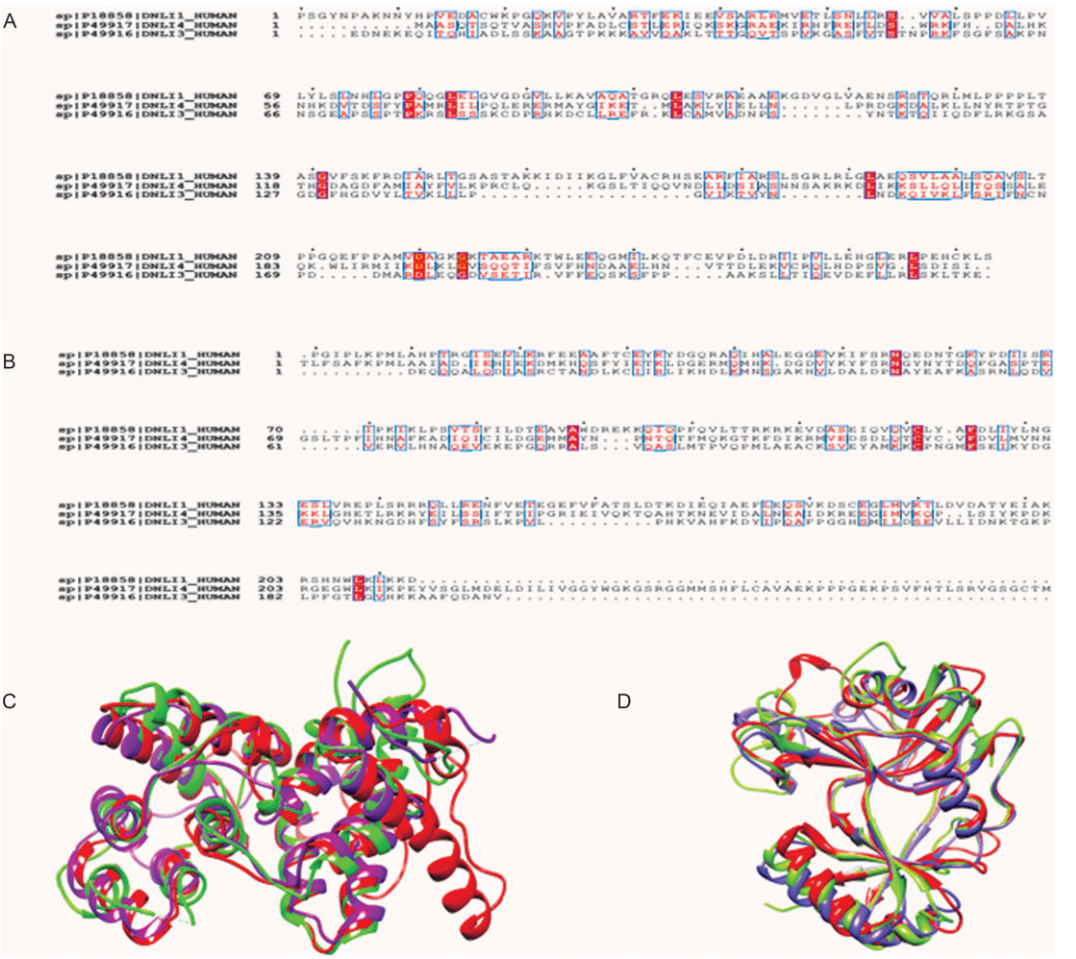


Figure 4. Domain analysis of the human ligase proteins. (A and B) The multiple sequence alignment of the DBD and catalytic domains of LigI, LigIII, and LigIV. Residues highlighted in red correspond to identical/conserved residues, while residues written in red depict residues that are similar but not identical. (C) The structural alignment of the DBD domains of human ligases. LigI-DBD (colored in red, residues 262–535; PDB ID-1×9N) superimposed on LigIII-DBD (colored in purple, residues 170–390; PDB ID-3L2P) and LigIV-DBD (colored in green, residues 1–240; PDB ID-3W1G). (D) The structural alignment of the catalytic domains of human ligases. LigI-DBD (colored in red, residues 536–748) superimposed on LigIII-DBD (colored in purple, residues 391–591) and LigIV-DBD (colored in green, residues 241–650).

(Table II), that have been reported to have potential-specific activity against human DNA ligases[48] (Fig. 5).

C. Approach III: Stepwise Inhibition of Ligation—A Mechanism-Based Approach

There are three steps of the ligation reaction as explained below. Inhibitors that target the enzyme may inhibit one or more steps of the ligation reaction. Although inhibitors specific to different steps of the ligation reaction may be desirable, most of the inhibitors reported so far target more than one step of the reaction. Figure 6 explains the three steps of the ligation reaction and shows the inhibitors that work at each step.

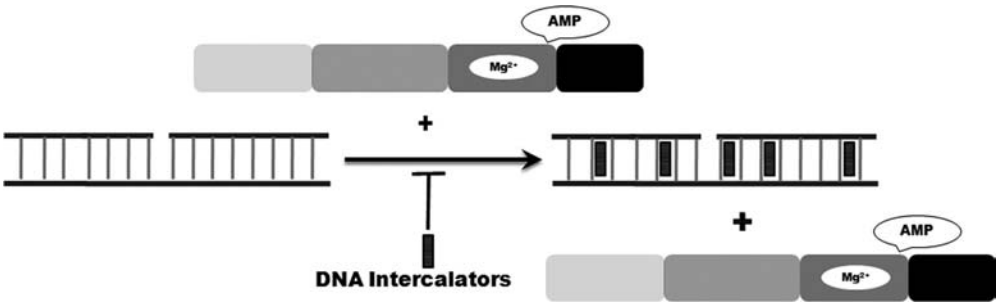


Figure 5. Inhibition of ligation activity by DNA intercalators such as anthracyclines. The molecules that intercalate with DNA near the nicks prevent ligase from binding to the nick, hence preventing ligation.

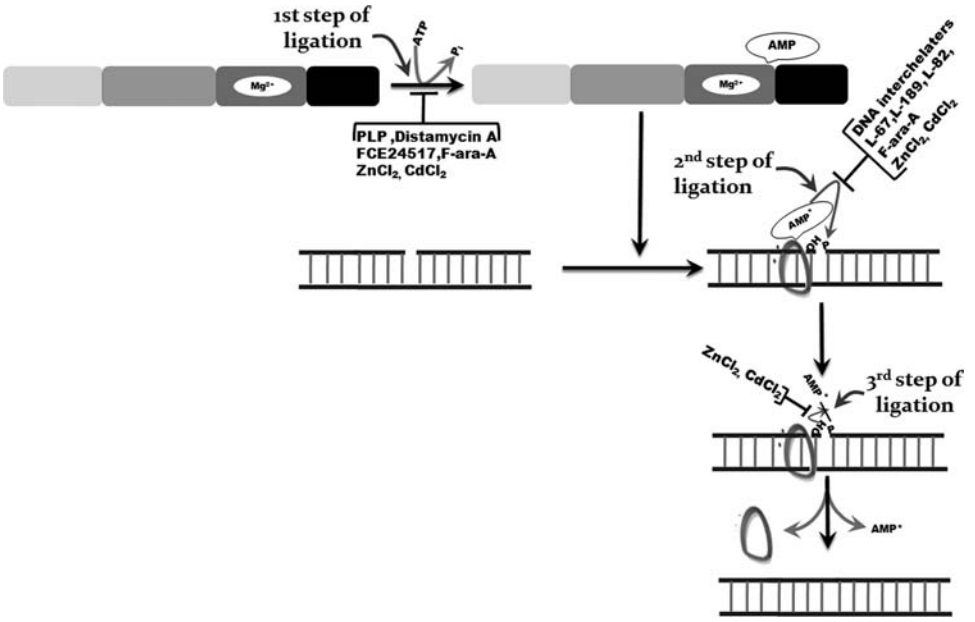


Figure 6. Different steps of the ligation reaction with a list of inhibitors known to block each specific step of the reaction are shown in this figure.

1. First Step of Ligation—Adenylation of Ligase

It is the first step of the ligation reaction; human ligases use ATP for adenylation where it is cleaved into AMP and inorganic phosphate (Pi). The AMP is linked to ε-amino group of lysine present at the active site of ligase. There are some compounds such as PLP[40] and nucleoside analogues such as distamycin A, FCE24517, F-ara-A (Fig. 7)[43,49] that have been reported to inhibit the adenylation step of ligation. However, these inhibitors show activity at high micromolar concentrations[40,43,49] and more efficient inhibitors of this step should be designed for efficient and specific inhibition of ligation.

2. Second Step of Ligation—Transfer of AMP from Ligase to the 5′-Phosphate of Nicked DNA

Most of the known ligase inhibitors target this step of the ligation reaction. In this step, the ligase first interacts with nicked DNA and then transfers its AMP to DNA. DNA intercalators such as anthracycline derivatives,[47,48] small molecule inhibitors targeting the DBD (e.g., L67, L189, and L82),[39,42] and some of the first step inhibitors such as F-ara-A[43] inhibit this step of

the ligation reaction. Inhibitors for this step may potentially be developed into highly specific ligase inhibitors with limited side effects, a property highly desirable for cancer therapy.

3. Third Step of Ligation—Formation of Phosphodiester Bond between 3′-OH and 5′-Phosphate of Nicked DNA

After the adenylation of DNA, the 3′-OH group of nicked DNA acts as a nucleophile and attacks the phosphodiester bond of the adjacent 5′-end of DNA. There are no inhibitors reported that specifically inhibit this third step of the ligation reaction. Although there are some inhibitors such as $ZnCl_2$ and $CdCl_2$ that are known to inhibit this step, but they also inhibit step 1 and step 2 of the ligation reaction.[41] Hence, specific inhibitors for this step are yet to be discovered.

4. SUMMARY OF KNOWN CLASSES OF HUMAN LIGASE INHIBITORS

Several classes of molecules have been tested for antiligase activity and a few of the more prominent ones are discussed here. The recent deciphering of the structure of hLigI bound to nicked DNA has been a major advance in the field of structure-based ligase inhibitor designing. Some small molecules have been designed and patented (Pub No.: US 2010/0099683 A1) using the structural information of the DBD of hLigI. The different classes of inhibitors as reported in the literature are discussed here in detail.

A. Metal Ions

It has been reported that heavy metal ions such as Zn (0.8 mM $ZnCl_2$) and Cd (0.04 mM $CdCl_2$) completely inhibit the hLigI activity purified from HeLa cells. They were shown to inhibit all the three steps of the ligation reaction.[41] A docking study done by us reveals the Zn^{++}-binding sites on hLigI (Fig. 2B). However, the nonspecific natures of binding of metal ions and the profound toxicity of cadmium[50] preclude their use as ligase inhibitors in vivo. However, other specific inhibitors targeted against these residues (the tertiary structure formed by these residues) may be developed for specific inhibition of ligase activity.

B. Pyridoxal Phosphate

PLP (Fig. 7A) is a prosthetic group of some enzymes. It is the active form of vitamin B_6. It has been reported that PLP forms a Schiff's base with the ε-amino group of a lysine residue of the adenylation domain of mammalian DNA ligase I (purified from calf thymus), by which they block the enzyme–AMP complex formation and hence the ligation activity of ligase I.[40] A docking study done by us reveals the conformation of PLP within the adenylation domain of hLigI and reveals the residues that it binds to (Fig. 2C). However, AMP-binding sites being common to various enzymes, the specificity of any molecule targeting this site may not be limited to ligase alone.

C. Nucleoside Analogues

F-ara-A triphosphate (Fig. 7B), an analogue of adenosine and deoxyadenosine, shows potential anticancer activity in experimental systems and activity against human ligase I purified from HeLa cells.[43] Inside the cell, F-ara-A is phosphorylated to F-ara-adenine triphosphate (F-ara-ATP) by cellular enzymes, which can inhibit nucleic acid biosynthesis by incorporating it into

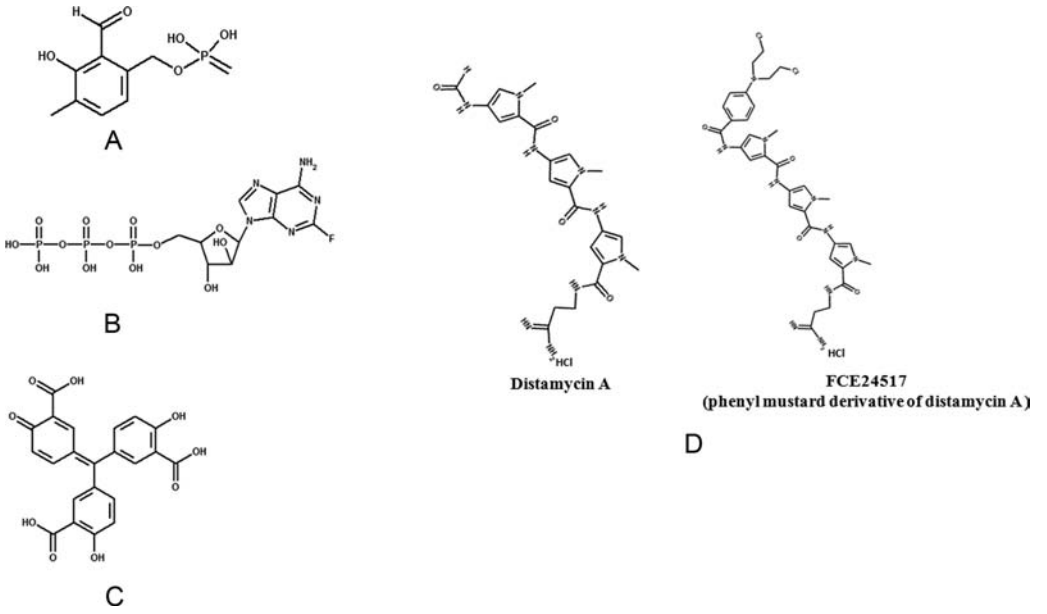


Figure 7. Structures of ligase inhibitors. (A) Structure of pyridoxal phosphate (PLP), an inhibitor of hLigI adenylation activity. (B) Structure of F-ara-ATP, an inhibitor of hLigI adenylation activity. (C) Structure of aurintricarboxylic acid (ATA). (D) Structure of distamycin A and its phenyl mustard derivative (FCE24517).

DNA and RNA. It shows dual mode of inhibition of hLigI (IC_{50}-42 μM). First, F-ara-ATP directly interacts with DNA ligase I and inhibits the formation of the ligase–AMP complex. Second, the activity of DNA ligase I is inhibited when F-ara-ATP is incorporated into the 3′-terminus of the DNA substrate. It has also been reported that when used alone, ara-ATP moiety is not able to show any inhibitory activity but in the presence of the 2-fluoro moiety of F-ara-ATP, it shows antiligase activity.[43]

D. Aurintricarboxylic Acid (ATA)

ATA, 5, 5′-((3-carboxy-4-oxocyclohexa-2,5-dien-1-ylidene)methylene)bis(2-hydroxybenzoic acid)) (Fig. 7C) is a well-known inhibitor of various DNA-metabolizing enzymes.[51] It also demonstrates a dose-dependent inhibition of hLigI activity.[52] Although it was shown to inhibit human ligase I purified from HeLa cells, it is actually a nonspecific inhibitor of protein–nucleic acid interactions and is known to be a potent inhibitor of ribonuclease and topoisomerase II by preventing the binding of the nucleic acid to the protein.[51]

E. Distamycin A and Its Derivatives

Distamycin A is an oligopeptide obtained from *Streptomyces distallicus.*[53] It inhibits nucleic acid biosynthesis by interacting with the minor groove of DNA[54] and inhibits the activity of both DNA and RNA polymerases as well as replicative DNA ligase purified from HeLa cells.[49] FCE24517 is a derivative of distamycin A (Fig. 7D) that is synthesized by the addition of a phenyl mustard residue at the N-terminus of distamycin A. It is effective against a broad spectrum of murine and human tumors, including those that are resistant to alkylating agents. Experimentally it has been proved that these drugs preferentially inhibit DNA synthesis.

Although these two drugs interfere with the action of all the proteins involved in DNA replication, but the adenylation step of DNA ligation is most sensitive to these two distamycins.[49]

It has also been reported that the DNA intercalating or DNA-binding drugs interfering with the minor groove of DNA can inhibit DNA ligases,[49,55] suggesting the possibility of determining the mechanism of inhibition of DNA ligases for distamycin A and FCE24517 by assaying their effects on different steps of the ligation reaction. These molecules have the potential to be developed into antiligase molecules with the proper kind of tweaking and molecular dynamics studies. Interestingly, in our own screening for ligase inhibitors, we have found a lipidated natural product derivative belonging to the glutarimide class that targets both the DBD domain of hLigI as well as the minor groove of DNA and is a potent inhibitor of hLigI. The molecule has shown selective anticancer activity in certain human cancer cell lines and seems to have the potential for drug development.

F. Anthracycline and Its Derivatives

Anthracyclines are an important class of antibiotics derived from *Streptomyces*. They are also a class of drugs used in cancer chemotherapy [e.g., doxorubicin (adriamycin) and daunomycin (daunorubicin)]. Nearly a hundred derivatives have been synthesized to try and reduce their toxic effect on cells and to improve their anticancer properties. The biological properties of these compounds are generally attributed to the intercalation and reversible binding to DNA, although stabilization of the DNA topoisomerase II-DNA complex may be an important factor in the cytotoxicity.[48] It is still unclear how anthracyclines attain their varied biological activities and this characteristic may be attributed to activity against multiple targets. Giovanni Ciarrocchi et al.[48] found that DNA ligases are resistant to several intercalating anthracyclines, but very sensitive to ones bearing a free amino group on the 3′-position of the sugar. The structure–activity relationship (SAR) studies of various anthracycline derivatives done by them revealed that maximal inhibition of DNA-joining activity require a 4′-deoxy-3′-amino sugar (compounds III, VIII, and XII; Table II).

Initially, 4′-deoxy derivatives (III, IV, VIII, X, XII, and XVI) were tested for their antiligase activity on replicative DNA ligase purified from HeLa cells. It was found that the derivatives that possess 4′-deoxy-3′-amino sugar (compound III, VIII, and XII) showed maximum inhibition of human DNA ligase as compared to their 4′-hydroxy counterparts (Table II).[48,56] Other derivatives having 4′-deoxy-4′-amino sugar (compound XVI with amino group present on 4′-epi position) show very low activity. If the 4′-deoxy-3′-amino sugar is replaced by 3′,4′-diamino sugar (compound X), this shows lesser activity than 4′-deoxy-3′-amino sugar derivatives, but similar activity as compared with 4′-hydroxy-3′-amino sugar counterparts (daunomycin, VIII) (Table II).

However, it is clear from the studies of Giovanni Ciarrocchi et al.[48] that anthracyclines are poor inhibitors of the first step of the DNA-joining reaction and that inhibition of DNA-joining activity is obtained via interaction with the substrate (DNA), as proposed for some other DNA binders such as ethidium bromide. However, although DNA intercalation is necessary, it is not in itself sufficient for the activity against human replicative DNA ligase. Crystallographic studies revealed that during DNA intercalation, while the anthracycline aglycone-chromophore moiety lies between base pairs perpendicular to the axis of the double helix, the sugar residue hangs in the minor groove with the 3′-amino group exposed.[48] Although it is still unclear what kind of interactions occur between the hanging amino group and the ligase, studies suggest that the interaction must be very specific.[48] It was also found that closely related anthracyclines with different sensitivity to ligases did not show the same variations to the stability of the DNA topoisomerase II-DNA complex. If this hypothesis is correct then it should be possible to find some anthracyclines that have specific activity for ligases.[48]

Table II. This Table Shows the Structure–Activity Relationship of Anthracycline Derivatives that Were Tested for Anti-Human Ligase Activity

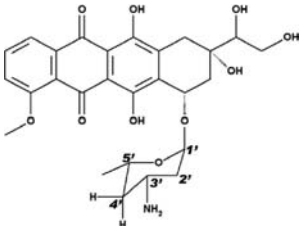
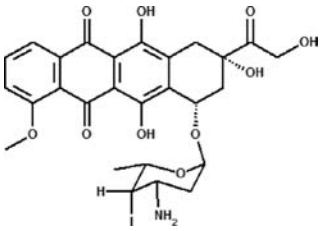
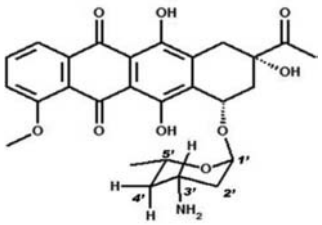
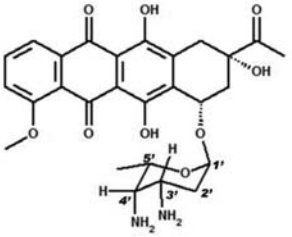
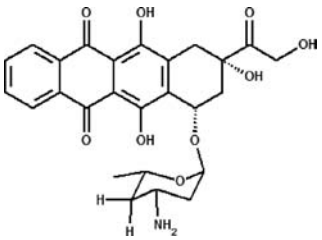
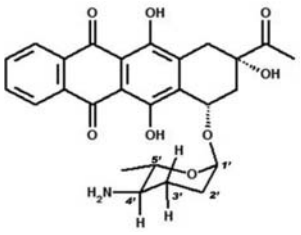
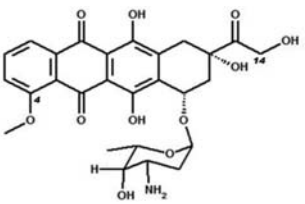
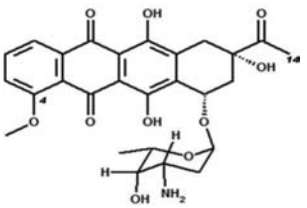
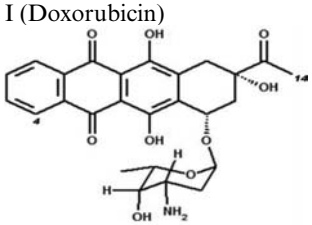
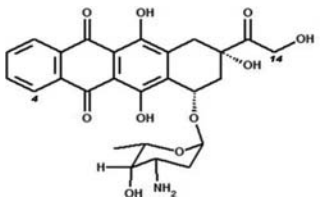
Anthracyclines showing activity against human DNA ligases	Anthracyclines inactive/less active against human DNA ligases	Possible explanation for the structure–activity relationship
 III (Esorubicin)	 IV (4′-Deoxy-4′-iododoxorubicin)	Derivatives that possess 4′-deoxy-3′-amino sugar (compounds III, VIII, and XII) showed maximum inhibition of the replicative human DNA ligase, as compared to their 4′-deoxy counterparts (compounds IV, X, and XVI).
 VIII (4′-Deoxydaunorubicin)	 X (4′-Deoxy-4′-aminodaunorubicin)	
 XII (4′-Deoxy-4-demethoxydoxorubicin)	 XVI (3′-Deamino-4′-deoxy-4′-epiamino-4 -demethoxydaunorubicin)	
 I (Doxorubicin)	 VII (Daunorubicin)	C-14 hydroxylation of derivatives having 4-methoxy group increases the antiligase activity of anthracyclines.
 XIV (Idarubicin)	 XI (4-Demethoxydoxorubicin)	In the absence of 4-methoxy group, hydroxylation of C-14 decreases the antiligase activity of anthracyclines.

Table II also lists other derivatives of anthracyclines that were created by modifications in the C-14 position of the carbon chain, by the addition of hydroxyl group or methyl group at that position. The addition of hydroxyl group at C-14 position of daunorubicin (VII) creates a new molecule doxorubicin (I) that is more active than daunorubicin. This suggests that hydroxylation promotes the inhibitory activity but 4-demethoxy molecule carrying same modifications (XI, XIV) shows negative effect of hydroxylation, that is, decreases the activity of the inhibitors. When the new analogues were created only by 4′-demethoxy modification like the 4′-demethoxy-doxorubicin (XI), differences were seen in their activity. Doxorubicin (I) was more active than its 4′-demethoxy counterpart whereas daunorubicin (VII) was less effective than its 4′-demethoxy derivative (XIV).

G. Arylamino Compounds

While a number of effective DNA ligase inhibitors have been synthesized from this class of compounds, most are unable to discriminate between NAD^+-dependent and ATP-dependent DNA ligases. For example, doxorubicin, chloroquine (CQ), hydroxyl-chloroquine, quinacrine, 6bisQ0, 6bisQ6, 6bisQ8, 8bisQ0, 8bisQ4, *N*,*N*9-bis[4-chloroquinolin-8-yl]succinamide, quinine, cinchonidine, 2-amino-5-diethylaminopentane, spermidine, putrescine inhibit both *Escherichia coli* ligase and human ligase I. All of these compounds have a higher IC_{50} value for hLigI than for *E. coli* ligase.[57] Hence, these are not ideal human ligase inhibitors at present and may need a lot of tweaking before specific human ligase inhibitors from this class of compounds can be achieved. See Table III for structures and IC_{50} values for different arylamines that show inhibition of *E. coli* and human ligase I activity.

H. Natural Product Inhibitors

There are several natural products reported that somewhat inhibit the hLigI activity among the human ligases (Table IV). They belong to various categories such as triterpenes, benzophenanthridine alkaloids, flavonoids, flavanoxanthones, aliphatic γ-lactones, iridoids, and vicia alkaloids.[56,58,59] A brief description of these inhibitors follow.

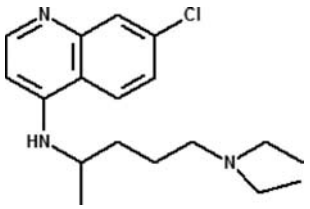
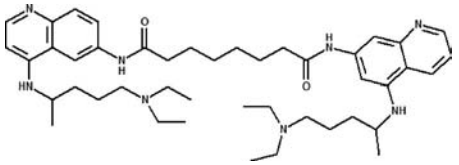
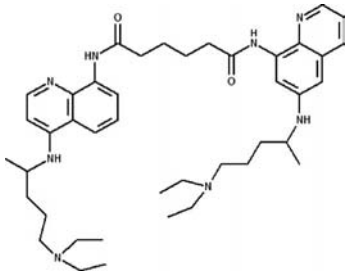
1. Triterpenes

Ursolic acid, oleanolic acid, betulinic acid, aleuritolic acid are reported in this category as human DNA ligase I inhibitor. Ursolic acid (IC_{50}-216 μM) and oleanolic acid (IC_{50}-216 μM) were isolated from *Tricalysia niamniamensis hiern* (Rubiaceae). Of the four triterpenes, aleuritolic acid (IC_{50}-205 μM) shows maximum ligase inhibition but its exact mechanism of action is still unclear.

2. Benzophenanthridine Alkaloids

A number of benzophenanthridine alkaloids are reported that inhibit hLigI; examples include fagaronine chloride, nitidine chloride, chelerythrine chloride, sanguinarine nitrate, chelidonine, and *N*-demethylfagaronine. Among these, fagaronine chloride and nitidine chloride, which contain the iminium ion ($-C{=}N^+-CH_3$) in their active site, intercalate with DNA and inhibit various RNA- and DNA-polymerizing enzymes, whereas chelidonine and *N*-demethylfagaronine are inactive due to absence of iminium ion from their active sites. Thus, fagaronine chloride (IC_{50}-27 μM) and nitidine chloride (IC_{50}-69 μM) can inhibit hLigI by intercalating with DNA. They are reported to show cytotoxic activity against P388 leukemia in mice.[60] The L1210 and P388 leukemia's, developed in 1948 and 1955, respectively, played a major role in both screening and

Table III. List of Arylamino Compounds Known to Inhibit Ligation. These Compounds Could Be Modified to Produce Better and More Specific Human Ligase Inhibitors

Arylamines	Structure	IC_{50} (μM) value		References
		For *E. coli* ligase	For human ligases	
Chloroquine		53 ± 63	720 ± 80	[57]
6bisQ6		2.2 ± 0.3	14.8 ± 3	[57]
8bisQ4		9 ± 2	43 ± 7	[57]

detailed evaluations of candidate anticancer agents by the division of cancer treatment of the National Cancer Institute (NCI).[60]

3. Flavonoids

Myricetin [(IC_{50}-28 mg/mL (91 μM)] and morin [IC_{50}-68 mg/mL (236 μM)] are well-known flavonoids that inhibit hLigI by intercalating with the nucleic acid.[56,59]

4. Flavanoxanthone

Swertifrancheside is a flavonoxanthone that is isolated from *Swertia franchetiana*. It inhibits ligation activity of hLigI [IC_{50}-8 μg/mL (11 μM)] as well as adenylation of hLigI [IC_{50}-76 μg/mL (105 μM)]. This suggests that it interacts with hLigI as well as with DNA.[56,59]

5. Iridoids

They belong to a class of secondary metabolites having cyclopentane ring fused with a six-membered oxygen heterocycle. Fulvoplumerin is an iridoid obtained from *Plumeria rubra* and shows antiligase activity (IC_{50}-88 μM). It also shows activity against HIV-RTs (human deficiency syndrome reverse transcriptases). This means that there must be some structural similarity between the human ligase and HIV-RT proteins and that it must interact with a region common to both proteins.[59]

Table IV. List of Different Categories of Known Natural Product Human Ligase Inhibitors

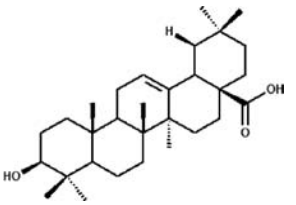
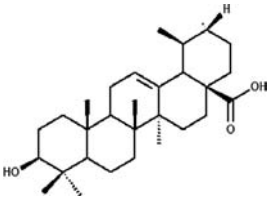
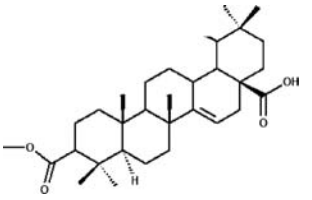
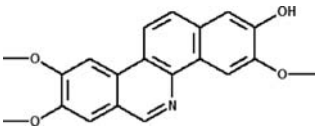
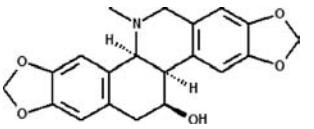
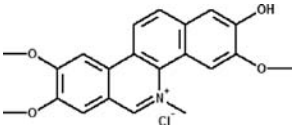
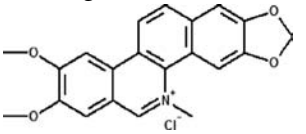
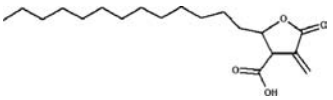
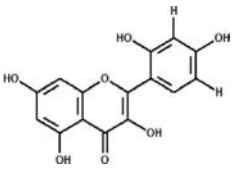
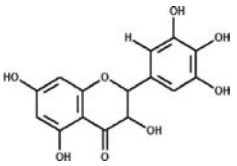
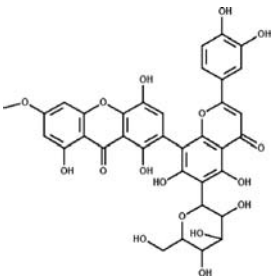
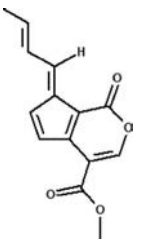
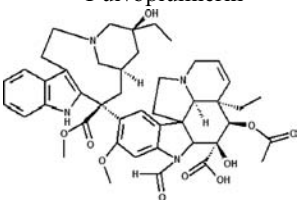
Natural product inhibitor classes	Examples with chemical structures	IC_{50} (μM) values	Possible explanation for their activity	References
Triterpenes	 Oleanolic acid	216	Triterpenes directly interact with ligases, but the exact mechanism of inhibition (possible interaction and structural features) is not known.	56, 59
	 Ursolic acid	216		
	 Aleuritolic acid	205		
Benzo-phenanthridine alkaloids	 *N*-Demethylfagaronine	Not available	In the presence of iminium ion, nitidine chloride and fagaronine chloride intercalate with DNA and inhibit DNA ligases. However, *N*-demethyl-fagaronine and chelidonine are inactive up to 200 μM concentration in the absence of iminium ion.	56, 59, 60
	 Chelidonine	Not available		
	 Fagaronine chloride	27		
	 Nitidine chloride	69		

Table IV. Continued

Natural product inhibitor classes	Examples with chemical structures	IC_{50} (μM) values	Possible explanation for their activity	References
Aliphatic γ-lactone	 Protolichesterinic acid	20	In case of HIV reverse transcriptases, these compounds interact with the allosteric site of the protein. This may be true for hLigI as well, but the actual mechanism is not known.	56, 59
Flavonoids	 Morin	236	The molecular planarity of flavonoid molecules facilitates their intercalation with DNA, and is the predominant mechanism of ligation inhibition by morin and myrcetin.	56, 59
	 Myrcetin	91		
Flavano-xanthone	 Swertifrancheside	11	Two mechanisms of ligation inhibition are seen, viz., intercalating with DNA (which is the predominant mechanism) and interactions with hLigI (alternate mechanism).	56, 59
Iridoid	 Fulvoplumierin	88	They inhibit both HIV reverse transcriptases and hLigI. This suggests that they interact with a structural feature that is common to both proteins.	56, 59
Vicia alkaloids	 Vincristin	Not available	It is showing competitive inhibition against ligase purified from normal lymphocytes and blasts	58

6. Aliphatic γ-Lactone

Protolichesterinic acid is an aliphatic α-methylase-γ-lactone obtained from the lichen *Cetraria islandica.* It inhibits hLigI activity at an IC_{50} value of 20 μM and blocks its adenylation at a much higher concentration (387 μM).[59] Although the molecule binds to ligase, it also binds to HIV-1 RT, highlighting its nonspecific nature of binding. The molecule does not interact or intercalate with DNA and should therefore be potentially nonmutagenic in nature. The molecule holds promise if it can be tweaked to specifically target hLigI or the HIV-1 RT for use in anticancer or antiretroviral therapy, respectively.

7. Vicia Alkaloids

They are obtained from *Catharanthus roseus.* Various vicia alkaloids such as vincristine, vinblastin, and vindesine have been tested for antiligase activity. Only vincristine (at 100 μM concentration) showed activity against ligase purified from thymocytes and normal lymphocytes, while having little or no effect on the enzyme purified from lymphoblast cells.[58]

Although several categories of natural products exhibit ligase inhibition, compounds showing specific inhibition at low micro- or nanomolar concentrations still remain elusive. We suggest that natural products with modified synthetic adages may be developed to achieve the required parameters of specificity, activity, stability (along with high bioavailability and low toxicity), and other features that are essential for drug-like molecules. This is a relatively unexplored area with a lot of potential, although unfortunately, an area where not much research is known to be happening.

I. Ligase Inhibitors from Structure-Based Drug Designing

With the availability of the crystal structure of hLigI bound to nicked DNA,[38] several chemically synthesized small molecules have been reported with activity against one or more human DNA ligases. They were derived by computer-aided drug designing using the structure of hLigI bound to nicked DNA.[39] Some of them are briefly described below and also listed in Table V.

1. L82

It inhibits the human DNA ligase I by an uncompetitive mode of action (IC_{50}-12 ± 2 μM). It is reported to inhibit the DNA–adenylate formation step of the ligation reaction and has been shown to be cytostatic in nature.[42]

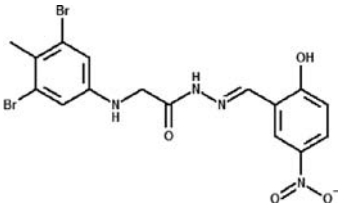
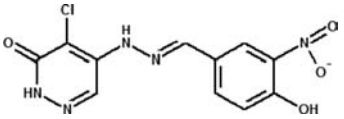
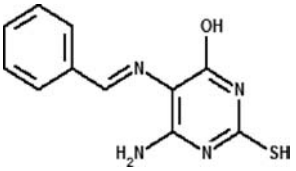
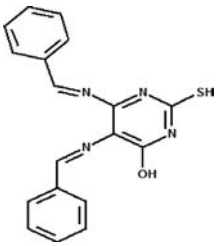
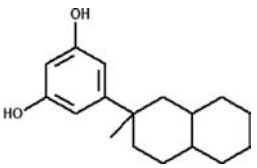
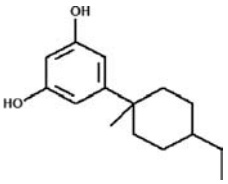
2. L67 and L189

These compounds show competitive mode of inhibition and are cytostatic in nature. L67 shows activity against hLigI and hLigIII with an IC_{50} value of 10 ± 3 μM for both ligases, whereas L189 shows activity against hLigI, hLigIII, and hLigIV with IC_{50} values of 5 ± 2, 9 ± 2, and 5 ± 2 μM, respectively. Both these compounds inhibit step 2 of the ligation reaction, that is, the DNA–adenylate formation step.[42]

3. SCR7

This is a very recently reported small molecule that was derived from L189, and specifically inhibits hLigIV by interacting with its DBD and inhibits hLigIV-mediated NHEJ pathway that is a part of the DSBR pathway.[45] This compound has reported IC_{50} values of 8.5, 120, and 10 μM for T47D (breast cancer), A2780 (ovarian cancer), and HT1080 (fibrosarcoma) cell lines, respectively. This inhibitor has been shown to be able to reduce tumor size in mouse at 10 mg/kg dose, when six doses were given every alternative day for 12 days. A significant reduction in tumor size was observed after 25 days.

Table V. List of Small Molecule Inhibitors Derived from Rational Structure-Based Designing for Human Ligase Inhibitors after the Elucidation of hLigI-Nicked DNA Crystal Structure.[39,42] The Compounds LI-01 and LI-02 were However Found from Random Screening of a Chemical Library from Micro-Source Discovery Systems by Sun et al.[52]

Compound	Structure	IC_{50} (μM) value (in human cancer cell lines)	Mechanism of action	Reference
L67		10 ± 3 for hLigI and hLigIII	It inhibits the second step of ligation by hLigI and hLigIII in a competitive manner.	42
L82		12 ± 2 for hLigI	It inhibits second step of ligation by hLigI in an uncompetitive manner.	42
L189		• 5 ± 2 for hLigI and hLigIV. • 9 ± 2 for hLigIII.	It inhibits the second step of ligation by hLigI, hLigIII, and hLigIV in a competitive manner.	42
SCR7		8.5	It interacts with the DBD of hLigIV and inhibits its activity competitively.	45
LI-01		Not available	It inhibits activity of hLigI by an unknown mechanism.	52
LI-02		Not available	It inhibits activity of hLigI by an unknown mechanism.	52

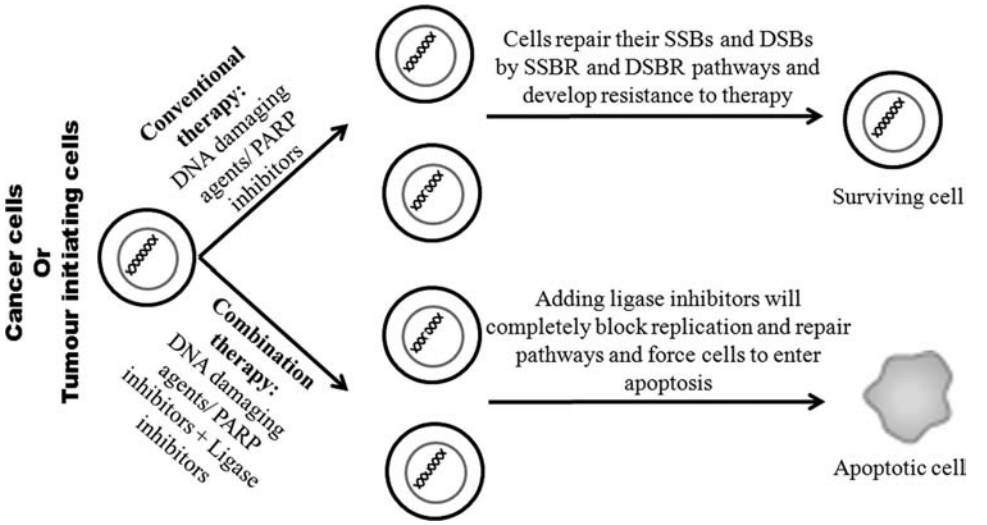


Figure 8. Schematic representation of the potential use of ligase inhibitors for successful combination therapy in treating cancers (SSBs, single-strand breaks; DSBs, double-strand breaks; SSBR, single-strand break repair; DSBR, double-strand break repair).

J. Ligase Inhibitors Discovered from Random Screening

Apart from the above, some molecules such as LI-01 (2-(3,5-dihydroxyphenyl)-2 methyldecalin) and LI-02 (2-(3,5-dihydroxyphenyl)-4-ethyl-1-methylcyclohexane) show hLigI inhibition. These compounds were found from a random screening of chemical libraries from Micro-Source Discovery Systems.[52] LI-01 was found to be more active than LI-02 but the exact IC_{50} values are not known.

5. STRATEGIES IN CANCER THERAPY AND THE FUTURE OF LIGASE INHIBITORS

The current state of knowledge clearly shows that human DNA ligases are crucial enzymes for DNA replication and repair pathways. It has been reported that hLigI is highly expressed in cancer cells.[14,61] Hence they are potentially good targets for development of anticancer drugs. However, there may be problems of specificity and drug resistance, problems that can be overcome by selective targeting and combination therapy of drugs, respectively (Fig. 8). Here we discuss how ligase inhibitors may be useful in combination cancer therapy and how hLigI can be targeted for development of more specific inhibitors for future therapy.

A. Ligase Inhibitors in Combination Therapy

Ligase inhibitors should work well in combination with DNA damaging and intercalation agents that can first cause breaks in the DNA and then help sustain the damage through inhibition of ligation. Hence, radiation therapy to induce ss and ds breaks, followed by antiligase therapy should be more effective than radiation therapy alone where such damages may be repaired by the DNA repair processes (involving ligases I, III, and IV). In fact, a combination of molecules, or a single molecule that can inhibit all three ligases (and hence inhibit all DNA repair processes), may be used for a limited time to ensure cancer cell killing and then withdrawn from therapy to avoid too much damage to normal cells over the long term. Again, DNA

intercalators such as doxorubicin and daunorubicin that are already in therapy and can inhibit ligation by physical occlusion of ligases (from binding the DNA), should become more effective when used in combination with other ligase inhibitors that can take care of any residual ligation activity.

Among the recent lot of promising anticancer drugs, several poly-ADP ribose polymerase (PARP) inhibitors were recently in the second and third phase of clinical trials being conducted by Astra-Zeneca (olaparib), Sanofi-Aventis (iniparib), Pfizer (PF-01367338), and others.[62] It is understood that inhibiting PARP-1 leads to the accumulation of ss DNA lesions, which during DNA replication can degenerate to form ds breaks. When PARP-1 is blocked, backup DNA recombination mechanisms involving BRCA1/2 (that are critical players in the homologous-recombination repair pathway), kick in. PARP inhibition in BRCA-defective cancer cells would therefore lead to massive genetic damage and cell death (a synthetic lethal effect).[62] However, recent reports suggest that PARP-1 inhibitors have failed to considerably prolong lifespan in the third phase of clinical trials.[62] Given the fact that the strand breaks left behind by PARP inhibitors need to be sealed by ligases for the cells to survive, we believe that human ligase inhibitors (specially hLigI inhibitors) would be very effective when used in combination with PARP-1 inhibitors in keeping DNA damage levels (strand breaks) high enough to induce cell death. PARP inhibitors, when used alone, work best in BRCA-negative cells (or in cells with altered DNA damage response),[63] whereas normal tissue with at least one functional *BRCA1* or *BRCA2* allele will be able to repair its DNA by alternate repair mechanisms and survive. At the same time, if ligase inhibitors are used simultaneously, then the alternate mechanisms will be halted for want of ligation, hence blocking the complete repair of damaged DNA. This will perhaps lead to a faster accumulation of DNA damage and induce apoptosis (Fig. 8).[63,64]

B. Specific Targeting of Ligase Inhibitors

As with all chemotherapeutic agents, targeting of ligase inhibitors to sites of tumors or to cancer cells dissipated in the body will be a major challenge and will depend on the discovery of specific identifiers of cancer cells that can be attached to the delivery modules. We suggest that chemotherapeutic agents, including ligase inhibitors can be packaged inside lipid micelles that carry target identifiers if possible. Lipid-based drug delivery is a very well established field and pharmacists/chemists in our group are currently working on ways to molecularly target lipids for drug delivery. The idea is that once these micelles reach the targeted cells, they can fuse with the cell membrane and release their load inside the cells, hence delivering the drugs.[65]

However, even in the absence of specific targeting, issues of side effects for ligase inhibitors should be minimal since several fast replicating cancer cells have high levels of hLigI compared to normal cells.[14] Also, most normal adult cells in the body do not replicate at all or replicate only sporadically (e.g., hematopoietic stem cells). Thus, the high proliferative index of cancer cells compared to the sporadic nature of normal stem cell multiplication should make an antiligase I strategy clinically viable. This combination of specificity and selectivity (perhaps in combination with targeted radiation therapy to induce DNA strand breaks in cancer cells) could become successful in anticancer therapy.

For issues of specific targeting of inhibitors to individual ligases, structure-based approaches highlighted by recent publications[39,45] indicate that it may be possible to design inhibitors with activity against an individual ligase (e.g., L82 and SCR7 against hLigI and hLigIV, respectively), or against a combination of ligases (e.g., L67 against hLigI and hLigIII and L189 against all three ligases). Our group is presently engaged in design and synthesis of specific ligase inhibitors.

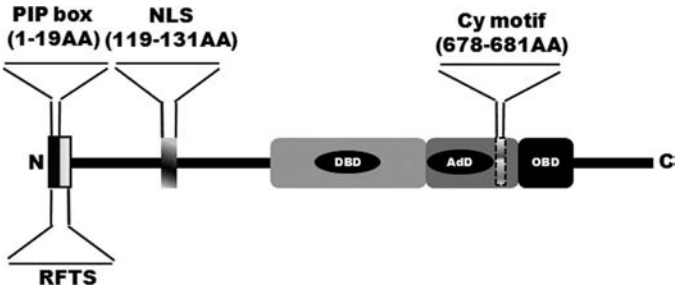


Figure 9. Schematic representation of hLigI domain structure showing PIP box, NLS, and Cy-motifs as potential new targets for development of specific hLigI inhibitors.

C. New Targets for Ligase Inhibitors

1. The PIP Box

In human DNA ligase I, the first 19 amino acids from the N-terminal region acts as a proliferating cell nuclear antigen (PCNA)-binding motif, also known as the PIP box (Fig. 9). It is unique among ligases in being found only in the ligase I sequence. Mutational studies show that if phenylalanine residues at the eighth and ninth position are replaced by alanine, PCNA binding with hLigI is abolished.[66] It is known that interaction of hLigI with PCNA is necessary for efficient ligation in vivo.[9] Hence, it is expected that if we can block the PIP box of ligase I by designing an inhibitor against it, it will block hLigI–PCNA interaction, and lead to a considerable reduction in ligation in vivo. Currently, no synthetic or natural product inhibitors have been reported against this region and it could potentially be an excellent target for the development of specific inhibitors against hLigI. As a proof of concept, it has recently been reported[67] that T2-amino alcohol (T2AA; IC_{50}-1 μM), which is a derivative of 3,3′,5-triiodothyronine (T3, thyroid hormone), is a synthetic compound that inhibits PCNA binding with PIP-box sequence peptide by competing for the same binding site. The inhibitor also inhibits PCNA binding with interaction partners such as p21 and Pol δ, leading to de novo DNA synthesis inhibition and cell cycle arrest in the S phase. This results in inhibition of growth and replication of cancer cells.[67]

2. Cyclin-Binding Motif (Cy Motif) and Nuclear Localization Signal (NLS Motif)

At the C-terminal region of hLigI, cyclin-binding site (RRQL) is found at amino acid position 678-681. This region plays two important roles. First, it acts as a cyclin A binding site that helps phosphorylation of Ser51 (at G1/S phase), Ser76 (at G2/M phase), and Ser91 (at G1/S phase). These phosphorylation events play a very important role in interaction of hLigI with other proteins involved in DNA replication such as PCNA and RFC.[68,69] Second, deletion studies of the nuclear localization sequences (NLS) and Cy-motif revealed that binding of cyclin A at Cy-motif is enough for nuclear translocation of hLigI from the cytosol (in the G1/S phase), even in the absence of the NLS.[69] Hence, the Cy-motif could be an excellent target for specific inhibition of hLigI by designing peptides mimicking the Cy-motif binding region of cyclin A and thus restricting the binding of cyclin A to hLigI. This will restrict the phosphorylation of hLigI and its interaction with partner proteins, thus limiting its activity in vivo.[69] The NLS is another obvious target for peptide inhibitors as binding of peptides to this region will preclude the translocation of hLigI from the cytoplasm into the nucleus, thus limiting the interaction of hLigI with either its interaction partners (PCNA, RFC, etc.) or its substrate (DNA), both of which are present in the nucleus (see Fig. 9).

6. CONCLUDING REMARKS

Given the fact that combination therapies are more effective for cancer treatment than single agent therapy,[70] we believe that adding ligase inhibitors to the existing or new arsenal of anticancer (or antibiotic) compounds might prove to be the missing link in broad-spectrum anticancer (or antibiotic) therapy. Having said that, a lot of research still needs to go into discovering or designing ligase inhibitors that are specific and effective enough to be used in therapy.

ACKNOWLEDGMENTS

The authors thank the Department of Science and Technology (DST), India (grant no. SB/FT/LS-163/2012), Department of Biotechnology (DBT), India (grant no. BT/PR6421/GBD/27/436/2012), and the Council for Scientific and Industrial Research (CSIR), India (funds from BSC0106) for funding and support in writing this review. D.K.S., S.C., S.K., M.S., and A.D. thank CSIR for their JRF's (Junior Research Fellowships). We acknowledge Dr. M.I. Siddiqi, Scientist, Molecular and Structural Biology Division, CSIR-CDRI for his comments and suggestions in improving the manuscript. The CSIR-CDRI institutional number allocated for this manuscript is 8488.

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