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NAD^+ -dependent DNA ligase: A novel target waiting for the right inhibitor

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Abstract

DNA ligases (EC.6.5.1.1) are key enzymes that catalyze the formation of phosphodiester bonds at single stranded or double stranded breaks between adjacent 5'phosphoryl and 3' hydroxyl groups of DNA. These enzymes are important for survival because these are involved in major cellular processes like DNA replication/repair and recombination. DNA ligases can be classified into two groups on the basis of their cofactor specificities. NAD^+ dependent DNA ligases are present in bacteria, some entomopox viruses and mimi virus while ATP -dependent DNA ligases are ubiquitous. NAD^+ -dependent ligases have recently been drawing a lot of attention as novel targets with the potential to overcome current drug resistance issues. Currently a diverse range of inhibitors have been identified including several by our group. There are several issues to be addressed in the quest for optimized inhibitors of the enzyme. In the first part of the review we summarise current structural work on these enzymes. Subsequently we describe the currently available classes of inhibitors. We also address modalities to improve the specificity and potencies of new inhibitors which can be identified using protein structure based rational approaches. In conclusion, NAD^+ -dependent ligases show great promise and represent a novel drug target whose time has come.

1. Introduction

DNA ligases (EC.6.5.1.1) are key enzymes that catalyze the formation of phosphodiester bonds at single stranded or double stranded breaks between adjacent 5'phosphoryl and 3' hydroxyl groups of DNA. These enzymes are important for survival because they are involved in major cellular processes like DNA replication/repair and recombination[1]. Although widely-referred to as DNA ligases today, previously these enzymes have also been known as DNA joinase, DNA repair enzyme, poly (deoxyribonucleotide) ligase, polynucleotide ligase (ATP) or sealases *etc*.

DNA ligases can be classified into two groups on the basis of their cofactor specificities. NAD^+ -dependent DNA ligases are present in bacteria, some entomopox viruses and mimi virus while ATP -dependent DNA ligases are ubiquitous.[2, 3] NAD^+ -dependent ligases are known to be essential for bacterial growth and have recently drawn attention as novel drug targets.[4, 5] This has been aided by structure based rational inhibitor identification. Since there are many structural and functional commonalities between ATP- and NAD^+ ligases, we shall summarize the relevant structural work in the first part of the review. Subsequently we shall focus on recent developments in inhibitor identification, challenges faced therein and future prospects.

2. Mechanism of DNA ligases

The mechanism of ligase action can be broken down to three steps (see Fig.1).[6]

1. The activated AMP-Enzyme adduct is generated by the formation of a phosphoamide bond between AMP moiety of ATP or NAD^+ and α- amino group of an active site lysine residue by releasing PPi/NMN respectively .
2. The 5'-phosphate group of AMP is transferred from the active site lysine to the phosphorylated 5'end of the nick through a pyrophosphate bond (P'-P'). The AMP activates the 5' phosphate of DNA to form a phosphodiester bond.
3. During the third step the 3' hydroxyl group attacks on the 5' phosphorylated DNA end and forms covalent bond after displacing the AMP.

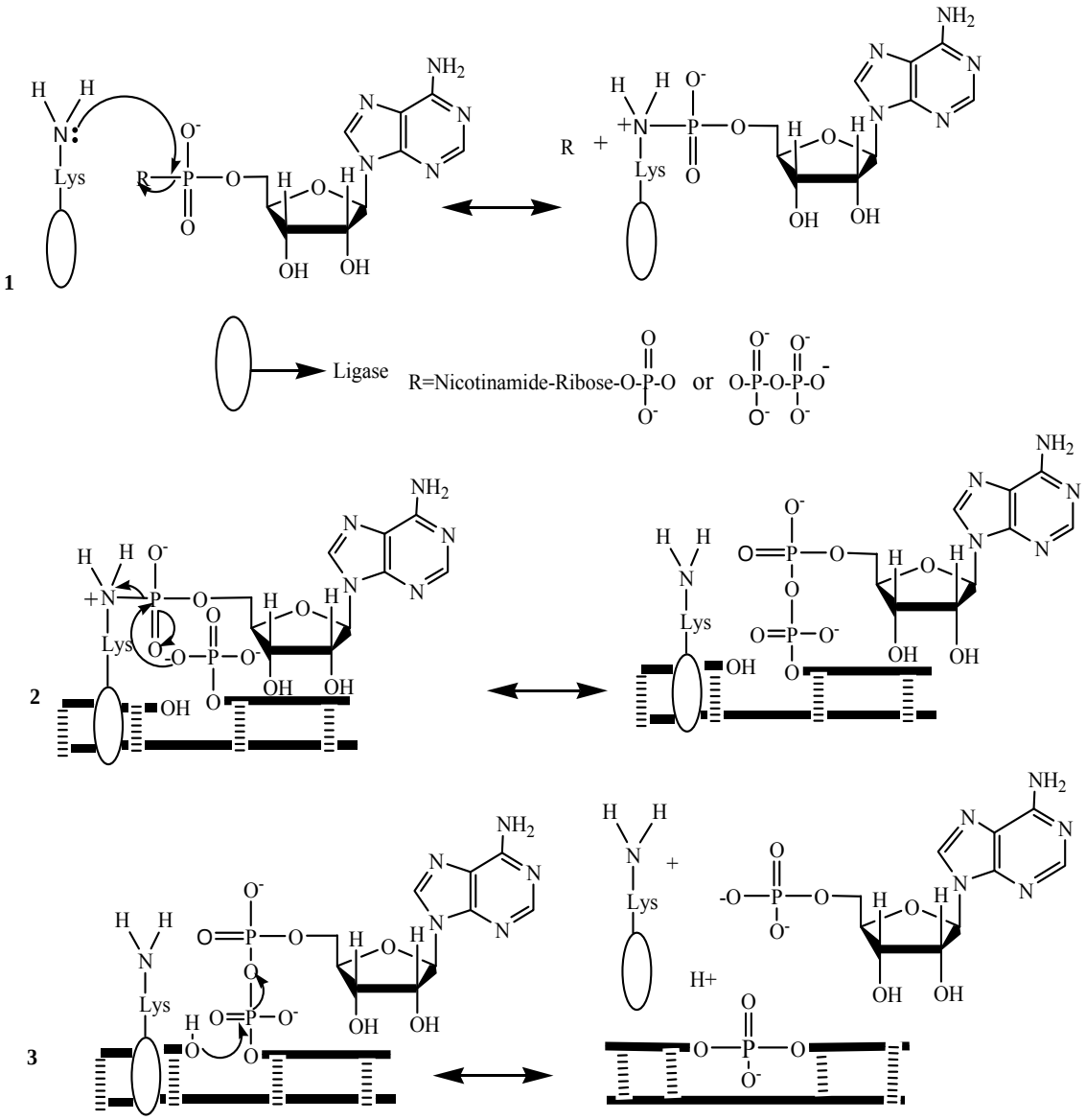

Figure1. The reaction mechanism of DNA ligases.

3. ATP -dependent DNA ligases

A. Eukaryotic ATP -dependent DNA ligases

ATP -dependent DNA ligases have been isolated from various sources and full-length genes of three different human isozymes, *viz.* DNA ligases I, III and IV have been cloned. [7a,b,c] These isozymes differ in sizeand also in their respective abilities to ligate nucleic acid substrates. DNA ligase III occurs in a variety of organisms including mammals while DNA ligases I and IV have been found in mice, humans, *S. cerevisiae*

and *A. thaliana*[8a]. While eukaryotic DNA ligases catalyze various specific functions inside the cell, DNA ligase IV is involved specifically in non-homologous end-joining processes.[8b]

DNA ligase I : ligates Okazaki fragments during lagging strand DNA replication events

DNA ligase III: forms complexes with the DNA repair protein XRCC1 and aids in sealing base excision mutations.

DNA ligase IV: forms complexes with XRCC4. It catalyzes the final step in the non-homologous end joining process. It is also required in the V(D)J recombination process, which generates diversity in immunoglobulin and T-cell receptor loci during immune system development.

The participation of DNA ligases in different DNA transactions is carried out by protein-protein interactions. In the following sections, we will focus on the protein partners of mammalian DNA ligases I, III, and IV identified to date (see Table.1)

Table. I Human DNA ligases and their partner proteins

Gene partners	protein		protein, partners
LIG1	DNA ligase I		PCNA, pol β , RFC
LIG3	DNA ligase III α	nuclear	XRCC1, PARP-1,NEIL-1
		mitochondrial	-
	DNA ligase III *β*		-
LIG4	DNA ligase IV		XRCC4

The genomes of archaebacteria code for at least one DNA ligase, and have been characterized from many thermophiles.[9] Among the best-studied DNA ligases are those encoded by various viruses.[6] Detailed biochemical analysis of the bacteriophage T4 DNA ligase has been carried out and the enzyme is widely used in molecular biology procedures that involve recombinant DNA. X-ray structures of DNA ligases from bacteriophage T7 and human sources have also been reported.[10]

B. Prokaryotic ATP dependent DNA ligases

ATP -dependent DNA ligases are now known to be found in all kingdoms of life.[2,3] The simplest DNA ligases, encoded by viruses, have a two-domain organization as

revealed by crystal structures of the enzyme from the T7 bacteriophage (Fig. 2).[11] The nucleotide-binding and OB-fold domains of these enzymes which occur adjacent to each other are structurally analogous to the catalytic core of more complex multi-domain DNA ligases found in bacteria.[11]

Some bacteria have multiple ATP -dependent DNA ligases; for instance, *M. tuberculosis* codes for three different such enzymes[12] *viz. ligB* (*Rv3062*), *ligC* (*Rv3731*) and *ligD (Rv0938)* respectively. It has been recently shown that the latter enzyme forms a non-homologous end joining repair 'machine' along with the Ku enzyme of the pathogen.[13] These ligases are in addition to the NAD^+ dependent enzyme, *ligA* (*Rv3014c*) of the pathogen.[4] *B. subtilis* harbors two ATP-dependent ligases, YkoU and YoqV, in addition to its NAD^+ dependent ligase YerG [14]. YoqV is contained within the lysogenic bacteriophage SPβ that is resident in this genome, and it is possible that other bacterial ATP dependent DNA ligases may have originated from this pathogen.

It is not completely clear as to why multiple copies of the ATP –dependent enzyme occur in bacteria. Eukaryotic DNA ligases are believed to function in specific pathways, such as DNA replication, repair or recombination. As many reactions occur during DNA metabolism in bacteria, the different bacterial DNA ligases may also function in specific pathways or during different times during the cell cycle or may be active under different growth conditions. There is some evidence that the isoforms are influenced differently by the sequence and structure of the DNA ends, [15] but it is important to identify whether such differences translate to any impact within bacterial cells. Both types of DNA ligases not found in all bacteria. For example, the genomes of many bacteria encode only a single NAD^+-dependent DNA ligase, but they can perform specific functions like DNA repair reactions in addition to their involvement in recombination and replication pathways. Nevertheless, despite the observation of multiple copies of the ATP –dependent enzyme in bacteria, NAD^+-dependent DNA ligases have been found to be essential in them.

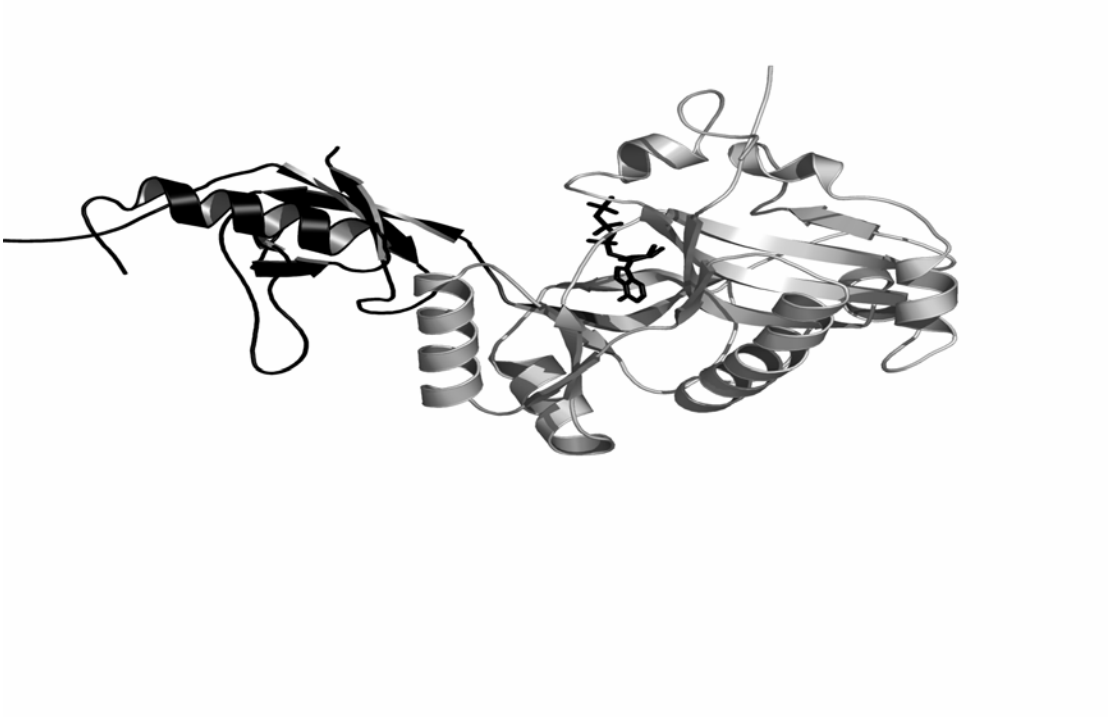

Figure 2. The ATP-dependent DNA ligase from bacteriophage T7 is a two-domain ligase: the adenylation or nucleotide-binding domain (dark) binds ATP and is connected to an OB-fold domain (light) by a flexible linker.

4. NAD^+-dependent DNA ligases

The first ligase that was purified biochemically was from *E. coli* containing 671 amino acid residues and encoded by the *ligA* gene.[6a] The deletion of the *E.coli* enzyme resulted in growth arrest of species but could be compensated by over-expression of the eukaryotic ATP -dependent enzyme.[16] DNA ligases with this co-factor specificity are not found in humans, so they are drawing attention as drug targets for the development of novel antibiotics.[17] Crystal structures of the adenylation domain of several bacterial ligases, including that from the *M. tuberculosis*, have been reported.[18, 4] The structure of *T. filiformis* DNA ligase (*Tfi*LigA)[19] (Fig. 3) is the only example of a crystal structure of an *apo* full length LigA. Recently the structure of *E. coli* enzyme complexed to nicked DNA was reported and this structure exhibited significant differences *vis-à-vis* the DNA binding mode of the human ligaseI[19]. Interestingly, the BRCT-domain in the C-terminus of the enzyme was found to be disordered in the *apo* and complexed structures.

The adenylation domain contains all the necessary residues for AMP/NAD^+ binding. The structural and biochemical work carried out so far have clarified various aspects of co-factor interaction. Important residues have also been identified through the studies.[4] The adenylation domain itself consists of two subdomains (Fig. 3). Subdomain 1a consists of residues 1–76 in the *M. tuberculosis* enzyme and contains residues involved in NMN recognition; this subdomain consists mainly of two helical stretches. The subdomain 1a is flexible and adopts different spatial orientations with respect to 1b in the various structures available. Subdomain 1b contains the AMP binding site and consists of residues 77–328 and contains many conserved co-factor binding features (Fig 4).

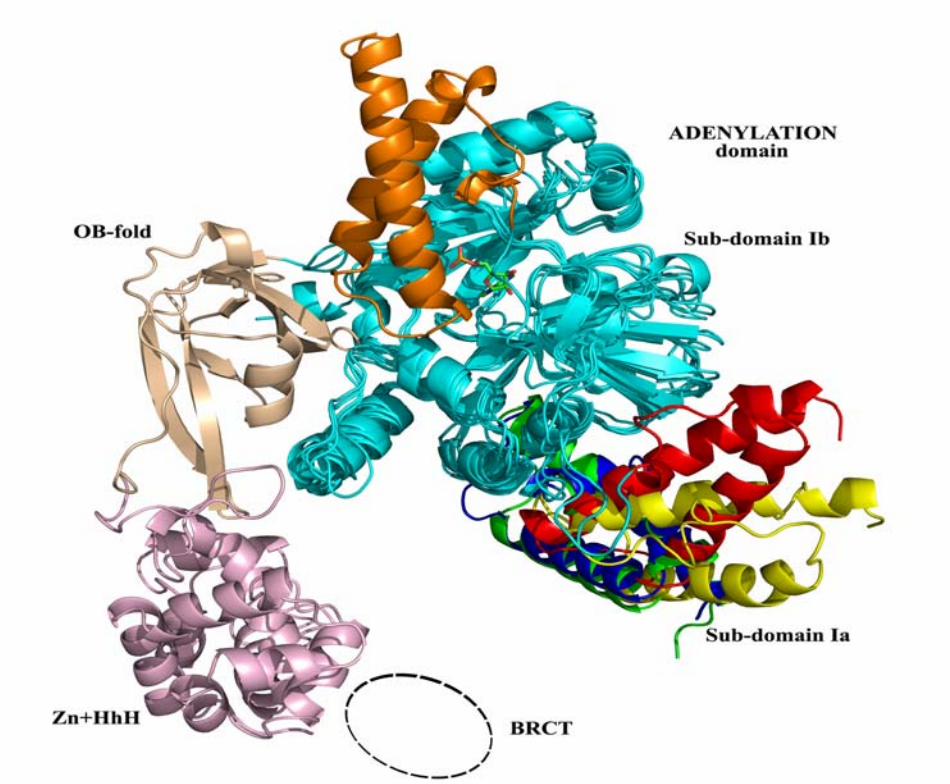

Figure 3. Superposition of NAD^+-dependent DNA ligase structures available in the Protein Data Bank (http://www.rcsb.org/pdb). The adenylation domains (Subdomain Ib is in cyan and Subdomain Ia is coloured as below) of full length ligase encoded by *T. filiformis* (red, PDB code: 1V9P) is superposed onto the adenylation domains of *M. tuberculosis* (yellow, 1ZAU), *B. stearothermophilus* (blue, 1B04), open and closed conformations of *E. faecalis* (green, 1TA8 & orange, 1TAE). *Homology* module implemented in InsightII (M/S Accelrys Inc.) was used for superpositions. The OB-fold and HhH domains are depicted in golden and light pink respectively. The BRCT domain at the C-terminus was found to be disordered in this structure as well as in the *E. coli* LigA-DNA complex[14]. It is indicated by a dotted balloon.

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Bst     ELKIDGLA -44- LEARGEAF -45- DLFVYGLADAEA -51- DGIVI -20- AIAYKFPAEEV
Efa     ELKIDGLA -44- VEVRGECY -46- TFLYTV-ADFGP -52- DGIVI -20- AIAYKFPPEEA
Tfi     EHKVDGLS -45- LEVRGEVY -47- TFYALGLGLEES -52- DGVVV -20- ALAYKFPAEEK
Mtu     ELKIDGVA -51- LEVRGEVF -46- MICHGL-GHVEG -51- DGVVV -20- AIAYKYPPEEA
Hum     EYKYDGQR -42- FILDTEAV -32- CLYAFDLIYLNG -52- EGLMV -17- WLKLKKDYLDG
           I            II           III            IV          V
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Fig. 4. Conserved Sequence motifs across NAD^+ -dependent DNA ligases. Five sequence elements, designated motifs I, III, III, IV, V, conserved in NAD^+- and ATP-dependent DNA ligases are shown. The alignment includes the enzymes from *M. tuberculosis* (*Mtu*), *T. filiformis* (*Tfi*), *B. stearothermophilus* (*Bst*), *E. faecalis* (*Efa*) and human ligases I (*Hum*). The numbers of amino acid residues separating the motifs are indicated. The active site lysine is shown in 'bold'.

5. Inhibitors of DNA ligases

DNA ligases are of paramount importance in many cellular reactions. Its pivotal role in DNA repair and replication as well as in recombination is well established. Gene knockout and other studies have shown that the enzyme is essential. Recent efforts, including those of our group (Figure 5), have resulted in the identification of many classes of ligase inhibitors and these are summarized in the next sections. The available evidence and body of work suggest that NAD^+ dependent ligases can serve as a valuable target in the development of chemotherapeutics for the treatment of numerous human ailments.[4,5] These therapies are expected to not only be specific but also selective as the enzyme is absent in eukaryotic genomes.

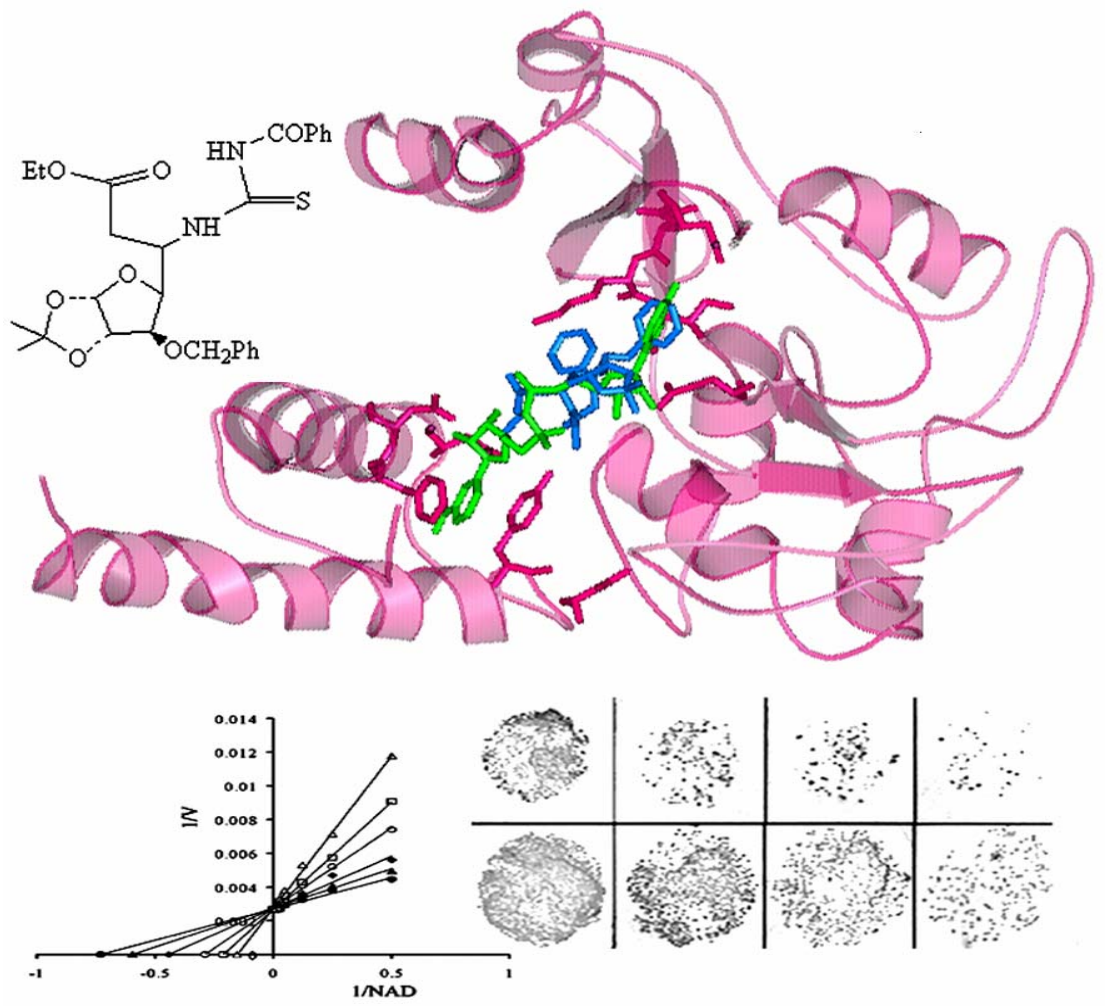
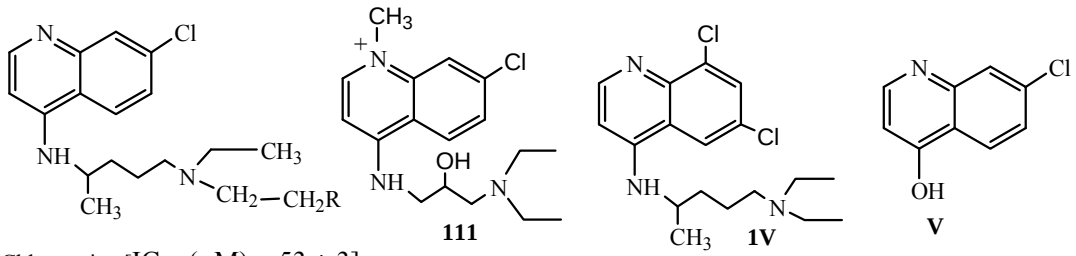


Figure 5. Adenylation site of the *M. tuberculosis* enzyme shown in pink. Modeled NAD^+ is shown in 'green' while a glycosyl ureide inhibitor identified by our group[4] through virtual screening is shown in 'blue'. The inhibitor was found to be a competitive inhibitor of NAD^+ as demonstrated by *in vitro* enzyme assays (bottom left panel). The compound exhibited higher inhibition of the *M. tuberculosis* enzyme (IC_{50} 4 μM) compared to human DNA ligase I (IC_{50} 127 μM) *in vitro*. Similarly, *S. typhimurium* LT2 cells harboring its natural NAD^+ ligase were more susceptible to the compound (top row, bottom right panel) compared to its null strain (bottom row of the panel) rescued by T4 ATP -dependent ligase. The columns of the panel correspond to the addition of the inhibitor in amounts corresponding to 0, 0.5, 1 and 2.5 MIC respectively (*See Ref. 4 for more details*).

A. Alkaloids

Many alkaloids from the simple quinoline, isoquinoline berberine and others are known for their inhibitory effects on NAD^+-dependent DNA ligases.

1. Quinoline analogues [20]



I.R=H, Chloroquine [IC_{50} (μM) = 53 ± 3]
II.R=OH, Hydroxychloroquine [IC_{50} (μM) = 63 ± 6]

I. Chloroquine. **II**. Hydroxychloroquine.e. **I11**. 1-Methy-7-Chloro-4(3-diethylamino-2-hydroxypropylamino)-quinoline. **IV**.6,8-Dichloro-4(4-diethylamino-1-methylbutylamino)-quinoline. **V**. 7-Chloro-4-amino quinoline

Figure 6. Quinoline derivatives

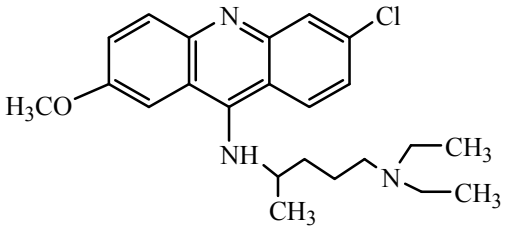
Various quinoline analogues have been tested for DNA ligase activity inhibition to date. Amongst these (*Fig 6 I-V*), chloroquine (*Fig 6 I*) and hydroxyquinoline (*Fig 5II*) are known as good DNA-binding agents [21]. Chloroquine is an effective inhibitor of the *E. coli* NAD^+ dependent enzyme but not of human and bacteriophage T4 ligases; for example it inhibits the *M. tuberculosis* enzyme with IC_{50} of about 46 μM. However it is not a competitive NAD^+ inhibitor and apparently acts by binding to other sites in the enzyme.[21c] The specificity factor, *viz* the ratio of IC_{50} values of bacteriophage and *E. coli* enzymes is >28 for this compound. Chloroquine is a diprotic weak base with pK_{a1}=8.1 and pK_{a2} =10.2 respectively. It was observed that activities against NAD^+ and ATP - dependent ligases increased with an increase in pH.

It has been observed that substitution of OH (**V**) for the amino group at the 4th position abolished the DNA interactions. [21] Methylation at 1 position (**III**) prevented the molecule from acting as a hydrogen bond donor. The 6, 8 dichloro compound (**IV**) showed increased ability to interact with DNA. The proton accepting nitrogen as a part of aliphatic side chain also assists binding.

Another quinoline derivative hydroxychloroquine (**II**) which is widely used as a non steroidal anti-inflammatory drug[22] has also been shown to be a specific inhibitor of the *E. coli* enzyme.

2. Quinacrin

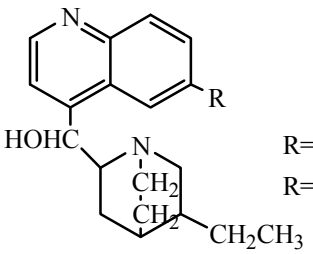
Quinacrine is a 9-aminoacridine (*Fig 7 VI*) that binds to DNA even more tightly than chloroquine. [23] Studies suggest that the quinoline ring structure is not very important for activity. Quinacrine is a potent inhibitor of the *E. coli* DNA ligase with IC_{50} of 1.5±0.2 μM. On the other hand, it exhibits 16-fold lower inhibition of the bacteriophage enzyme.



VI

Figuere 7. Quinacrine [IC_{50} (μM) = 1.5 ± 0.2]

3. Cinchondine and Quinine[20,21]

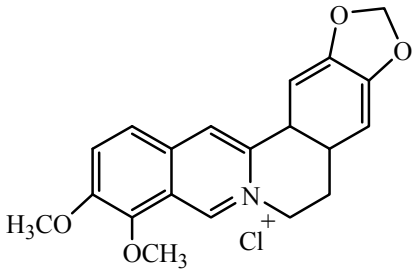


R=H , Cinchondine **VII** [IC_{50} (μM) >5000]
R=OCH_3 , Quinine **VIII** [IC_{50} (μM) > 2000]

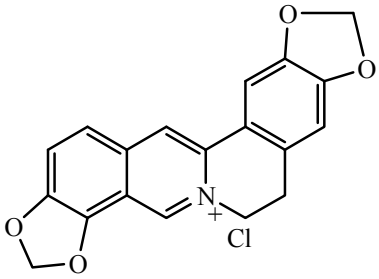
Figure 8. Cinchonodine and ananlogs

These compounds are also quinoline derivatives, but they do not inhibit ATP or NAD^+ -dependent ligases from *E. coli*. These have been included here for their relevance in inhibitor optimization; the results suggest that quinoline compounds lacking the diamine side chain (cinchonidine, quinine) lack inhibitory activity *eg*. The IC_{50} for quinine is > 2000 μM while that of cinconadine is >5000 (see Fig. 8).

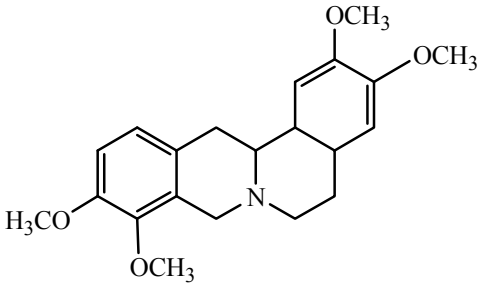
4. Protoberberine alkaloid



IX. Berberine Chloride [IC_{50} (µg/ml) > 200]



X. Coptisine Chloride [IC_{50} (µg/ml) > 200]



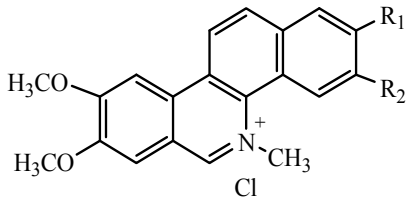
XI. Tetrahydropalmatine [IC_{50} (µg/ml) > 200]

Figure 9. Proberberine alkaloids

Protoberberine alkaloids (*Fig. 9 IX-XI*) which are structurally related to the benzophenanthridine alkaloids, have shown a wide range of biological activities including antimicrobial and antitumour activities.[24] Although berberine (*IX*) and coptisine chloride (*X*) has a quaternary nitrogen atom to interact with the nucleic acid [25] this alkaloid was inactive in the human ligase I with IC_{50} > 200 µg/ml. Tetrahydroberberine and tertahydropalmatine (*XI*) did not display any significant activity against human ligase I.

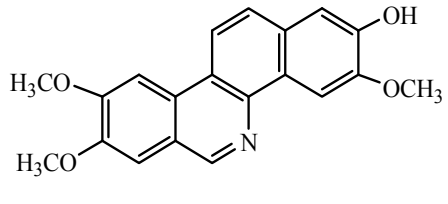
(What about activity against NAD+ ligases?)

5. Benzophenanthridine alkaloids

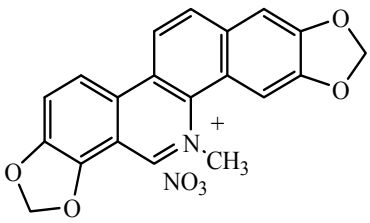


XII. .Fagaronine chloride R_1=OH, R_2= OCH_3 [IC_{50} (μg/ml) = 10]

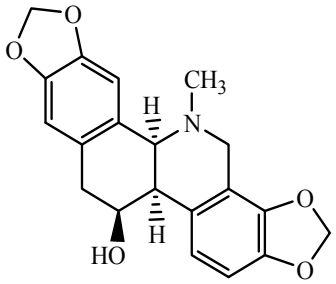
XIII. .Nitidine chloride R_1, R_2= OCH_2O [IC_{50} (μg/ml) =25]



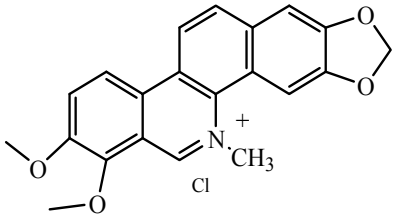
XIV. N-Demethyl Fagaronine [IC_{50} (μg/ml) > 200]



XV . Sanguinarine Nitrate [IC_{50} (μg/ml) = 322]



XVI. Chelidonine [IC_{50} (μg/ml) > 200]



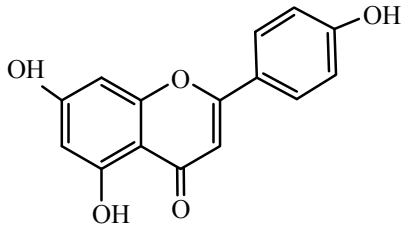
XVII. Chelerythrine Chloride [IC_{50} (μg/ml) = 75]

Figure 10. Benzophenanthridine alkaloids

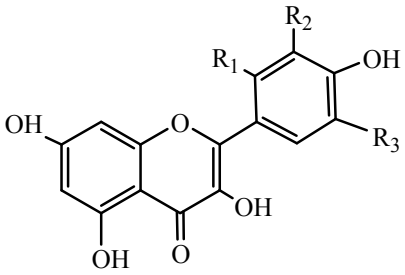
A number of benzophenanthridine alkaloids such as fagaronine chloride and nitidine chloride (see Fig. 10) have demonstrated significant cytotoxic activity against P-388 leukaemia in mice.[26] These alkaloids reportedly inhibit various RNA- and DNA-polymerizing enzymes *via* interactions with nucleic acid template-primers. [27]

The most potent ligation-inhibitory activity against human ligase I was demonstrated by fagaronine chloride and nitidine chloride, which exhibited [IC_{50} = 10 μg/ml (27 μM)]) and [IC_{50} = 25 μg/ml (69 μM)]. Sanguinarine (*XV*) and Chelerythrine Chloride (*XVII*) were considerably less active and exhibited [IC_{50} = 127 μg/ml (322 μM)]) and [IC_{50} = 75 μg/ml (226 μM)] only respectively. Chelidonine (*XVI*) and *N*-demethylfagaronine (*XIV*) were inactive, with IC_{50} > 200 μg/ml. This is presumably due to the absence of the quaternary nitrogen atom in the latter molecules.

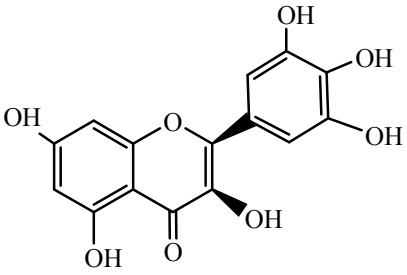
B. Flavonoids



XVIII. Apigenin [IC_{50} (µg/ml) > 200]



XIX. Mortin R_1=OH, R_2, R_3=H [IC_{50} (µg/ml) = 68]
XX Myrecitin R_1=H, R_2, R_3 = OH [IC_{50} (µg/ml) = 28]



XXI. Catecin [IC_{50} (µg/ml) > 200]

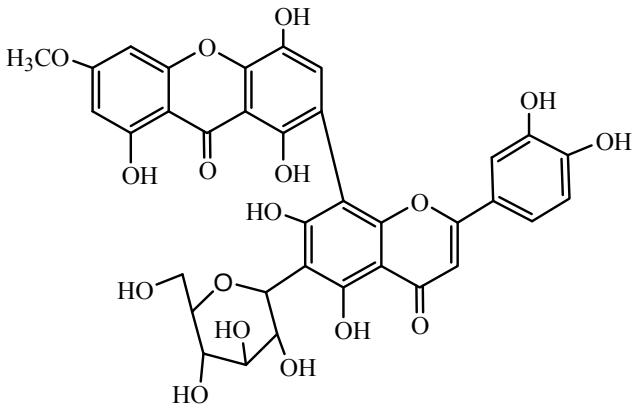
Figure 11. Flavanoids tested against Human DNA Lig 1

Flavonoids (*Fig.11*) are a class of low-molecular-mass phenolic compounds exhibiting a wide spectrum of pharmacological properties[28] including interference with a variety of mammalian enzyme systems. The molecular planarity of flavones, demonstrated by myricetin (*XX*) and morin, (*XIX*) is thought to facilitate the intercalation of these molecules into double-stranded nucleic acids. [29] Furthermore, the number of phenolic hydroxy groups on flavonoids bears a striking correlation with the enzyme inhibitory potential of these compounds.[30] Consistent with these observations, myricetin and morin were found to be more potent inhibitors of human ligase I ([IC_{50} = 28µg/ml (91 µM)]) and [IC_{50} = 68 µg/ml (236 µM)]) as compared with apigenin (*XVIII*) and (-) – catechin (*XXI*) which are inactive having IC_{50} values > 200 µg/ml.

C. Flavonoxanthone

Swertifrancheside (*Fig.12 XXII*) is a flavonoxanthone glucoside previously isolated from *Swertia franchetiana.* [31] The disruption of the ligation activity of human ligase I [IC_{50} = 8 µg/ml (11 µM)] correlated with the ability of the compound to interact with nucleic acids. [32] The decreased formation of DNA ligase-adenylate complexes in the

presence of swertifrancheside [IC_{50} = 76µg/ml ml (105 µM)] also suggests a possible inhibitory role of human ligase I binding



XXII [IC_{50} (µg/ml) = 8]

FIgure 12. Swetifrancheside

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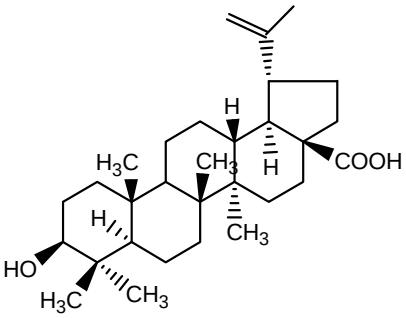
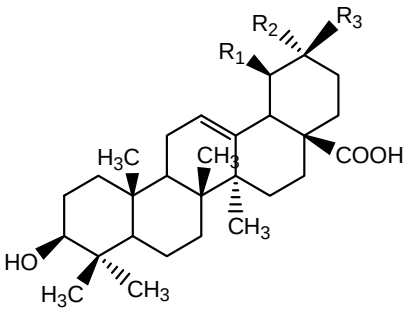
D.Triterpenes

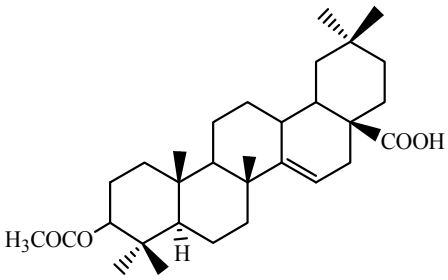
Two triterpenoids, ursolic and oleanolic acids (*Fig.13. XXV, XXIV*) were earlier isolated from *T. niamniamensis* (family rubiaceae). These compounds exhibited IC_{50} = 100 µg/ml (216 µM) and IC_{50} = 100 µg/ml (216 µM) respectively against human ligase I. On the other hand, betulinic acid, (*XXIII*) another triterpenoid is inactive against this enzyme having (IC_{50} >200 µg/ml),[31] which indicates specific structural requirements for the action of these molecules. Aleturolic acid (*XXVI*) having IC_{50} = 95 µg/ml (205 µM) also showed considerable ligation inhibitory activity



XXIII. Betulinic acid [IC_{50} (µg/ml) > 200]



XXIV. Oleanolic acid (R_1= H, R_2, R_3= CH_3) [IC_{50} (µg/ml) = 100]
XXV.Ursolic acid (R_1, R_2= CH_3, R_2, R_3= H) [IC_{50} (µg/ml) = 100]



XXVI..Aleuritolic acid [IC_{50} (µg/ml) = 95]

Figure 13. Triterpenoids analogs

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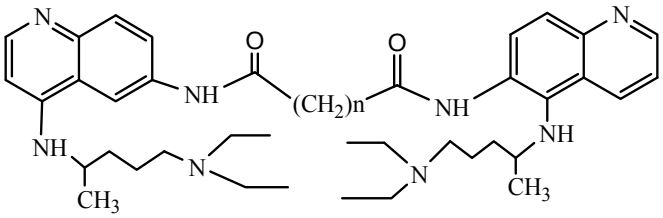
E. Bisquinoline compounds

The members of a series of novel bisquinoline compounds were prepared by reacting aliphatic diacid with 6-amino and 8-amino [(4-(4-(diethylamino)-1-methylbutyl) amino)-quinoline-6-yl]. [33, 34]

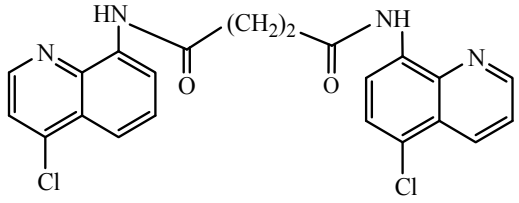
The compounds were tested against *E. coli* ligase and compound *N,N'*- bis[4-((4-(diethylamino)-1-methylbutyl)amino)-quinoline-6-yl]sabecamide, (*Fig.14. XXVII*) 6bisQ8 which has an eight-carbon linker showed good activity against the *E. coli* enzyme with IC_{50} (µM) 1.8±0.2. Two other members of this 6-linked bisquinoline series, with zero and six carbon spacers, showed very similar inhibitory characteristics. These compounds have IC_{50} (µM) 2.6±0.4, 2.2±0.3 respectively.

Similarly out of several members of 8-linked bisquinoline series, *N*, *N*'- bis[4-((4-(diethylamino)-1-methylbutyl)amino)-quinoline-8-yl]adipamide (8bisQ4) (*Fig.14. XXIX*) showed good activity and specificity against [IC_{50}(µM) 9±2] the bacterial ligase.[35] On the other hand, *N*, *N*'-bis[4chloroquinolin-8-yl]succinamide (*XXVIII*) showed no

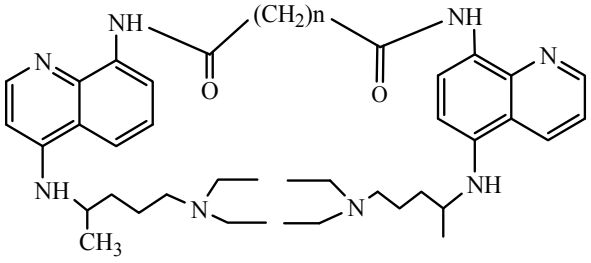
measurable inhibitory activity against DNA ligase suggesting that it is not the quinoline ring but the diamine side chain that is necessary for activity.



XXVII. 6bisQ n =0 [IC_{50} (μM) = 2.6±0.4.
n=6 [IC_{50} (μM) = 2.2±0.3.
n= 8 [IC_{50} (μM) = 1.8±0.2.



XXVIII. N, N'-bis(4-quinoline-8-yl)succinamide [IC_{50} (μM) >2000



XXIX. 8bisQn n=0 [IC_{50} (μM) = 10.2±2.
n=4 [IC_{50} (μM) = 9±2.

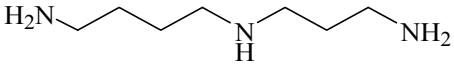
Figure 14. Bisquinoline analogs showing Eubacterial DNA ligase inhibitory activity

F. Simple polyamines

It was previously reported that polyamines[36] were known to stimulate ATP dependent DNA ligases and accordingly a few simple alkyl and aryl amines were tested against this particular enzyme. Putrescine, (*XXX*) the simplest alkyl amine with a NCCCCN structure, preferentially inhibited *E. coli* DNA ligase, with no significant inhibition of the T4 ligase being observed at the highest concentration examined. By contrast, 2-amino-5-diethylaminopentane (*XXXI*) enhanced the activity of the ATP-dependent T4 DNA ligase at concentrations above 2 mM.

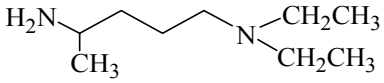
More recently, simple aryl amines[37] (see Fig. 15A), which showed considerable anti-tubercular activity, were tested against *E.coli* GR501 ligA[ts] temperature sensitive mutant rescued with the *M. tuberculosis* enzyme or alternatively with the T4 ligase. But

none of the compounds showed any inhibitory activity. The same results were noted against the *Salmonella typhimurium* LT2 and its DNA ligase minus mutant also.



Putrescine [IC_{50} (μM) = 92000±950]

XXX



2-Amino-5-diethylaminopentane [IC_{50} (μM) = 21,000±180]

XXXI

Figure 15. Simple alkyl amines

These data indicate that a structure with four carbon spacer separating two nitrogen atoms is the minimum inhibitory element and that this particular inhibitory moiety can be presented on a variety of scaffolds without losing its capacity for specific inhibition of the eubacterial ligase.

G. Anthracycline analogs [37]

The structural formula of compounds for which inhibition of human ligase activity was tested is shown in Fig. 16. Different classes of carbohydrate based anthracyclins were identified as inhibitors of this particular enzyme. Doxorubicin (*XXXII*) is a more potent inhibitor than its 4-demethoxy counterparts (*XLII*). On the other hand, daunorubicin (*XXXVIII*) is less potent compared to its 4-demethoxy derivative idarubicin (*XLV*). Again, no significant differences were found between the two very active 4'-deoxy (*XXXIV*, *XLIII*) and between the two inactive 3'-deamino-3'-hydroxy-4'-epihydroxy (*XXXVI*, *XLIV*) molecules. Doxorubicin which is an antitumor drug has previously been shown to be a potent inhibitor of ATP-dependent DNA ligases. [38] Using a slightly modified assay of poly (dA-dT) joining activity, it was confirmed that doxorubicin inhibits the ATP-dependent DNA ligase of bacteriophage T4 and also that it inhibits the *E. coli* NAD^+-dependent ligase with a similar potency.

Among 4'deoxy sugars *viz* (*XXXIV, XXXV*, *XXXIX*, *XLI*, *XLIII*, and *XLV*) which were tested, compounds having 3'amino 4'deoxy sugars (*XXXIV, XXXIX*, *XLIII*) exhibited more potent inhibition of human DNA ligase as compared to the 4' hydroxyl counterparts. The modifications in aglycon units have very little effect on the inhibitory activity. On the other hand, 4'deoxy 4' amino sugars with no modification on sugars

showed a low activity profile. While the inhibition patterns of 3'4'diaminosugars (*XLVI*) is similar to 3'amino 4'hydroxy counterparts (*XXXVIII*), substitution of the hydroxyl group with an iodine atom in 3' further reduced the activity.

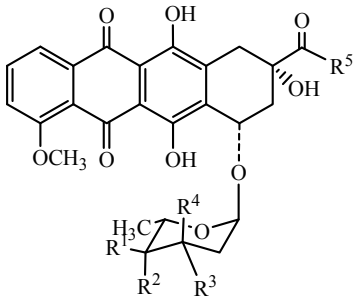
It was noted that the C-14 hydroxylation that transforms daunorubicin (*XXXVIII*) to doxorubicin (*XXXII*) had a positive effect on inhibitory activity. In contrast, 4-demethoxy molecules carrying these modifications at C-14 (*XLII*, *XLV*) exhibit reduced inhibition. In the case of very active 4'deoxy (*XXXIV*, *XXXIX*) and the inactive 3'deamino-3'-hydroxy-4'epihydroxy molecules (*XLIII*, *XLVIII*) no effects of C-14 modifications are evident.

Table 2 Showing effects against human replicative DNA ligase of tested antharacyclines

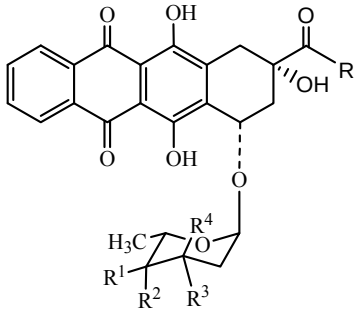
S.no	Compound	ID_{50} joinase activity (drug/bp)	ID_{50} Self adenylation (μM)
1	**XXXII**	0.055±0.006	690±50
2	**XXXIII**	0.041±0.005	450±85
3	**XXXIV**	0.025±0.003	570±70
4	**XXXV**	0.050±0.004	540±65
5	**XXVI**	1.475±0.350	>>1000
6	**XXXVII**	0.085±0.011	221±45
7	**XXXVIII**	0.120±0.023	>1000
8	**XXXIX**	0.023±0.003	327±51
9	**XL**	0.235±0.058	>1000
10	**XLI**	0.115±0.020	620±120
11	**XLII**	0.117±0.015	760±95
12	**XLIII**	0.022±0.003	>1000
13	**XLIV**	0.370±0.110	>>1000
14	**XLV**	0.049±0.012	n.d
15	**XLVI**	1.170±0.350	690±85
16	**XLVII**	1.170±0.045	65±17
17	**XLVIII**	0.780±0.210	>>1000
18	**XLIX**	0.725±0.200	n.d
19	**L**	0.128±0.039	770±100

When the activity of Doxorubicin (*XXXII*) was compared with derivatives containing inverted configuration of sugars (*XXV*) or bearing a hydroxyl group at the *epi* position (*XXXIII*), very little difference in the activity was observed. The activity of 4-demethyl-6-deoxydaunorubicin (*L*) was comparable to that daunorubicin. On the other hand, an

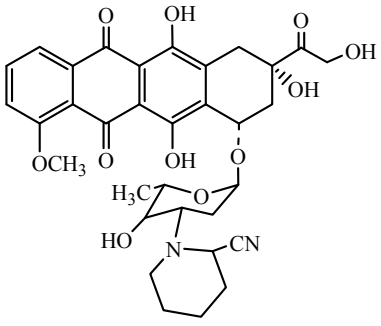
analogue with a modified amino group in the 3' position showed no significant inhibitory activity and (7R, 9R) -4' methoxydaunorubicin [(7R, 9R)-idarubicin] (*XLVI*) exhibits relatively less activity.



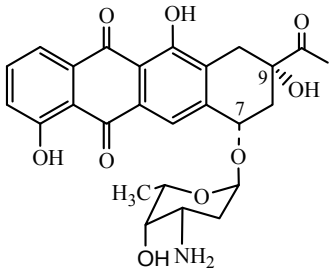
XXXII: R^1=H, R^2=OH, R^3=NH_2, R^4=H, R^5= CH_2OH
XXXIII: R^1=OH, R^2=H, R^3=NH_2, R^4=H, R^5= CH_2OH
XXXIV: R^1=R^2=H, R^3=NH_2, R^4=H, R^5= CH_2OH
XXXV: R^1=H, R^2=I, R^3=NH_2, R^4=H, R^5= CH_2OH
XXXVI: R^1=OH, R^2=H, R^3=OH, R^4=H, R^5= CH_2OH
XXXVII: R^1=H, R^2=NH_2, R^3=OH, R^4=H, R^5= CH_2OH
XXXVIII: R^1=H, R^2=OH, R^3=NH_2, R^4=H, R^5= CH_3
XXXIX: R^1=H, R^2=H, R^3=NH_2, R^4=H, R^5= CH_3
XL: R^1=H, R^2=NH_2, R^3=H, R^4=OH, R^5= CH_3
XLI: R^1=H, R^2=NH_2, R^3=NH_2, R^4=H, R^5= CH_3



XLII: R^1=H, R^2=OH, R^3=NH_2, R^4=H, R^5= CH_2OH
XLIII: R^1=H, R^2=H, R^3=NH_2, R^4=H, R^5= CH_2OH
XLIV: R^1=OH, R^2=H, R^3=OH, R^4=H, R^5= CH_2OH
XLV: R^1=H, R^2=OH, R^3=NH_2, R^4=H, R^5= CH_3
XLVI: Same as LVII but inversion of configuration at C-7, C-9
XLVII: R^1=NH_2, R^2=H, R^3=H, R^4=H, R^5= CH_3
XLVIII: R^1=OH, R^2=H, R^3=OH, R^4=H, R^5= CH_3



XLIX

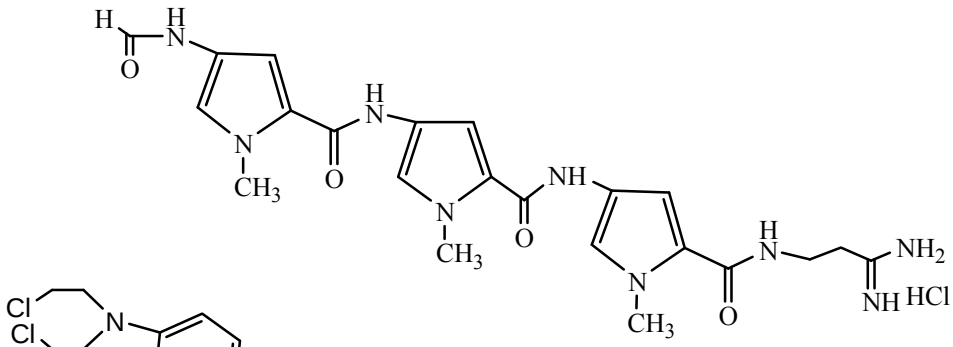


L

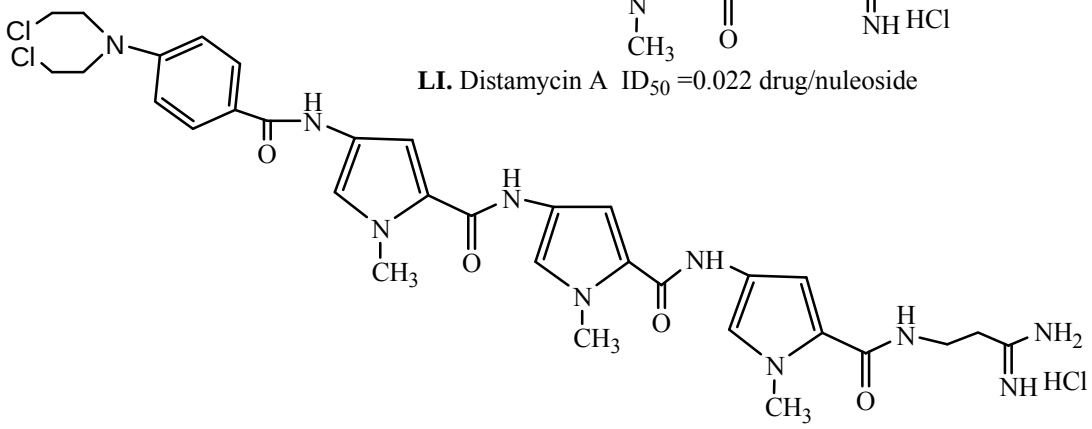
Figure 16. Anthracycline analogs as inhibitors of human DNA ligase

H. Distamycin A and its analogue

Distamycin A, is an oligopeptide obtained from *Streptomyces distallicus.*[39] It not only inhibits nucleic acid synthesis, but also binds to the DNA template. It prevents nucleic acid synthesis by inhibition of DNA and RNA polymerase as evidenced from *in vivo* studies. It acts by binding within the minor groove of AT-rich sequences of B-DNA[40] and directly affects the conformation of bound and flanking nucleotides. [41]



LI. Distamycin A ID_{50} =0.022 drug/nuleoside



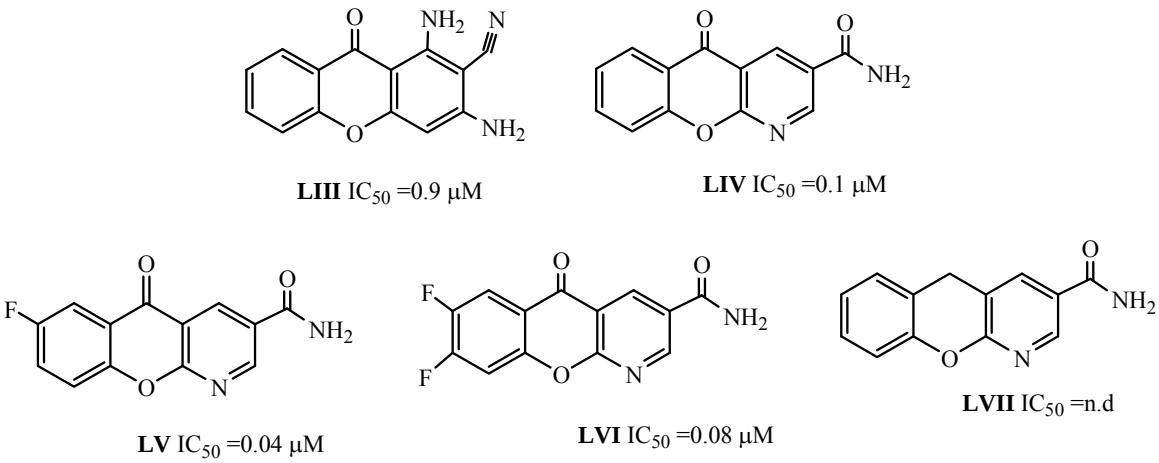
LII. FCE 24517 ID_{50} =0.032 drug/nuleoside

Figure 17. Distamycin and its analogue

The compound FCE24517 which is similar to distamycin was synthesized by treatment of phenyl mustard residues to the N-terminus of distamycin A.[42] The molecule is effective against a broad spectrum of murine and human tumors, including those which are resistant to alkylating agents.[43] The two drugs are enzyme specific and they interact with the DNA and are responsible for cell lysis. These compounds (Fig. 17) were reportedly tested against human DNA ligase and the results suggest that FCE24517 can preferentially act on DNA ligase while distamycins can bind within the minor groove of DNA.[42,44] The ligase inhibition activity of the two oligopeptides was confirmed by *in vitro* assays involving a poly d(AT) substrate. The compounds were also observed to inhibit the bacteriophage T4 DNA ligase (ID_{50}: 0.022 and 0.032 for distamycin A and FCE24517 expressed in drug /nucleoside respectively, ID_{50} accounts for the dose causing 50 % of maximum inhibition) suggesting that the strong inhibition of the overall ligation reaction is probably mediated by an interaction with the substrate rather than with the protein itself. These results agree with the conclusion that was derived from studies of anthracycline derivatives where it was suggested these molecules bind with minor groove of DNA and thus inhibit DNA ligase activity.[45]

I. Pyridochromanones

Pyridochromanones (Fig.18) were identified as powerful inhibitors of the *E. coli* NAD^+-dependent DNA ligase and exhibited good inhibition at nanomolar concentrations[46]. In contrast, purified human DNA ligase I was not affected (IC50 > 75 μM), demonstrating remarkable specificity for the prokaryotic target.



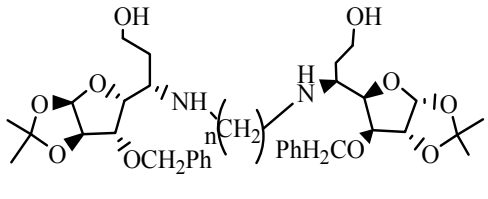
Figurer 18. Pyridochromanones derivatives

The compounds bind competitively to the NAD^+ binding site and furthermore no intercalation into DNA was detected. The compounds exhibited bactericidal activity against the prominent human pathogen *Staphylococcus aureus* in the low μg/ml range, whereas eukaryotic cells were not affected up to 60μg/ml. The hypothesis that inhibition of DNA ligase is the antibacterial principle was proven in studies involving a temperature-sensitive[47,48] ligase-deficient *E. coli* strain. In summary, the pyridochromanones demonstrated that a single inhibitor can address diverse eubacterial DNA ligases without affecting eukaryotic ligases or other DNA-binding enzymes.

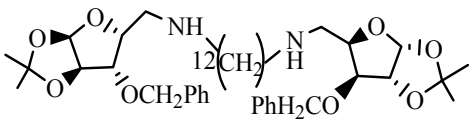
J. Glycosyl amines and ureides

The crystal structure of the adenylation domain of *M. tuberculosis* DNA ligase solved by us recently has been used in virtual screening experiments to identify classes of molecules that can distinguish between related binding sites in NAD^+ and ATP ligases.[4,5] Novel compound classes including glycosyl ureides and glycosylamines were

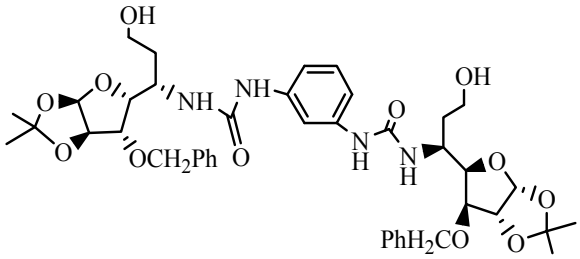
identified as inhibitors of the enzyme in this exercise.[4,5] Out of several compounds tested we identified about seven compounds (Fig.19) which were able to distinguish between bacterial and human ligases. These identified compounds were synthesized as per our earlier reported protocols. [49-53]



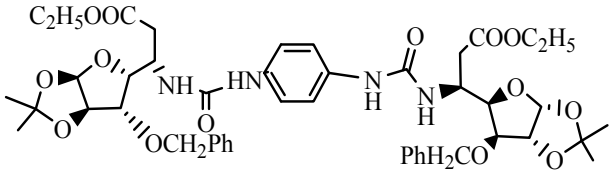
LVIII n =3, IC_{50} =46.2 ±1.6 μM
LIX n=10 IC_{50} =260 ±4.0 μM



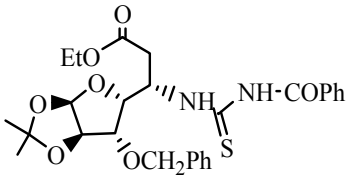
LX IC_{50} =11.4 ±1.2 μM



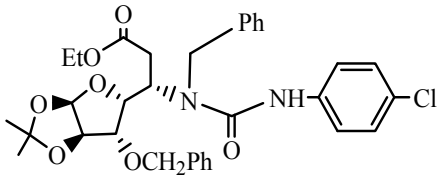
LXI 1,3-Phenylene IC_{50} =85.0 ±1.3 μM



LXII, 1,4-Phenylene IC_{50} =225 ±62 μM

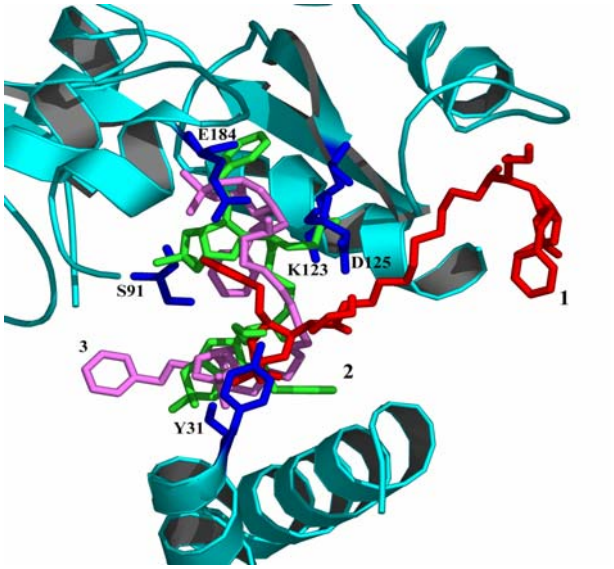


LXIII IC_{50} =4.0 ±03 μM

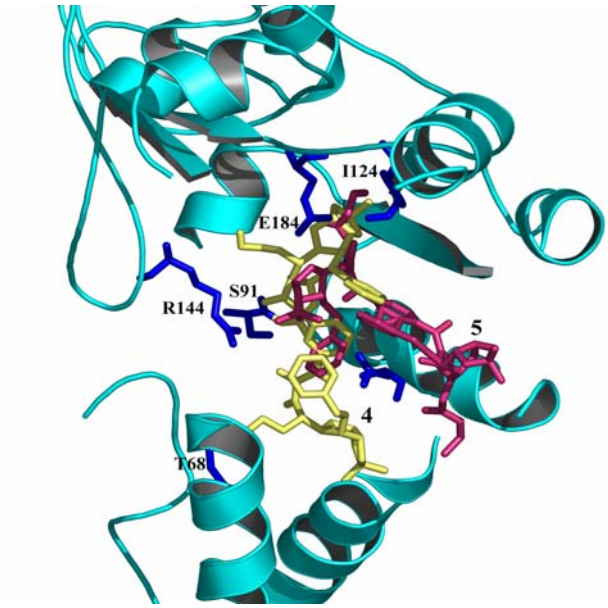


LXIV IC_{50} =9.65 ±0.5 μM

Figure 19. In vitro inhibition of *M. tuberculosis* NAD+ -dependent DNA ligase, by the respective compounds



(A)



(B)

Figure 20. (A) Interactions of compounds 1, 2 and 3 (those with aminoalkyl spacers) in the NAD^+ binding site of the enzyme from *M. tuberculosis*. The compounds are shown in green, red and pink respectively. Key interacting residues are shown as blue sticks and labeled for clarity. (B) Interactions of compounds 4 and 5 (those with phenylene carbamoyl spacers) in the NAD^+ binding site of the *M. tuberculosis* enzyme. The compounds are depicted in yellow and pink, respectively.

The identified glycofuranosylated diamines could be divided further into two subclasses *viz*. *bis* xylofuranosylated diamines with aminoalkyl spacers compounds

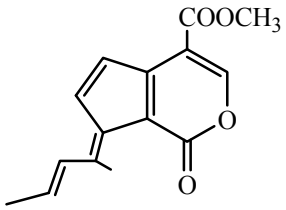
LVIII, LIX and LX (**1, 2, 3**) and those with phenylene carbamoyl based spacers (compounds **4** and **5)** respectively (Fig. 19). Docking experiments (*Fig 20*) suggest that they interact with several essential residues in the NAD^+ binding site among other interactions. Compounds with aminoalkyl spacers (**1**, **2** and **3**), *LVIII* and *LX* have predicted electrostatic interactions with K123 and E184. They also interact with essential residues like Y31 and D45 from subdomain 1a which binds to the NMN moiety of NAD^+ Compound *LIX* however exhibits polar interactions only with Y31, D41 and D45 in the NAD^+ binding site although it exhibits *van der Waals* contacts with E184 also. These are in addition to other interactions with conserved residues not directly interacting with bound NAD^+.

Among the compounds with phenylene carbamoyl spacers *viz*. compounds *LXI* and *LXII* respectively, the former interacts more extensively with the residues involved directly and indirectly in NAD^+ recognition. The interactions include those with the essential E184 and D45 residues. Compound *LXII* on the other hand has less extensive interactions with residues in the NAD^+ binding site, although it also interacts with E184 and D45. Of the two compounds *LXI* and *LXII* with phenylene carbamoyl based spacers, compound *LXII* with the 1, 4-linker had higher affinity for the ATP-dependent enzymes while compound *LXI* with the 1,3-linker could distinguish between the human and *M. tuberculosis* enzymes exquisitely and exhibited a higher affinity for the latter.

Of the three *bis* xylofuranosylated diamines with 3, 10 and 12 carbon spacers respectively, compound *LVIII* with 3-carbon spacer was able to distinguish NAD^+ and ATP-dependent ligases up to 9-fold and exhibited specificity for the *M. tuberculosis* enzyme. Compound *LX* could also distinguish between the human and *M. tuberculosis* enzymes by a factor of 2 and has higher affinity for the latter with IC_{50} values in the low micromolar range. Compound *LIX*, on the other hand, inhibited ATP-dependent enzymes from human and bacteriophage sources better.

The identified glycosyl ureide-based inhibitors (*LXIII, LXIV*) were also able to distinguish between NAD^+- and ATP-dependent ligases quite well *in vitro*. They inhibited *MtbLigA* in the single digit micromolar range, whereas the major human DNA ligase I was inhibited in the 100–200 μM range.[11] Optimization and synthesis of derivatives are in progress.

K. Pyrones

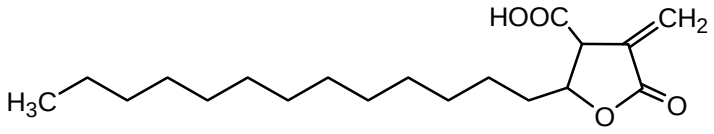


LXV IC_{50} (μM) = 357

Figure 21. Fulvoplumierin

Many pyrones are known to inhibit DNA ligases[54]. Fulvoplumierin (see *Fig 21*) iridoid obtained from *Plumeria rubra*[55] has previously been shown to inhibit HIV-1 [IC_{50} = 98 μg/ml (400 μM)] and HIV-2 [IC_{50} = 87 μg/ml (357 μM)] RTs to similar extents.[56] Interestingly, adenylation and the ligation activities of human ligase I were inhibited, with similar IC_{50} = 87 μg/ml (357 μM). The observed inhibition of activity against human ligase I and the unrelated HIV RTs, implies that fulvoplumierin interacted with structural features common to both enzyme proteins.

L. Protolichesterinic acid [57]

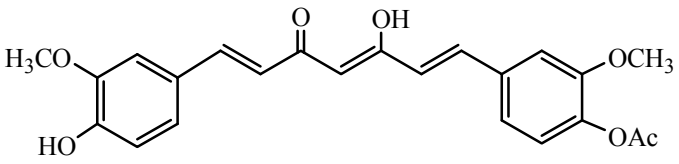


LXVI IC50 (μM) = 387

Figure 22. Protolichesterinic acid

The compound was reportedly obtained from a lichen *Centralia islandica* and is an aliphatic α-methylene-β-lactone (Fig. 22). It has been observed to interfere with ligation [IC_{50} = 6 μg/ml (20 μM)] and adenylation [IC_{50} = 116 μg/ml (387 μM)] activities of human ligase I..[58]

M. Curcumin derivatives



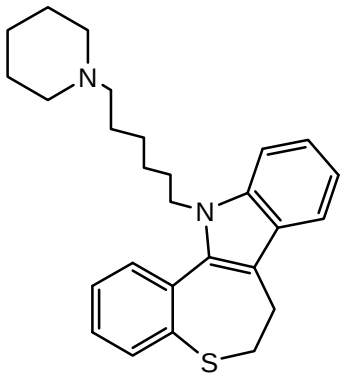
LXVII

Figure 23 Monoacetylcurcumin

Out of the several compounds[59] tested (see Fig. 23) monoacetylcurcumin (*LXVII*) was reported to inhibit human pol λ at concentrations of 1–10 μm. This class of compounds is the first one which is specific to mamamalian pol λ. Since the specificity was extremely high, compound *LXVII* could be useful as a pol λ-specific inhibitor because it inhibit pol λ activity indirectly by acting at the BRCT domain.[60]

It has been shown that the BRCT domain of pol λ is involved in interaction with non-homologous end joining (NHEJ) factors, and therefore, it can be predicted that compound 13 inhibits the capacity of pol λ to participate in an NHEJ mechanism. At present, the BRCT domain structure of pol λ has not been determined by X-ray crystal or nuclear magnetic resonance analysis, however, the three-dimensional structures of the BRCT domains in human XRCC1, bacterial NAD^+-dependent DNA ligase and human DNA ligase III α are available. [61] Compounds which bind to the BRCT-domain of the NAD^+ dependent ligase should have novel modes of enzyme activity inhibition. These compounds offer promise and need to be evaluated further as specific ligase inhibitors.

N. Tetracyclic indoles



LXVIII IC50 (μM) 13.5 ± 0.6

Figure 24. Tetracycline indole derivative

Following virtual screening experiments, several N-substituted dihydrobenzothiepino dihydrobenzoxepino & tetrahydro benzocyclohepta indoles were evaluated for inhibition of ligase activity of the *M. tuberculosis* and T4 enzymes by one of our groups [62]. Out of the several tested compounds N-substituted tetracyclic indoles (*LXVIII Fig. 24*) were identified as a novel class of inhibitors that competes with NAD^+ and inhibits the enzyme with IC_{50} in the low μM range. It exhibited around 15-fold better inhibition of the *M. tuberculosis* enzyme compared to human DNA ligase I. *In vivo* assays using ligase deficient *S. typhimurium* and *E. coli* strains suggest that the observed antibacterial activity of the inhibitor arises from specific inhibition of NAD^+ dependent ligases over ATP ligases in bacteria. *In silico* ligand-docking studies suggest that the exquisite specificity of the inhibitor arises on account of its mimicking the interactions of NAD^+ with the enzyme.

O. Succinyl acetone

The precise influence of succinyl acetone on different mammalian DNA ligases was reportedly determined to correlate the level of activity with defects observed in HT1 cells (Hereditary tyrosinemia type I) [63.] These results suggest that accumulation of succinyl acetone reduces the overall observed ligase activity in HT1 cells. It also indicates that metabolism errors may play a role in regulating enzymatic activities involved in DNA replication and repair.

The first step of the ligation reaction is the formation of a covalent ligase–AMP intermediate with a lysine-adenylate phosphoamide bond. The influence of succinyl acetone on this step of the reaction was measured by incubating the T4 DNA-ligase for 15 min at 25°C with increasing compound concentrations. The formation of the DNA-ligase adenylate complex is inhibited in the presence of the compound. The amount of adenylylated protein decreases with increase in succinyl acetone concentration with an IC_{50} of about 20 mM, showing that the inhibition occurs at the first step of the ligation reaction.

6. Strategies in inhibitor design and future prospects

DNA ligases are essential elements in many DNA metabolic pathways in bacterial cells, but their mode of interaction with other components of these reactions is largely unknown. Bacterial NAD^+-dependent DNA ligases have been studied for more

than 30 years, but surprisingly few genetic and biochemical details are known about their regulation. The recent determination of several DNA ligase structures by X-ray crystallography has provided an increasingly detailed understanding of the molecular mechanisms and conformational changes involved in the three-step ligation reaction. However, more experiments are needed to fully understand different mechanistic aspects. Significant breakthroughs in studies of DNA ligases have occurred as a result of the utilization of a broad range of techniques encompassing molecular biology and protein biochemistry. Application of these techniques to proteins from a wider range of organisms will, undoubtedly, provide further insights into these ubiquitous and important enzymes.

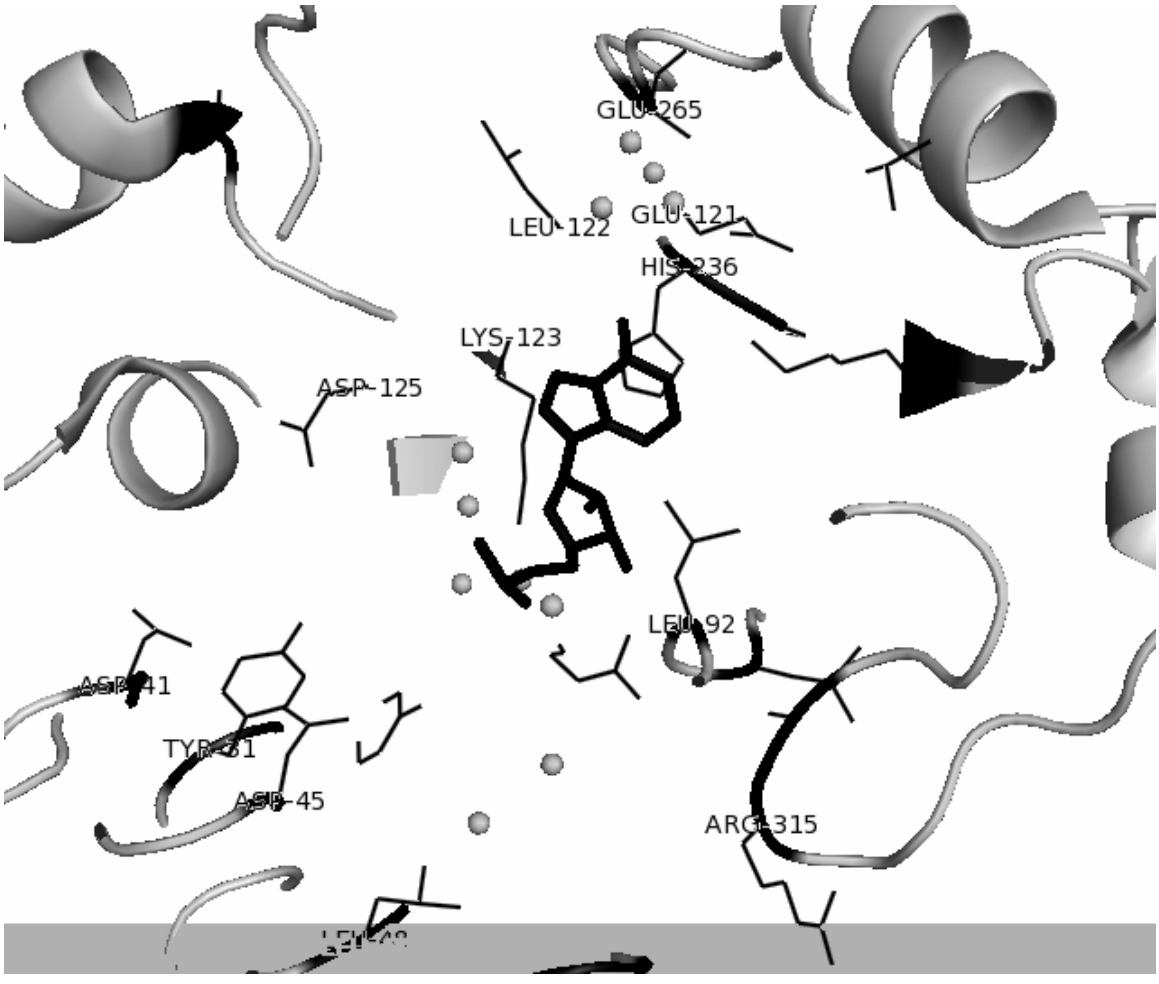


Figure 25. The active site of LigA with cofactor AMP from the *M. tuberculosis* structure (PDB: 1ZAU) is shown. The circles indicate water clusters which are being exploited for *de novo* inhibitor design[62].

Since DNA ligases in bacteria and eukaryotes use different cofactors these are being considered as a potential targets to be explored. In this respect, a diverse range of inhibitors has been identified against DNA ligases. Our group in the first instance has searched for diverse compound families which inhibit *Mtb*LigA with several fold specificity compared to ATP-dependent ligases including for the human DNA ligase I. and have successfully identified three novel classes of compounds which possess IC_{50} values in the low μM range. In the quest for identifying better LigA inhibitors, two important issues must be taken into account. Firstly the identified inhibitors should be able to discriminate between NAD^+ and ATP -dependent ligases and secondly the inhibitor should possess strong specificity towards the target species rather than being a broad-spectrum anti-bacterial compound. The present crop of inhibitors mostly block the binding of the co-factor and thereby the enzyme activity although other modes of enzyme inhibition can also be possible. Given the conserved nature of the co-factor binding site, expectedly most of the inhibitors exhibit some degree of general anti-bacterial activity too. Therefore better approaches should be employed for improving the specificity of the identified compounds. Two such approaches being pursued by our group for *MtbLigA* involve utilizing active site water where inhibitors designed to mimic the interactions of displaced water oxygen are expected to be better than the first generation of inhibitors. In an analysis of available structural information on LigA, we identified several water clusters at the adenylation site of potential interest for inhibitor optimisation[62] (Figure 25). The region occupied by the cluster at the top was also identified for development of new structure based inhibitor design in the recent *E. coli* LigA–DNA complex structure by the authors[14]. The other approach involves the development of inhibitors, which can disrupt other steps of the mechanistic cycle by binding to other regions of the molecule.

It is important in this context to evaluate further already identified inhibitors like those enumerated here, for their ability to distinguish between ATP and NAD^+ -dependent ligases. It should then be possible to design derivatives of these parent inhibitors with improved properties using recently identified principles. Where it has not

been already carried out, it should be probed as to which step of the catalytic cycle is affected by the inhibitor for development of inhibitors capable of affecting different steps of the catalytic cycle. We recently demonstrated that the BRCT domain of MtbLigA is essential for bacterial survival in contrast to LigA from *E. coli* and *T. thermophilus* sources.[62] Compounds designed to bind to this domain to prevent the interactions of its key residues should have better specificity for a given pathogen because of the suggested finer variations in individual enzyme action.

In conclusion, NAD^+ -dependent ligases show great promise for the development of novel chemical entities with the potential to overcome current drug resistance issues. These enzymes represent a novel drug target whose time has come.

8. Acknowledgements

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10. Legends to the figures

Figure1. Reaction mechanism for DNA ligases.

Figure 2. The ATP-dependent DNA ligase from bacteriophage T7 is a two-domain ligase: the adenylation or nucleotide-binding domain (cyan) binds ATP and is connected to an OB-fold domain (green) by a flexible linker.

Figure 3. Superposition of NAD^+-dependent DNA ligase structures available in the Protein Data Bank (http://www.rcsb.org/pdb). The adenylation domains (Subdomain Ib is in cyan and Subdomain Ia is coloured as below) of full length ligase encoded by *T. filiformis* (red, PDB code: 1V9P) is superposed onto the adenylation domains of *M. tuberculosis* (yellow, 1ZAU), *B. stearothermophilus* (blue, 1B04), open and closed conformations of *E. faecalis* (green, 1TA8 & orange, 1TAE). *Homology* module implemented in InsightII (M/S Accelrys Inc.) was used for superpositions. The OB-fold and HhH domains are depicted in golden and light pink respectively. The BRCT domain at the C-terminus was found to be disordered in this structure as well as in the *E. coli* LigA-DNA complex[14]. It is indicated by a dotted balloon.

Fig. 4. Conserved Sequence motifs across DNA ligases. Five sequence elements, designated motifs I, III, III, IV, V, conserved in NAD^+- and ATP-dependent DNA ligases are shown. The alignment includes the NAD^+-dependent ligases encoded by M.tuberculosis (*Mtu*), T.filiformis (*Tfi*), B.stearothermophilus (*Bst*), E.faecalis (*Efe*) and human ligases I (*Hum*). The numbers of amino acid residues separating the motifs are indicated. The active site lysine is shown as red.

Figure 5. (I). Compound's structure is shown in stick representation. (II). Adenylation site in MtbLigA shown in pink. Modelled NAD^+ is shown in green while a glycosyl ureide inhibitor is shown in blue. The inhibitor was found to be a competitive inhibitor and apparently mimics the interactions of NAD^+. It also demonstrated greater efficacy against cells harboring NAD^+ ligase compared to those harboring only ATP -dependent ligase (*See Ref. 4 for more details*). (III). The double-reciprocal plot is an indicant of competitive inhibition of the enzyme by the compound. (IV). The *in vivo* bactericidal activity of the compound is shown by reduced cfu units of *S .typhimurium* strain.

Figure 6. Quinoline derivatives

Figure 7. Quinacrine

Figure 8. Cinchondine and analogs

Figure 9. Proberberine alkaloids

Figure 10. Benzophenanthridine alkaloids

Figure 11. Flavanoids tested against Human DNA Lig I

Figure 12. Swetifrancheside

Figure 13. Triterpenoids analogs

Figure 14. Bisquinoline analogs showing eubacterial DNA ligase inhibitory activity

Figure 15. Simple polyamines

Table 2 Showing effects against human replicative DNA ligase of tested antharacyclines

Figure 16. Anthracycline analogs as inhibitors of human DNA ligase

Figure 17. Distamycin and its analogue

Figurer 18. Pyridochromanones derivatives

Figure 19. In vitro inhibition of M. tuberculosis NAD+ -dependent DNA ligase by the respective compounds

Figure 20. (A) Interactions of compounds 1, 2 and 3 (those with aminoalkyl spacers) in the NAD^+ binding site of the enzyme from *M. tuberculosis*. The compounds are shown in green, red and pink respectively. Key interacting residues are shown as blue sticks and labeled for clarity. (B) Interactions of compounds 4 and 5 (those with phenylene carbamoyl spacers) in the NAD^+ binding site of the *M. tuberculosis* enzyme. The compounds are depicted in yellow and pink, respectively.

Figure 21. Fulvoplumierin

Figure 22. Protolichesterinic acid

Figure 23. Structure monoacetylcurcumin

Figure 24. Tetracycline indole derivative

Figure 25. The active site of LigA with cofactor AMP from the *M. tuberculosis* structure (PDB: 1ZAU) is shown. The circles indicate water clusters which are being exploited for *de novo* inhibitor design[59].