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Targeting DNA Polymerase β for Therapeutic Intervention

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Abstract

DNA damage plays a causal role in numerous disease processes. Hence, it is suggested that DNA repair proteins, which maintain the integrity of the nuclear and mitochondrial genomes, play a critical role in reducing the onset of multiple diseases, including cancer, diabetes and neurodegeneration. As the primary DNA polymerase involved in base excision repair, DNA polymerase β (Polβ) has been implicated in multiple cellular processes, including genome maintenance and telomere processing and is suggested to play a role in oncogenic transformation, cell viability following stress and the cellular response to radiation, chemotherapy and environmental genotoxicants. Therefore, Polβ inhibitors may prove to be effective in cancer treatment. However, Polβ has a complex and highly regulated role in DNA metabolism. This complicates the development of effective Polβ-specific inhibitors useful for improving chemotherapy and radiation response without impacting normal cellular function. With multiple enzymatic activities, numerous binding partners and complex modes of regulation from post-translational modifications, there are many opportunities for Polβ inhibition that have yet to be resolved. To shed light on the varying possibilities and approaches of targeting Polβ for potential therapeutic intervention, we summarize the reported small molecule inhibitors of Polβ and discuss the genetic, biochemical and chemical studies that implicate additional options for Polβ inhibition. Further, we offer suggestions on possible inhibitor combinatorial approaches and the potential for tumor specificity for Polβ-inhibitors.

Keywords

Base excision repair; chemotherapy; DNA polymerase β; lyase; polymerase; protein-protein interactions; small-molecule inhibitors

INTRODUCTION

DNA polymerase β (Polβ) is a member of the X family of DNA polymerases [1, 2]. The *POLB* gene spans 14 exons across 33 kb and is localized to chromosome 8. A summary of genetic and physical characteristics of Polβ, along with links to several databases with additional details on Polβ, is shown in Table 1. Polβ has been implicated in several cellular

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functions, including genome stability [3], telomere maintenance [4–6] and meiosis [7]. Defects in Polβ have been linked with cancer [8, 9], aging [10], neurodegeneration [11, 12] and its expression is critical for the cellular response to environmental and chemotherapeutic genotoxins [13]. This latter function involves its primary role as the major DNA polymerase in the base excision repair (BER) pathway. A model for the BER proteins involved in the repair of temozolomide (TMZ)-induced lesions is depicted in Fig. (1), along with the chemical nature of each repair intermediate. In mammalian BER, a damaged base residue, such as those induced by the chemotherapeutic alkylating agent TMZ [14] is removed by a lesion-specific DNA glycosylase [15, 16]. Alkylation-induced base adducts, such as the N7-MeG and N3-MeA base lesions induced by TMZ, are removed by methylpurine (alkyladenine) DNA glycosylase. This protein has several designations, including MPG, AAG or ANPG but for clarity we will refer to it herein as MPG. The resulting abasic site is incised by apurinic/apyrimidinic endonuclease (APE1), leaving a single-nucleotide gap in the DNA strand with 3'-OH and 5'-deoxyribose phosphate (5'dRP) groups at the margins. Poly(ADP-ribose)polymerase-1 (PARP1), together with poly(ADP-ribose)polymerase-2 (PARP2) and the catabolic enzyme poly(ADP-ribose)glycohydrolase (PARG), are then suggested to be recruited to the APE1-mediated strand-break. It has been postulated that low-level activation of PARP1 and the resultant synthesis of poly(ADP-ribose) (PAR) facilitates recruitment of the downstream BER proteins XRCC1, DNA ligase IIIa (LigIIIa) and Polβ to stimulate and complete DNA repair [17].

Although the *POLB* gene is quite large, the protein encoded by *POLB* is the smallest of the human DNA polymerases [3, 18]. Polβ is a bi-functional, two-domain, single-polypeptide 39kDa enzyme [18]. Structurally, Polβ is similar to other DNA polymerases in which the polymerase domain is further divided into sub-domains referred to as the fingers, palm and thumb (Fig. (2)). Detailed structural characterization of Polβ has been summarized elsewhere [18, 19]. The polymerase or nucleotidyltransferase activity, responsible for gap-filling DNA synthesis in BER, resides in the C-terminal 31kDa domain and contains three aspartic acid (D) residues (190, 192 and 256) required for activity (Fig. (2)). A second active site in the N-terminal domain of Polβ conducts the essential gap-tailoring step in BER, the removal of the 5'dRP moiety [18, 20–24] (Fig. (2)). A close examination of the molecular mechanism of the 5'dRP lyase activity of Polβ reveals the formation of a Schiff base intermediate during the reaction, as originally reported by Wilson and colleagues [25]. In this earlier report, it was demonstrated that the Polβ 5'dRP lyase reaction proceeded through an imine-intermediate, implicating one or more of 4 lysine amino acid residues (K35, K68, K72 and K84) as the 5'dRP lyase active site catalytic nucleophile [25], although later studies have defined K72 as the essential nucleophile [26]. Once the epsilon-amino group of the active site lysine has begun the nucleophilic attack on the C-1' atom of the sugar within the sugar-phosphate BER intermediate (5'dRP), the BER reaction intermediate may be covalently trapped by reduction with sodium borohydride ($NaBH_4$), resulting in a covalently bound substrate-enzyme complex, as depicted in Fig. (3). The 5'dRP lyase activity of Polβ is the predominant BER-dependent 5'dRP lyase activity observed in mammalian cells for the repair of alkylated [27] and oxidized [28] bases. However, biochemical and genetic analyses have identified additional proteins with 5'dRP lyase activities that may contribute to gap tailoring in nuclear and mitochondrial BER, including DNA polymerases theta (θ), lambda (λ,), gamma (γ) and iota (ι) [24, 29–33]. Additional enzymes with similar 5'dRP lyase activities include the catalytic subunit of the HSV-1 DNA polymerase [34–36], the *S. cerevisiae* Trf4 protein [37], NEIL family glycosylases [38, 39], DNA polymerase β from *Leishmania infantum* [40], Ku [41], DNA polymerase X from African swine fever virus [42] and *Deinococcus radiodurans* [43], mitochondrial DNA polymerase β from *Crithidia fasciculate* [44], the RecJ and Fpg proteins of *E. coli* [45, 46], HMGA2 [47] and HMGB1 [48].

Polβ has two active sites and several critical functional domains that may be considered as targets to inhibit Polβ and BER. In addition, Polβ-dependent BER is a highly complex process involving several proteins and requires multiple protein-protein interactions for repair (depending on the base lesion) [3], suggesting that interference with DNA binding or with the formation of one or more essential protein-protein interactions may in effect inhibit Polβ-mediated repair. Further, cancer mutations or cancer specific truncations of Polβ offer additional opportunities for BER-specific modulation and recently, it has been suggested by us and others that inhibition of Polβ may provide an opportunity for synthetic lethality associated with cancer specific genotypes. BER and Polβ can also be regulated by protein modifications but an understanding of this aspect of BER and Polβ regulation is in its infancy [3, 49]. For example, Polβ can be modified by acetyl, methyl and phosphate groups, yet little is known about the functional impact of these modifications [50, 51]. More recently, it has been suggested that Polβ is modified by ubiquitin, leading to proteasome-mediated degradation [52–54]. As will be discussed throughout this review, the central and pivotal role of Polβ in BER implicates this protein as a prime target to enhance the response to chemotherapeutic agents or radiation. The complexity of BER and the ubiquitous role of Polβ in cellular metabolism suggest that the design and effectiveness of a robust Polβ inhibitor will be a significantly complex but rewarding challenge.

INHIBITING POLβ-DEPENDENT DNA SYNTHESIS

Initial attempts to regulate cancer cell growth focused on inhibiting DNA synthesis in order to regulate the uncontrolled replication of the cancer cell [55, 56]. Such inhibition can be accomplished directly or indirectly [55]. Indirect methods include damaging the DNA sufficiently to prevent replication [57] or depletion of nucleotide pools with anti-metabolites such as methotrexate [58]. Alternatively, components of the DNA replication machinery can be inhibited directly. This idea has led to numerous inhibitors of DNA polymerases useful in the treatment of diseases associated with cellular hyperproliferation, including cancer and viral infection [55, 56]. However, the requirement for normal cellular replication severely limits the utility of general DNA polymerase inhibitors. The identification of 15 mammalian DNA polymerases [1, 59] involved in nuclear and mitochondrial DNA replication, repair, recombination and translesion DNA synthesis complicates the utility of DNA polymerase inhibitors, requiring a high degree of selectivity and specificity. However, the unique role of repair and translesion DNA polymerases, especially in response to radiation and chemotherapy [56, 60, 61] suggests an opportunity for selectivity and specificity of DNA polymerases in response to some anti-cancer agents. The unique role of Polβ in BER, in particular in response to chemotherapy [60, 62], suggests that targeting the DNA synthesis activity of Polβ may offer a selective advantage in the treatment of cancer. A discussion on the role of Polβ in translesion DNA synthesis is presented by Sharma and colleagues elsewhere in this Special Edition and will not be covered here. Below, we have summarized past and present advances in the development of small molecule inhibitors specific to the DNA polymerase activity of Polβ.

Natural Products as Inhibitors of Polβ-Mediated DNA Synthesis

Hecht and colleagues utilized a standardized approach of ‘bioassay-guided fractionization’ to identify a series of natural-product-derived Polβ inhibitors isolated from crude plant extracts (*e.g.*, aqueous or methyl ethyl ketone extracts) [63–70]. The assay used was a standard *in vitro* DNA polymerase assay using either purified calf thymus Polβ or recombinant rat or human Polβ, in the presence or absence of bovine serum albumen (BSA), monitoring the incorporation of [^{3}H]dTTP in response to the presence or absence of the inhibitor or extract. In some cases, the compounds were resynthesized to validate the results.

The earliest studies yielded the identification of several alkylresorcinol analogs [65, 69], including one previously identified as bis-5-alkylresorcinol [65]. In addition, two novel alkylresorcinol analogs were identified, depicted in Fig. (4). The most potent of these exhibited IC_{50} values as low as 5.3 μM [65, 69]. However, specificity and selectivity of these compounds has yet to be evaluated. Interestingly, these two alkylresorcinol analogs, as shown in Fig. (4), initiate Cu^{2+}-dependent DNA cleavage, prompting Hecht and colleagues to evaluate additional classes of compounds that can both inhibit Polβ and cleave DNA. Recently, Hecht and colleagues isolated a set of flavinoids [(+)-myristinin A and D], shown in Fig. (5), from *knema elegans* [71] and later they accomplished the complete chemical synthesis of (+)-myristinin A [72]. These also have potent Polβ inhibitory activity and mediate Cu^{2+}-dependent DNA cleavage. When evaluated for an impact on cellular viability at 10 μM, each appeared to be as effective as 0.075μM bleomycin. The combination of bleomycin plus the alkylresorcinol compound or the flavinoids yielded either a similar impact or slightly greater impact on cell growth and viability. It was therefore suggested that these alkylresorcinol and flavinoid compounds damage DNA and then inhibit the repair of the induced DNA damage. It remains to be determined if the damage induced by either the alkylresorcinol or flavinoid compounds is repaired by Polβ.

The same group also reported the isolation of several triterpenoids and similar multicyclic compounds isolated from several plant species that can inhibit DNA synthesis mediated by Polβ. Compounds were isolated from *Brackenridgea nitida* [68], *Bleasdalea bleasdalei* [68], *Freziera sp*. [66], *Baeckea gunniana* [64], *Tetracera boiviniana* [63] and *Sandoricum koetjape* [70]. None yielded compounds with IC_{50} values lower than 2 μM. More recently, Cazaux and colleagues utilized a high-throughput approach, screening over 8,000 natural products (extracts) to identify an inhibitor of Polβ-mediated DNA synthesis. In this screen, they identified masticadienonic acid (MA), demonstrating a small degree of specificity as MA does not appear to inhibit the enzyme activity of Polδ. Since MA enhanced the cell killing effect of cisplatin [73], it was suggested that MA may affect the translesion-synthesis role of Polβ (See article by Sharma and colleagues elsewhere in this Special Edition). Other natural products found to inhibit Polβ include fatty acid derivatives [74], azaphilones isolated from cultures of *Talaromyces sp*. [75] and 1-deoxyrubralactone [76], among others we may have inadvertently missed. It remains to be established if any of these are selective or specific to Polβ, as compared to other Pol X family members or other DNA polymerases.

Sulfolipids

Using a similar functional screening paradigm, Sakaguchi and colleagues have identified several novel classes of compounds that inhibit Polβ. By analyzing extracts of the pteridophyte *A. niponicum*, they isolated a series of sulfolipids with inhibitory activity towards Polα and Polβ [77], with IC_{50} values of 6 and 8 μM, respectively. This discovery was followed by several reports describing a series of sulfolipids of the sulfo-quinovosyl-acyl-glycerol (SQAG) class [78–81]. All seem to function as competitive inhibitors of Polβ (with respect to template/primer or dNTP) whereas some exhibit non-competitive inhibition of Polα. As with Lithocholic acid (LCA), MA and related fatty acid or steroid-based lipid derivatives (see below), the sulfolipids appear to bind to the N-terminal 8kDa domain and interfere with DNA binding, preventing nucleotidyltransferase (polymerase) activity [82]. Since many of these sulfolipids contain an esterified form of fatty acids similar to those known to inhibit Polβ (see below), it is speculated that both fatty acids and sulfolipids may inhibit Polβ *via* a similar mechanism [78, 80]. However, it is noted that both the sulfate in the quinovose moiety as well as the fatty acid component of these sulfolipid compounds are critical for inhibition of Polβ-mediated nucleotidyltransferase activity [78–80]. Similarly, it was established that sulfated glycoglycerolipids (*e.g*., KN-208) are effective in inhibiting

Polβ (Ki = 0.05 μM), yet as with the others mentioned here, this compound also inhibits Polα although at a 10-fold higher concentration [83].

Interestingly, many of these sulfolipids have additional cellular effects that may need to be considered if these are to be developed clinically. For example, Sulfo-quinovosylmonoacylglycerol (SQMG), also shown to inhibit Polβ [82], triggers cell cycle arrest [84] and has anti-angiogenic effects, likely *via* down-regulation of Tie2 [85]. Further, SQMG functions as a radiosensitizer [86], although it has not been determined if any of the radiosensitizing effect is *via* inhibition of Polβ or other DNA polymerases.

KA-A

Kohamaic acid A (KA-A) is a sesterterpenic acid that was isolated from *Ircinia sp*., a marine sponge, and was first reported to inhibit cell division of fertilized sea urchin eggs [87]. The effect on cell division was suggested to proceed *via* a block or inhibition of sea urchin replication-essential DNA polymerases. More detailed analysis of KA-A and a series of analogs [88, 89] revealed broad spectrum inhibition of human DNA polymerases α, β, δ and γ, suggesting a possible universal mechanism of inhibition of DNA polymerases but a clear lack of specificity for Polβ. The structure of the natural compound KA-A and the most effective derivative [Compound #11; (1S*, 4aS*, 8aS*)−17-(1,4,4a,5,6,7,8,8a–octahydro-2,5,5,8a–tetramethylnaphthalen-1-yl)heptanoic acid] [88] is shown in Fig. (6). Compound #11 acts as a competitive inhibitor of Polβ with Ki values of 1.9 μM (template/primer) and 2.3 μM (nucleotide). Molecular modelling studies suggest KA-A derivative #11 binds to the 8kDa domain of Polβ along the interface that interacts with the ssDNA of a template/primer. Such inhibition would be similar to that of other fatty acid derivatives [90].

Cloretazine

The nucleotidyltransferase activity of Polβ has also been reported to be inhibited by the inter-strand DNA crosslinking agent Cloretazine [91] or 1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-2-[(2-methylamino)carbonyl] hydrazine. This agent, also referred to as Laromustine or VNP-40101M [92] is currently in several stages of clinical evaluation [93–95]. Cloretazine is activated under aqueous conditions, yielding the two reactive molecules 90CE and methylisocyanate, as shown in Fig. (7). The 90CE derivative is responsible for alkylation of DNA at the O^6 position of guanine bases, triggering cytotoxicity resulting from the formation of interstrand cross-links to cytosine. However, cytotoxicity is alleviated in cells expressing the direct-reversal repair protein O^6-methylguanine-DNA methyltransferase (MGMT) [96]. The methylisocyanate compound induces carbamoylation of protein sulfhydryl groups [97]. Rice and colleagues observed that the Cloretazine derivative 90CE has no effect on Polβ whereas the analog 101MDCE, which retains the carbamoylating activity, has an IC_{50} value of 92 μM, suggesting Cloretazine inhibits Polβ by methylisocyanate-mediated carbamoylation of an active-site sulfhydryl group, attenuating DNA polymerase activity. We await validation of this initial report.

Lithocholic Acid

One small molecule that has received considerable attention as a purported specific inhibitor of Polβ is Lithocholic acid (LCA) − see Fig. (8). Originally identified by DeClercq and colleagues as a cholic acid derivative with selectivity for HIV-1-mediated viral replication [98], LCA was subsequently shown to promote N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-mediated tumor growth [99–101]. Since MNNG induces DNA damage that is repaired by Polβ and the BER pathway [13], Yoshida and colleagues then hypothesized that the tumor promoting action of LCA may be mediated by inhibiting Polβ and preventing repair of the MNNG-induced DNA damage. After evaluating seventeen related bile acids, it

was determined that LCA and select derivatives were potent inhibitors of Polβ. Note however, that LCA was also found to inhibit Polα, Polδ, Polε and Polγ although Polβ was reported as the most sensitive [99]. In follow-up analyses by Sakaguchi and colleagues, it was determined that LCA bound to the N-terminal 8kDa domain of Polβ [102]. Although the binding affinity of LCA to Polβ was sufficient to allow modified forms of LCA (*e.g.*, biotinylated) for use in the purification and affinity capture of Polβ [103, 104], this approach can also be used to purify Polα [103, 104] and LCA also binds tightly to DNA Topoisomerase II [105], suggesting some level of off-target effects of LCA with regard to Polβ.

Recently, LCA was shown to potentiate the cell killing effect of TMZ, a clinical alkylating agent previously shown to induce BER specific DNA damage requiring Polβ for cell survival [14, 60, 62, 106]. Importantly, it was shown that potentiation of LCA on TMZ was enhanced in cells with a null mutation in the BRCA2 gene [107]. In this latest report, Glazer, Sweasy and colleagues very clearly demonstrate that LCA inhibits both the nucleotidyltransferase and the 5'dRP lyase activity of purified Polβ, as well as inhibiting Polβ in a complete BER reaction. Under the assumption that LCA is specific for Polβ, this recent study was then extended to cells deficient or proficient for BRCA2, showing synergy between LCA and TMZ. However, these studies failed to effectively demonstrate that the synergy between LCA and TMZ is the result of specific or selective Polβ inhibition. For example, LCA treatment yields a similar level of synergy with TMZ in both wild-type (WT) and MPG(AAG) knockout (KO) cells [107]. Since it was earlier demonstrated that Polβ/AAG double-KO cells are resistant to the cell killing effects of alkylating agents (as compared to Polβ KO cells) [108], the reported synergy of LCA and TMZ in AAG KO cells [107] likely involves at least some level of cell killing resulting from either non-specific effects of LCA or inhibition unrelated to Polβ. As with many of the compounds discussed herein, further tests are required to clearly demonstrate if LCA is selective or specific to Polβ in human cells, especially given the previous evidence for inhibition of other human DNA polymerases [99]. Further, LCA has additional cellular effects, likely both related and unrelated to its function as a ligand for the Vitamin D receptor, including regulation of NF-κB and Vitamin D [109, 110] as well as induction of the urokinase-type plasminogen activator receptor (uPAR) [111]. Most recently, LCA was identified in a large-scale chemical and genetic screen to extend yeast chronological life span [112], an effect clearly not mediated through Polβ.

DNA BINDING INHIBITION

NSC666715

As depicted in Fig. (2), Polβ has two essential DNA binding domains [18]. The 8kDa domain, corresponding to amino acid residues 1–89, binds to ssDNA [18] with a preference for a small gap with a 5'phosphate [23]. The fingers sub-domain, corresponding to amino acid residues 90–149, encodes the dsDNA-binding domain [18]. However, there is likely some level of cooperative binding since the C-terminal 31kDa Polβ fragment, corresponding to amino acid residues 90–335, has minimal DNA binding and DNA polymerase activity [27]. Given the extraordinary number of proteins capable of binding DNA plus the potential for a high degree of similarity among proteins that bind DNA in a non-sequence dependent manner, it was surprising that a relatively specific Polβ inhibitor was recently reported that appears to function precisely by inhibiting the binding of Polβ to the damaged DNA under repair [113]. In this report, Narayan and colleagues reported promising results with the compound NSC666715 [113], depicted in Fig. (9). This compound (NSC666715; IUPAC designation = 4-chloro-N-[5-(4-chloroanilino)-1H-1,2,4-triazol-3-yl]-5-methyl-2-sulfanylbenzene-sulfonamide) was first synthesized as an anti-HIV compound, designed for inhibition of viral integration [114–116]. Narayan and colleagues used a molecular docking

approach to identify small molecules that would bind to a recently identified APC-binding site on Polβ [117, 118]. Using purified proteins, they find that NSC666715 inhibits both the 5'dRP lyase and DNA synthesis steps catalyzed by Polβ, resulting in an overall block to BER. Treatment with NSC666715 was effective in enhancing the efficacy of TMZ in a mouse xenograft model using the HCT-116 colon tumor model [113]. In this report, the data suggests that NSC666715 prevents Polβ from binding damaged DNA. The specificity and selectivity of NSC666715 remains to be determined, as treatment with NSC666715 alone resulted in the inhibition of tumor cell growth in the absence of TMZ, an effect not likely to be the result of Polβ inhibition. Regardless, this initial report is a promising example of targeting Polβ by preventing the ability to recognize and bind to damaged DNA.

DISRUPTING POLβ-DEPENDENT DNA REPAIR COMPLEXES

As our understanding of the BER pathway has evolved, especially the molecular details of each step in the mechanism of BER, it has become clear that repair of base lesions and related BER substrates is facilitated *via* a series of protein complexes that assemble at the site of the DNA lesion [3, 18, 49, 119]. This should not be surprising as essentially all proteins either function by or are regulated by specific protein-protein interactions. In fact, protein-binding interfaces may offer a greater number of targets for the development of highly specific and selective drugs. For example, one recent accounting of the number of protein-protein interactions in human cells suggest as many as 150,000 protein interaction pairs [120] and this number may be greatly enhanced when considering the increase in the number of protein products that result from alternative splicing events [121]. Further, as with other repairosomes or transient DNA Repair complexes, post-translational modification (PTM) of BER proteins impacts both function and repair complex formation [49].

Targeting the Polβ-XRCC1 Interface

A recent example of targeted disruption of a proteinprotein interaction is the disruption of the c-Myc/Max heterodimer. The oncoprotein c-Myc is over-expressed in many cancers and forms a heterodimer with Max to function as a transcription factor [122]. It has been demonstrated that small molecules can disrupt the c-Myc/Max dimer, eliminating the oncogenic potential of c-Myc overexpression [123, 124]. Similar protein-protein disrupting molecules or peptide mimetics have also been developed to target SMAC in an attempt to induce apoptosis [125, 126] although other targets have been proposed [127, 128]. In this same vein, we propose that given the number of specific protein-protein interactions that are formed during the repair of base lesions by Polβ and the BER pathway, it might also be possible to develop selective and specific BER inhibitors by targeting specific protein-protein interfaces that arise during repair. As summarized in Table 2, there are a number of proteins that functionally interact with Polβ or modulate Polβ function. These proteins have been reported to either modify Polβ (*e.g.*, Mule) or participate in BER (*e.g.*, LigI). However, for many of these proteins, the functional significance of binding to Polβ is unknown (*e.g.*, TAF1D). For those that form complexes with Polβ to facilitate BER (*e.g.*, XRCC1), we propose that targeted disruption of this complex or protein-protein interaction may lead to inhibition of Polβ-mediated BER and hence the Polβ/XRCC1 interface may provide a unique drug development target. Polβ forms a tight complex with XRCC1 [129], amenable to both NMR analysis [130, 131] and crystallographic analysis [132]. The region of interaction with Polβ is located within residues 84–183 in the N-terminal half of the XRCC1 protein [133]. Conversely, the Polβ domain that binds to XRCC1 is the C-terminal or thumb domain (see Fig. (2)). As was demonstrated using NMR spectroscopy, Polβ mutants with a L301R/V303R/V306R triple point mutation cannot interact with XRCC1 [131, 134]. These amino acid residues form part of a protusion (referred to as the V303 hairpin) that appears to insert within the Polβ/XRCC1 binding interface, as determined by crystallographic analysis

[132] – see Fig. (10). Interestingly, the Polβ/XRCC1 binding interface appears to be regulated by oxidation [132]. Given the significance of the Polβ/XRCC1 interaction in BER [135–137], it is therefore likely that disruption or inhibition of BER may be accomplished by small molecules or peptide mimetics targeted to this location within the Polβ/XRCC1 binding interface.

Modulating POLβ Function By Regulating PTMs

As discussed previously [3, 49] and mentioned above, Polβ helps facilitate BER *via* the formation of transient protein complexes at the site of the DNA lesion. Further, it appears that, as with many proteins, post-translational modification (PTM) of BER proteins can either enhance or disrupt BER, in some cases by modulating protein complex formation [49]. Like many BER proteins, Polβ is modified at multiple amino acid residues – summarized in Table 3. For example, Dianov and colleagues have suggested that Polβ levels are kept in check by ubiquitylation and subsequent proteosome-mediated degradation [52, 54]. Therefore, it is conceivable that a targeted approach of Polβ ubiquitylation and degradation might be feasible. Alternatively, enhancing the acetylation of K72 [51] would inhibit the 5'dRP lyase activity of Polβ. In both cases, the result would be a targeted disruption of BER. However, we concede that selective drugs to facilitate enhanced modification of Polβ is highly speculative at this time, especially considering that many of the reported Polβ-specific PTMs (Table 3) have not been validated in multiple systems and little is known regarding the functional impact of these PTMs. It is possible that many of the PTMs reported to date are not universal and/or do not impact Polβ function or Polβ-mediated BER complex formation. In addition, the protein modifiers (*e.g.*, CHIP, PKC, p300) have multiple targets unrelated to Polβ and BER and therefore inhibition of these enzymes is not likely to be Polβ specific. We look forward to future studies in this regard.

DOMINANT-NEGATIVE INTERFERENCE OF POLβ FUNCTION AND THE IMPACT OF TUMOR MUTATIONS

An essential aspect of any enzyme inhibitor to be used in cells or *in vivo*, as we have discussed throughout, is specificity and selectivity. Unfortunately, none of the small molecule inhibitors of Polβ discussed so far have been shown to be highly specific or selective. Many of these small molecule inhibitors can inhibit other DNA polymerases with equal or greater affinity, have multiple biological effects unrelated to Polβ or are directly cytotoxic, an effect not expected to result from Polβ inhibition since Polβ null cells are viable [13, 27, 108, 138].

Interestingly, Polβ expression or primary sequence appears to be altered in a significant percentage of human tumors so far evaluated [139]. High levels of Polβ expression have been demonstrated in several human cancers and tumor cell lines [140]. We, and others, have observed elevated expression of Polβ in gastrointestinal tumors and cancers from the esophagus, colon and pancreas [9, 141–143]. In addition, chronic myelogenous leukemia (CML) [144] and infection by human papillomavirus 16 (HPV16) [145] or Epstein-Barr virus (EBV) [146] leads to elevated expression of Polβ. In many cases, mutations within the Polβ coding region results in over-expression of dysfunctional Polβ proteins [8]. Overall, approximately 30% of human cancers express mutant or aberrant forms of Polβ proteins [8, 147–149], leading to genomic instability and possibly conferring a mutator phenotype to cells [108, 140, 150]. An updated list of disease-associated Polβ mutants is shown in Table 4. These range from single-point mutants such as the Leu22Pro mutant found in gastric cancer that is deficient in 5'dRP lyase activity to deletion mutants that function as dominant negative inhibitors of Polβ activity (Table 4). It is intriguing to consider the possibility that small molecules may be developed to specifically target cancer specific mutants of Polβ,

although the likelihood of such inhibitors may be low. However, as we, and others, have shown, Polβ targeting by siRNA or shRNA gives rise to an almost complete loss of Polβ expression and increased sensitivity to DNA damaging agents [60, 62, 106, 151]. Hence, RNA interference could be utilized to specifically target mutant forms of Polβ, similar to that reported recently to target mutant forms of the Huntingtin alleles without affecting the normal allele [152]. Alternatively, it has been suggested that inhibiting the normal form of Polβ may be synthetically lethal with some cancer genotypes such as MSH2 mutations found in Hereditary NonPolyposis Colorectal Cancer (HNPCC) [153]. However, synthetic lethality of Polβ with a null mutation in MSH2 remains to be validated in a separate study.

One interesting method that might be more amenable to a gene-therapy type approach is the identification and utilization of mutants of Polβ that function as dominantnegative inhibitors. Such an approach could take advantage of cell penetrating peptides for delivery of a highly specific Polβ and BER inhibitor [154]. The first report of a naturally occurring dominant-negative mutant of Polβ was from Banerjee and colleagues in which they identified a splicevariant of Polβ, missing 87 bp (exon 11) corresponding to amino acid residues 208–236 [155]. This mutant form of Polβ (PolβΔ87) is defective in BER [156] and functions as a dominant-negative inhibitor of Polβ [157]. Although suggested to be a cancer-specific form of Polβ [155], the PolβΔ87 mutant is expressed in both normal and tumor tissue [158].

Earlier, Wilson and colleagues demonstrated that the 14kDa N-terminal fragment of Polβ, corresponding to amino acid residues 1–140 (PolβN140), inhibited recombinant Polβ *in vitro* [159]. Interestingly, the inhibitory effect of PolβN140 was specific in that other N-terminal fragments, corresponding to fragment sizes of 8, 27 and 31 kDa did not inhibit the activity of Polβ and conversely, PolβN140 did not inhibit Polα [159]. Capitalizing on the dominant-negative phenotype of PolβN140, Vens and colleagues have shown repeatedly that this mutant form of Polβ can be utilized to specifically and selectively inhibit BER in human cells, likely by competing with the WT form of Polβ, as originally suggested by Wilson and colleagues [159]. Using this approach, they initially reported that PolβN140 revealed a role for Polβ in sensitization to ionizing radiation [61]. This dominant-negative mutant of Polβ acts as a radiosensitizer *via* XRCC1 dependent mechanisms that is independent of Polβ expression [160] and it's action appears cell cycle dependent [161]. It is interesting to speculate that PolβN140 may function by interfering with XRCC1-dependent DNA repair complex formation. However, the mechanism of this interference is not obvious, as PolβN140 does not contain the XRCC1 binding domain. As might be expected, cell death due to radiation combined with expression of the dominantnegative PolβN140 correlates with accumulation of DNA double-strand breaks [161]. DNA double-strand break induction might result from interference with the repair of clustered lesions characteristic of ionizing radiation-induced damage [162]. We await the evaluation of the dominant-negative PolβN140 protein in xenograft studies and suggest that PolβN140 might be an excellent candidate for delivery using a cell penetrating peptide such as those described by Bitler and Schroeder [154].

INHIBITION OF POLβ-DEPENDENT 5'dRP LYASE ACTIVITY AND THE CELLULAR CONSEQUENCES

DNA Polβ has always been considered a "DNA Repair polymerase" [138] yet it was not until the development and characterization of Polβ knockout (KO) mouse embryonic fibroblasts (MEFs) that its role in BER was clearly defined where it was demonstrated that Polβ KO MEFs are sensitive to DNA alkylating agents due to a BER defect [13]. However, with the discovery that Polβ conducts two critical enzyme activities to complete BER, nucleotidyltransferase activity [18, 163] and 5'dRP lyase activity [20], it was important to determine the enzyme activity that was the most essential for BER and therefore if inhibited,

could increase sensitivity to DNA alkylating agents. In a follow-up study, it was then determined that the alkylation sensitivity of Polβ KO MEFs was the result of a failure to repair the 5'dRP group, an intermediate in BER [27]. Subsequently, we have demonstrated that loss of Polβ-mediated 5'dRP lyase activity enhances sensitivity to the chemotherapeutic agent TMZ in human cells derived from breast and glioma tumors [60, 62, 106]. Although there appears to be several other enzymes that can repair the 5'dRP lesion [24, 29–33, 38, 39, 41, 47, 48], cellular hypersensitivity to alkylating agents can be achieved if one eliminates or attenuates the 5'dRP lyase activity of Polβ [27, 106]. In this final section, we present a summary of progress towards the development of effective inhibitors of the 5'dRP lyase activity of Polβ and discuss our recent insights into the cellular consequences that result from 5'dRP lyase inhibition.

Although not initially designed as such, many of the nucleotidyltransferase and DNA binding inhibitors discussed above also inhibit the 5'dRP lyase activity of Polβ. Earlier studies by Hecht, Sakaguchi and others (see above) focused on the polymerase activity of Polβ but the impact of many of these compounds on the associated 5'dRP lyase activity was not reported. However, it is likely that MA [73] and the sulfolipids (described above) may inhibit the 5'dRP lyase activity of Polβ since these and related compounds bind to the 8kDa domain of Polβ that encodes the 5'dRP lyase active site [82] (see Fig. (2)). Cloretazine is suggested to inhibit Polβ by modifying one of three cysteine amino acid residues. These all lie within the C-terminal or nucleotidyltrans-ferase domain and therefore if such modifications do occur, Cloretazine will likely effect the nucleotidyltransferase as opposed to the 5'dRP lyase activity of Polβ. On the other hand, LCA, originally identified as a DNA polymerase inhibitor, inhibits both the nucleotidyltransferase and the 5'dRP lyase activity of Polβ [107]. In addition, it was also determined that NSC666715 not only prevents binding to the damaged DNA but as a result, inhibits the 5'dRP lyase activity of Polβ [113]. From many of these studies, one may be able to extrapolate that tight binding to the 8kDa domain may be a signature or pre-requisite for inhibition of 5'dRP lyase activity.

In a completely surprising finding, Vijayanti and colleagues have reported that the dementia drugs donepezil hydrochloride, rivastigmine tartrate and Nootropil bind to the 8kDa domain of Polβ as determined in an *in silico* analysis to evaluate potential macromolecular docking sites on the protein [164]. Using *in vitro* DNA polymerase and 5'dRP lyase assays, they show no significant impact on polymerase activity but suggest that rivastigmine tartrate and Nootropil both have a significant impact on Polβ dependent 5'dRP lyase activity. However, there was little or no impact on a complete BER reaction containing DNA ligase. Additional studies are therefore suggested to validate the inhibitory effect of donepezil hydrochloride, rivastigmine tartrate and Nootropil on Polβ.

In what appears to be the first dedicated effort to identify and develop Polβ specific 5'dRP lyase inhibitors, Wilson and Hecht and colleagues have discovered a number of compounds that inhibit both the nucleotidyltransferase and 5'dRP lyase activity of Polβ, such as koetjapic acid (KJA), isolated from *Sandoricum koetjape* [70]. Using a bioassayguided fractionization approach and a standardized 5'dRP lyase activity assay, Hecht and Kingston identified a large number of 5'dRP lyase inhibitory compounds from natural products, including lupanetriterpinoids, (−)epicatechin, sesquiterpinoids, biscoumarin derivatives, plant sterols, oleananetriterpinoids, ursanetriterpenes and neolignans [165– 172]. In some cases, these compounds were reported to enhance or potentiate the cytotoxicity of the alkylating agent methyl methanesulfonate (MMS) [170], as might be expected from an inhibitor of the 5'dRP lyase activity of Polβ [27, 106]. The sulfolipid KN-208, a reported polymerase activity inhibitor (see above), enhanced cellular sensitivity to MMS [83] and is likely to inhibit the 5'dRP lyase activity of Polβ since the general class of sulfolipids are known to bind to the 8kDa domain of Polβ, although it has yet to be evaluated.

Selectivity for many of these compounds remains an issue since potentiation of MMS in a WT cell could result from the inhibition of many cellular functions. Wilson and Hecht approached this problem using a combined NMR, biochemical and cellular approach to identify lead compounds for specific inhibition of the 5'dRP lyase activity of Polβ [173]. In this initial study, they used their previously reported NMR structure of the 8kDa domain of Polβ [130, 131] to map the binding site and critical contacts (amino acid residues and chemical groups) for binding of the 5'dRP lyase inhibitor KJA [70]. Subsequently, thirty-four structurally similar compounds were analyzed using this NMR chemical shift mapping approach and ten compounds, including KJA, were identified that yielded appropriate (micromolar) binding constants. These and other 5'dRP lyase inhibitor compounds are listed in Table 5. To address the specificity issue, each of these compounds were then evaluated for the ability to potentiate MMS-induced cell death, comparing WT and Polβ KO MEFs [173], with the expectation that a highly specific Polβ 5'dRP lyase inhibitor would potentiate MMS in WT cells but would yield little or no potentiation in the Polβ KO cells. Any potentiation in the Polβ KO cells would likely be the result of non-specific or off-target effects. By comparing potentiation in both cell lines (WT and Polβ KO), Wilson and colleagues were then able to evaluate the specificity and efficacy of each compound by calculating an enhancement ratio (ER) [173], the most effective and specific compounds yielding the highest ER. By this criteria, it was determined that Pamoic Acid (PA) is the most effective and specific of the inhibitors tested, with a binding constant of 9 μM [173]. In line with this result, PA was also shown to inhibit both the nucleotidyltransferase and 5'dRP lyase activities of Polβ, when tested in an *in vitro* assay using purified, recombinant Polβ [173]. The binding interface of PA and the 8kDa domain of Polβ was then independently confirmed by Milon and colleagues [174] to further development of PA analogs and related compounds with greater affinity and selectivity for Polβ. Finally, a summary of the development and characterization of these compounds (Table 5) by Wilson and colleagues has also been reported [175].

The increased alkylating agent sensitivity of Polβ KO or deficient cells (or Polβ 5'dRP lyase deficient/inhibited cells) has been well documented [13, 27, 60, 62, 106, 108, 176, 177]. However, the mechanism of cell death initiated by alkylating agents in a Polβ null cell was only recently revealed. Using a series of human tumor cell lines depleted of Polβ (*via* RNA interference) together with isogenic lines complemented with WT and mutant forms of Polβ, we reported that failure to repair the 5'dRP lesion (termed *BER Failure*) initiates hyperactivation of PARP1/PARP2 leading to the depletion of NAD^+/ATP pools, the release of the RAGE ligand HMGB1 and the onset of necrosis [106]. In fact, we find that cell death from Polβ inhibition (in concert with DNA damage) is highly dependent on the availability of cellular bioenergetic metabolites (NAD^+ and NAD^+ precursors) and the capacity of the NAD^+ biosynthesis machinery [106]. In that vein, we suggest that the efficacy of Polβ inhibitors, especially 5'dRP lyase inhibitors, to potentiate DNA damage-induced cell death (e.g., anti-tumor effect) can be improved by simultaneous disruption or inhibition of cellular NAD^+ biosynthesis [178].

SUMMARY

In summary, we have discussed past, present and future options for inhibiting Polβ as a means to enhance response to chemotherapy and radiation. The obvious target, to inhibit the nucleotidyltransferase activity of Polβ, has not resulted in any highly effective or specific inhibitors to date. The most effective compounds appear to be those that result in inhibition of both the nucleotidyltransferase and 5'dRP lyase activities of Polβ such as LCA, NSC666715 and PA. However, reported binding constants are still too high but NSC666715 and PA appear to provide a high level of specificity and offer promise, each targeting a unique site on Polβ. Future studies may also include novel targets such as the Polβ/XRCC1

interface. It has not yet been determined if interrupting the Polβ/XRCC1 interaction results in *BER Failure* or BER inhibition in cells, but studies are underway to determine if targeting BER-dependent protein-protein interfaces is a viable approach to inhibit BER. Another option is to modulate PTMs to inhibit Polβ. However, more information is needed about the functional impact of Polβ specific PTMs before considering this as a target. In addition, one report has proposed a potential for synthetic lethality between Polβ loss and mutations in MSH2, suggesting that targeting Polβ alone (in the absence of DNA damage) may be considered for MSH2-deficient tumors. Finally, as we discussed above, combinations of inhibitors may prove most effective in the short term. Since inhibiting Polβ triggers DNA damage-induced necrosis *via* NAD^+ depletion [106], one might consider using a DNA damaging agent, a Polβ inhibitor and an NAD^+ biosynthesis inhibitor. Such an approach might be most effective in tumors with defects in NAPRT1-mediated NAD^+ biosynthesis to allow normal tissue rescue with nicotinic acid [179, 180].

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ABBREVIATIONS

5'dRP	5'-deoxyribose phosphate
APC	Adenomatous polyposis coli
APE1	Apurinic/apyrimidinic endonuclease
ATP	Adenosine-5'-triphosphate
BER	Base excision repair
BSA	Bovine serum albumen
CML	Chronic myelogenous leukemia
dNTP	Deoxynucleotide triphosphate
dsDNA	Double-stranded DNA
EBV	Epstein-Barr virus
ER	Enhancement ratio
HIV	Human Immunodeficiency virus
IUPAC	International Union of Pure and Applied Chemistry
KA-A	Kohamaic acid A
KJA	Koetjapic acid
KO	Knockout
LCA	Lithocholic acid
LigIIIa	DNA ligase IIIa
MA	Masticadienonic acid

MEF	Mouse embryonic fibroblast
MGMT	O^6-methylguanine-DNA methyltransferase
MMS	Methyl methanesulfonate
MNNG	N-methyl-N'-nitro-N-nitrosoguanidine
MPG	Methylpurine (alkyladenine) DNA glycosylase
$NaBH_4$	Sodium borohydride
NAD^+	Nicotinamide adenine dinucleotide
NMR	Nuclear magnetic resonance
PA	Pamoic Acid
PAR	Poly(ADP-ribose)
PARG	Poly(ADP-ribose)glycohydrolase
PARP1	Poly(ADP-ribose)polymerase-1
PARP2	Poly(ADP-ribose)polymerase-2
Pol	Polymerase
Polβ	DNA polymerase β
PolβΔ87	Polβ missing 87 bp (residues 208–236)
PolβN140	Amino acid residues 1–140 of Polβ
PTM	Post-translational modification
SQAG	Sulfo-quinovosyl-acyl-glycerol
SQMG	Sulfo-quinovosyl-monoacyl-glycerol
ssDNA	Single-stranded DNA
TMZ	Temozolomide
uPAR	Urokinase-type plasminogen activator receptor
WT	Wild type

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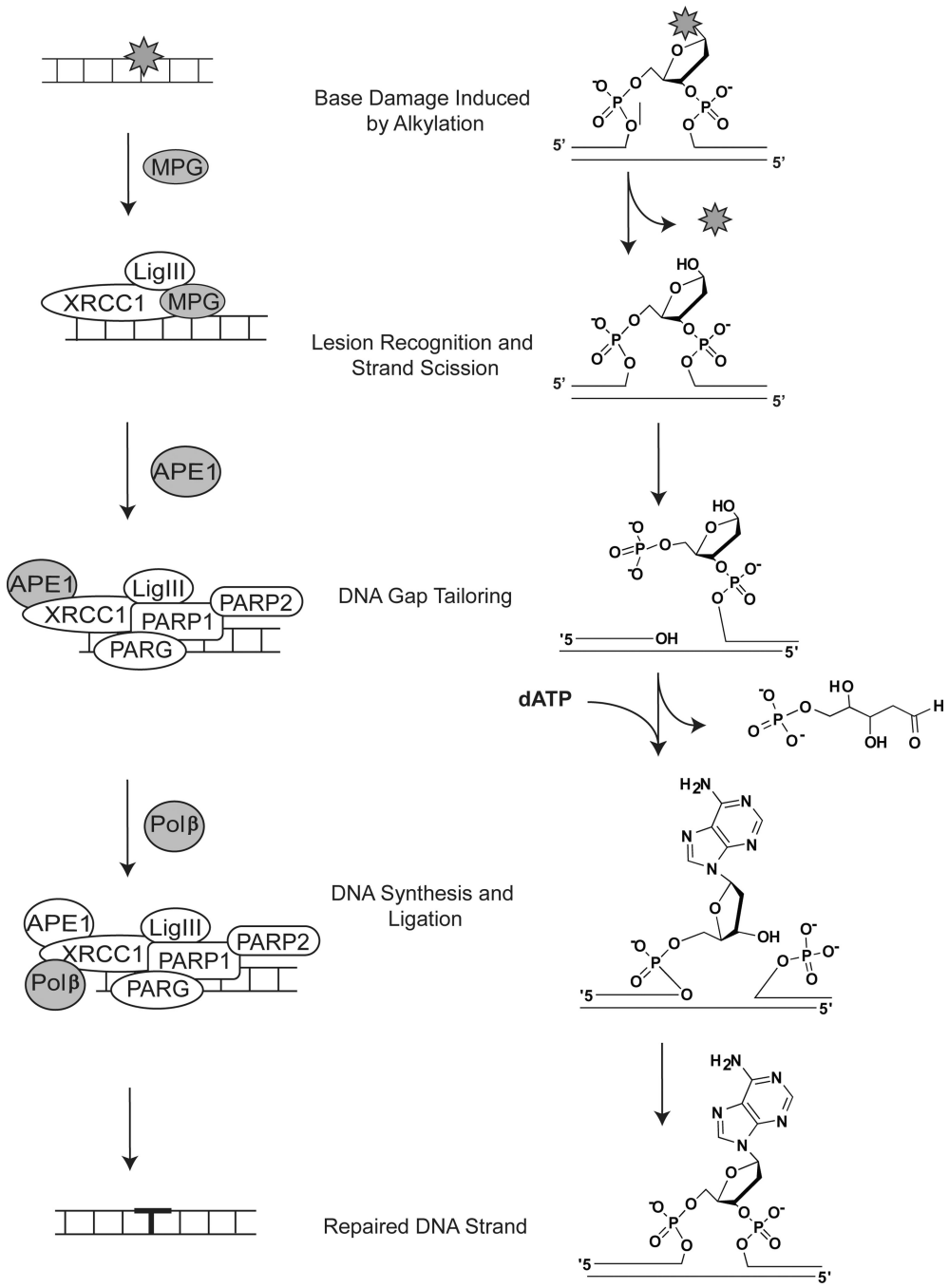

Fig. 1. Model for MPG-initiated BER

This model depicts the proteins and chemical structures of a TMZ-induced base lesion (N3-MeA) and the corresponding BER intermediates following BER initiated by the methylpurine DNA glycosylase, MPG. The chemistry of the lesion and the repair intermediates throughout the repair process are shown on the right, highlighting the three major steps for BER: Lesion Recognition/Strand Scission, Gap Tailoring and DNA Synthesis/Ligation. The structures on the left depict the protein complexes required for completion of each step in BER initiated by MPG.

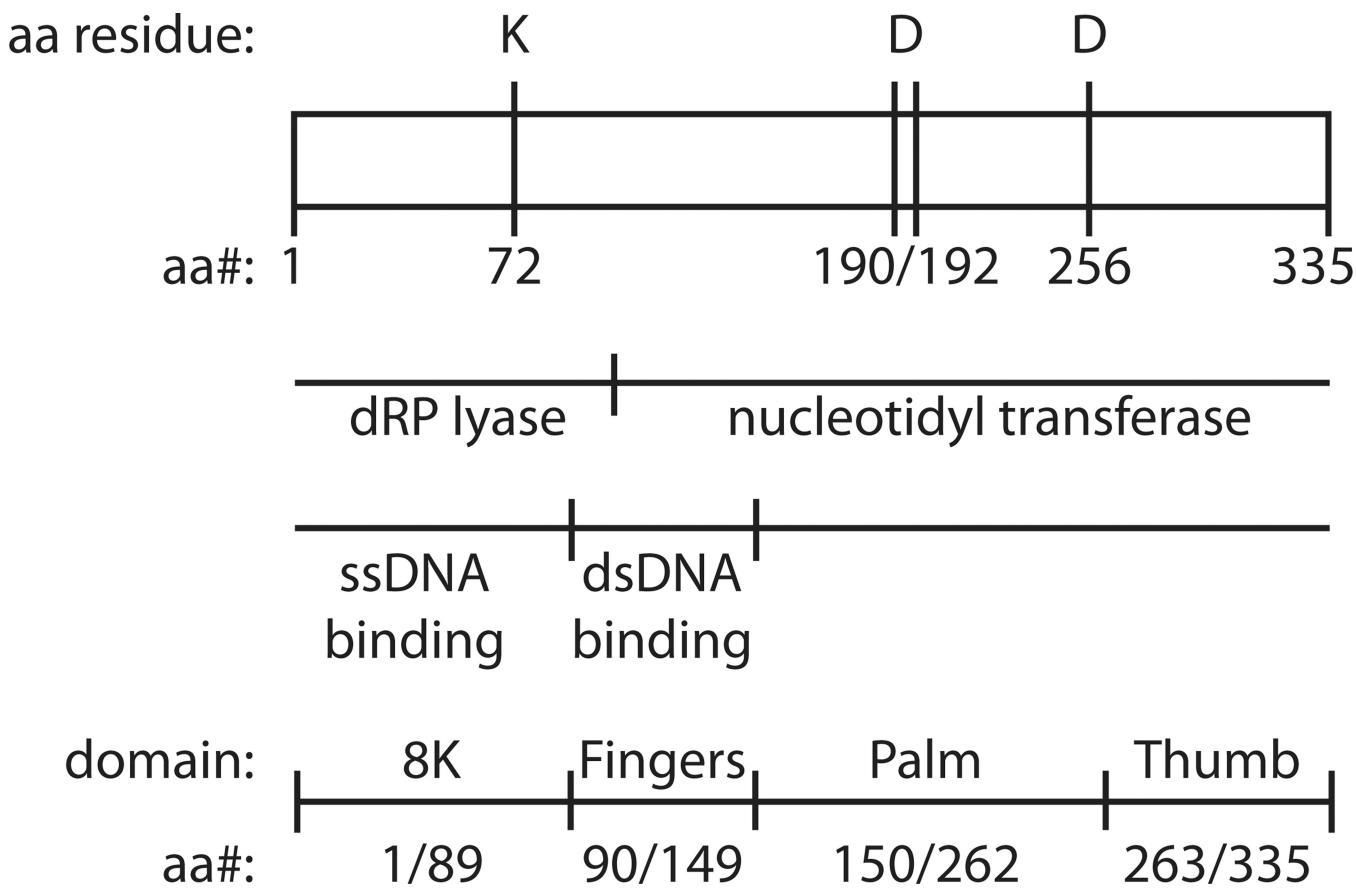

Fig. 2. DNA Polymerase β

A linear depiction of the amino acid residues (335) of DNA Polymerase β: indicating the functional domains (dRP lyase and nucleotidyltransferase), the essential active site residues (K72 and D190/192/256), regions involved in ssDNA and dsDNA binding and the structural sub-domains as determined by crystallographic analysis (8K, fingers, palm and thumb).

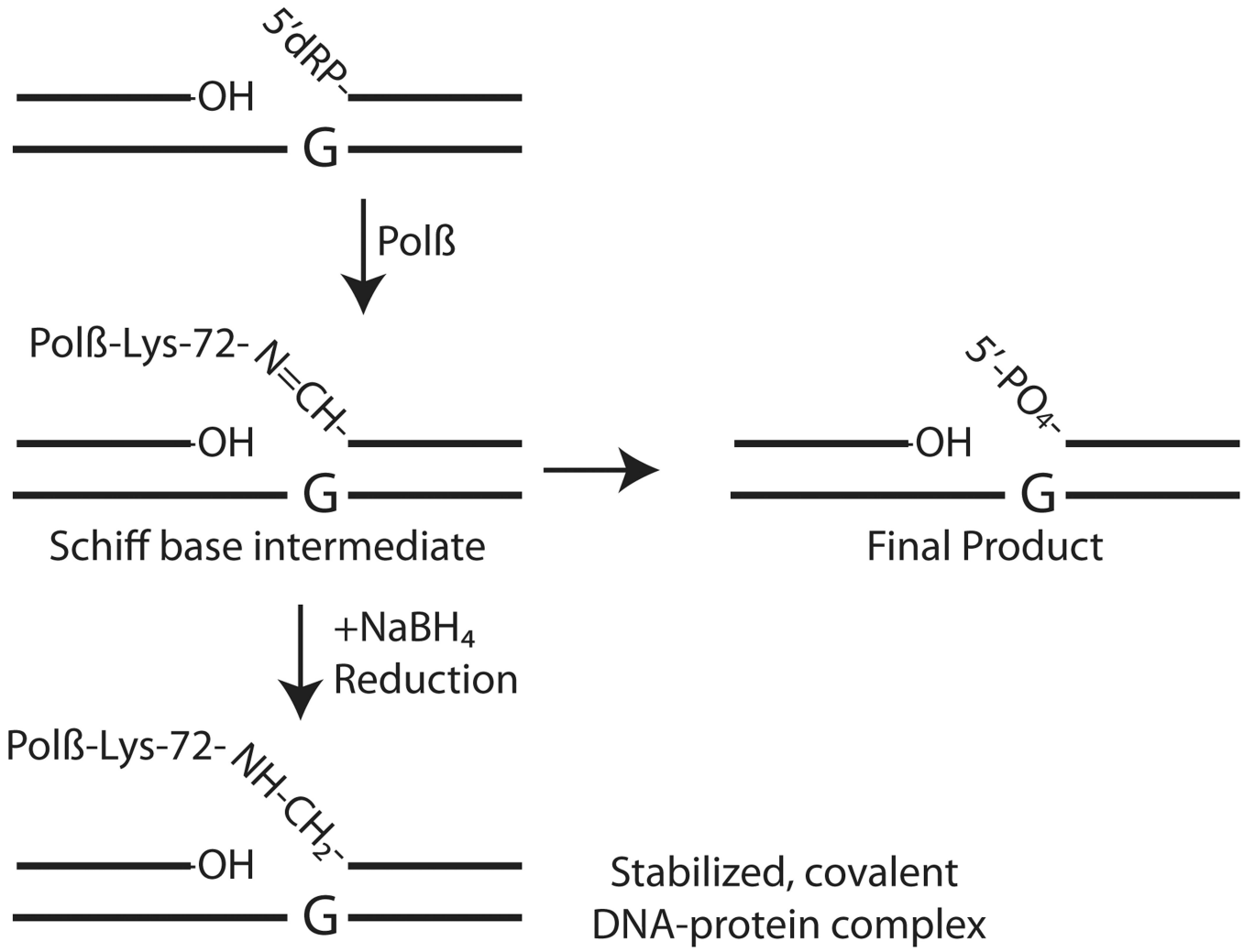

Fig. 3. Schiff base formation and the 5'dRP lyase activity of Polβ

Schematic demonstrating the Schiff base formed as an intermediate in the 5'dRP lyase reaction conducted by Polβ, mediated by the nucleophile, K72. Completion of 5'dRP removal yields a final product (right side) that is further processed by Polβ by addition of a single nucleotide and ligation mediated by the XRCC1/LigIII heterodimer (see Fig. (1)). Evidence of the Schiff base is demonstrated by the stabilized DNA-protein complex formed upon reduction with $NaBH_4$ (bottom left).

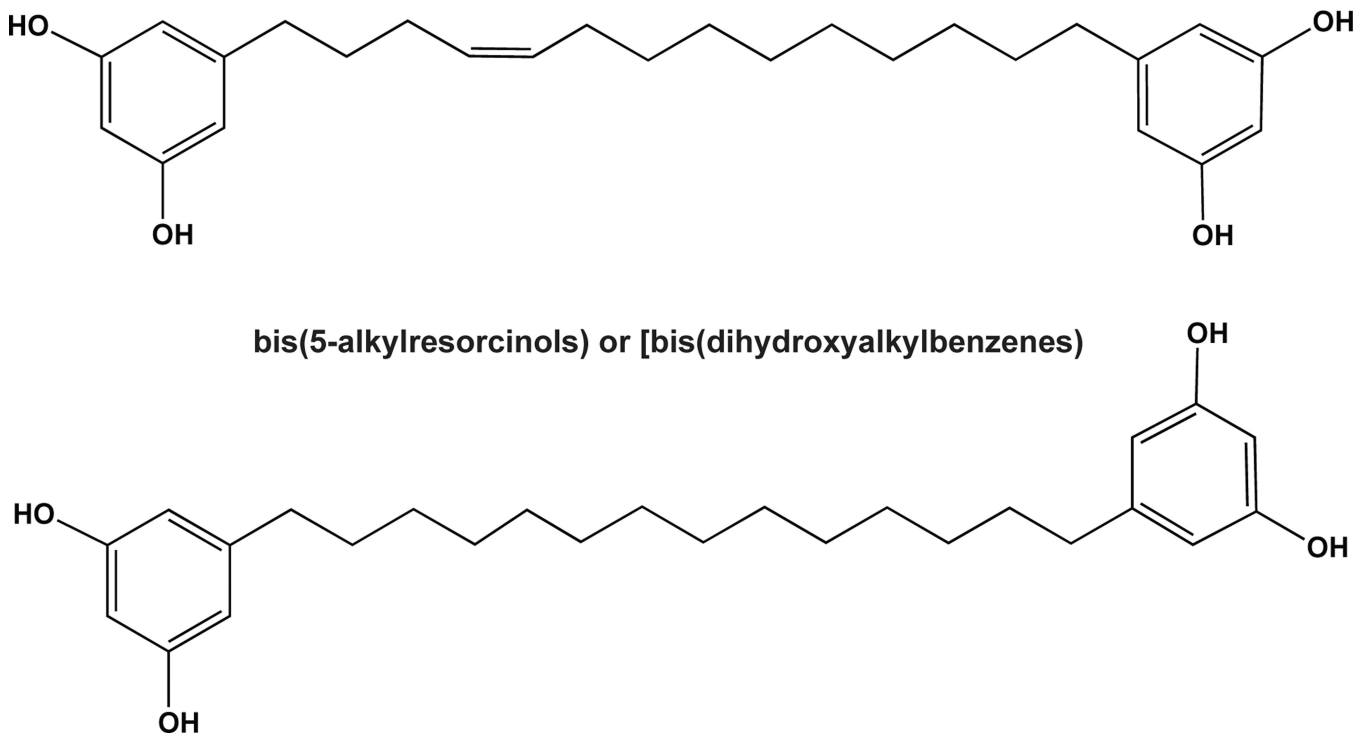

Fig. 4. Novel alkylresorcinol inhibitors of Polβ

Schematic depicting two novel alkylresorcinol analogs found to inhibit Polβ.

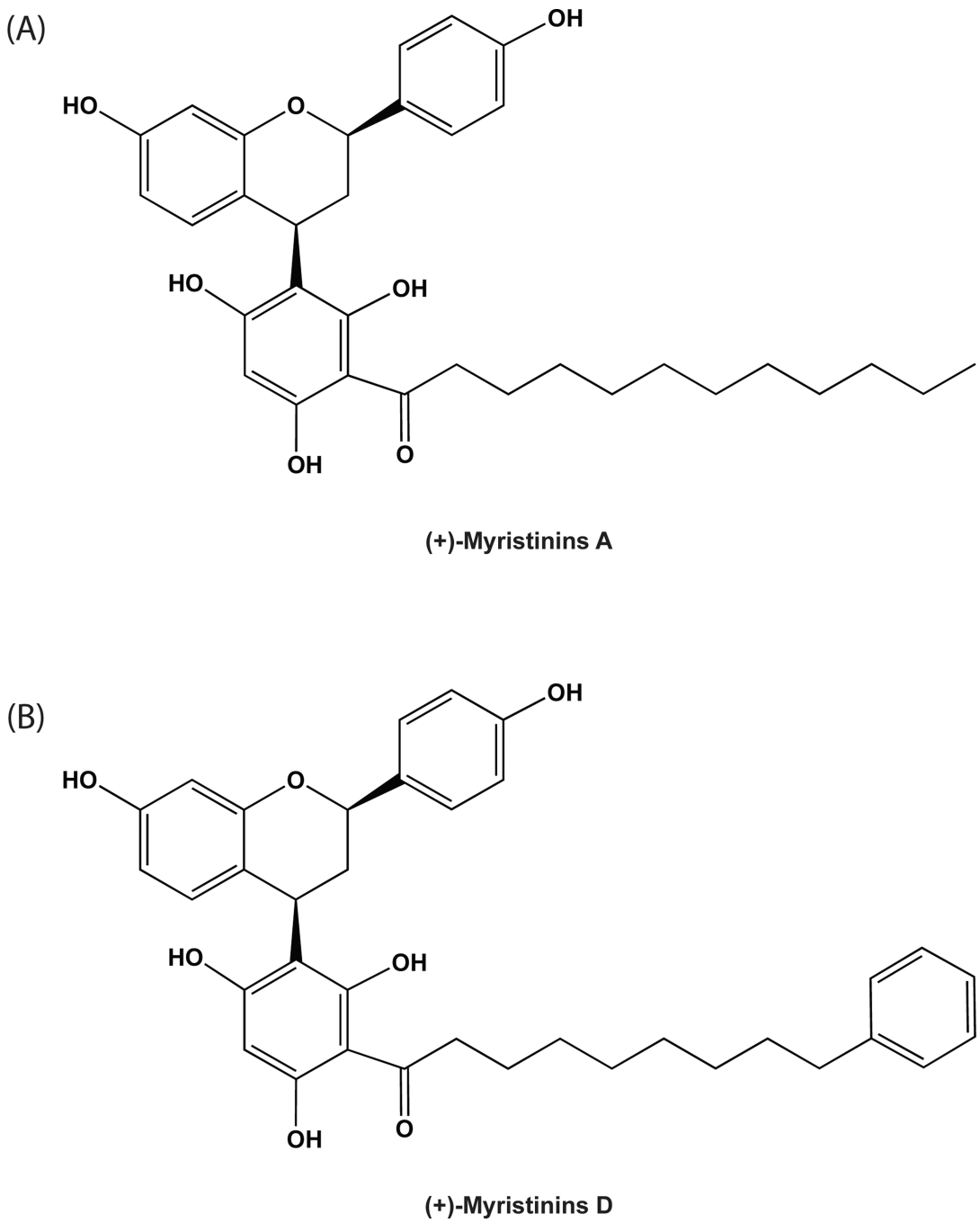

Fig. 5. Flavinoid inhibitors of Polβ

Structure of two flavinoids isolated from *Knema elegans* and identified as inhibitors of Polβ. The parent structure is shown, with varying R groups, depicting **(A)**(+)-myristinin A and **(B)** (+)-myristinin D.

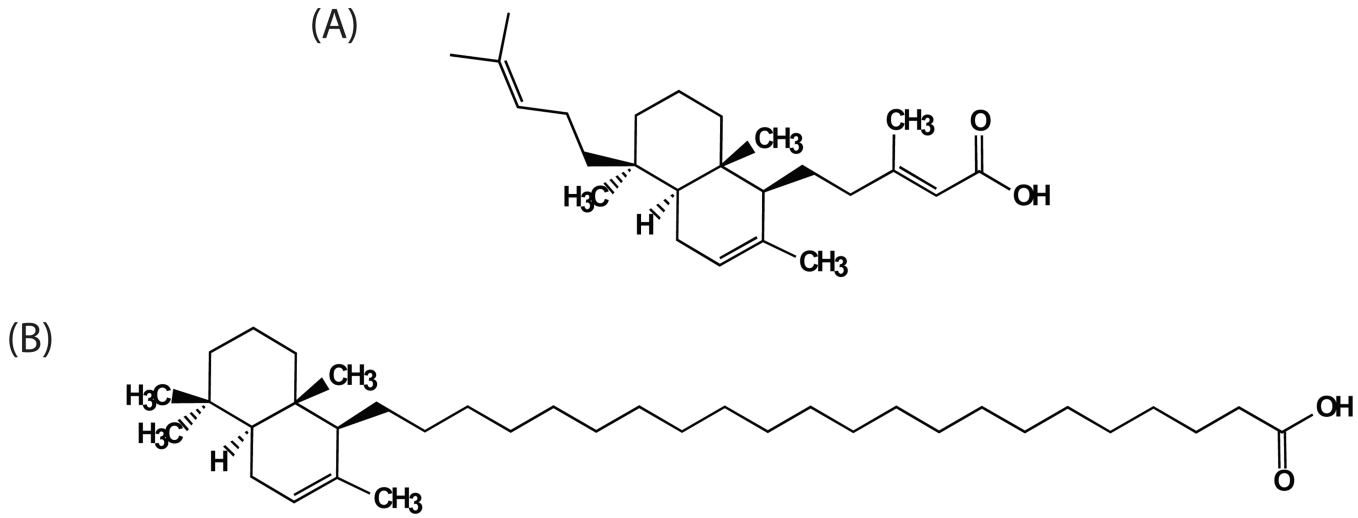

Fig. 6. Kohamaic acid A and a chemically derived fatty acid derivative

The structure of **(A)** the *Ircinia sp*. derived compound Kohamaic acid A (KA-A) and **(B)** the fatty acid derivative Compound #11; [1S*,4aS*,8aS*)-17-(1,4,4a,5,6,7,8,8a–octahydro-2,5,5,8a–tetramethylnaphthalen-1-yl)heptanoic acid] [88].

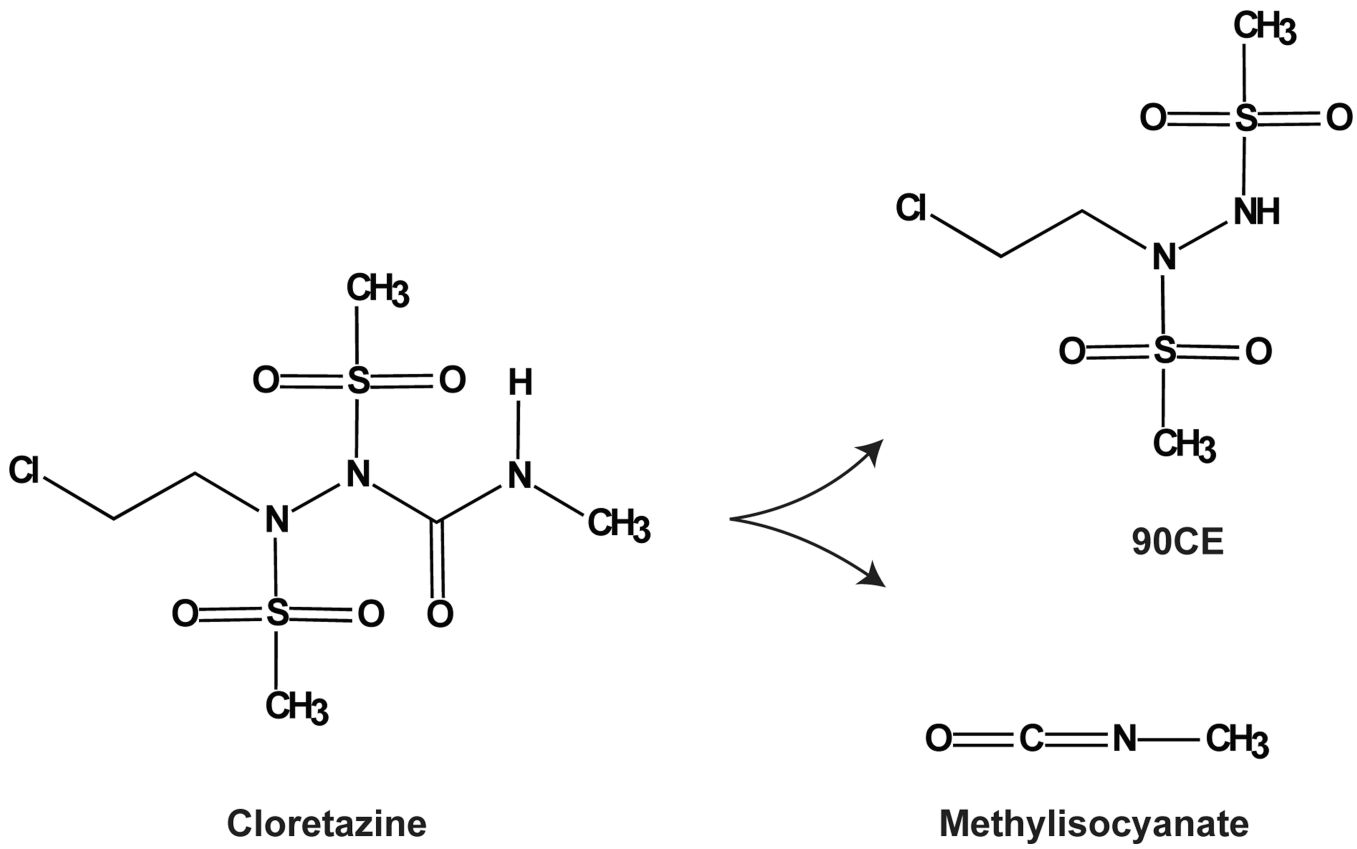

Fig. 7. Cloretazine and reactive metabolites

Shown is the structure of Cloretazine and the reactive breakdown products 90CE and methylisocyanate [91, 96].

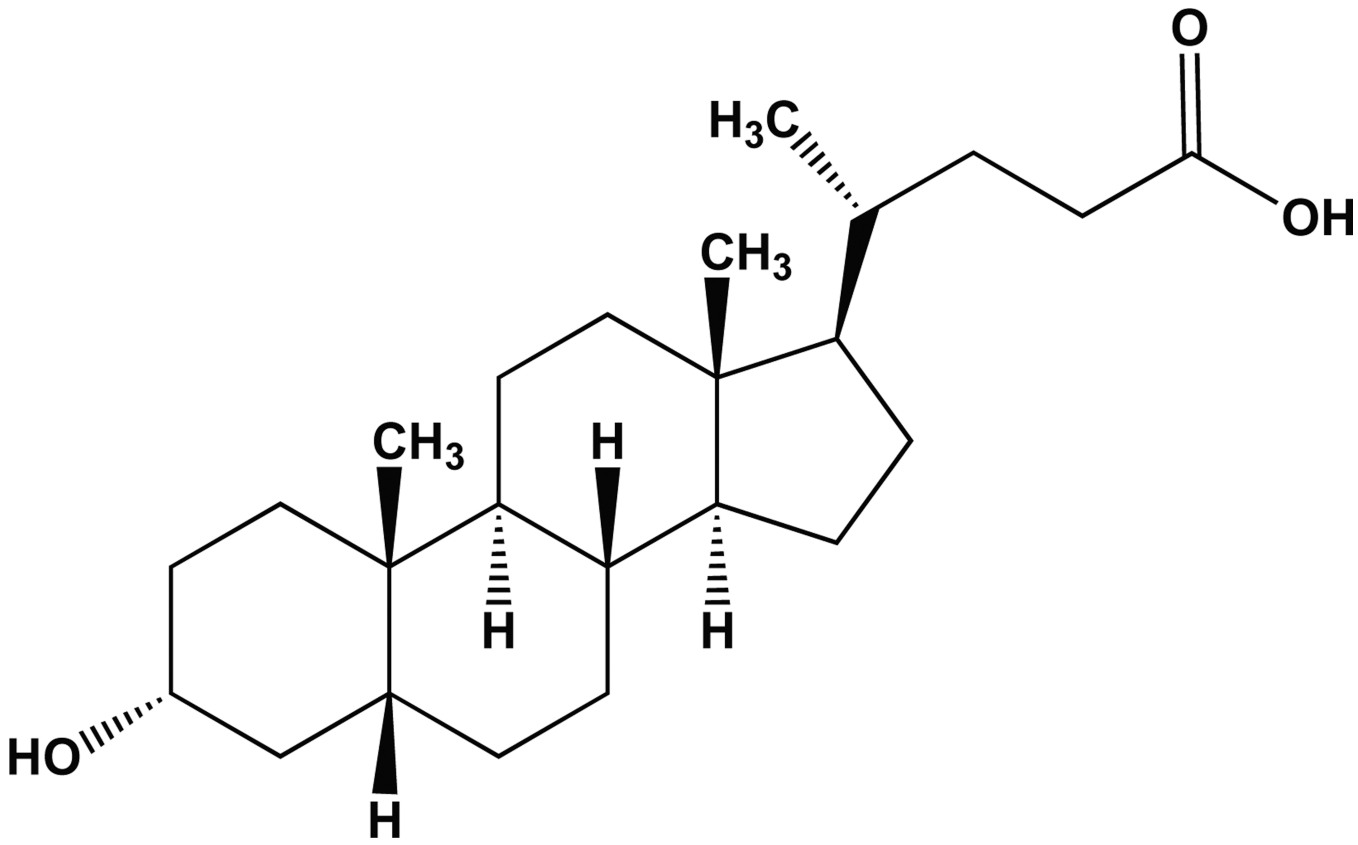

Fig. 8. Lithocholic acid

Structure of Lithocholic acid: (4R)-4-[(3R,5R,8R,9S,10S,13R,14S, 17R)-3-hydroxy-10,13-dimethyl-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1H–cyclopenta[a]phenanthren-17-yl]pentanoic acid.

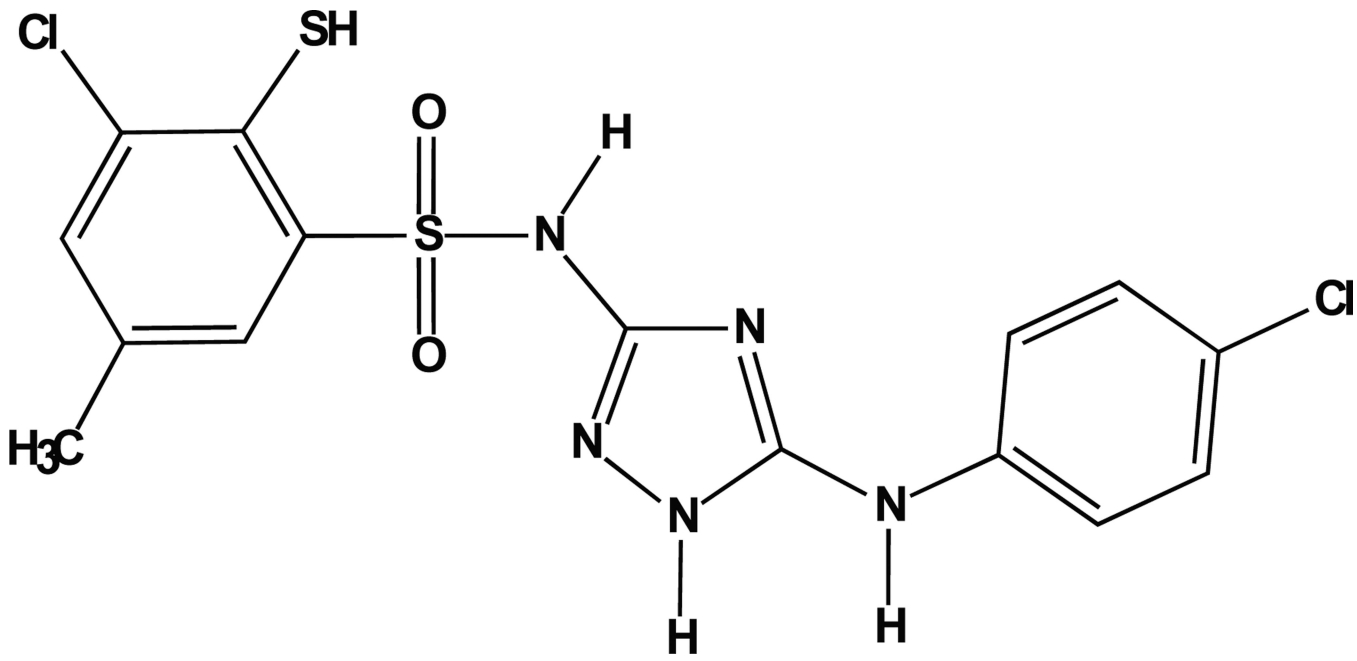

Fig. 9. Structure of NSC666715

Schematic depicting the chemical structure of 4-chloro-N-[5-(4-chloroanilino)-1H-1,2,4-triazol-3-yl]-5-methyl–2-sulfanylbenzenesulfonamide (NSC666715), initially reported as a potential inhibitor of HIV integrase [114–116] and later identified as an inhibitor of Polβ [113].

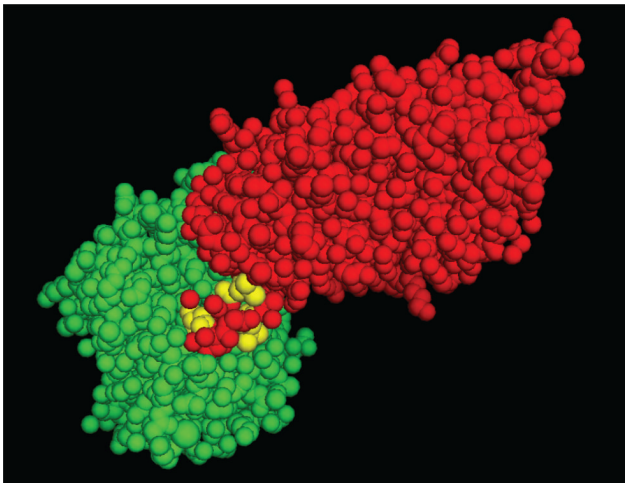

Fig. 10. Model depicting the Polβ/XRCC1 interaction

Structure of a complex formed by the thumb sub-domain of Polβ and the N-terminal domain of XRCC1 [132]. The image is a spacefilling rendition of the amino acid residues of XRCC1 in green and the thumb sub-domain of Polβ in red. The residues in the V303 hairpin, corresponding to L301, V303 and V306 are shown in yellow. The image was generated using PyMOL (Molecular Graphics System, Version 1.2r3pre; Schrödinger, LLC; http://pymol.org/).

Table 1

Genetic and physical characteristics of Polβ*.

Gene Name	**POLB**	**[1]**
NCBI Gene ID	5423	http://www.ncbi.nlm.nih.gov/gene/5423
NCBI-gi	4505931	http://www.ncbi.nlm.nih.gov/protein/4505931
Accession Code	NM_002690	http://www.ncbi.nlm.nih.gov/nuccore/NM_002690
GenBank	NP_002681	http://www.ncbi.nlm.nih.gov/protein/NP_002681
Uniprot	P06746	http://www.uniprot.org/uniprot/P06746
STRING	P06746	http://string.embl.de/newstring_cgi/show_network_section.pl?identifier=P06746
HPRD	7517	http://www.hprd.org/summary?hprd_id=07517&isoform_id=07517_1&isoform_name=isoform_1
Chromosomal location	8p11.2	[181, 182]
Gene length	33 kb	[183]
Protein size (MW)	39 kDa	[3, 18]

* Additional details of Polβ and other DNA Repair genes may be found at: https://dnapittcrew.upmc.com/ and http://sciencepark.mdanderson.org/labs/wood/DNA_Repair_Genes.html

Table 2

DNA polymerase β interacting proteins.

Protein abbreviation	Protein name	Polβ interaction domain	Method*	Functional impact	Citation
AAG (MPG)	N-methylpurine DNA glycosylase	-	IP	-	[184]
APC	Adenomatous polyposis coli	8K domain (Thr79, Lys81, and Arg83)	FW, Y2H	Inhibition of Polβ lyase activity, inhibition of Polβ strand displacement synthesis	[118, 185, 186]
APE1	DNA-(apurinic/apyrimidinic site) lyase	-	Y2H	Stimulation of the 5'dRP lyase activity	[187]
CHIP	E3 ubiquitin-protein ligase CHIP	8K domain	-	Polyubiquitylation of Polβ	[54]
FEN1	Flap structure-specific endonuclease 1	-	IP	Stabilization of FEN1 binding to DNA, stimulation of strand displacement and flap hydrolysis	[188, 189]
HMGB1	High-mobility group box 1	-	IP	-	[48]
HSP27	Heat shock protein 27	-	IP	Thermoprotection of Polβ	[190]
HSP70	Heat shock protein 70	-	IP	Stimulation of Polβ activity, thermoprotection of Polβ	[190, 191]
Lig1	DNA ligase 1	-	IP, PD	Inhibition of strand displacement synthesis	[192]
MGC5306 (JOSD3; TAF1D)	TATA box-binding protein-associated factor RNA polymerase I subunit D	-	Y2H, IP	-	[193]
Mule	Mcl-1 ubiquitin ligase	8K domain	-	Monoubiquitylation of Polβ	[52]
MYST1	MYST histone acetyl transferase 1	-	Y2H	-	[194]
NEIL1	Nei endonuclease VIII-like 1 (E. coli)	Amino acids 1–140	FW	NEIL1 initiated APE-1 independent BER	[195]
NEIL2	Nei endonuclease VIII-like 2 (E. coli)	Amino acids 1–140	IP, FW	NEIL2 initiated APE-1 independent BER	[196]
OGG1	8-oxoguanine DNA glycosylase 1	-	IP	-	[184]
p300	Histone acetyltransferase p300	8K domain	IP, PD	Acetylation of Polβ	[51]
p53	Cellular tumor antigen p53	-	FW, IP	Enhanced stabilization of Polβ to AP site DNA	[197]
PARP1	Poly(ADP-ribose) polymerase 1	Amino acids 124–335	PD	Stimulation of Polβ strand displacement synthesis activity, enhanced BER activity	[198]
PARP2	Poly(ADP-ribose) polymerase 2	-	PD	Enhanced BER activity	[199]
PCNA	Proliferating cell nuclear antigen	31K domain (Palm)	IP, Y2H	-	[200]
PNKP	Polynucleotide kinase 3'- phosphotase	-	IP	Enhanced single strand break repair	[201]

Protein abbreviation	Protein name	Polβ interaction domain	Method*	Functional impact	Citation
PRMT1	Protein arginine methyltransferase 1	8K domain	IP, PD	Arginine methylation of Polβ	[202]
PRMT6	Protein arginine methyltransferase 6	8K domain	IP, PD	Arginine methylation of Polβ	[203]
Rad9/Rad1/Hus1 (9-1-1 complex)	RAD9 homolog/RAD1 homolog/HUS1 checkpoint homolog	-	IP	Stimulation of Polβ strand displacement synthesis activity, increased Polβ affinity for 3'OH primer end	[204]
RPA	Replication protein A	-	PD	Reduction of Polβ gap filling efficiency opposite of 8-oxo-G	[205]
TLE1	Transducin-like enhancer of split 1	-	Y2H	-	[194]
Trf2	Telomeric repeat binding factor 2	31K domain		Stimulation of primer extension, stimulation of strand displacement synthesis	[5, 6]
WRN	Werner syndrome, RecQ helicase	-	IP, PD	Stimulation of Polβ strand displacement synthesis, unwinding of Polβ single strand intermediates	[206]
XRCC1	X-ray repair complementing defective repair in Chinese hamster cells	31K domain (thumb-palm domains)	FW, Y2H, PD	Scaffolding of BER complex, inhibition of strand displacement	[133]

*IP, immunoprecipitation; PD, GST- or other tagged protein pulldown; Y2H, Yeast two-hybrid; FW, Far-Western.

Table 3

Post-translational modifications of Polβ.

Modification	Amino acids modified	Modified by	Functional impact	Citation
Phosphorylation	Ser 44, Ser 55	PKC alpha	Inhibition of polymerase activity	[207]
Phosphorylation	Tyr 250	n.d.	n.d.	[208, 209]
Acetylation	Lys 72	p300	Inhibition of 5'dRP lyase activity	[51]
Methylation	Arg 137	PRMT1	Inhibition of binding to PCNA	[202]
Methylation	Arg 83, Arg152	PRMT6	Stimulation of DNA binding and polymerase activity	[203]
Polyubiquitylation	*n.d.*	E3 ligase CHIP	Degradation of Polβ not involved in BER complex	[54]
Monoubiquitylation	Lys 41, Lys 61, Lys 81	E3 ligase Mule	Further polyubiquitylation by CHIP and protein degradation	[52]

n.d. = not determined.

Table 4

Disease-Associated Polβ Mutants

Mutation	Effect of mutation	Associated disease	Citation
Glu295Lys	Decreased polymerase activity, acts as a dominant negative	Gastric cancer	[210]
Leu22Pro	Loss of 5'dRP lyase activity	Gastric cancer	[211, 212]
Cys239Arg	Reduction in polymerase accuracy	Gastric cancer	[211]
Tyr39Cys	*n.d.*		
Asn294Asp	*n.d.*		
Asp160Asn	*n.d.*		
Lys289Met	Reduction in polymerase accuracy	Colon cancer	[213, 214]
Δ208–236	Decreased polymerase activity, acts as a dominant negative	Colon cancer	[8, 214]
Δ213–219	*n.d.*		
Δ249–262	*n.d.*		
Δ17 bp (frameshift and truncation = 209 amino acid protein)	*n.d.*		
ΔT at codon 152 (frameshift and truncation = 162 amino acid protein)	*n.d.*	Prostate cancer	[8, 215]
Ile260Met	Misalignment-mediated errors in dipyrimidine sequences	Prostate cancer	[216]
Δ208–236	Decreased polymerase activity, acts as a dominant negative	Lung cancer	[155, 214]
Δ546 bp (deletion of codons 20–202)	*n.d.*	Lung cancer	[155, 214]
Δ208–236	Decreased polymerase activity, acts as a dominant negative	Breast cancer	[217]
Pro242Arg	*n.d.*	Breast cancer	[218]
Lys289Met	*n.d.*		
Val215Pro	*n.d.*	Bladder cancer	[219]
Lys248Gln	*n.d.*		
Ser229Pro	*n.d.*		
A insertion at nucleotide 744	*n.d.*		
Arg183Gly	*n.d.*	Esophageal cancer	[220]
Glu177Stop	*n.d.*		
Δ177–234	*n.d.*		
Gly179Arg	*n.d.*		
Phe114Ser	*n.d.*		
Gly118Glu	*n.d.*		
Ile88Val	*n.d.*		
Phe114Ser	*n.d.*		
Lys167Ile	*n.d.*		
Glu186Gly	*n.d.*	Esophageal cancer	[221]
Δ177–234 bp (frameshift)	*n.d.*	Esophageal cancer	[220, 221]

Mutation	Effect of mutation	Associated disease	Citation
Δ208–236	Decreased polymerase activity, acts as a dominant negative	Werner syndrome	[222]
Arg182Gly	*n.d.*	Cervical Cancer	[223]

n.d. = not determined.

Table 5

DNA Polβ 5'dRP lyase inhibitors

Compound Name	Abbreviation	Citation
Lithocholic acid	LCA	[107]
4-chloro-N-[5-(4-chloroanilino)-1H-1,2,4-triazol-3-yl]-5-methyl-2-sulfanylbenzenesulfonamide	NSC666715	[113]
Koetjapic acid	KJA*	[70, 173]
Biquinoline-dicarboxylic acid	BQD*	[173]
Naphthochrome green	NCG*	[173]
Mordant blue	MB*	[173]
Glycyrrhizic acid	GA*	[173]
4,4′-(hexafluoroisopropylidene) bis(benzoic acid)	HFPB*	[173]
3-(4-carboxyphenyl) 2,3-dihydrotrimethyl 1-indene-5-carboxylic acid	CPIC*	[173]
Carbenoxolone	CBX*	[173]
4′4′-biphenyl dicarboxylic acid	BPDC*	[173]
Pamoic Acid	PA*	[173, 174]

* The structures have been reported – see [173].