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DNA polymerase beta (pol β) inhibitors: A comprehensive overview

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Base excision repair (BER) is the fundamental pathway responsible for the elimination of damaged DNA bases and repair of DNA single-strand breaks generated spontaneously or produced by DNA-damaging agents. Among the essential enzymes that are required to achieve the BER reaction is DNA polymerase beta (pol β), which has been regarded as a potential therapeutic target. More than 60 pol β-inhibitors have been identified so far; however, most of them are either not potent or not specific enough to become a drug. In this article we compile an essential knowledge base regarding the structures, the modes of inhibition and the activities of these pharmacologically interesting molecules.

Introduction

Base excision repair (BER) (Fig. 1) is the major cellular pathway that is responsible for the recovery of single-strand breaks (SSB) and removal of damaged bases such as oxidized-reduced, alkylated and deaminated bases [1]. These DNA modifications can occur spontaneously due to thermodynamical hydrolysis events or by exposing cells to environmental mutagens [2–5]. Furthermore, other DNA modifications can be induced synthetically as a result of anticancer treatments using alkylating agents or ionizing radiation. However, in the latter case, BER constitutes a prevailing way that is usually adopted by cancer cells to reduce the efficacy of and to promote resistance against radiotherapy and a growing list of DNA-damaging agents [2,3] including bleomycin [6] and monofunctional alkylating agents [7]. Therefore, it has been broadly proposed that regulating the BER pathway through small molecule inhibitors can reduce the required dosage of such DNA-damaging agents while potentiating their efficacy in eradicating cancer cells [8]. Fortunately, most of the proteins involved in and coordinating the BER process have been identified, cloned and crystallized enabling the rational design of small molecule inhibitors for their activity [9]. One of these proteins, DNA polymerase beta (pol β), has been recognized as a vital element in completing the BER pathway [10,11]. In addition, there is a large body of evidence that suggests that pol β has an important role during the life of a cell. For example, a 'knock-out' of the gene that encodes for pol β in mice results in embryonic lethality, confirming the importance of the protein during fetal development [12].

Pol β has a significant role in chemotherapeutic agent resistance, because its overexpression reduces the efficacy of anticancer drug therapies [13]. As a result of bypassing errors, the enzyme can help cancer cells tolerate DNA damage [14]. Furthermore, small-scale studies on different types of cancer showed that pol β is mutated in approximately 30% of tumors, which in turn reduces pol β fidelity in DNA synthesis exposing the genome to serious and often deleterious mutations [15–17]. Based on these findings, pol β has been seriously considered as a promising therapeutic target for cancer treatment.

Many inhibitors of DNA pol β have been identified during the past two decades. To name but a few classes of these inhibitors, this list includes polypeptides [18], fatty acids [19], triterpenoids [20], sulfolipids [21], polar lipids [22], secondary bile acids [23], phenalenone-derivatives [24], anacardic acid [25], harbinatic acid [26], flavonoid derivatives [27], and pamoic acid [28]. However, most of these inhibitors are not potent enough or lack sufficient specificity to eventually become approved drugs.

The objective of this review is to provide a comprehensive classification of known DNA pol β-inhibitors discovered so far. Furthermore, we have compiled an extensive knowledge base

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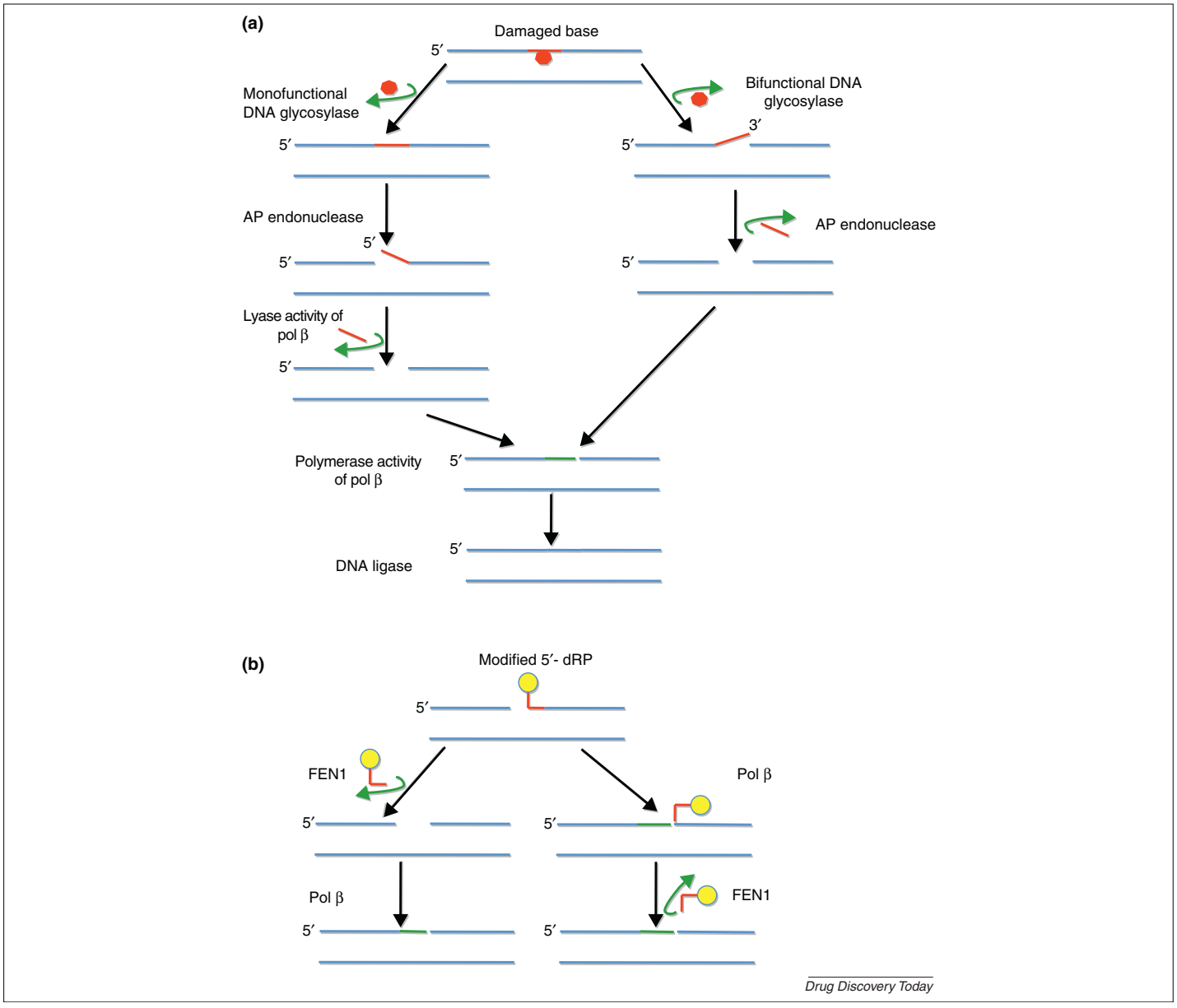

FIGURE 1

Base excision repair (BER). Two different types of BER mechanisms. (a) Short patch BER replaces a single damaged nucleotide by a new correct nucleotide. It starts with damage-specific DNA glycosylases which recognize and cleave the N-glycosylic bond between the irregular base and the sugar-phosphate backbone. If a bifunctional DNA glycosylase was used, the lyase activity of pol β removes the 5′-sugar-phosphate residue. If a monofunctional glycosylase was used, the incision step is performed by AP endonuclease. During the next step, the polymerase activity of pol β fills the generated gap with the correct nucleotide leaving the final step to DNA ligase I or III to ligate the nicked DNA termini. (b) Long patch BER incorporates patches greater than one-nucleotide. In this case, bifunctional glycosylases are commonly used and pol β adds a single nucleotide to the repaired gap and then is replaced by pol δ/ε, which extends the repair patch and displaces several nucleotides to create a 50-flap junction. The flap is then detached from the structure with the help of flap endonuclease-1 (FEN1). *Abbreviation*: AP: apurinic/apyrimidinic.

regarding the structure, the mode of inhibition and molecular-level activity of these molecules. It is hoped that the application of this knowledge will lead to the discovery of novel, more potent and ultimately cancer cell-specific drug candidates resulting in the improvement of existing cancer therapies, including ionizing radiation, and chemotherapy based on bleomycin and monofunctional alkylating agents.

Activity of DNA pol β

The faithfulness of BER is dependent on the polymerization step, where the major BER DNA polymerase, pol β, must incorporate the correct Watson–Crick base paired nucleotide into the one-nucleotide repair gap. The enzyme has been identified as a 39-kDa protein with 335 amino acids in its sequence (Fig. 2) [10]. Its small size compared to other polymerases, has made it the smallest and simplest cellular DNA polymerase found. Although pol β lacks the proof-reading 3′- or 5′-exonuclease activities, which are usually found in high fidelity enzymes, it possesses 5′-dRP lyase and AP lyase activities instead [11,29].

The active pol β enzyme is a stable monomer in solution, folded into distinct domains and subdomains that exhibit a variety of functions essential for its activity. These functions include

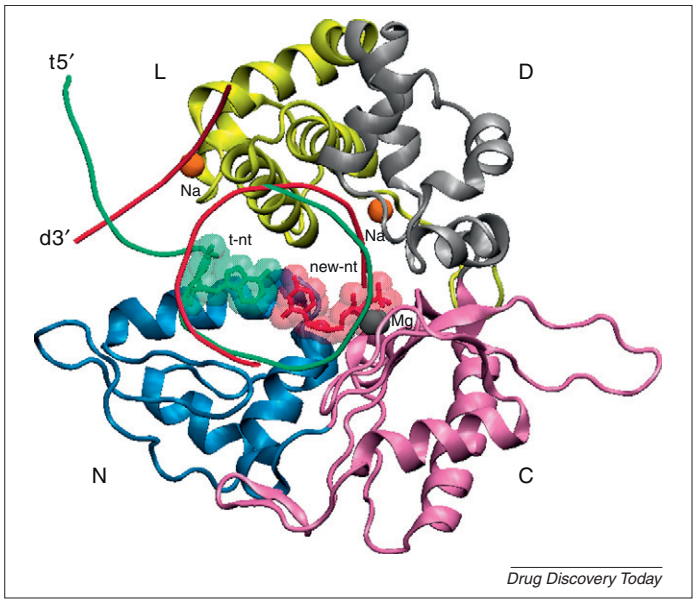

FIGURE 2

Structure of DNA polymerase beta. The enzyme is a 39-kDa protein with 335 amino acids in its sequence. The full-length enzyme consists of an amino-terminal lyase domain (8 kDa) (L), shown in yellow, connected by a short protease-sensitive fragment to a carboxyl-terminal polymerase domain (31 kDa). The 31-kDa domain is further subdivided into, D- (duplex DNA binding subdomain), shown in gray, C-(catalytic subdomain), shown in mauve and N- (nascent base pair binding subdomain), shown in blue. The overall structure of pol β resembles the shape of a right hand, with fingers (C-subdomain), thumb (D-subdomain) and palm (N-subdomain) arrangements. The active enzyme requires a single-stranded DNA template (green) and two divalent metal Mg^{2+} ions (gray) for its polymerase activity. It employs two major substrates, namely, a 2′-deoxynucleoside 5′-triphosphate (new-nt), shown in red and a template-primer DNA, shown in green. The 8-kDa domain interacts with the downstream duplex (red), where the 5′-phosphate on the downstream strand is located close to the dRP lyase active site. The lyase domain cooperates with the N-subdomain to form a doughnut-shaped structure that surrounds the DNA molecule. The enzyme also uses two helix-hairpin-helix (HhH) motifs that unspecifically interact with the DNA backbone. The two HhH motifs, located within the lyase domain (residues 55–79) and the D-subdomain (92–118), incorporate Na^{+} ions (orange) and interact with each end of the incised DNA strand, namely, the downstream and primer strands.

single-stranded (ss) and double-stranded (ds) DNA binding, nucleoside triphosphate (dNTP) binding, and the dRP lyase and nucleotidyl transferase catalytic activities [30,31]. Essentially, the full-length enzyme consists of an amino-terminal lyase domain (8 kDa), connected by a short protease-sensitive fragment to a carboxyl-terminal polymerase domain (31 kDa). The 31-kDa domain is further subdivided into C-(catalytic), D- (duplex DNA binding), and N- (nascent base pair binding) subdomains. Interestingly, similar to other DNA polymerases, the overall structure of pol β resembles the shape of a right hand, with fingers (C-subdomain), thumb (D-subdomain) and palm (N-subdomain) arrangements (Fig. 2) [10,32]. The active enzyme requires a primer-template, a ssDNA template and divalent metal ions for its polymerase activity.

Structure of DNA pol β

Analogous to most DNA-binding proteins, pol β uses the well-known helix-hairpin-helix (HhH) motifs that unspecifically interact with the DNA backbone. These HhH motifs are located within the lyase domain (residues 55–79) and the D-subdomain (92–118) and interact with each end of the incised DNA strand, namely, the downstream and primer strands. The crystal structures of pol β at different catalytic stages revealed the significant conformational changes that take place within the various subdomains of the protein [33]. These conformational dynamics processes are obvious when comparing the structure of the apoenzyme [34] to other structures that encompass DNA and the two substrates of the enzyme [35]. The fully loaded pol β structure implied that the 8-kDa domain interacts with the downstream duplex, where the 5′-phosphate on the downstream strand is located close to the dRP lyase active site. Furthermore, the lyase domain cooperates with the N-subdomain to form a doughnut-shaped structure that surrounds the DNA molecule (Fig. 2). These notable interactions and functions of the lyase domain indicate that a small molecule that can bind to the lyase active site, especially within the ssDNA-binding pocket, should be able to affect the polymerization activity of pol β as well. Besides the lyase domain dynamics, the N-subdomain seems to exhibit considerable movements once the correct dNTP substrate is bound to the enzyme [35]. Additionally, as illustrated in Fig. 2, there is a major conformational change within the structure of the DNA substrate.

Initial investigations of DNA pol β inhibitors

Although the structure of pol β reveals some conserved regions that would be suitable for designing a specific inhibitor for its activity [34], the synthesis of such compounds seems to be difficult and most of the identified inhibitors were derived from naturally occurring molecules (see below). In fact, the first attempt to inhibit and understand the activity of pol β employed portions of the protein itself [18]. This earliest *in vitro* study by Husain and coworkers [12] tried to examine the enzyme as a potential therapeutic target by investigating its interaction with various pol β domains. Although this study used a large peptide as a potential pol β inhibitor, which may seem to be an impracticable and unreasonable drug candidate, this work provided the much needed proof-of-concept that a pol β inhibitor can impede the BER pathway.

In parallel to this pioneering effort, many attempts were made to isolate and identify a small molecule inhibitor that can specifically bind to pol β. In regard to these endeavors, one can recognize at least two research groups that contributed to a large extent to the discovery of more than 60 molecules that can bind to DNA polymerases in general and a few of them that can target pol βin particular. Table 1 and Fig. 3 list the structure and activities of a selected number of the identified inhibitors (see below for more details).

First DNA pol β inhibitors

The origin of the studies that were focused on screening for small molecule pol β-inhibitors can be attributed to Mizushina *et al.* in the midnineties [19]. This team represents collaboration among research groups in Japan, and their first study was the screening of microbial fermentation for structures that can inhibit DNA polymerases activity. During the analysis part of the project, they isolated linoleic acid (LA) (**1**), a well-known fatty acid, as an inhibitor for calf thymus DNA pol α and cloned purified rat DNA pol β. They also examined the effect of several commercially available fatty acids on the activity of DNA polymerases. Their

TABLE 1

Selected inhibitors of DNA pol β

ID	Given name	Binding site and affinity	Other targets	Refs
1	Linoleic acid (LA)	8-kDa domain (IC_{50} = 38 μM)	Pol α	[19,36,37]
2	Nervonic acid (NA)	8-kDa domain (IC_{50} = 5.8 μM)	Pol α	[19,36,37]
3	Fomitellic acid (FA) A	8-kDa domain (IC_{50} = 125 μM)	Pol α	[20,38]
4	Fomitellic acid (FA) B	8-kDa domain (IC_{50} = 90 μM)	Pol α	[20,38]
5	BreMP	31-kDa domain (IC_{50} = 20 μM)	Pol α	[40]
6	Sulfolipid derivative 1	Not identified (IC_{50} = 3 μg/mL)	Pol α	[21]
7	KN-208	Not identified (K_i = 0.05 μM)	Pol α, *E. coli* pol I, HIV RT	[22]
8	Lithocholic acid (LCA)	8-kDa domain (IC_{50} = 11 μM)	Pol α	[23,41]
9	Solanapyrone A	8-kDa domain (IC_{50} = 30 μM)	Pol λ	[42]
10	SCUL-A	Not identified (IC_{50} = 17 μM)	Pol λ, Pol α	[24]
11	DRB	8-kDa domain (IC_{50} = 28 μM)	Polymerases and glycosidases	[43]
12	Anacardic acid	Not identified (IC_{50} = 9 μM)	Not studied	[25]
13	Oleic acid	Not identified (IC_{50} = 25 μM)	Not studied	[25]
14	Bis-5-alkylresorcinols derivative	Not identified (IC_{50} = 5.8 μM)	Not studied	[50]
15	Triterpenoid-derivative	Not identified (IC_{50} = 5.6 μM)	Not studied	[51]
16	Koetjapic acid (KJA)	8-kDa domain (IC_{50} = 20 μM)	Not studied	[28,52]
17	Pamoic acid (PA)	8-kDa domain (K_D = 9 μM)	Not studied	[28]
18	Harbinatic acid	Not identified (IC_{50} = 2.9 μM)	Not studied	[26]
19	Betulinic acid	Not identified (IC_{50} = 14 μM)	Not studied	[54]
20	3-*cis*-p-Coumaroyl maslinic acid	Not identified (IC_{50} = 15 μM)	Not studied	[54]
21	3-*trans*-p-Coumaroyl maslinic acid	Not identified (IC_{50} = 4.2 μM)	Not studied	[54]
22	Oleanolic acid	Not identified (IC_{50} = 7.5 μM)	Not studied	[55]
23	2-α-Hydroxyursolic acid	Not identified (IC_{50} = 12.6 μM)	Not studied	[57]
24	Lupane triterpenoids derivative	8-kDa domain (IC_{50} = 3.8 μM)	Not studied	[58]
25	(−)-Epicatechin	8-kDa domain (IC_{50} = 18.5 μM)	Not studied	[59]
26	Edgeworin	8-kDa domain (IC_{50} = 22.5 μM)	Not studied	[60]
27	Neolignan-1	8-kDa domain (IC_{50} = 15.5 μM)	Not studied	[63]
28	Neolignan-3	8-kDa domain (IC_{50} = 18.6 μM)	Not studied	[63]
29	Myristinin A	Not identified (IC_{50} = 2.8 μM)	DNA	[27]
30	Stigmasterol	8-kDa domain (IC_{50} = 60.2 μM)	Not studied	[61]

The structures are shown in Fig. 3.

findings set up an important concept regarding the structure–activity relationship of these compounds; several fatty acids, particularly long chain fatty acids with a *cis*-configuration, interact with DNA polymerases and strongly suppress their activities. More importantly, the same group completed a more detailed study to understand the mode of inhibition of two different fatty acids that showed promising interaction with pol β, namely LA and nervonic acid (NA) (**2**) [36]. Their results showed that the binding of the two compounds to the lyase active site of pol β is much stronger than their binding to the polymerase site [36]. The two fatty acids compete with both the dNTP substrate and template-primer for pol β, whereas they bind to pol α without competing with these substances. Additionally, Mizushina *et al*. identified an ergosterol peroxide derivative that can enhance the efficacy of LA in inhibiting the activity of pol β [37].

Tanaka *et al*. reported the discovery of four triterpenoid compounds isolated from the mycelium of a basidiomycete and found that these compounds selectively inhibit the activities of mammalian DNA polymerase α and β *in vitro* [20]. The four compounds have been termed fomitellic acid (FA) A (**3**), B (**4**), C and D. Similar to fatty acids, on DNA pol β, the fomitellic acids competed with both the substrate and the template-primer, however, on DNA pol α, their mode of action was not competitive with either the template primer or the substrate. In fact, they found that the two FAs bind strongly to the lyase active site of pol β, but not to the 31-kDa fragment [38]. This group also identified the immunosuppressive drug, 5′-monophosphate form (breMP) (**5**) of bredinin as a selective inhibitor of both DNA polymerases α and β [39]. A comprehensive study of the mode of interaction of breMP with pol β showed that the drug not only competed with the substrate of the polymerase active site but also with the template-primer [40]. Their results suggest that breMP directly binds to the substrate-binding site of the catalytic domain, and indirectly perturbs the template-primer incorporation into its binding domain [40].

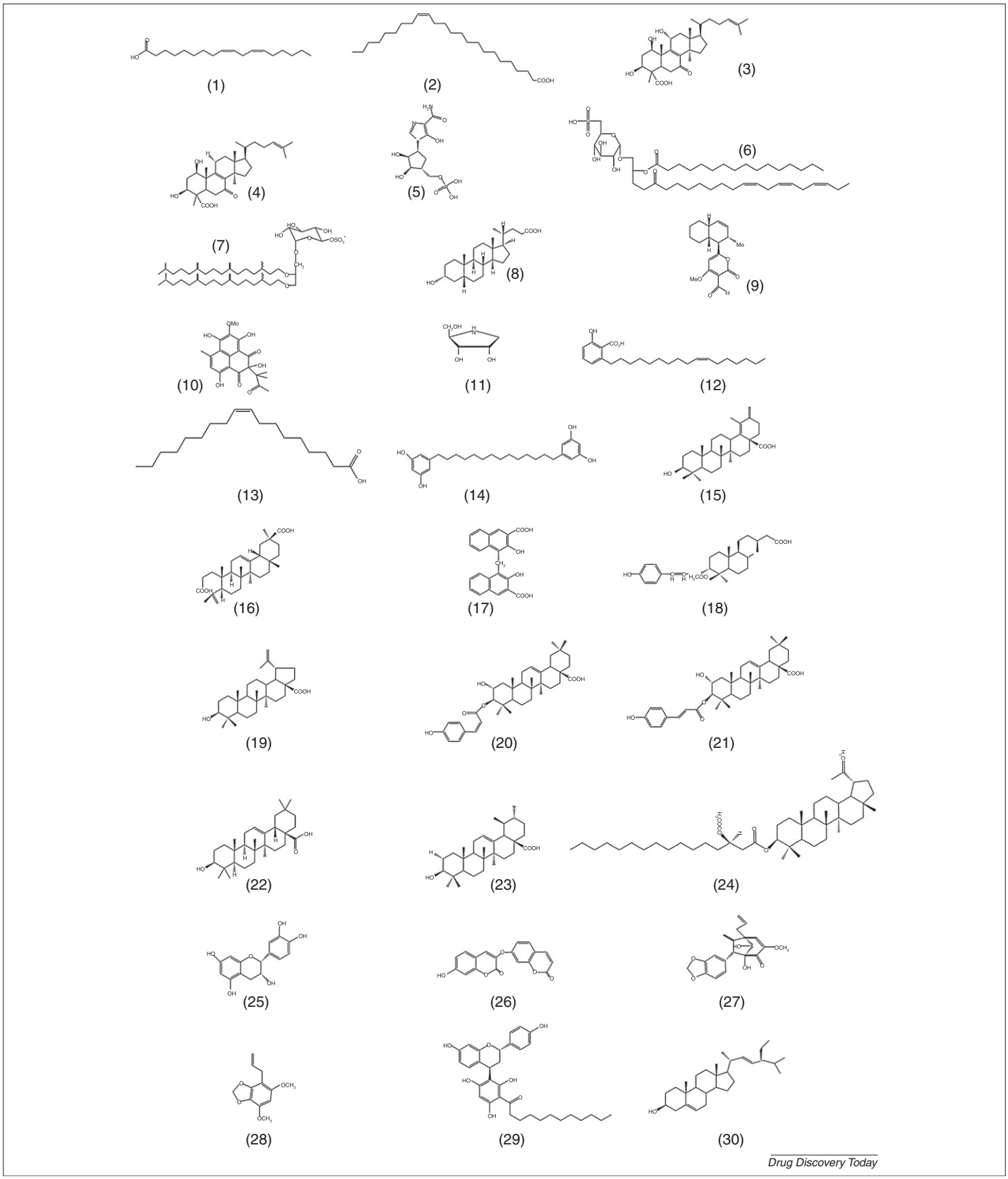

FIGURE 3

Structures of DNA polymerase beta inhibitors listed in Table 1.

DNA pol β inhibitors

Mizushina *et al.* isolated three sulfolipid compounds from a pteridophyte, *Athqrium niprmicum* (**6** is their most potent structure) [21]. Analogously to fatty acids, the three inhibitors competed with the DNA template and substrate of DNA pol β, and acted non-competitively on DNA pol α. More importantly, these compounds did not affect the activity of several other proteins. In a similar study, Mizushina *et al.* [15] extracted prunasin as a weak inhibitor of pol β but the compound was competitive with the substrate, dNTP. This inhibitory behavior was improved to approximately 40 μM in the presence of fatty acid, indicating that the fatty acid enabled easier access of the compound to the substrate-binding site.

Ogawa *et al.* isolated sulfated glycoglycerolipid (KN-208) (**7**), a polar lipid, from an archaebacterium and identified it as an inhibitor for both DNA polymerase α and β [22]. Its mode of action on these polymerases was only competitive with the binding of the DNA template primer and not competitive with the binding of the substrate.

In addition, these researchers studied 17 different kinds of bile acids with respect to their inhibition of mammalian DNA polymerases [23]. Intriguingly, their findings revealed that only lithocholic acid (LCA) (**8**), one of the major components among secondary bile acids, was able to suppress the activity of DNA polymerases. Ogawa *et al.* found that the C-7 and C-12 positions in the sterol skeleton are important for the inhibitory activity of LCA [23]. In a study by Mizushina *et al.* [41], the full-length pol β was separated proteolytically into two fragments, namely, the lyase active site (template-primer binding domain 8 kDa) and the polymerase active site (catalytic domain 31 kDa). Binding analysis revealed that LCA tends to bind strongly to the 8 kDa domain, and not to the 31 kDa domain. This important finding was confirmed using NMR analysis, where the 8 kDa domain was shown to associate with LCA as a 1:1 complex with a dissociation constant (K_D) of 1.56 mM. Docking analysis revealed that the binding sites of the two different compounds comprised the DNA binding pocket within pol β as an essential component for their binding.

Mizushina *et al.* identified solanapyrone A (**9**) as an inhibitor for both DNA polymerase β and λ [42]. Interestingly, solanapyrone A competed with both the DNA template and the nucleotide substrate. They also found that the compound could bind selectively to the N-terminal 8-kDa domain of pol β. In fact, the Mizushina group showed that solanapyrone A inhibits the binding of DNA to the ssDNA-binding site within pol β and does not affect the other two activities of the 8-kDa domain, namely, recognition of the 5′-phosphate in gapped DNA structures and AP lyase activity [38].

Perpelescu *et al.* tested the effects of two phenalenone-skeleton-based compounds, sculezonone-B (SCUL-B) and sculezonone-A (SCUL-A) (**10**), upon the activity of several DNA polymerases [24]. The two compounds were found to exhibit diverse interactions with the different tested polymerases. SCUL-A was found to be more selective against pol β than SCUL-B. This is apparent by comparing the IC_{50} values of the two compounds with respect to their interaction with pol β (17 μM for SCUL-A and 90 μM for SCUL-B). Similarly, Mizushina *et al.* showed that a pyrrolidine alkaloid, termed as DRB (**11**), was able to suppress the activity of several eukaryotic DNA polymerases [43]. Although such compounds are widely known to inhibit other enzymes, such as glycosidases, DRB had almost no effect on the activities of prokaryotic DNA polymerases.

Recent work on DNA pol β inhibitors

Recent studies revealed several compounds that can target DNA polymerases and in some cases interact with several other enzymes as well. Examples of such compounds include isosteviol, which targets mammalian polymerases and human DNA topoisomerase II (topo II) [44]; several sulfolipid derivatives that can interact with both pol α and β [45]; epolactaene derivatives that can target DNA polymerases and topo II [46]; catechin derivatives that can target pol α and λ [47]; and finally the two azaphilone derivatives, kasanosins A and B that were specifically found to target pol β and λ [48].

Starting from the late nineties, one can recognize the emergence of a new team representing the National Cancer Institute-sponsored National Cooperative Drug Discovery Groups (NCDDG) that has entered the field of screening for novel inhibitors of pol β [49]. One of the first studies focusing on pol β inhibitors performed by this group was the work by Chen *et al.*, who isolated five compounds that showed inhibitory activities against pol β [25]. The five compounds were anacardic acid (**12**) with its four structurally related derivatives and oleic acid (**13**). In a different study, Deng *et al.* isolated three bis-5-alkylresorcinols compounds from *Panopsis rubescens* [50], the three compounds showed strong binding to calf thymus pol β (**14** is the most potent structure). Moreover, Deng *et al.* used *Baeckea gunniana* and extracted a methyl ethyl ketone extract, which was identified as a potent inhibitor of rat pol β [51]. This study revealed four active ursane and oleanane triterpenoid compounds that can bind to pol β, in the presence of bovine serum albumin (BSA) and in the absence of BSA to a lesser degree (**15** is the most potent structure).

Natural products as pol β inhibitors

Sun *et al.* isolated three active natural products [52] and ten more derivatives and examined their interactions with the protein. Only three derivative compounds were active against pol β. The authors found that the compounds exhibited a mixed-type inhibition pattern for both the substrate dNTP, in addition to the DNA template-primer. When altering the concentrations of both dNTP and the DNA template-primer separately, the inhibition pattern was intermediate between competitive and noncompetitive inhibition for the two substances. Comparing the performance of the three compounds along with their derivatives, one can notice that one of these molecules, known as koetjapic acid (KJA) (**16**), showed reasonable activity in interacting with pol β and was the subject of a later study that was carried out by Hu *et al.* from the same NCDDG team [28]. In this study, Hu *et al.* used NMR analysis to identify the binding interface between KJA and the 8-kDa domain of pol β and to decompose its residue contributions. Their findings suggest that the binding pocket of the compound within the surface of pol β is located between the two helices, helix-2 and helix-4 of the 8-kDa domain. Interestingly, the same region has been recognized in different studies to be essential in the DNA binding and deoxyribose phosphate lyase activities of the enzyme [35]. Hu *et al.* also examined nine structurally related synthetic compounds that are similar to KJA for their activity

against pol β. These compounds were also able to enhance the efficacy of methyl methanesulfonate (MMS), a monofunctional methylating agent that targets the DNA whose induced damage is mainly repaired by BER. More importantly, the most potent compound and the one that the authors of the current review suggest as the best pol β inhibitor so far is pamoic acid (PA) (**17**). PA was one of the tested derivatives and was found to inhibit both the deoxyribose phosphate lyase and DNA polymerase activities of purified pol β on a BER substrate. The findings were further pursued by a different group from the Centre National de la Recherche Scientifique (CNRS) in France, to understand and identify the precise interactions between PA and the 8-kDa domain of pol β [53] and confirmed findings of Hu *et al.* [28]. In this study, Hazan *et al.* used a combination protocol of blind docking and NMR analysis to identify the binding site of PA within the surface of the lyase domain of pol β and to suggest its binding conformation. These results confirmed the earlier findings of Hu *et al.* and revealed that PA binds to a site formed by helix 2 and helix 4, which also corresponds to the ssDNA-binding site. Particularly, the aromatic groups of pamoic acid formed favorable hydrophobic interactions with the residues Tyr39, Ala42, Gly64 and Gly66 within the identified binding site. Furthermore, the presence of many lysine residues in the binding pocket enabled favorable electrostatic interactions for the two carboxyl groups of PA. In their proposed model, one of the carboxyl groups is oriented towards His34 and Lys35 making close contacts with Ile69 amide proton, whereas the other carboxyl group formed hydrogen bonds with the amide proton of Lys68 and with the hydroxyl group of Thr67.

In a series of similar studies, the NCDDG group identified a considerable number of pol β inhibitors that can bind to the enzyme with reasonably high affinities. This list includes harbinatic acid (**18**) [26]; the three triterpenoid compounds betulinic acid (**19**), 3-*cis*-p-coumaroyl maslinic acid (**20**) and 3-*trans*-p-coumaroyl maslinic acid (**21**) [54]; an additional six pentacyclic triterpenoids compounds extracted from *Freziera* sp. (**22** is the most potent structure) [55]; a sesquiterpenoid derivative targeting the lyase activity of pol β [56]; four lyase inhibitors comprising a triterpene, ursolic acid, hydroxyursolic acid, and β-sitosteryl- β-D-galactoside (**23** is 2-α-hydroxyursolic acid, their most potent inhibitor) [57]; four lupane triterpenoids targeting the lyase activity of pol β (**24** is their best inhibitor) [58]; the lyase-inhibitor, (−)-epicatechin (**25**), which also potentiated the efficacy of monofunctional methylating agent in cultured human cancer cells [59]; the biscoumarin derivative, Edgeworin, which inhibited the lyase activity (**26**) [60]; and finally, two neolignan lyase inhibitors (**27, 28**). However, for all of these listed inhibitors, the exact binding locations, mode of inhibition against pol β, or the possibility of targeting other DNA polymerases or enzymes within the cell were not identified.

Additional pol β inhibitors

Maloney *et al.* investigated the synthesis of three flavonoids derivatives, namely myristinin A (**29**), B and C, which exhibited a distinctive characteristic besides their ability to inhibit the activity of pol β [27]. That is, these compounds can also cleave and induce a considerable damage to DNA, enabling one to exploit their dual activity as an innovative therapeutic strategy in cancer treatments. Myristinin A showed more potent Cu^{2+}-dependent DNA-damaging activity and pol β inhibition than the inseparable mixture of myristinin B and C. Similarly to the interesting behavior of the above-mentioned flavonoid derivatives, Starck *et al.* identified several 5-alkylresorcinols that also mediated Cu^{2+}-dependent DNA damage and also suppressed the ability of pol β to restore the DNA damage that they cause. Interestingly, one of these alkylresorcinols, namely bis(dihydroxyalkylbenzenes), showed potent activity both as an inhibitor for pol β and as a DNA-damaging agent.

In their most recent study, the NCDDG group isolated oleanolic acid, edgeworin, betulinic acid, and stigmasterol [61]. Interestingly, although stigmasterol (**30**) was not as strong as the other compounds, it was the most specific structure for the lyase activity, which inhibited both the lyase and polymerase activities of the enzyme. More importantly, Gao *et al.* showed that, the four inhibitors potentiated the efficacy of the anticancer drug bleomycin in cultured A549 cells, without any influence on the expression of pol β in these cells.

Concluding remarks

Family-X of DNA polymerases and pol β in particular are the foremost elements of BER [29]. This is mainly due to their ability to fill short gaps within the damaged DNA molecule. Fortunately, a large body of structural data and biological information about pol β is currently available, making it the first DNA polymerase enzyme whose structural description is complete [33,62]. These observations encouraged researchers to look for regulators of BER through the discovery of inhibitors of the polymerization step of the pathway. The fundamental principle behind this objective is to preserve the ionizing radiation or chemotherapeutic-induced damage within the genome to potentiate the efficacy of these DNA-damaging agents and hence, force the cell to undergo apoptosis [49]. Although, these efforts resulted in a large number of DNA pol β-inhibitors listed and discussed here in this review, these inhibitors are not specific or potent enough to be pursued as drug candidates. This is mainly because most of the identified compounds target other polymerases or enzymes and a considerable number of them cannot enter the cell due to solubility problems.

Therefore, we propose that alternative drug discovery avenues that have been only slightly touched upon in a few of the above-mentioned studies should be extensively applied in future searches for novel inhibitors of pol β [59]. These approaches include computational experiments such as *de novo* drug design, receptor-based virtual screening or pharmacophore search techniques. Moreover, as indicated in this review, most of the listed pol β inhibitors can target other enzymes. For that reason, it is important in the future to study the off-target interactions of newly discovered pol β inhibitors to minimize their side effects and enhance their potential applications as therapeutic agents.

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