

Base excision repair pathways of bacteria: new promise for an old problem

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Infectious diseases continue to be a major cause of human mortality. With the emergence of drug resistance, diseases that were long thought to have been curable by antibiotics are resurging. There is an urgent clinical need for newer antibiotics that target novel cellular pathways to overcome resistance to currently used therapeutics. The base excision repair (BER) pathways of the pathogen restore altered bases and safeguard the genomic integrity of the pathogen from the host's immune response. Although the BER machinery is of paramount importance to the survival of the pathogens, its potential as a drug target is largely unexplored. In this review, we discuss the importance of BER in different pathogenic organisms and the potential of its inhibition with small molecules.

First draft submitted: 11 September 2019; Accepted for publication: 14 November 2019; Published online: 7 February 2020

Maintenance of the integrity of the genomic DNA is of paramount importance for the survival of living cells. The cellular DNA constantly encounters damaging challenges from the intracellular and extracellular environmental agents. High energy radiations such as cosmic rays, gamma rays, x-rays and UV rays are the predominant extracellular agents that cause DNA damages such as pyrimidine dimers, DNA strand breaks or interstrand cross-links [1,2]. The intracellular environment of the cell is also harmful to DNA as the reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) generated during the metabolism can penetrate through cell membranes/cell wall [3,4] and inflict damages to the DNA [5–8]. Inability to correct the damages inflicted on the DNA results in changes in the DNA sequence (mutations) that can be deleterious or even fatal to the cell [9].

The ozone layer of the earth's atmosphere serves as a natural barrier against the impact of UV radiation reducing DNA damages [10–12] and the ROS generated within the cells are neutralized by antioxidants within the cells. In spite of these preventive mechanisms, the DNA does get damaged. The DNA damages can be classified into base modifications, depurination/depyrimidination, base mismatches, breaks in the DNA strands, DNA cross-links and DNA-protein cross-links. The organisms also undergo self-inflicted DNA damages as a result of replication errors, which is known to occur at higher rates upon the activation of error-prone DNA polymerases [13]. To identify the damages and restore the DNA to the normal state, cells have evolved elaborate DNA repair mechanisms, which have been broadly classified into birect reversal pathway, base excision repair pathway (BER), nucleotide excision repair pathway (NER), mismatch DNA repair pathway (MMR), recombination repair pathway (RR) and non-homologous end joining (NHEJ) pathway. The entire repair and replication machinery works in synchrony to reverse the DNA damages. Many cancers, neurodegenerative disorders, and premature aging have been linked to defective DNA repair, highlighting the key role of DNA repair in preventing human diseases [14].

During microbial infection, one of the host's defense mechanisms is to generate a genotoxic environment through ROS or RNI [15–17] which are proven causes of DNA damage. Successful intracellular pathogens employ an extensive repertoire of DNA repair pathways to not only survive the host generated genotoxic onslaught but also to cause disease.

Table 1. DNA glycosylases and their substrates.

DNA glycosylase	Base recognised in DNA
Uracil-DNA-glycosylase	Uracil
3-Methyladenine-DNA-glycosylase	3-Methyladenine
Hydroxymethyluracil-DNA-glycosylase	Hydroxymethyluracil
Hypoxanthine-DNA-glycosylase	Hypoxanthine
5-Methylcytosine-DNA-glycosylase	5-Methylcytosine
Mismatch specific thymine DNA glycosylase	Thymine from G:T mismatches
MutY DNA glycosylase	Adenine from A:G, A:C and A:GO mispair
Formamidopyrimidine (FaPy) DNA glycosylase	Formamidopyrimidine and 8-oxoguanine
Thymine glycol DNA glycosylase	Pyrimidine adducts such as thymine glycol, urea, uracil glycol, etc.
EndoVIII	Pyrimidine adducts
Pyrimidine-dimer DNA glycosylase	Pyrimidine dimers

Currently used antibacterial therapies mainly target key cellular processes such as cell wall biosynthesis, DNA replication, RNA transcription, protein synthesis, respiration, and DNA topology. However, genetic drug resistance has emerged at an alarming rate across all bacteria. For example, there are case reports of total drug resistance in *Mycobacterium tuberculosis*, the causative agent of tuberculosis (TB), making it incurable with the existing therapeutics [18–20]. Hence, there is an urgent need for discovering new cellular pathways and molecular targets that can be inhibited by the new molecules to control bacterial growth independently or in combination with the existing drugs. Given the important role of the bacterial DNA repair mechanisms in the establishment of the infection, the DNA repair mechanisms also represent vulnerabilities that can be exploited for the development of new antimicrobials.

Here, we have reviewed the potential of one of the key DNA repair mechanisms in bacteria, the BER pathway for its possible use in drug development. The BER pathway deals with the repair of base modifications such as alkylation, oxidation or deamination. Identification of a suitable drug target among the enzymes involved in the BER pathways of the bacterial pathogens may provide a new strategy in controlling and treating infectious diseases.

BER pathway of DNA repair

The discovery of the BER pathway is credited to Thomas Lindahl [21–23]. The key enzymes involved in BER are a DNA Glycosylase, an AP Endonuclease, a dRPase, a DNA polymerase, and a DNA ligase. The prokaryotes, as well as the eukaryotes, possess a very similar functional homolog of each enzyme in this pathway with similar mechanism, but there are additional enzymes that are specific to eukaryotes. The outcome of decades of research on the BER pathway has yielded a wealth of mechanistic details about the repair pathway which is well published [24–28], and summarised in Figure 1. In the first step of the BER pathway, the damaged DNA base is identified and specifically excised by a DNA glycosylase which cleaves the N-glycosidic bond between the damaged base and the deoxyribose sugar, leaving behind an apurinic/apyrimidinic site (AP site). Specific DNA glycosylases are recruited depending on the type of base damage involved (Table 1). The DNA glycosylases can be divided into two classes: those possessing only a glycosylase activity or monofunctional (e.g., 3-methyladenine DNA glycosylase, hydroxymethyluracil DNA glycosylase, hypoxanthine DNA glycosylase, 5-methylcytosine DNA glycosylase, uracil DNA glycosylase [Ung] and mismatch specific thymine DNA glycosylase) and the ones that possess an associated AP lyase activity or bifunctional (e.g., MutY DNA glycosylase [MutY], formamidopyrimidine DNA glycosylase [Fpg or MutM], thymine glycol DNA glycosylase, Endo VIII [Nth] and pyrimidine dimer DNA glycosylase). The removal of damaged bases by monofunctional DNA glycosylases generates AP sites which are cleaved by apurinic/apyrimidinic endonucleases (APE). The APE incises at 5' to the abasic site, generating a 3'-OH terminus and a 5'-abasic sugar that is removed by a dRPase (such as RecJ in *Escherichia coli*) to produce a 5' phosphate end (and a single nucleotide gap). This single nucleotide gap is filled by the DNA polymerase and the remaining nick in the DNA is sealed by a DNA ligase. The bifunctional DNA glycosylases have an associated AP lyase activity responsible for incising the phosphodiester bond 3' to the abasic site in a process called β -elimination, which generates a 5'-phosphate end, and a 3'- α , β -unsaturated aldehyde on the 3' end requiring further processing by an

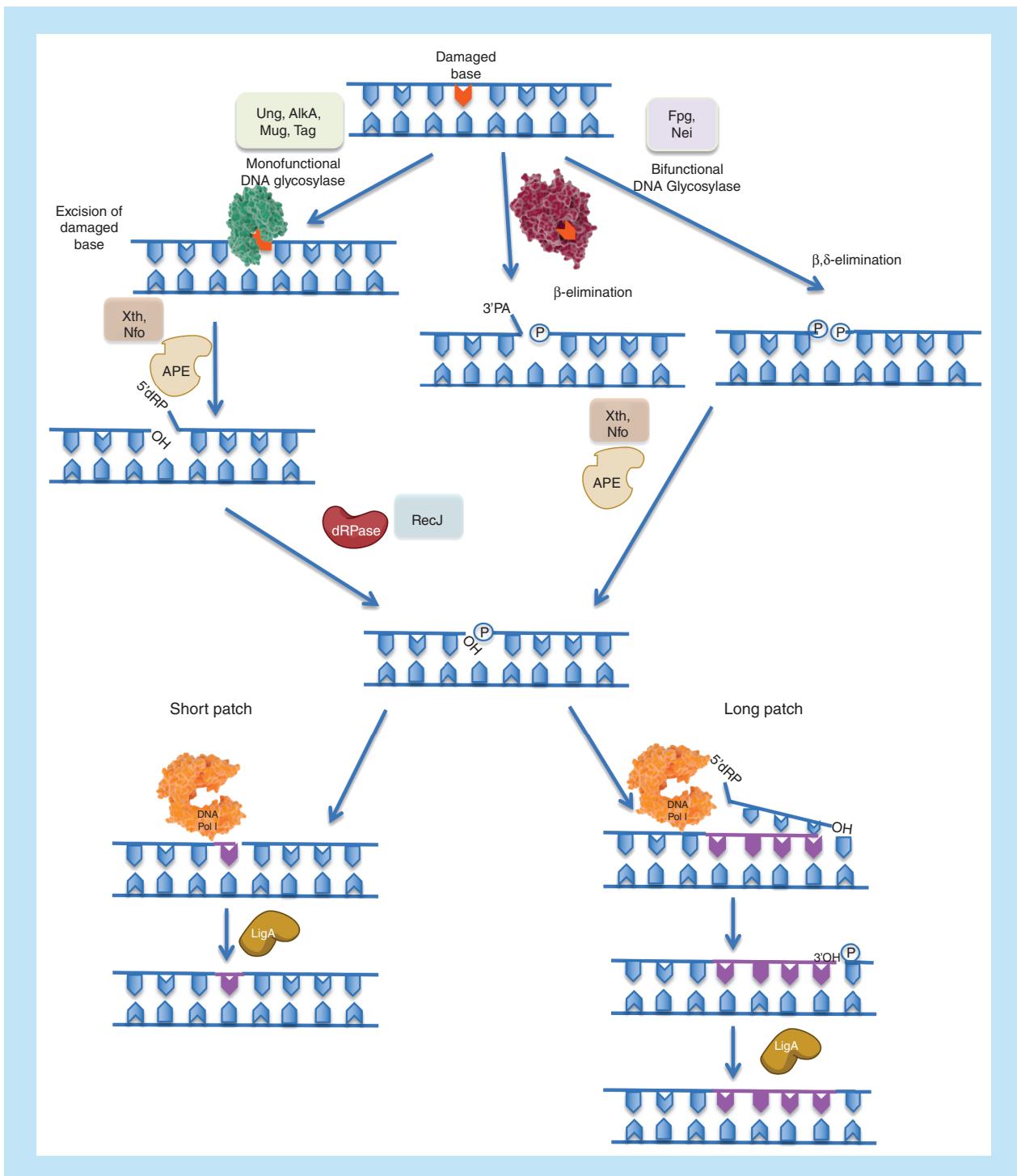


Figure 1. Schematic of bacterial base excision repair pathway. Base excision repair in bacteria is initiated by a DNA glycosylase which hydrolyzes the N-glycosidic bond between the base and the sugar. The DNA glycosylases fall into two groups: monofunctional and bifunctional. The monofunctional DNA glycosylases generate abasic (AP) sites. The AP site is further processed by AP Endonuclease (APE) generating 3' OH and 5' dRP ends. The 5' dRP end is further processed by dRase to generate 5' phosphate end. On the other hand, the bifunctional enzymes cleave the AP sites they generate to result in a 3'- α,β unsaturated aldehyde (3' PA) and a 5'-phosphate ends. However, some of the bifunctional DNA glycosylases (Fpg and Nei) are able to further process the 3' PA via δ -elimination to a 3'-phosphate end. AP endonuclease is then required to process the 3' phosphate end to 3' OH end. The DNA polymerase then adds the correct nucleotide, and the nick is sealed by DNA ligase. Alternatively, DNA polymerase I can displace the strand and polymerize tracts of DNA longer than one base producing flapped substrates that are refractory to ligation and require further processing.

AlkA: 3-Methyladenine-DNA-glycosylase; APE: AP endonuclease; Fpg: Formamidopyrimidine DNA glycosylase; LigA: DNA ligase A; Mug: Mispair specific Uracil DNA glycosylase; Nei: Endonuclease VIII; Nfo: Endonuclease IV; Ung: Uracil DNA glycosylase; Xth: Exonuclease III.

APE prior to the gap-filling and ligation [28]. There is also another process involving two consecutive elimination steps (β , δ -elimination) that generates a 3'-phosphate which must also be removed by an APE [28].

E. coli harbours two major evolutionarily conserved families of APEs, exonuclease III (XthA) and endonuclease IV (Nfo). XthA removes the 3' phosphoryl group generating 3' OH, which is used by DNA polymerase I for the gap-filling [29]. XthA also possess a 3'-phosphodiesterase activity that removes 3' phosphoglycolate residue from DNA [30]. It also removes the 3' unsaturated aldehyde residues generated after β -elimination at the AP sites [31]. The mutant strains lacking *xthA* are extremely sensitive to H₂O₂ and near-UV radiation (320–400 nm), indicating its (*xthA*) role in repairing oxidative DNA damages [32]. Endonuclease IV (Nfo) attacks the phosphodiester bonds 5' to the AP sites leaving 3' OH group [33]. The *nfo* null mutant of *E. coli* shows increased sensitivity to alkylating agents such as MMS, mitomycin-C, *tert*-butyl hydrogen peroxide and bleomycin [34]. The APE in mammalian cells is known as APE1. The AP sites can also arise due to spontaneous hydrolysis of the N-glycosidic bonds in DNA, particularly those connected to the alkylated bases [35]. The presence of abasic sites in the DNA is both mutagenic and cytotoxic [36,37]. The AP sites in DNA can pause or inhibit the functioning of RNA as well as DNA polymerases [38]. However, DNA polymerases may bypass the lesion and preferentially insert adenine opposite to it [39].

Since the action of the repair machinery is limited to removal of a single damaged residue and replacing it with the correctly base-paired nucleotide, this mode of repair is also known as short-patch repair (Figure 1, short-patch), where the correct nucleotide is incorporated for example by bacterial (*E. coli*) DNA polymerase (Pol) I or eukaryotic Pol β and finally ligated by bacterial DNA ligase (LigA) or mammalian LigIII α which form a complex with XRCC1. An alternate route of repair following the removal of damaged residue (referred to as long-patch repair) involves replacement synthesis by DNA Pol I [25,40] or eukaryotic Pol β , Pol δ and Pol ϵ utilizing the 3' OH generated by the cleavage of phosphodiester bond by an APE. In this process, the parental strand containing 5'dRP gets displaced by DNA Pol I forming a flap structure which is then cleaved by its 5'-3' exonuclease activity, but in mammals, another enzyme called FEN-1 endonuclease removes the flap structure. In eukaryotes, PARP1 also regulates the activity of Pol β in long patch repair [41]. Subsequently, the action of LigA or mammalian LigI and LigIII α results in the sealing of the nick to complete the repair (Figure 1, long-patch) [25].

In bacteria (*E. coli*) and mammalian cells, under normal physiological conditions, short-patch repair pathway is employed in BER [21,42]. Interestingly, in pathogens such as *M. tuberculosis* and *Plasmodium falciparum*, long-patch repair has been observed [43,44], while *Trypanosoma cruzi* repairs uracils in DNA exclusively via short-patch repair [45]. More recently, proteins that specifically bind to uracil and 5-hydroxymethylcytosine DNA and form a covalently cross-linked DNA protein complex have been reported [46,47]. The primary role of these proteins appears to protect the AP sites and prevent the formation of DNA strand breaks. Due to their unique property of forming a highly stable DNA protein complex, the subsequent repair pathway also differs from the classical BER, and it requires RecA mediated pathway and the protein is subjected to degradation by proteasomal machinery [46,47].

BER of pathogenic bacteria

In the following section, we discuss the base excision repair system known in the pathogenic bacteria and understanding of these pathways critical to harness the potential of the BER as a potential drug target.

Mycobacterium tuberculosis: *M. tuberculosis* is the causative agent of tuberculosis which is estimated to have infected one-quarter of world population according to the current WHO report (www.who.int/tb/en/). It enters the host respiratory tract by aerosol route, where it is engulfed by the host macrophages [48,49]. As a defense mechanism against this infection, the host macrophages generate ROS and RNI which will eventually kill the cell. *M. tuberculosis* has a repertoire of DNA repair systems that is responsible for repairing any damage caused to its DNA. Although the functional homologs of mismatch repair have not been reported in *M. tuberculosis* [50], a recent study has shown the evidence of non-canonical mismatch repair in *M. smegmatis* [51]. Additionally, the NHEJ repair pathway has also been reported in *Mycobacteria* [52,53]. *M. tuberculosis* being a G+C rich organism is more susceptible to oxidation of guanine and deamination of cytosine, hence *M. tuberculosis* maintains multiple enzymes with redundant functions [50]. It has two functional uracil DNA glycosylases, MtuUng and MtuUdgB [54]. It also maintains an enzyme called dUTPase which hydrolyzes any intracellular dUTP preventing its incorporation into the DNA by the promiscuity of DNA polymerase. MtuUng can excise uracil from single-stranded, double-stranded DNA, and also from loop substrates. MtuUdgB is a Fe-S cluster containing protein which excises uracil only from double-stranded DNA substrate. However, it has broad specificity for the modified bases in DNA. It has the ability to rescue the mutator phenotype of *E. coli ung* deficient strain [54]. The deletion of *ung* and *udgB* has

an additive effect on the mutation frequency, underscoring their individual importance in mutation prevention in *M. tuberculosis* [55]. For repair of oxidatively damaged bases, *M. tuberculosis* employs Fpg, Fpg2, Nei1, Nei2 and Nth DNA glycosylases [56]. The incorporation of 8-oxo-dGTP into the DNA is also minimized by the presence of 8-oxo-dGTPases (MutT enzymes). *M. tuberculosis* possesses four MutT homologs (MutT1, MutT2, MutT3, MutT4) [57,58]. *M. tuberculosis* can successfully repair alkylation of the DNA bases using enzymes such as a 3-methyladenine-DNA glycosylase (TagA) or direct and irreversible methyltransferase (AlkA). *M. tuberculosis* also possesses the Xth and Nfo AP endonuclease [59] that are similar to the *E. coli* enzymes. It is also of note that *M. tuberculosis* utilizes long-patch repair when it encounters uracil in DNA unlike *E. coli* that mostly utilizes short-patch repair. This is attributed to the absence of a RecJ homolog (dRPase) in *M. tuberculosis*, however, MtuFpg possesses intrinsic dRPase activity and pathway may be different for repair of nonuracil DNA damages [43].

Neisseria meningitidis: *N. meningitidis* also known as meningococcus is known to cause a life-threatening sepsis and meningitis. Unlike other organisms, it has a limited repertoire of DNA repair enzymes and lacks an SOS response pathway [60]. Its DNA glycosylase, Nth, and Fpg are bifunctional enzymes that are known to act on a broad range of oxidized substrate [61,62]. Another DNA glycosylase, MutY which in an adenine DNA glycosylase is also reported to have antimutator role and has been shown to play a significant role in combating oxidative DNA damages [63,64]. The APE, NApe, and NExo are also required for resistance to oxidative stress and are necessary for virulence. Targeted deletion of both genes had a cumulative deleterious effect [65].

Pseudomonas aeruginosa: *P. aeruginosa* is another G+C rich organism which is an important opportunistic pathogen in hospitals (nosocomial infections) and also one of the ESKAPE pathogens. Like *E. coli* it contains the Ung protein for excision of uracil from the DNA and depletion of Ung leads to an increase in mutation frequency and decreased survival in mouse infection model [66]. It also contains multiple enzymes involved in 8-oxo-G repair (Fpg, MutY and MutT) and it was shown that inactivation of these enzymes increased frequency of resistance to the antibiotic ciprofloxacin which inhibits DNA gyrase and is known to induce DNA damage [67].

Salmonella typhimurium: *S. typhimurium*, similar to *E. coli* possesses all the enzymes required for efficient BER pathway (i.e., Ung, Nei, Nth, Fpg, Xth and Nfo). *S. typhimurium* glycosylase and endonuclease mutant strains show a comparable phenotype to *E. coli* mutant strains [68]. The BER system is important for the maintenance of its genomic integrity under nitric oxide stress [69].

Helicobacter pylori: *H. pylori* is the causative agent of the gastric ulcer which can result in stomach cancer. It is currently estimated that nearly 50% of the human population could be harboring this bacterium [70]. Owing to its smaller genome size, *H. pylori* does not possess the complete repertoire of DNA glycosylases as found in *E. coli* [71]. The canonical mismatch repair has also not been identified in *H. pylori* and it lacks the NHEJ pathway [72]. Two type III endonucleases, Nth and MagIII, have been identified and are responsible for the repair of oxidized pyrimidines and 3-methyladenine in DNA, respectively [73]. A homolog of MutY has also been identified and seems to be important in preventing transversion mutations [74]. It also contains the *ung* gene in the genome but has not been biochemically characterized.

BER pathways as potential targets for drug therapy in infectious diseases

Despite the availability of a wide range of antibiotics, infectious diseases continue to be a major cause of human mortality. Increased incidence of drug resistance among the pathogenic bacteria have further complicated and constrained treatment options. Therefore, the discovery of new drugs, identification of novel druggable targets and a better understanding of causes of drug resistance is a critical consideration toward fighting global epidemics of infectious diseases. The pathogens, especially intracellular pathogens require their extensive DNA repair machinery to survive genotoxic host defense mechanisms to establish disease. Hence, these pathways also highlight the vulnerable points of these pathogens. Therefore, DNA repair processes present with novel pathways that may be a rich source of potential new drug targets and may also provide candidate genes that can be explored and exploited for vaccine development programs. To successfully target a bacterial pathogen, it is essential to identify druggable enzymes that are genetically vulnerable and crucial for their survival in hosts. There is increasing evidence indicating that DNA repair processes harbor such components, for example, UvrB in mycobacteria [75]. Also, it has been reported that most antibiotics facilitate bacterial killing with the assistance of ROS [76,77], hence inhibition of DNA repair pathways may provide synergistic action that can potentiate existing antibiotics leading to sterilizing cures.

Importance of uracil excision repair in G+C rich bacteria

Several intracellular pathogens such as *Mycobacteria*, *Pseudomonas* and *Leishmania* possess high G+C content in their genomes and are at high risk of cytosine deamination (to uracil) and oxidation of guanine (to 8-oxo-G) resulting from RNI and ROS generated by the host. Exposure of the BER deficient strains of *Mycobacteria* and *Pseudomonas* to exogenous stress such as RNI and ROS makes them hypermutable as well as hypersusceptible [66]. Additionally, these strains showed compromised survival in activated mouse macrophages [66]. Interestingly, saturation transposon mutagenesis identified *ung* as an essential gene for the survival of *M. tuberculosis* in mouse [78]. Other genetic studies have found that deletion of *dut* in bacteria has a deleterious or lethal effect *in vitro* survival [79,80]. It has been shown that the lethality of *dut* deficiency is partly due to increased levels of uracil incorporation that results in abnormally high AP sites and heightened risk of double-strand breaks in DNA. In *M. tuberculosis* dUTPase (*rv2697c*) is essential and cannot be deleted indicating it could serve as a potential drug target (detailed in the structure-based design section) [78]. Recently, in *M. smegmatis*, an unusual uracil DNA glycosylase encoded by *MSMEG_0265* that shows close similarity to the Family 4 UDG has been identified. The gene product (now referred to as *UdgX*) also contains the Fe–S cluster and displays specific tight binding to uracil containing DNA [46,81]. Structural analysis of the *UdgX* in complex with uracil containing DNA revealed that a histidine in a unique motif rich in basic residues makes a direct nucleophilic capture of the reaction intermediate compared to the conventional water-based mechanism utilized by closely related UDGs [81].

Importance of Fpg & MutY

In addition to uracil excision repair, Fpg mediated repair pathway has also been shown to play a significant role in the survival of mycobacteria under *in vivo* stress conditions. It is desirable that the drug target aspect of these key repair enzymes is further explored. Rex et al. have reported that mycobacteria lacking Fpg, MutY or Ung had increased sensitivity to DNA damaging agents in the Wayne's hypoxia model of persistence [82]. Further, transposon mutants of *M. tuberculosis* CD1551 lacking Fpg or Nei showed approximately 30% attenuation in the non-human primate lung indicating the importance of BER toward survival within the host [83]. The hypoxic condition has been widely recognized as an important factor in mycobacterial dormancy [84]. Therefore, a combination of drugs inhibiting multiple steps in various BER pathways may help achieve sterilizing activity and potential elimination of persisting or latent bacteria. In the case of *H. pylori*, a micro-aerophilic bacterium which causes gastric ulcer, MutY plays an important role in preventing mutagenesis. A mutant strain of *H. pylori* lacking MutY showed approximately 300-fold increase in mutation rate and reduced bacterial burden in mouse stomach [64,85] suggesting MutY to be a potential drug target. Although RNI and ROS are the predominant agents of DNA damage *in vivo*, BER enzymes involved in repairing alkylating bases have also been shown to be important. In *Salmonella enterica*, inactivation of alkylation repair pathway enzymes showed sensitivity to alkylating agents and when combined with enzymes involved in the nucleotide excision repair pathway, the bacteria showed reduced fitness within infected mice [86]. Genes involved in GO repair pathway were among the earliest induced in macrophage infected with *Brucella* indicating that the BER pathway to be necessary for bacterial adaptation [87]. In members of *Neisseria*, MutY shows excision activity on A:8-oxoG and A:C substrates, and loss of MutY is associated with a high mutation rate *in vitro* [63]. Importantly, biochemical activity analysis of cell lysate from clinical strains of *Neisseria* demonstrated robust activity for Fpg and MutY indicating their constitutive expression in bacteria [88].

Accessory BER targets

A combination of drugs inhibiting multiple targets in BER pathways may lead to sterilizing activity and the potential elimination of the pathogen. In addition to the DNA glycosylases, downstream enzymes in the pathway may also be attractive targets. Enzymes such as APEs, DNA polymerases and ligases are common to various glycosylase initiated BER. It has been shown in *S. typhimurium*, an intracellular pathogen, that deletion of APEs such as *xth* and/or *nfo* significantly impairs the bacterial survival both in cultured and primary macrophages activated with IFN- γ and the pathogen was attenuated for virulence in C3H/HeN mice [68,69]. In the human pathogen *H. pylori*, the *xthA* performs the classical APE activity and is also competent to repair α anomer lesions generated during oxidative damage [86]. Interestingly, whole-genome sequencing revealed that *H. pylori* contains a single copy of APE and encoded by *xthA* making it a potential target for small molecule-based inhibition [89]. It has also been observed that in *H. pylori*, *HP1470* encodes for the DNA polymerase I which lacks the proofreading activity necessary for

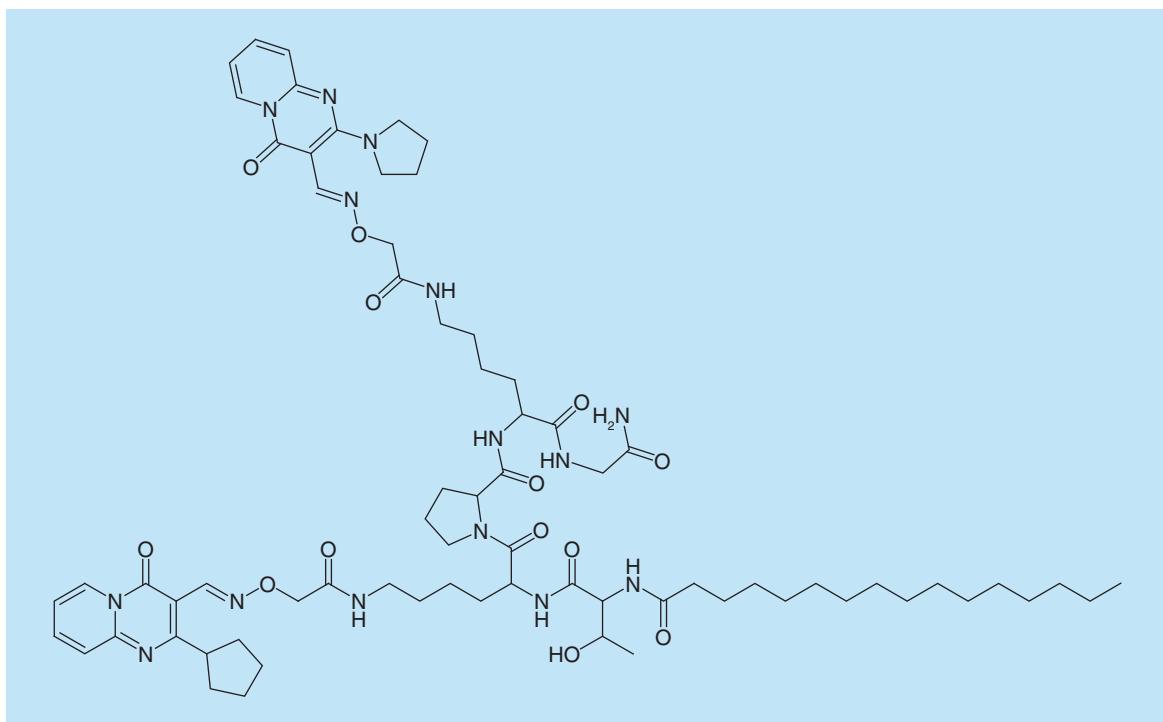


Figure 2. Structure of dUTPase inhibitor. Structure of TB820 a peptide conjugate of pyridopyrimidine derivative that targets mycobacterial dUTPase. The molecule was reported in Horvati *et al.* [93] and the structure of TB820 was generated using ChemBiodraw software.

the completion of repair synthesis following excision of the damaged base. As a result, over-expression of *HP1470* resulted in increased mutagenesis and strain variability [90].

Structure-based drug design

Through an *in-silico* docking method, Horvati *et al.*, performed docking studies of small molecules on the known structure of *M. tuberculosis* Dut and identified pyridopyrimidine based compounds that showed strong binding to the enzyme [91,92]. A derivative of this compound TB820 (4-oxo-2-(pyrrolidin-1-yl)-pyr- ido[1,2-a]pyrimidine-3-carbaldehyde, (Figure 2), when delivered through peptide conjugated nanoparticle, displayed anti-tuberculosis activity [93]. In another human pathogen *Plasmodium falciparum* (Pfa), the causative agent of malaria, inhibition of uracil DNA glycosylase (PfaUNG) by uracil analogs such as 1-methoxyethyl-6-(p-n-octylanilino) uracil and 6-(phenylhydrazino) uracil resulted in growth inhibition of the parasite [94]. Interestingly, these analogs did not show cytotoxicity in liver cell line HepG2 at concentrations required to inhibit the parasite (IC_{50} for cytotoxicity was ~tenfold higher than the IC_{50} for inhibiting the plasmoidal growth). Although it should also be said that PfaUNG had both lower affinity and turnover rates on uracil containing DNA substrates compared to human UNG. Efficacy and specificity of novel UNG inhibitors in anaerobic pathogen *P. falciparum* suggest that it has the potential to inhibit latent *M. tuberculosis* that survives low oxygen conditions in the host.

The last enzyme in a BER reaction is the NAD⁺ dependent bacterial ligase, LigA. LigA predominantly functions in ligating the Okazaki fragments generated during the replication of the lagging strand and is essential for bacterial survival. In addition to its role in replication, LigA performs the final step in the DNA repair pathway by establishing the phosphodiester bond following repair synthesis by DNA polymerases [95]. In a target-based high-throughput screen, Mills *et al.* identified substituted adenosine analogs that inhibited LigA of *H. influenzae* (Figure 3) [96]. The inhibition occurred at nanomolar quantitates on purified enzyme and addition of the compound to bacterial culture resulted in the accumulation of smaller DNA fragments, consistent with inhibition of DNA ligase activity. Notably, the molecule showed antibacterial activity against other Gram-positive bacteria such as *Staphylococcus* and *Streptococci*. Administration of adenosine analogs to mice infected with *S. aureus* or *S. pneumoniae* led to bacterial

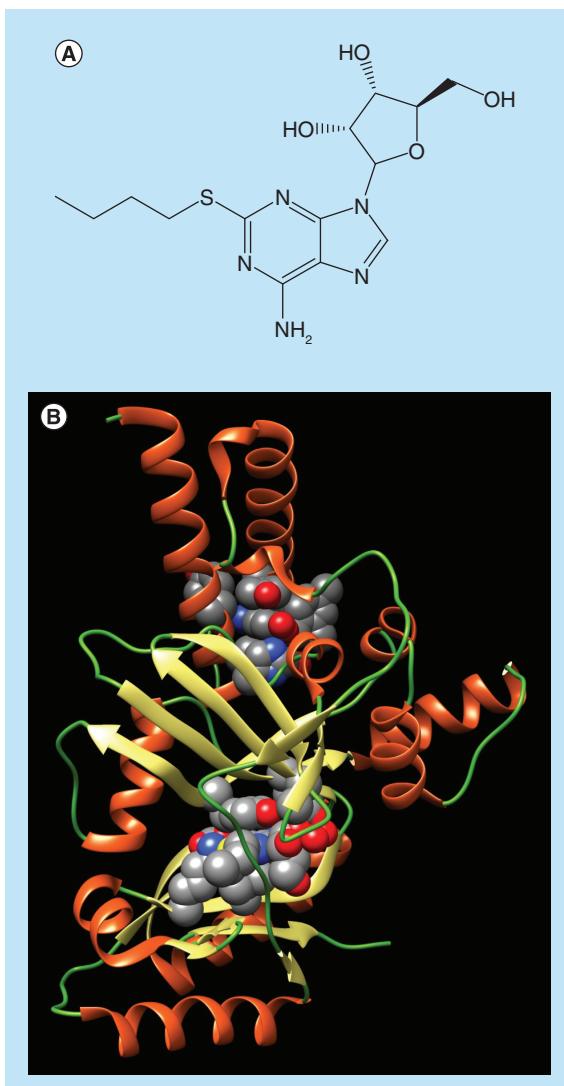


Figure 3. Small molecule inhibitor of bacterial ligase inhibitor. Chemical structure of LigA inhibitor (A). Adenosine analog inhibitors of LigA of *H. influenzae* were identified by high throughput screening of molecule library. (B) Cocrystal structure of *H. influenzae* LigA with compound 1. The protein is depicted in ribbon format while the compound 1 bound to LigA is shown in space filling. The image is reproduced using the co-ordinates for 3PN1 deposited in the PDB bank from Mills *et al.* [96] using UCSF Chimera, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from NIH P41-GM103311 [120].

clearance in a dose-dependent manner. Most importantly, the compound did not inhibit human ligase, indicating that these analogs could be potential drug candidates [96].

More recently, molecules with thienopyridine scaffold have been reported to be better inhibitors as modification of the C2 in adenosine analogs resulted in significant loss of activity [97,98]. In addition to the NAD⁺ dependent ligase, eubacteria encode ATP dependent ligases [97,99]. In *Mycobacteria*, in addition to the house-keeping LigA three additional ATP dependent ligases have been identified but they could not substitute the function of LigA indicating that these enzymes have a limited role in bacterial physiology [100].

Application of BER inhibitors developed for anticancer therapy in treating infectious diseases: a drug repurposing approach

Inhibitors targeting the human BER pathway have gained considerable significance for cancer therapy. Chemotherapy and radiotherapy used in cancer treatment result in DNA damage both to cancerous and normal cells. Cancerous cells, by their very nature, are highly proliferative and the increased DNA damage signals cell cycle arrest or death. In order to mitigate the effects of DNA damage, cells induce DNA repair wherein BER forms an important component. In this context, the use of BER inhibitors could reduce the repair of DNA damages induced during therapy and prevent the cancerous cell from recovery.

In the case of intracellular pathogens, the deamination of cytosines to uracil is often the result of RNI and ROS generated by the host. The loss of *ung* significantly increases the mutation frequency in bacteria [66,101]. The

catalytic mechanism of Ung action is known in considerable details [102,103]. Based on this mechanism, nitrogen substituted sugars (aza-deoxyribose) were tested for their ability to inhibit Ung wherein the N⁺ would mimic the oxocarbenium intermediate. Indeed, the binding affinity of a 1-aza-deoxyribose containing oligonucleotide was ~4000-fold tighter for enzyme-uracil anion intermediate and is shown to be the tightest inhibitor of Ung [104]. It has been shown that the administration of a thymidylate synthesis inhibitor, pemetrexed, increased double-strand breaks in cells that were deficient in UNG, leading to the death of the cancerous cells. Interestingly, it has been reported that in mycobacteria under hypoxic conditions, levels of Ung are significantly reduced to mitigate the toxicity of increased AP sites [105]. In latent TB infection, dormant mycobacteria reside within the granuloma and experience hypoxia. In this context, pemetrexed administration could be bactericidal and a potential drug candidate against dormant *M. tuberculosis*.

In addition to analogs that inhibit BER, small molecule inhibitors against BER enzymes involved in oxidative repair are also being tested in human cancers [106]. Jacobs et al., performed a primary screen for inhibitory molecules and identified CGP-74514A that inhibited NEIL1 activity at micro molar concentrations (Figure 4) [107]. CGP-74514A is a known Cdk inhibitor leading to apoptosis and the molecule chemically consists of a purine backbone [108]. Based on these findings, the authors performed a secondary screen using modified purine analogs and identified four additional compounds that showed better inhibition of NEIL1 activity than CGP-74514. Although the complete details of the mechanism of inhibition are not known, it is believed that the molecules bind to the enzyme and inhibits them. Further, these compounds showed inhibition against other glycosylases related to NEIL1 such as OGG1, NTH, and Fpg. It would be interesting to check the effect of these molecules on bacterial Fpg. In an independent study, nearly 50,000 compounds were screened for their inhibitory activity on OGG1, and 13 of these compounds showed promising inhibitory activity [109]. Many of these molecules belong to the hydrazide class of compounds, and five compounds that showed IC₅₀ in the submicromolar range were further analysed. Detailed analysis of the mechanism of action revealed that these compounds did not interfere with the substrate binding but rather inhibited the Schiff base intermediate formed during the catalysis of OGG1 [109]. Although the compounds showed exclusive selectivity to OGG1 and did not inhibit bacterial Fpg, it may be worth testing the derivatives of these inhibitors on a wider spectrum of bacterial Fpg enzymes.

Following the action of DNA glycosylases, AP sites are generated that are acted upon by APEs. In addition to inhibiting the BER glycosylase, APEs are also being explored as possible targets for inhibiting BER. Inhibition of APE affects the processing of AP sites and thus the downstream steps of BER. Many small-molecule inhibitors targeting the APE1 have been identified and tested for their ability to increase sensitivity to chemotherapeutic drugs. For example, methoxyamine, which binds to abasic sites generated by DNA glycosylases interferes with the processing of AP sites by APE, thus affecting BER [110]. Another molecule lucanthone, has been shown to inhibit exonuclease family of APE. Co-administration of lucanthone with alkylating agents such as MMS and TMZ increased the killing of cancerous cells [111]. Using a high throughput screen Madhusudan et al., identified CRT0044876, chemically known as 7-nitro-1H-indole-2-carboxylic acid that inhibited APE1 (Figure 5) with an IC₅₀ of 3.06 μM under *in vitro* conditions. *In silico* docking analysis revealed that the most favored enzyme-ligand complex was formed when the ligand (CRT0044876) was within the active site of APE1 [112]. In a more recent report Bapat et al., reported the identification of four compounds with IC₅₀ values less than 10 μM for Ape1 endonucleases in a high-throughput screen of 60,000 compounds [113]. Out of these compounds, AR03 (Ape1 repair inhibitor 03) showed the best inhibition activity against Ape1 within cells and inhibited the proliferation and viability of glioblastoma cells and enhanced the cytotoxicity of MMS and TMZ. Interestingly, AR03 was able to inhibit the activity of *E. coli* endonuclease IV, though the inhibition was less compared to mammalian Ape1. Derivatives of AR03 that are more potent inhibitors of bacterial endonuclease IV can be tested for antibacterial activity. The fact that small-molecule screens have the potential to identify specific inhibitors can be exploited to design screening platforms specific to bacterial enzymes.

The potential new inhibitors of DNA repair against pathogens also need to be extensively tested for cross-reactivity with the host's DNA repair enzyme. To overcome cross-reactivity, the potential drug molecules can be selectively delivered to the pathogen. Iron withholding is a well-recognized strategy by the host to restrict the growth of the pathogen. To overcome the iron limitation imposed by the host, the pathogens secrete high-affinity iron chelators (siderophores) that extract iron bound to proteins. Members of the *Streptomyces* group have capitalized on this arm's race to produce sideromycins that contain antibiotic moieties linked to siderophores. The sideromycins essentially function in a 'trojan horse' analogy by transporting antibiotics into pathogens. Albomycin and salmycin that are naturally occurring sideromycins are active against Gram-negative and Gram-positive bacteria [114,115].

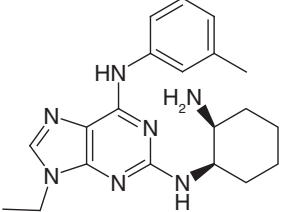
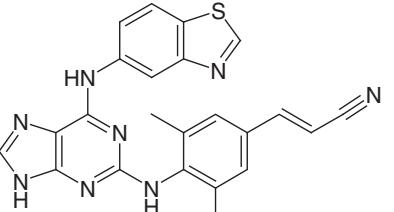
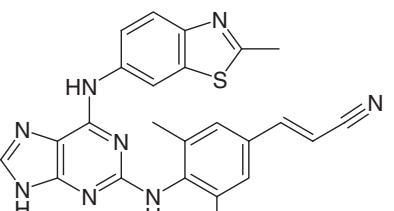
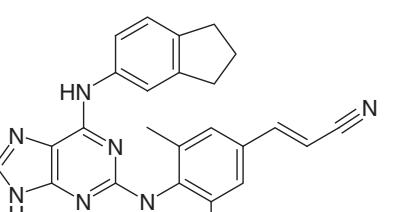
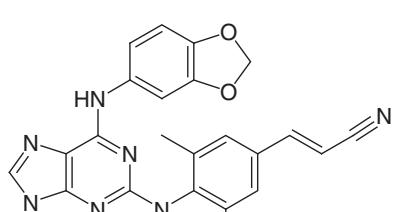
Structure and IDs	qHTS IC ₅₀ (μ M)
 CGP-74514A (NCGC0015229)	25.1
 P2 (NCGC00188618)	4.0
 P6 (NCGC00188616)	7.9
 P7 (NCGC00188617)	8.9
 P8 (NCGC00188619)	10.0

Figure 4. Structures of purine analogs that inhibit Nei such as DNA glycosylase I. NEIL1 small molecule inhibitors.
The structures of purine analogs that inhibit NEIL1 identified in a high-throughput chemical library screen.
The structures of inhibitors with their NIH Chemical Genomics Centre (NCGC) IDs and their corresponding IC₅₀ values are reproduced from [107] Jacobs *et al.* Inhibition of DNA glycosylases via small molecule purine analogs, *PLoS ONE* (8/e81667) 2013, with permission from *PLoS ONE* publication.

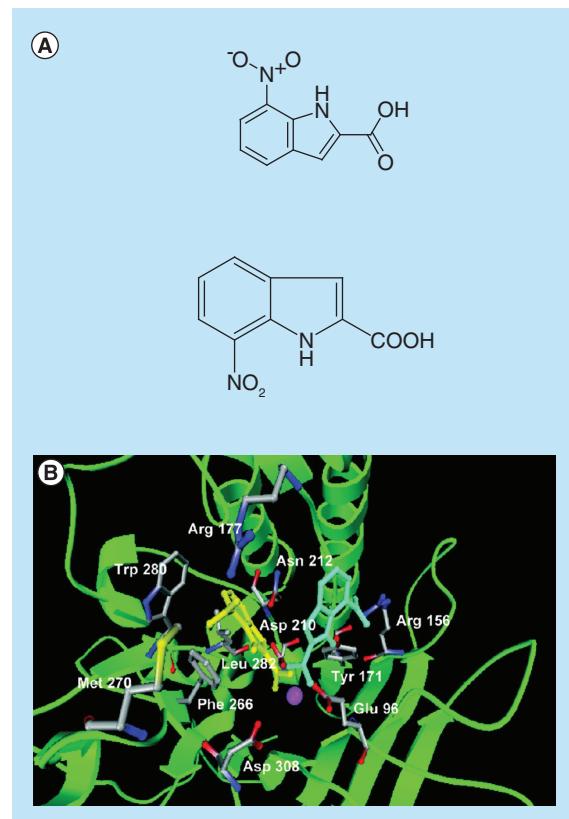


Figure 5. Small molecule inhibitor of DNA base excision repair pathway. (A) Chemical structure of 7-nitro-1H-indole-2-carboxylic acid referred to as CRT0044876. Molecular docking analysis of CRT0044876 to the active site of APE1 in two different low energy conformations (B) designated cluster I (in yellow) and cluster II (in blue). APE1 is shown as a ribbon structure. Selected active site amino acid residues are indicated.
Image reproduced from [112] with permission from Oxford University Press on behalf of Future of Science.

The active antimicrobial part of albomycin is an aminoacyl-tRNA synthetase inhibitor that is linked to iron siderophore moiety (Figure 6) [116]. This strategy has been utilized to rationally synthesize siderophore antibiotic conjugates [117]. In the case of *M. tuberculosis*, this strategy was successfully tested by conjugating artemisinin with synthetic mycobactin T derivative [118]. Artemisinin, a known antimalarial drug, has no reported activity on *M. tuberculosis*, but the mycobactin-artemisinin conjugate showed good anti mycobacterial activity against *M. tuberculosis* H37Rv, XDR strains, and *M. smegmatis*. Interestingly, the conjugate retained its antimalarial activity as well making it a dual-purpose drug.

Conclusion

There are several bacterial pathogens that remain challenging for infection control and treatment. The emergence of drug resistance among several established bacterial pathogens has necessitated the discovery of new anti-bacterials with novel molecular targets. A high magnitude of mortality due to bacterial pathogens such as *M. tuberculosis*, drug-resistant *Staphylococcus aureus* (MRSA) strains, Gram-positive bacteria, non-tuberculous mycobacteria (NTM) remains to be addressed. BER offers a novel and new toolbox to develop new therapeutics for these diseases. The traditional targets of anti-bacterial drugs that exist within cell wall biosynthesis, replication, transcription, and translation pathways are being nullified by rapidly emerging drug resistance. There is a toxic combination of emerging resistance and dwindling interest of big pharma in developing treatments for neglected bacterial diseases. DNA repair affords a new pathway that has the potential to provide multiple vulnerable drug targets. Since DNA repair pathways nullify the effects of oxidative DNA damage in bacteria, their inhibition can augment the efficacy of existing drugs that have been shown to increase ROS levels. However, drugs targeting DNA repair may also lead to increased mutation rates that can lower the genetic barrier of resistance to existing drugs and needs to be thoroughly explored.

Future perspective

The advancements in structure-based drug design *in vitro* screening based on docking affords exciting new possibilities for the selection of novel inhibitors of BER targets. There has been significant structural data for key enzymes that may allow primary screening and selection of new candidates. The key is to then find whole-cell

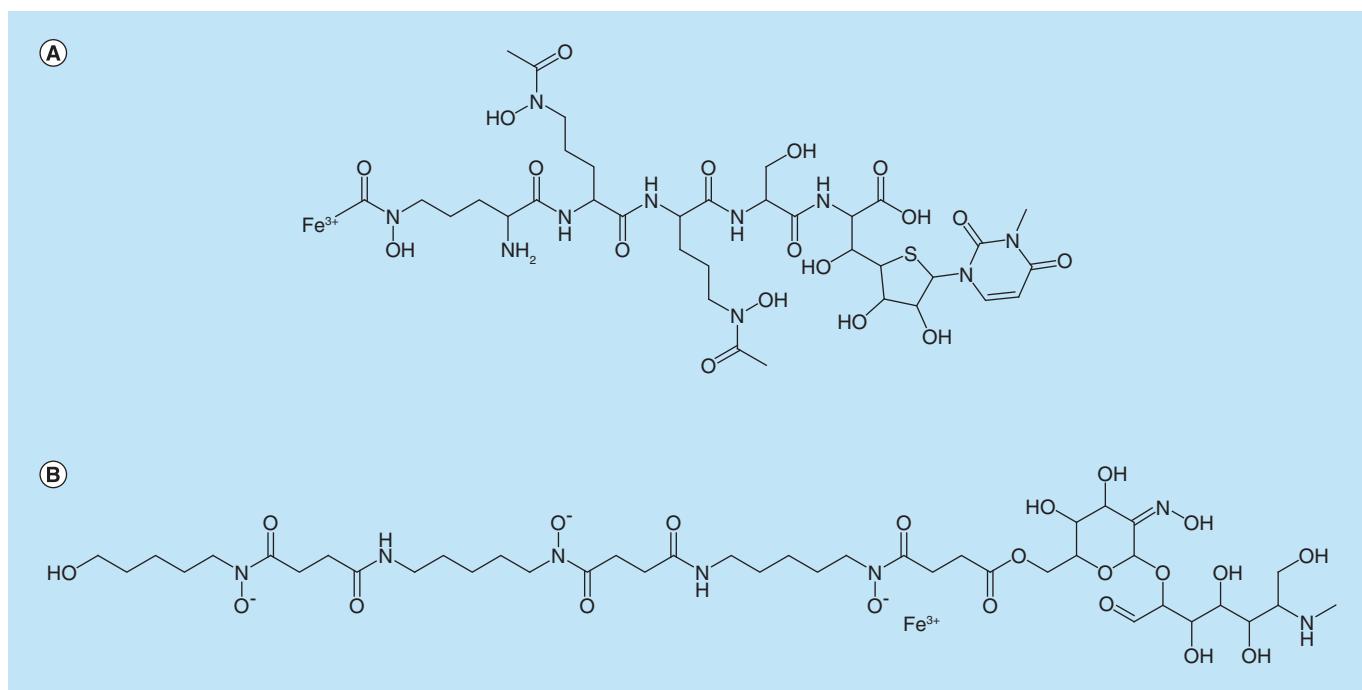


Figure 6. Sideromycins the trojan-horse antibiotic. Structure of natural sideromycins. The structures of (A) Albomycin and (B) Salmycin produced by were retrieved from Pubchem number 3083716 and 90479033, respectively, and generated using ChemBiodraw software.

actives with specificity for selected bacterial pathogens without inhibiting human or mitochondrial homologs. As discussed, it has already shown potential for dUTPase (Dut). Additionally, more genetic evidence for conditional essentiality is required for BER components to establish them as druggable and cidal targets. Since multiple DNA repair pathways operate within the pathogen, it is possible that none of BER targets are sterilizing in isolation, but combinations may be needed to find such potent regimens that are immune to genetic resistance, effective in killing drug-resistant bugs as well as counter latent TB in the case of mycobacteria.

DNA repair pathways play a key role in the intracellular survival of bacterial pathogens such as shown in *M. tuberculosis* [83,119] and *Salmonella* [68]. The gene encoding uracil DNA glycosylase is required for growth of *M. tuberculosis* *in vivo* in mouse spleen [78]. The currently used TB vaccine is based on *M. bovis* BCG that fails to provide long term protection to *M. tuberculosis* infections. With the increasing incidence of drug-resistant TB worldwide, an effective vaccine would play a key role in preventing infection and spread of the disease, hence paving the way for its eventual elimination. Most current strategies are based on highly attenuated strains that are deficient in efficient response. An alternative approach is highly desired. Although there is a lack of studies, evidence suggests that the *M. tuberculosis* strains lacking multiple DNA repair pathways that fail to establish active disease but elicit a strong immune response could provide an alternative or adjunct route to the development of a better vaccine candidate. The recently characterized UdgX and HMCES represent a new class of DNA repair proteins that act by covalently binding to damaged DNA and protecting from AP site mediated cytotoxicity. The fact that homologs of mammalian HMCES are found in *E. coli* suggests that this pathway is evolutionarily conserved across different domains of life [47]. Although the beneficial effects of these proteins cannot be ignored, their existence in the DNA bound state for an extended period can hinder other cellular processes such as transcription thereby generating undesired cascading effects. Interestingly, co-expression of UdgX and Ung inhibitor, Ugi in a *recA* mutant background attenuated the growth of a diverse group of bacteria such as *E. coli* and *M. smegmatis* [46]. It would be interesting to test if such engineered strains of *Salmonella* or *Mycobacteria* can work as vaccine strains.

Acknowledgments

The authors thank the lab members of U Varshney, IISc, and A Kumar, Mycobacterium Research Laboratory, RGCB for their valuable inputs and support during the preparation of the manuscript.

Financial & competing interests disclosure

U Varshney, a J. C. Bose Fellow (Science and Engineering Research Board, Government of India), acknowledges the financial support received from the Department of Biotechnology (DBT), Ministry of Science and Technology; and the Office of the Principal Scientific Advisor, Government of India. The authors acknowledge the support of DBT-IISc partnership programme, University Grants Commission, New Delhi for the Centre of Advanced Studies, and the DST-FIST level II infrastructure support to carry out this work. K Kurthkoti acknowledges the financial support from Ramalingaswami Re-entry fellowship from Department of Biotechnology, Government of India, intramural funds from Rajiv Gandhi Centre for Biotechnology and the extramural fund from Science and Engineering Research Board (CRG/2018/001209), Government of India. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary**Background**

- Currently used antibacterial therapies target key essential cellular processes in bacteria but are exceedingly being rendered ineffective due to rapidly evolving clinical drug resistance.
- Base excision repair (BER) of bacteria is crucial for reversing the DNA damages inflicted by the host immune responses that includes reactive oxygen species and reactive nitrogen intermediates, and critical for the survival of intracellular pathogens.

BER pathway as novel drug targets

- Deletion mutant strains of pathogenic bacteria lacking partial BER have reduced survival within *ex vivo* as well as *in vivo* host environments suggesting their strong but hitherto largely unharvested potential as drug targets.
- Utilizing the atomic structures of important BER enzymes, small molecule inhibitors have been designed and have shown promise in controlling bacterial pathogens in animal models of infection.
- Repurposing of anticancer molecules that target BER enzymes provides a starting point to initiate targeted design efforts to develop new antibiotics. Selective affinity for bacterial targets can be designed along with selective delivery of such molecules to bacteria can be achieved using siderophore conjugates.

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