ELSEVIER

DNA topoisomerase I and DNA gyrase as targets for TB therapy

Valakunja Nagaraja[1,2], Adwait A. Godbole[1], Sara R. Henderson[3] and Anthony Maxwell[3]

[1] Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560 012, India
[2] Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore 560064, India
[3] Department of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK

Tuberculosis (TB) is the deadliest bacterial disease in the world. New therapeutic agents are urgently needed to replace existing drugs for which resistance is a significant problem. DNA topoisomerases are well-validated targets for antimicrobial and anticancer chemotherapies. Although bacterial topoisomerase I has yet to be exploited as a target for clinical antibiotics, DNA gyrase has been extensively targeted, including the highly clinically successful fluoroquinolones, which have been utilized in TB therapy. Here, we review the exploitation of topoisomerases as antibacterial targets and summarize progress in developing new agents to target DNA topoisomerase I and DNA gyrase from *Mycobacterium tuberculosis*.

Introduction

Although the information content of DNA is essentially independent of how the DNA is knotted or twisted, the access to this information depends on the topology of the DNA. DNA topoisomerases are ubiquitous enzymes that maintain the topological homeostasis within the cell during these DNA transaction processes [1–3]. In the words of James C. Wang, 'DNA topoisomerases are the magicians of the DNA world' [4]; however, unlike the sleight of hand used by a magician, topoisomerases rely on the elegant chemistry of transesterification. Depending on their mechanism of action, these enzymes are broadly classified as type I (which make transient single-stranded breaks in DNA) and type II (transient double-stranded breaks). The enzymes are further subdivided into types IA and IB, distinguished by whether the transient covalent bonds are to the 5′- or 3′-phosphate, respectively, and types IIA and IIB, which differ in mechanistic and evolutionary aspects [5]. Given their functional importance, every species has at least one enzyme from each type. Although the presence of more than one topoisomerase from each type allows for a division of labor for supercoiling, relaxation, knotting/unknotting, and catenation/decatenation, this redundancy also provides for a certain degree of overlap in their functions. For instance, in *Escherichia coli*, there are four topoisomerases: two type I [topoisomerase (topo) I and topo III] and two type II (DNA gyrase and topo IV). *In vitro*, all four enzymes are capable of DNA relaxation, whereas, *in vivo*, their roles tend to be more specialized, for example, gyrase introduces negative supercoils, whereas topo IV is responsible for decatenation following replication [6]; these functions are critical for cell survival. By contrast, the *Mycobacterium tuberculosis* (*Mtb*) genome encodes a single type I (topo I; gene = Rv3646c) and a single type II topoisomerase [gyrase; genes = Rv0006 (gyrA) and Rv0005 (gyrB)] [7], which take care of the entire burden of decatenation, relaxation, and supercoiling. In organisms with additional topoisomerases, not all the topoisomerases are essential for cell survival. For example, in *E. coli*, topo I is not necessarily essential [8], whereas saturation mutagenesis studies suggested the essentiality of Mtb topo I (MttopoI) [9]. This essentiality is confirmed by generating conditional knockdown strains, wherein the intracellular level of the enzyme is downregulated [10]. The minimal composition of the topoisomerases in the Mtb genome necessitates the enzymes to carry out additional functions *in vivo*. Previous studies of mycobacterial topoisomerases have revealed these additional functions as well as their distinct features [11–18].

Corresponding author: Maxwell, A. (tony.maxwell@jic.ac.uk)

http://dx.doi.org/10.1016/j.drudis.2016.11.006

Please cite this article in press as: Nagaraja, V. et al. DNA topoisomerase I and DNA gyrase as targets for TB therapy, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.11.006

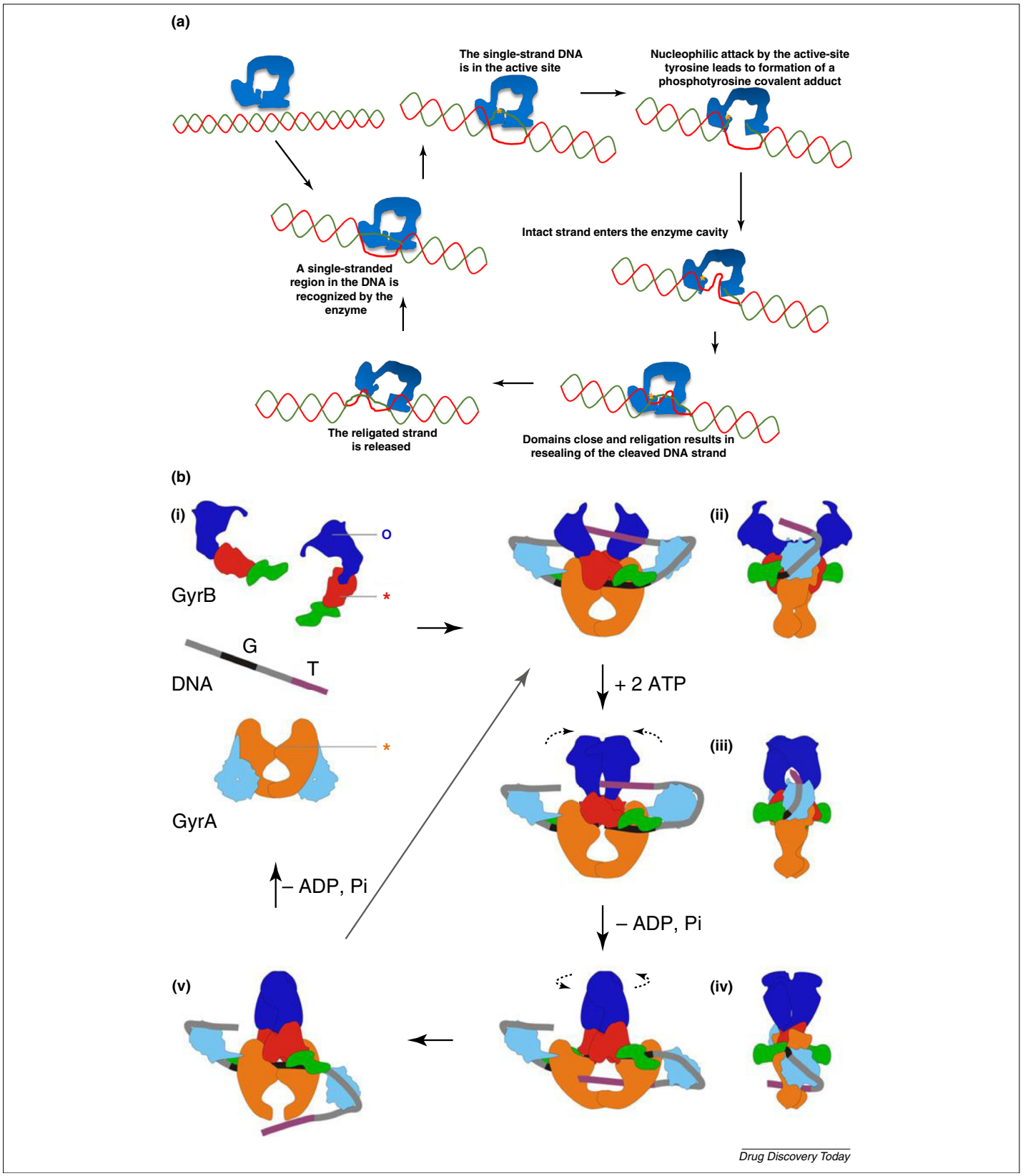

FIGURE 1

Topoisomerase reaction mechanisms. **(a)** Bacterial topoisomerase I (topo I) reaction cycle: the multistep reaction is initiated with the interaction and binding of the enzyme to negatively supercoiled DNA. DNA binding is followed by the first transesterification reaction in which the nucleophilic attack by the tyrosine of the active site on the phosphodiester backbone results in the formation of the topo I-DNA covalent complex and separation of the domains to form an enzyme–DNA gate. Next, the intact strand of DNA is passed through this enzyme–DNA gate, resulting in a change in linking number. After strand passage, during the second transesterification reaction, the separated domains close and the 3′-hydroxyl group targets the phosphotyrosine covalent adduct with a nucleophilic attack, resulting in the resealing of the cleaved DNA strand. During the reaction cycle, the various domains of the enzyme open and close like a clamp, facilitating the

Please cite this article in press as: Nagaraja, V. et al. DNA topoisomerase I and DNA gyrase as targets for TB therapy, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.11.006

DNA cleavage: the Achilles' heel of the topoisomerase mechanism

The reaction mechanism of type IA topoisomerases involves a series of coordinated steps. The reaction cycle, depicted in Fig. 1a, shows the noncovalent binding of the enzyme to the substrate, followed by a transesterification reaction involving a nucleophilic attack on the phosphodiester backbone by the active-site tyrosine, resulting in the formation of a phosphotyrosine covalent adduct. The intact strand of DNA is passed through the cleaved DNA followed by another transesterification reaction involving nucleophilic attack by the free 3′-OH on the phosphotyrosine covalent adduct resulting in the resealing of the cleaved DNA. A cleavage–religation equilibrium between the two transesterification reactions is an important feature of the overall reaction. Perturbation of this equilibrium can lead to the accumulation of enzyme–DNA covalent adducts, which are potentially cytotoxic. Topoisomerase poisons are molecules that perturb the cleavage–religation equilibrium, resulting in the accumulation of enzyme–DNA covalent adducts [19]. The cleavage of the DNA is a vulnerable step during the reaction mechanism of the enzyme that could be exploited to develop inhibitors that can act as topoisomerase poisons.

In the case of type II topoisomerases, such as bacterial gyrases, the reaction cycles proceed via transient double-stranded breaks in DNA. In the general case, as represented by eukaryotic topo II and bacterial topo IV, the dimeric enzyme binds two segments of DNA, a G (or gate) segment and a T (or transported) segment. Double-stranded cleavage of the G segment facilitates passage of the T segment through the G segment (and, thus, through the protein interfaces of the dimeric enzyme), in a reaction driven by the binding and hydrolysis of ATP [20]. Gyrase is a special case of this mechanism (Fig. 1b) in which the G and T segments are colinear and the strand-passage event leads to the introduction of negative supercoils [1]. As with the type I enzymes, cleavage of the DNA (double stranded in this case) is a vulnerability that can be potentially exploited to develop inhibitors [21].

Thus, one of the most desired classes of molecule that target topoisomerases are topoisomerase poisons [19]. However, until recently, no such molecules had been discovered for bacterial type I enzymes, although it has been genetically and chemically validated as an anti-TB target [22]. By contrast, several molecules target type II topoisomerases (bacterial and eukaryotic) and type IB enzymes (typically found in eukaryotes). In the case of eukaryotic type IB enzymes, camptothecin [23,24] and its various derivatives target human topo I. Although an inhibitor that acts in a similar fashion is desired for type IA topoisomerases, catalytic inhibitors of the enzyme might also be useful in those organisms harboring a single type IA topoisomerase. DNA gyrase has been extensively exploited as a target for the development of antibacterial compounds (discussed below), prominent among them being the fluoroquinolones, which are one of the most effective cellular poisons, arresting the gyrase reaction after DNA cleavage leading to the generation of double-strand breaks [25,26].

Inhibitors of MttopoI

In work by the Nagaraja lab, in part supported by the MM4TB consortium (www.mm4tb.org), has pursued the design of proof-of-principle inhibitors for MttopoI. Biochemical characterization of topo I from both *Mycobacterium smegmatis* [11] as well as *Mtb* [27] revealed the site-specific nature of the enzyme, which recognizes a hexameric sequence, CG/TCT↓TC/G (where ↓ indicates the site of cleavage) [16]; these sites are referred to as 'strong topoisomerase sites' (STS). The binding as well as the cleavage reaction is sequence specific and, therefore, enabled the design of oligonucleotide substrates that can inhibit the DNA relaxation reaction. Incubation of the enzyme with the oligonucleotides harboring the STS causes the DNA-binding site of the enzyme to be occluded for the binding of negatively supercoiled DNA, leading to the inhibition of the DNA relaxation reaction [16]. Next, monoclonal antibodies (mAbs) were developed that specifically inhibit the mycobacterial topo I, an approach that had been used previously to develop specific inhibitors of mycobacterial gyrase [28]. Apart from being used as tools to understand the finer details of the reaction, these mAbs could also serve as valuable start points for the development of small-molecule inhibitors against mycobacterial topo I. One of the mAbs, 1E4F5, was especially interesting owing to its mechanism of action. Depending on the order of addition of the mAb, it could inhibit the reaction at multiple steps. Pre-incubation of the mAb with the enzyme led to the inhibition of the DNA-binding ability, while incubation of the mAb with a preformed topo I-DNA complex led to the formation of a mAb-topo I-DNA ternary complex [29]. This mAb was shown to close the clamp of the enzyme, thus stimulating the second transesterification step. Another mAb, 2F3G4, affected the cleavage–religation equilibrium of the reaction, leading to the accumulation of the topo I-DNA covalent complex; thus, 2F3G4 behaves like a topoisomerases poison [30].

All topoisomerases, irrespective of their class, transiently cleave DNA during their reaction cycle. As discussed, this step has been extensively exploited in the development of antibacterial as well as anticancer agents, which target gyrase and eukaryotic topoisomerases, respectively. Thus, it is surprising that there is a dearth of such small molecules that target bacterial topo I. However, in the recent past, several studies have addressed this shortcoming. Using an high-throughput screening (HTS) assay based on SOS induction in *E. coli*, a phenanthrene compound, stephenanthrene, was identified as a bacterial topo I poison [31]. More recently, derivatives of bolden, seconeolitsine, and *N*-methyl-seconeolitsine (Fig. 3) were also identified to target the DNA relaxation activity of bacterial topo I [32]. Apart from derivatives of bolden, bisbenzimidazole analogs of Hoechst dyes, 3,4-dimethoxyphenyl bisbenzimidazole

formation of the topo I-DNA gate and religation, respectively. **(b)** Model for the mechanism of DNA supercoiling by DNA gyrase. Gyrase domains are colored as follows: GyrB-NTD, dark blue; GyrB-TOPRIM domain, red; GyrB-tail, green; GyrA-NTD, orange; GyrA-CTD, light blue. The G and T DNA segments are colored black and purple, respectively. (i) The subunits and DNA in their free states in solution; stars indicate the active site residues for DNA cleavage and the circle indicates an ATP-binding pocket. (ii) The G segment binds between GyrA-NTD and GyrB-TOPRIM, at the dimer interface, and the GyrA CTDs wrap DNA to present the T segment in a positive crossover. (iii) ATP is bound, closing the GyrB clamp (GyrB-NTD) and capturing the T segment; the G segment is transiently cleaved. (iv) Hydrolysis of one ATP molecule allows GyrB to rotate, the DNA gate to widen, and the transport of the T segment through the cleaved G segment. (v) The T segment exits through the C gate, and the G segment is religated. The hydrolysis of the remaining ATP molecule resets the enzyme. Reprinted, with permission, from [73].
Abbreviations: CTD, C-terminal domain; NTD, N-terminal domain; TOPRIM, topoisomerase primase domain.

Please cite this article in press as: Nagaraja, V. et al. DNA topoisomerase I and DNA gyrase as targets for TB therapy, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.11.006

(DMA) [33] and DPA 153 [34] with a terminal alkyne substitution have been identified as potential inhibitors of topo I (Fig. 3). DMA was shown to act as a topoisomerase poison, both *in vitro* as well as, to a limited extent, in whole-cell assays [33]. More recently, analogs of bisbenimidazoles, such as bisbenzimidazole 12b (Fig. 3), were shown to be an improvement over DMA in terms of IC_{50} values and having lower MICs among the compounds tested against several *E. coli* strains [35].

Exploring natural product chemical space yielded anziaic acid (Fig. 3) derived from the lichen *Hypotrachyna* sp. and shown to be a dual inhibitor of type IA as well as type IIA topoisomerases. Anziaic acid was demonstrated to act like a topoisomerase poison [36]. However the molecule exhibited toxicity toward human pulmonary artery endothelial cells, possibly because of its dual-targeting ability [36]. Another small molecule identified by exploring the natural product spectrum was 2,4-diiodoemodin (Fig. 3), a haloemodin derived from the bioactive natural product emodin. This molecule is a dual inhibitor of topoisomerase I and DNA gyrase [37]. The dual inhibition capability could have important implications in suppressing resistance in pathogenic bacteria, such as *Mtb*, which has only two topoisomerases.

An alternate approach was used to find small-molecule inhibitors for MttopoI. Initially, a structural model of the enzyme was generated to screen *in silico* a large number of molecules followed by testing these molecules for enzyme inhibition directly (Fig. 2). Using this target-based approach, three molecules were shown to inhibit the DNA relaxation activity of MttopoI as well as to affect the growth of *Mtb* cells in a whole-cell assay (Fig. 3). One of the three molecules, m-AMSA (amsacrine; Fig. 3), is a well-known type IIA topoisomerases poison [38]. The other two molecules, imipramine and norclomipramine (Fig. 3), are used clinically as antidepressants [39,40]. These two molecules inhibited the DNA relaxation activity at lower concentrations [41]. Notably, they arrested the reaction and formed cleavage complexes with a higher efficiency and were found to be bactericidal. Moreover, a decrease in the MIC for the compounds in cells overexpressing topo I validated topo I as the intracellular target and their action as intracellular topo I poisons [41]. Although their potency was not comparable to that of fluoroquinolones, they are the most potent anti-topo I inhibitors reported so far. This study also highlighted the potential to repurpose clinically relevant compounds to target mycobacterial topo I [38]. Recent efforts starting from a polyamine scaffold have led to the identification of four more compounds that inhibit the DNA relaxation activity of MttopoI and also affect the growth of mycobacterial cells; however, none of these molecules acts as a topoisomerase poison [42].

To summarize, the search for potent inhibitors of bacterial type IA enzymes has begun, with some indications of success. Although these efforts using different approaches have yielded a few hits, the efficacy of these molecules in *in vivo* models of infection needs to be tested. Such studies are underway with imipramine. Clearly, more efforts are needed to develop better inhibitors using a variety of approaches and a combination of strategies, including synthesis of new derivatives from the early leads. Perhaps a high-throughput assay for screening a large number of compounds in a short time might not be out of place. The X-ray structure of the truncated MttopoI published recently is a way forward in finding potent inhibitors of the enzyme [43]. The availability of this structure will provide major insights into the rational design of small-molecule inhibitors of MttopoI.

Inhibitors of *M. tuberculosis* gyrase

In contrast to topo I, *Mtb* gyrase (Mtgyrase) has been extensively exploited as a target for potential antibacterials. This is in part

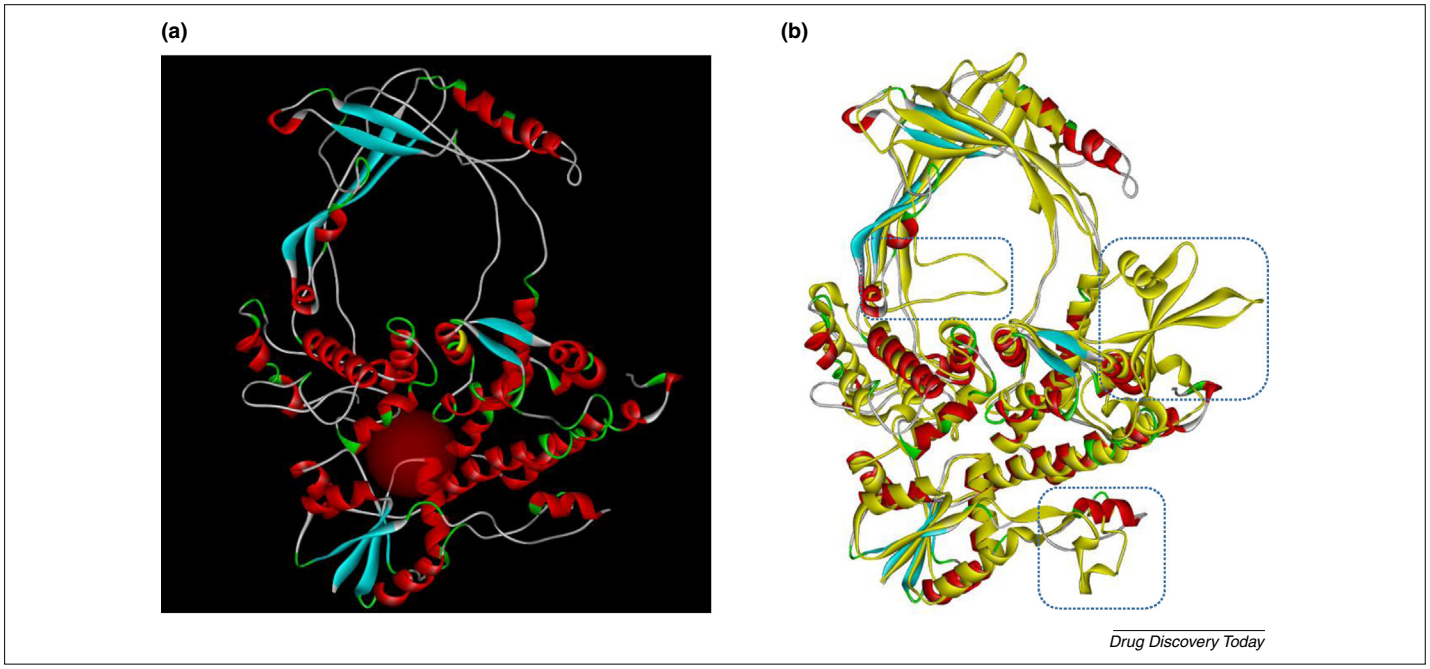

FIGURE 2

Structural model of *Mycobacterium tuberculosis* (*Mtb*) topoisomerase I (Mttopol). Structural model of Mttopol **(a)** with the imipramine docking region (filled sphere), and the superimposition of the crystal structure shown in yellow with the homology model colored by atom **(b)**; the differences highlighted between the structure and the model of the enzyme are outside the docking region.

Please cite this article in press as: Nagaraja, V. et al. DNA topoisomerase I and DNA gyrase as targets for TB therapy, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.11.006

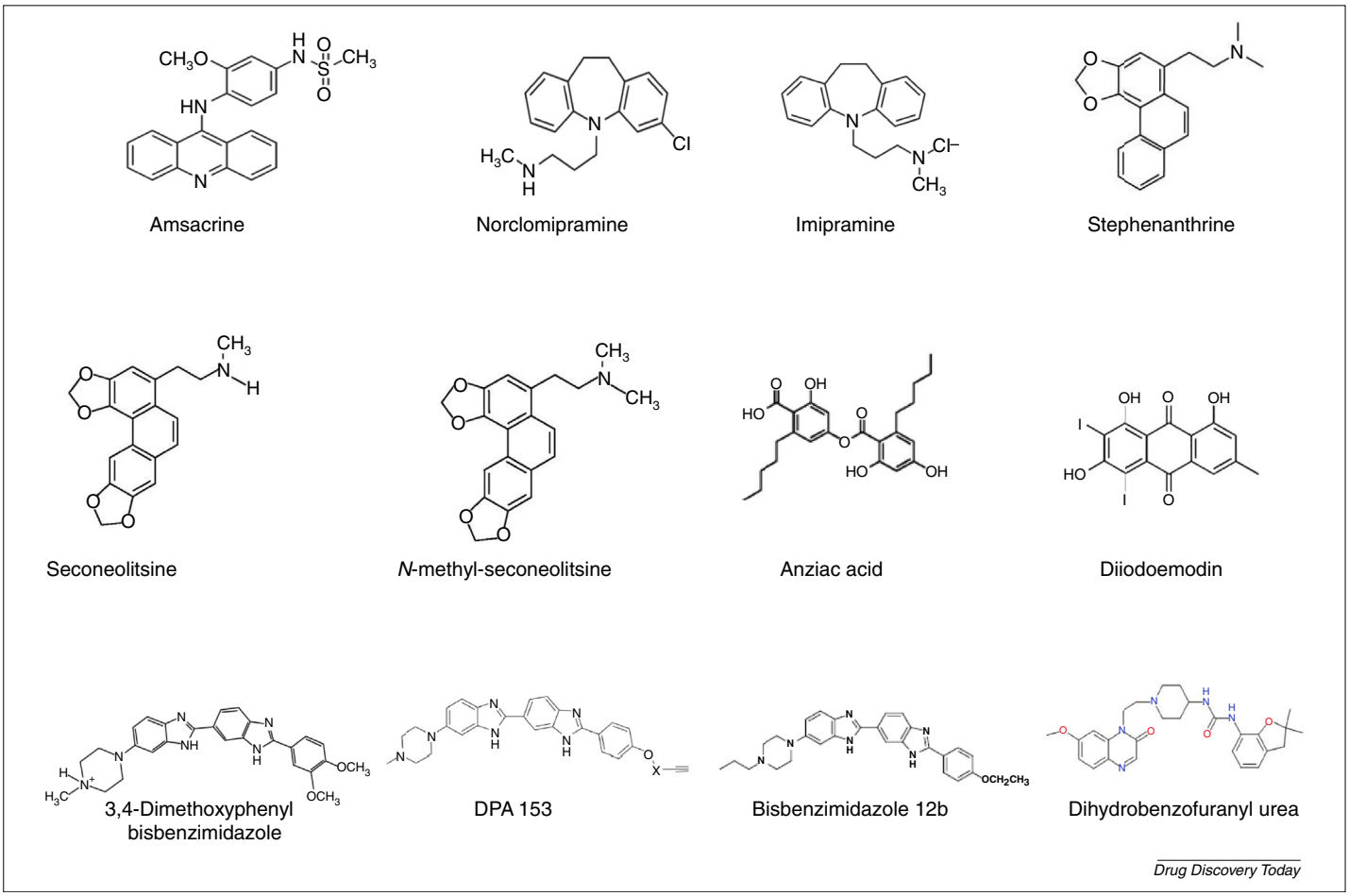

FIGURE 3

Small-molecule inhibitors of topoisomerase I.

because of the high degree of success of gyrase as a target for antibiotics effective against a range of pathogenic bacteria [21]. In addition, the extensive structural information on gyrase from several bacteria, including *Mtb* [44–48], has potentiated *in silico* methods for developing new gyrase-specific inhibitors; examples of this approach are given below. Perhaps surprisingly, there is currently no complete crystal structure of gyrase (A_2B_2) for any organism. However, it is possible to build such a structure (in this case for Mtgyrase) using available information from domains and protein fragments (Fig. 4).

The most successful gyrase-targeted antibacterial agents are the fluoroquinolones and several of these compounds have proved efficacious against TB; these include gatifloxacin and moxifloxacin (Fig. 5) [49–51], which are being evaluated both for first-line therapeutics and second-line therapy for multi-drug resistant (MDR) and extensively drug-resistant (XDR) TB (Fig. 5). In relation to moxifloxacin, which is currently the most promising fluoroquinolone targeted to TB, the results from a recent large-scale Phase 3 trial demonstrated that this drug could not be substituted for isoniazid or ethambutol in a 4-month treatment regimen [52,53]. However, it is clear that moxifloxacin will still have an important role in TB therapy, particularly for MDR-TB.

Work on understanding quinolone action on Mtgyrase and developing new inhibitors has been greatly assisted by X-ray crystallography. The structures of the GyrA N-terminal domain (NTD) and the catalytic core [C-terminal domain (CTD) of GyrB fused to the GyrA NTD] were initially determined [47,48]. Given that these regions contain amino acid residues that interact with quinolones, this gave some initial insight into drug–protein interactions. Work on gyrase and topo IV from other organisms revealed crystal structures with bound DNA and quinolones, specifically, the structure of the catalytic core fusion of *Streptococcus pneumoniae* topo IV complexed with moxifloxacin and clinafloxacin [54], the structure of the catalytic core fusion of *Staphylococcus aureus* gyrase complexed with ciprofloxacin [55], and the catalytic core fusion of *Acinetobacter baumannii* topo IV complexed with moxifloxacin [56]. These structures provided molecular insight into the mechanism of inhibition by quinolones. In more recent work, structures have been obtained for the *Mtb* GyrB-GyrA catalytic core with moxifloxacin, gatifloxacin, and other quinolones [45]. These structures highlight the details of Mtgyrase–quinolone–DNA interactions and how these differ from those seen in other organisms, and will potentiate the development of new compounds with increased potency against TB.

However, despite success in understanding the molecular basis of gyrase–quinolone–DNA interactions, resistance to quinolones is a serious problem in TB and other bacterial infectious diseases. Therefore, we need to develop other agents that can avoid the resistance issues of existing antibiotics.

Please cite this article in press as: Nagaraja, V. et al. DNA topoisomerase I and DNA gyrase as targets for TB therapy, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.11.006

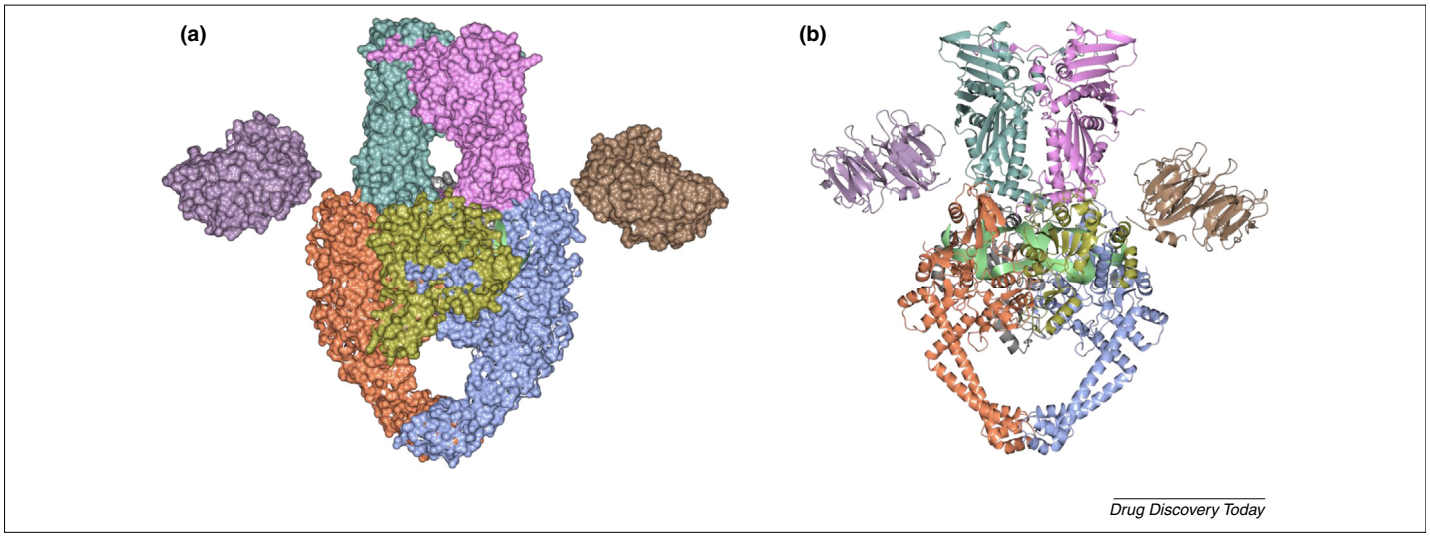

FIGURE 4

Structural model of *Mycobacterium tuberculosis* DNA gyrase (Mtgyrase). The figure shows space-filling **(a)** and ribbon **(b)** representations of Mtgyrase. The models were made by taking existing crystal structures (5BS8, 1ZVU, and 3ZKB) and using the program COOT to assemble a model of the A_2B_2 complex. Domains are colored as follows: GyrB-NTDs, pink and sea-green; GyrB-CTDs, gold and gray; GyrA-NTDs, coral and ice blue; GyrA-CTDs, purple and pale brown. DNA is in a green ribbon representation. *Abbreviations*: CTD, C-terminal domain; NTD, N-terminal domain.

In work supported by the MM4TB consortium, we investigated a large number of compounds for their potential as new gyrase-targeted antibiotics (unpublished data). Specifically, we were seeking compounds that show efficacy against *Mtb* (growing and/or dormant) and that inhibit the gyrase supercoiling reaction. Ideally, the compounds should have a good IC_{50} against gyrase (<50 μM) and, if possible, should stabilize the cleavage complex but not show crossresistance to quinolones. We received >2000 compounds (mostly synthetic) from other partners and evaluated their efficacy versus Mtgyrase. We found >60 compounds with IC_{50} <50 μM and evidence for compounds with new modes of action; fully characterizing these compounds and establishing their prospects as potential TB agents is the subject of ongoing work.

In connection with the MM4TB project, we also investigated naphthoquinone natural products as inhibitors of Mtgyrase. Extracts from the South African 'toothbrush' tree, *Euclea natalensis*, have been extensively used in traditional medicine and were found to be active against *Mtb* [57]. One active component of this extract was shown to be the naphthoquinone diospyrin (Fig. 5) [58], which was found to be an inhibitor of DNA gyrase [60]. Moreover, diospyrin and other naphthoquinones were found to have a novel mechanism of action, binding to the GyrB-NTD, but not at the ATPase site [59]. The molecular details of this binding pocket remain to be determined. Interestingly, naphthoquinone compounds have also been found to inhibit *Mtb* ThyX, an essential thymidylate synthase [60]. Whereas some compounds, such as 7-methyljuglone (Fig. 5), inhibit both enzymes, most affect ThyX without inhibiting gyrase. This suggests that the pharmacophores for the two targets (ThyX and GyrB) are distinct [60].

Other research groups have been developing new TB agents targeted to gyrase, particularly using *in silico* methods based on the new and emerging structural information on Mtgyrase. The crystal structure of the GyrB-NTD (ATPase domain) of *E. coli* gyrase complexed with an ATP analog, and that of the 24-kDa GyrB N-terminal subdomain with novobiocin [61,62], paved the way for structure-based drug design based around these ligand pockets. More recently, the corresponding structure (GyrB-NTD) for Mtgyrase has further promoted such work [44]. The group of Sriram investigated a large number of inhibitors of Mtgyrase, following computationally based approaches [63,64]; for example, using this methodology, 28 2-amino-5-phenylthiophene-3-carboxamides were synthesized, the best one having an MIC against *Mtb* of ~4 μM and an IC_{50} for Mtgyrase supercoiling of ~0.8 μM [65]. In another study, 48 quinoline-aminopiperidine-based urea and thiourea derivatives were designed, synthesized, and evaluated; the best compound had an MIC against *M. tuberculosis* of ~3.5 μM and an IC_{50} for Mtgyrase supercoiling of ~0.7 μM [66]. Similar work has been carried out with substituted benzofuran compounds and pyrrolamides [67,68].

Astra-Zeneca have also published a series of papers describing compounds that target the ATP-binding site. Aminopyrazinamides (Fig. 5) were identified from a HTS using the *M. smegmatis* GyrB ATPase reaction [69]. A crystal structure of one of these compounds complexed with the *M. smegmatis* GyrB-NTD revealed the specifics of ligand–protein interactions in this case. Aside from having good IC_{50}, these compounds also show promising MICs. *In silico* approaches led to thiazolopyridine ureas [70] and thiazolopyridone ureas [71] as compounds that also had good activities and properties.

Although a significant amount of effort has been expended on discovering new Mtgyrase inhibitors, most of this has centered on the ATP- and quinolone-binding sites. Exceptions to this are the naphthoquinone studies described above [59,60] and work from GSK [72], which identified novel bacterial topoisomerase inhibitors (NBTIs) with activity against Mtgyrase and that lack cross-resistance to fluoroquinolones (Fig. 5). More inhibitors acting at sites different from the well-known binding pockets would be desirable.

Please cite this article in press as: Nagaraja, V. et al. DNA topoisomerase I and DNA gyrase as targets for TB therapy, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.11.006

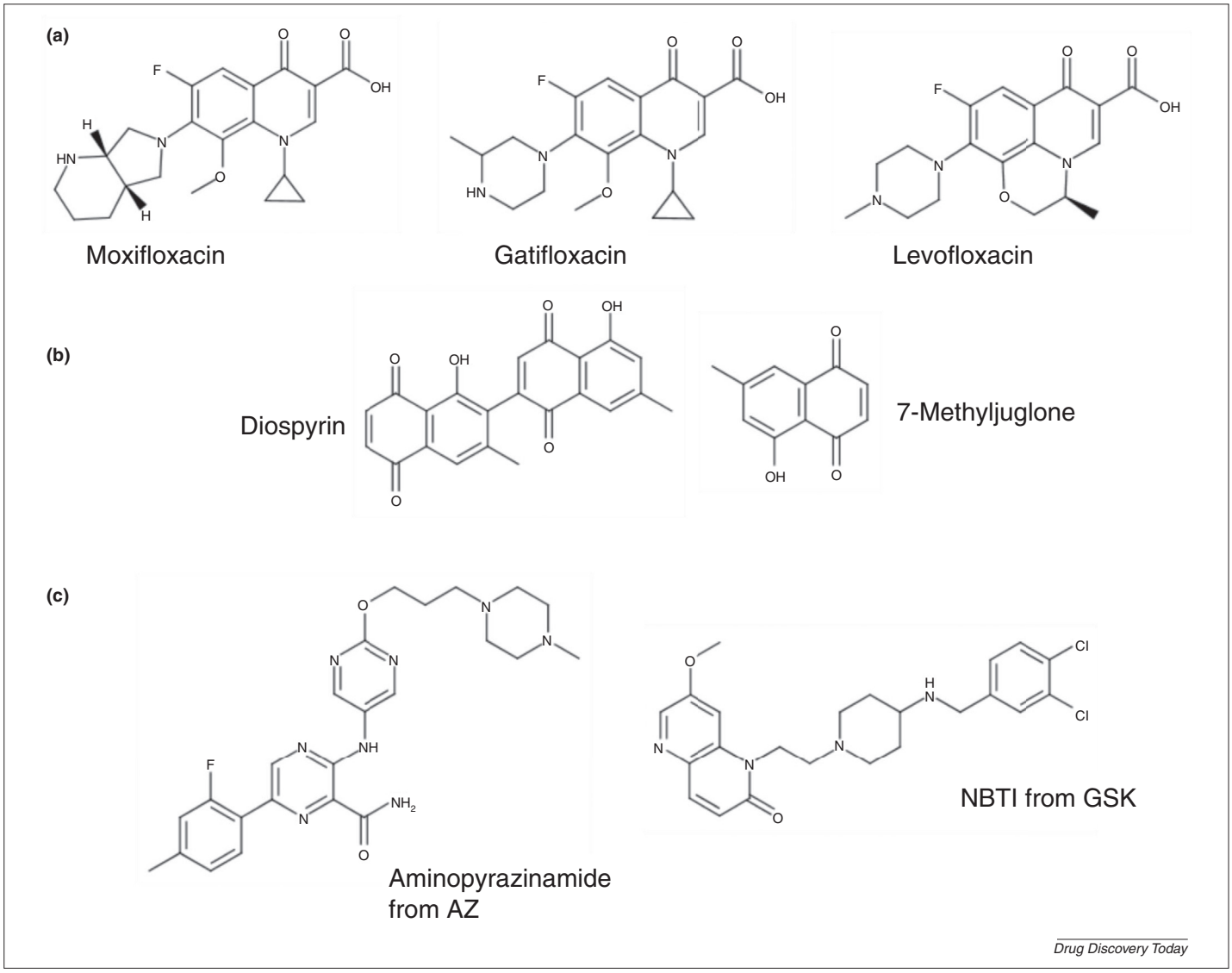

FIGURE 5

Small-molecule inhibitors of *Mycobacterium tuberculosis* DNA gyrase (Mtgyrase): **(a)** fluoroquinolones; **(b)** naphthoquinones; **(c)** a representative aminopyrazinamide from Astra-Zeneca [69], and a representative novel bacterial topoisomerase inhibitor (NBTI) from GSK [72].

Concluding remarks

There is no question that topoisomerases have been effective targets both for anticancer and antibacterial chemotherapy and there appears to be every reason to persist in the search for new agents that target MttopoI and Mtgyrase. These two enzymes can be regarded separately. In the case of MttopoI, it can be regarded as a 'new' target that remains to be exploited clinically. However, given the urgent need to find new antibacterial agents and the depth of knowledge about this enzyme, it is justifiable to expend more effort in seeking new inhibitors. In the case of Mtgyrase, the success of fluoroquinolones and the existence of other exploitable ligand-binding pockets in gyrase suggest that work on seeking new gyrase-specific inhibitors will continue to be important. Whether these searches are target-based or follow phenotypic screens is a moot point; the evidence from recent studies suggests that both approaches are valid. In any case, it is probably that combined academic and pharma efforts will be needed to achieve success.

Acknowledgements

We thank Sean Ekins for help in generating Fig. 2, and David Lawson for help in generating Fig. 4. The work was partially supported by a grant from the European Community's Seventh Framework Program (grant 260872, MM4TB Consortium) to A.M. and V.N., and by a UKIERI grant from the British Council (DST-2013-14/091) to A.M. and V.N. Work in A.M.'s lab is also supported by grant BB/J004561/1 from BBSRC (UK) and the John Innes Foundation. S.R.H. is supported by a CASE studentship from BBSRC and Redx.

Please cite this article in press as: Nagaraja, V. et al. DNA topoisomerase I and DNA gyrase as targets for TB therapy, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.11.006

References

1 Bush, N.G. *et al.* (2015) DNA topoisomerases. In *EcoSal – Escherichia coli and Salmonella: Cellular and Molecular Biology* (Böck, A. *et al.* eds), ASM Press
2 Tse-Dinh, Y.C. (1998) Bacterial and archeal type I topoisomerases. *Biochim. Biophys. Acta* 1400, 19–27
3 Vos, S.M. *et al.* (2011) All tangled up: how cells direct, manage and exploit topoisomerase function. *Nat. Rev. Mol. Cell Biol.* 12, 827–841
4 Wang, J.C. (2002) Cellular roles of DNA topoisomerases: a molecular perspective. *Nat. Rev. Mol. Cell Biol.* 3, 430–440
5 Champoux, J.J. (2001) DNA topoisomerases: structure, function, and mechanism. *Annu. Rev. Biochem.* 70, 369–413
6 Zechiedrich, E.L. and Cozzarelli, N.R. (1995) Roles of topoisomerase IV and DNA gyrase in DNA unlinking during replication in *Escherichia coli*. *Genes Dev.* 9, 2859–2869
7 Cole, S.T. *et al.* (1998) Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* 393, 537–544
8 Stupina, V.A. and Wang, J.C. (2005) Viability of *Escherichia coli* topA mutants lacking DNA topoisomerase I. *J. Biol. Chem.* 280, 355–360
9 Sassetti, C.M. *et al.* (2003) Genes required for mycobacterial growth defined by high density mutagenesis. *Mol. Microbiol.* 48, 77–84
10 Ahmed, W. *et al.* (2014) Conditional silencing of topoisomerase I gene of *Mycobacterium tuberculosis* validates its essentiality for cell survival. *FEMS Microbiol. Lett.* 353, 116–123
11 Bhaduri, T. *et al.* (1998) DNA topoisomerase I from *Mycobacterium smegmatis*. An enzyme with distinct features. *J. Biol. Chem.* 273, 13925–13932
12 Bhaduri, T. *et al.* (1998) Sequence specific interaction of *Mycobacterium smegmatis* topoisomerase I with duplex DNA. *Nucleic Acids Res.* 26, 1668–1674
13 Kumar, R. *et al.* (2012) Binding of two DNA molecules by type II topoisomerases for decatenation. *Nucleic Acids Res.* 40, 10904–10915
14 Manjunatha, U.H. *et al.* (2002) Functional characterisation of mycobacterial DNA gyrase: an efficient decatenase. *Nucleic Acids Res.* 30, 2144–2153
15 Sikder, D. and Nagaraja, V. (2001) A novel bipartite mode of binding of *M. smegmatis* topoisomerase I to its recognition sequence. *J. Mol. Biol.* 312, 347–357
16 Sikder, D. and Nagaraja, V. (2000) Determination of the recognition sequence of *Mycobacterium smegmatis* topoisomerase I on mycobacterial genomic sequences. *Nucleic Acids Res.* 28, 1830–1837
17 Aubry, A. *et al.* (2004) *Mycobacterium tuberculosis* DNA gyrase: interaction with quinolones and correlation with antimycobacterial drug activity. *Antimicrob. Agents Chemother.* 48, 1281–1288
18 Aubry, A. *et al.* (2006) First functional characterization of a singly expressed bacterial type II topoisomerase: the enzyme from *Mycobacterium tuberculosis*. *Biochem. Biophys. Res. Commun.* 348, 158–165
19 Pommier, Y. *et al.* (2010) DNA topoisomerases and their poisoning by anticancer and antibacterial drugs. *Chem. Biol.* 17, 421–433
20 Schoeffler, A.J. and Berger, J.M. (2008) DNA topoisomerases: harnessing and constraining energy to govern chromosome topology. *Q. Rev. Biophys.* 41, 41–101
21 Collin, F. *et al.* (2011) Exploiting bacterial DNA gyrase as a drug target: current state and perspectives. *Appl. Microbiol. Biotechnol.* 92, 479–497
22 Ravishankar, S. *et al.* (2015) Genetic and chemical validation identifies *Mycobacterium tuberculosis* topoisomerase I as an attractive anti-tubercular target. *Tuberculosis (Edinb)* 95, 589–598
23 Dexheimer, T.S. and Pommier, Y. (2008) DNA cleavage assay for the identification of topoisomerase I inhibitors. *Nat. Protoc.* 3, 1736–1750
24 Hsiang, Y.H. *et al.* (1985) Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J. Biol. Chem.* 260, 14873–14878
25 Chen, C.R. *et al.* (1996) DNA gyrase and topoisomerase IV on the bacterial chromosome: quinolone-induced DNA cleavage. *J. Mol. Biol.* 258, 627–637
26 Gellert, M. *et al.* (1977) Nalidixic acid resistance: a second genetic character involved in DNA gyrase activity. *Proc. Natl. Acad. Sci. U. S. A.* 74, 4772–4776
27 Godbole, A.A. *et al.* (2012) Characterization of DNA topoisomerase I from *Mycobacterium tuberculosis*: DNA cleavage and religation properties and inhibition of its activity. *Arch. Biochem. Biophys.* 528, 197–203
28 Manjunatha, U.H. *et al.* (2001) Monoclonal antibodies to mycobacterial DNA gyrase A inhibit DNA supercoiling activity. *Eur. J. Biochem.* 268, 2038–2046
29 Leelaram, M.N. *et al.* (2013) Type IA topoisomerase inhibition by clamp closure. *FASEB J.* 27, 3030–3038
30 Leelaram, M.N. *et al.* (2012) Inhibition of type IA topoisomerase by a monoclonal antibody through perturbation of DNA cleavage-religation equilibrium. *FEBS J.* 279, 55–65
31 Cheng, B. *et al.* (2007) Compounds with antibacterial activity that enhance DNA cleavage by bacterial DNA topoisomerase I. *J. Antimicrob. Chemother.* 59, 640–645
32 Garcia, M.T. *et al.* (2011) New alkaloid antibiotics that target the DNA topoisomerase I of *Streptococcus pneumoniae*. *J. Biol. Chem.* 286, 6402–6413
33 Bansal, S. *et al.* (2012) 3,4-Dimethoxyphenyl bis-benzimidazole, a novel DNA topoisomerase inhibitor that preferentially targets *Escherichia coli* topoisomerase I. *J. Antimicrob. Chemother.* 67, 2882–2891
34 Ranjan, N. *et al.* (2014) Selective inhibition of bacterial topoisomerase I by alkynyl-bisbenzimidazoles. *MedChemComm* 5, 816–825
35 Nimesh, H. *et al.* (2014) Synthesis and biological evaluation of novel bisbenzimidazoles as *Escherichia coli* topoisomerase IA inhibitors and potential antibacterial agents. *J. Med. Chem.* 57, 5238–5257
36 Cheng, B. *et al.* (2013) Identification of anziaic acid, a lichen depside from *Hypotrachyna* sp., as a new topoisomerase poison inhibitor. *PLOS ONE* 8, e60770
37 Duan, F. *et al.* (2014) Haloemodin as novel antibacterial agent inhibiting DNA gyrase and bacterial topoisomerase I. *J. Med. Chem.* 57, 3707–3714
38 Godbole, A.A. *et al.* (2014) Inhibition of *Mycobacterium tuberculosis* topoisomerase I by m-AMSA, a eukaryotic type II topoisomerase poison. *Biochem. Biophys. Res. Commun.* 446, 916–920
39 Glowinski, J. and Axelrod, J. (1964) Inhibition of uptake of tritiated-noradrenaline in the intact rat brain by imipramine and structurally related compounds. *Nature* 204, 1318–1319
40 Kelly, M.W. and Myers, C.W. (1990) Clomipramine: a tricyclic antidepressant effective in obsessive compulsive disorder. *DICP* 24, 739–744
41 Godbole, A.A. *et al.* (2015) Targeting *Mycobacterium tuberculosis* topoisomerase I by small-molecule inhibitors. *Antimicrob. Agents Chemother.* 59, 1549–1557
42 Sandhaus, S. *et al.* (2016) Small molecule inhibitors targeting topoisomerase I as novel antituberculosis agentS. *Antimicrob. Agents Chemother.* 60, 4028–4036
43 Tan, K. *et al.* (2016) Insights from the structure of *Mycobacterium tuberculosis* topoisomerase I with a novel protein fold. *J. Mol. Biol.* 428, 182–193
44 Agrawal, A. *et al.* (2013) *Mycobacterium tuberculosis* DNA gyrase ATPase domain structures suggest a dissociative mechanism that explains how ATP hydrolysis is coupled to domain motion. *Biochem. J.* 2, 263–273
45 Blower, T.R. *et al.* (2016) Crystal structure and stability of gyrase-fluoroquinolone cleaved complexes from *Mycobacterium* tuberculosis. *Proc. Natl. Acad. Sci. U. S. A.* 113, 1706–1713
46 Fu, G. *et al.* (2009) Crystal structure of DNA gyrase B' domain sheds lights on the mechanism for T-segment navigation. *Nucleic Acids Res.* 37, 5908–5916
47 Piton, J. *et al.* (2010) Structural insights into the quinolone resistance mechanism of *Mycobacterium tuberculosis* DNA gyrase. *PLoS ONE* 5, e12245
48 Tretter, E.M. *et al.* (2010) Crystal structure of the DNA gyrase GyrA N-terminal domain from *Mycobacterium tuberculosis*. *Proteins* 78, 492–495
49 Takiff, H. and Guerrero, E. (2011) Current prospects for the fluoroquinolones as first-line tuberculosis therapy. *Antimicrob. Agents Chemother.* 55, 5421–5429
50 Asif, M. *et al.* (2013) Quinolone derivatives as antitubercular drugs. *Med. Chem. Res.* 22, 1029–1042
51 Cheepsattayakorn, A. and Cheepsattayakorn, R. (2008) Prospects for new drugs and regimens in the treatment of tuberculosis. *J. R. Coll. Physicians (Edinb)* 38, 207–211
52 Gillespie, S.H. (2016) The role of moxifloxacin in tuberculosis therapy. *Eur. Respir. Rev.* 25, 19–28
53 Gillespie, S.H. *et al.* (2014) Four-month moxifloxacin-based regimens for drug-sensitive tuberculosis. *N. Engl. J. Med.* 371, 1577–1587
54 Laponogov, I. *et al.* (2009) Structural insight into the quinolone-DNA cleavage complex of type IIA topoisomerases. *Nat. Struct. Mol. Biol.* 16, 667–669
55 Bax, B.D. *et al.* (2010) Type IIA topoisomerase inhibition by a new class of antibacterial agents. *Nature* 466, 935–940
56 Wohlkonig, A. *et al.* (2010) Structural basis of quinolone inhibition of type IIA topoisomerases and target-mediated resistance. *Nat. Struct. Mol. Biol.* 17, 1152–1153
57 Lall, N. and Meyer, J.J.M. (1999) *In vitro* inhibition of drug-resistant and drug-sensitive strains of *Mycobacterium tuberculosis* by ethnobotanically selected South African plants. *J. Ethnopharmacol.* 66, 347–354
58 Lall, N. and Meyer, J.J.M. (2001) Inhibition of drug-sensitive and drug-resistant strains of *Mycobacterium tuberculosis* by diospyrin, isolated from *Euclea natalensis*. *J. Ethnopharmacol.* 78, 213–216
59 Karkare, S. *et al.* (2013) The naphthoquinone diospyrin is an inhibitor of DNA gyrase with a novel mechanism of action. *J. Biol. Chem.* 288, 5149–5156
60 Djaout, K. *et al.* (2016) Predictive modeling targets thymidylate synthase ThyX in *Mycobacterium tuberculosis*. *Sci. Rep.* 6, 27792
61 Lewis, R.J. *et al.* (1996) The nature of inhibition of DNA gyrase by the coumarins and the cyclothialidines revealed by X-ray crystallography. *EMBO J.* 15, 1412–1420

Please cite this article in press as: Nagaraja, V. et al. DNA topoisomerase I and DNA gyrase as targets for TB therapy, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.11.006

62 Wigley, D.B. *et al.* (1991) Crystal structure of an N-terminal fragment of the DNA gyrase B protein. *Nature* 351, 624–629
63 Jeankumar, V.U. *et al.* (2016) Structure-guided discovery of antitubercular agents that target the gyrase ATPase domain. *ChemMedChem* 11, 539–548
64 Saxena, S. *et al.* (2014) Mycobacterial DNA gyrB inhibitors: ligand based pharmacophore modelling and *in vitro* enzyme inhibition studies. *Curr. Top. Med. Chem.* 14, 1990–2005
65 Saxena, S. *et al.* (2015) Development of 2-amino-5-phenylthiophene-3-carboxamide derivatives as novel inhibitors of *Mycobacterium tuberculosis* DNA GyrB domain. *Bioorg. Med. Chem.* 23, 1402–1412
66 Medapi, B. *et al.* (2015) Design and synthesis of novel quinoline-aminopiperidine hybrid analogues as *Mycobacterium tuberculosis* DNA gyraseB inhibitors. *Bioorg. Med. Chem.* 23, 2062–2078
67 Hameed, S.P. *et al.* (2014) Optimization of pyrrolamides as mycobacterial GyrB ATPase inhibitors: structure-activity relationship and in vivo efficacy in a mouse model of tuberculosis. *Antimicrob. Agents Chemother.* 58, 61–70
68 Renuka, J. *et al.* (2014) Design, synthesis, biological evaluation of substituted benzofurans as DNA gyraseB inhibitors of *Mycobacterium tuberculosis*. *Bioorg. Med. Chem.* 22, 4924–4934
69 Shirude, P.S. *et al.* (2013) Aminopyrazinamides: novel and specific GyrB inhibitors that kill replicating and nonreplicating *Mycobacterium tuberculosis*. *ACS Chem. Biol.* 8, 519–523
70 Kale, M.G. *et al.* (2013) Thiazolopyridine ureas as novel antitubercular agents acting through inhibition of DNA gyrase B. *J. Med. Chem.* 56, 8834–8848
71 Kale, R.R. *et al.* (2014) Thiazolopyridone ureas as DNA gyrase B inhibitors: optimization of antitubercular activity and efficacy. *Bioorg. Med. Chem. Lett.* 24, 870–879
72 Blanco, D. *et al.* (2015) *Mycobacterium tuberculosis* gyrase inhibitors as a new class of antitubercular drugs. *Antimicrob. Agents Chemother.* 59, 1868–1875
73 Costenaro, L. *et al.* (2007) Modular structure of the full-length DNA gyrase B subunit revealed by small-angle X-ray scattering. *Structure* 15, 329–339

Please cite this article in press as: Nagaraja, V. et al. DNA topoisomerase I and DNA gyrase as targets for TB therapy, Drug Discov Today (2016), http://dx.doi.org/10.1016/j.drudis.2016.11.006