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Comprehensive review of DNA gyrase as enzymatic target for drug discovery and development

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ABSTRACT

DNA gyrase is a member of the DNA topoisomerase protein family that catalyzes the conversion of different topological forms of DNA into one another. It is the sole enzyme that causes DNA to negatively supercoil. The enzyme is tetrameric with two GyrA ("A") and two GyrB ("B") subunits. DNA gyrase is an ideal target for medication because of its basic properties in bacterial cells and the lack of gyrase activity in eukaryotes. Antibacterial medications, including quinolones and derivatives based on coumarins that specifically target DNA gyrase, underscore the significance of the enzyme in the fight against bacterial infections. In addition to the typical antibiotic-binding sites, including novobiocin and fluoroquinolones, several other areas are being used in drug discovery. Simocyclinone, thiophene, gepotidacin, halogen atoms in the para position of the phenyl right-hand side (RHS) moiety, and coupled cell division B (CcdB) are examples of novel bacterial type II topoisomerase inhibitors (NBTIs). These binding sites are structurally and chemically active and inhibit the supercoiling activity of topoisomerase. This article provides an overview of DNA gyrase inhibition using synthetic and natural precursors aimed at medication development and discovery.

1. Introduction

Remarkably, the bacterial enzyme DNA gyrase can catalyze the formation of negative super-helical twists into closed-circuit double-stranded DNA, as first mentioned in 1976. Gellert et al. [1] initial finding was that DNA gyrase was used to identify the *E. coli* host components required for bacteriophage X site-specific integration. DNA gyrase, an ATP-dependent enzyme, can add negative supercoils to closed-circular duplex DNA gyrase, an ATP-dependent enzyme [1]. The unusual potential to quicken interconversions between multiple topological forms of DNA is exhibited by a class of proteins called DNA topoisomerases, which also include DNA gyrase [2]. DNA supercoiling must exist for chromosome segregation, recombination in bacteriophages and eukaryotic viruses such as HIV, and transcription in prokaryotes [3] and eukaryotes. Since these enzymes are crucial for activity, pharmacological inhibition of many enzymes that influence bacterial DNA supercoiling is an efficient antibacterial approach [4,5].

Topoisomerases regulate the topographical state of DNA, which is constantly altered by biological processes, such as transcription, recombination, and replication. Eubacterial DNA gyrases are members of the type II subfamily, which also contain DNA topoisomerases. One characteristic of the type II subfamily of topoisomerases is that they cause a double-stranded break in the DNA [6]. It has been proposed that a protein from the posterior silk gland of Bombyx mori complements

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eukaryotic topoisomerase II, causing supercoiling activity. Prior to the supercoiling event, this component must be present over the DNA in a considerable molar abundance. One theory implies that this factor regulates the manner in which DNA is twisted around topoisomerase II, although it is unclear how it supercoils DNA. Rather than active supercoiling of DNA gyrase, these activities in eukaryotic cells are most likely instances of "passive" supercoiling [7]. Several gyrase-specific antibacterial drugs have been identified. Most chemicals can be categorized into two groups: quinolones and coumarins, whereas certain substances do not fall into either of these categories [8].

2. DNA topoisomerase

The enzyme class known as DNA topoisomerases can change the structural composition of closed-circular DNA molecules. Both in vitro and in vivo, these enzymes can catalyze changes in the tertiary structure of DNA [9]. They can be extracted from prokaryotic, eukaryotic, or viral sources. Prokaryotic topoisomerase I unwinds negatively supercoiled closed-circular DNA and was the first topoisomerase activity described [10]. Prokaryotic topoisomerase II is the only topoisomerase that has been shown to be capable of inserting negative supercoils into DNA, commonly known as DNA gyrase [11]. A "reverse gyrase" found in the thermophilic bacteria *Sulfolobus* generates positive super helical twists to DNA under the influence of ATP [12]. According to a few recent publications, prokaryotic DNA topoisomerase II may be involved in the unwinding of supercoils during the transcription of the DNA template. Chicken topoisomerase II immunofluorescence studies have revealed that this enzyme is a component of mitotic chromosomal scaffolding. According to recent research, topoisomerase II is necessary for chromosome condensation, and its function is most likely structural [13]. Examples of representative enzymes are listed in Table 1, which provides a succinct summary of these two categories.

3. DNA supercoiling

The DNA-induced negative supercoiling of bacterial DNA Gyrase is necessary for replication and affects all DNA-related metabolic processes. Gyrase supercoils DNA using a process known as sign inversion, in which a DNA segment experiences a brief double-strand break to convert a positive supercoil into a negative one [14]. Cairns initially recognized a possible topological issue inherent to circular double-stranded DNA molecules in 1963 [15]. Several microorganisms, such as most bacteria, microbial plasmids, and organelles including mitochondria and chloroplasts, have circular genomes. Early gyrase models proposed the addition of negative supercoils in two stages. A DNA molecule first separates its positively and negatively supercoiled sections, and then the positively supercoiled portions are selectively relaxed, leaving only the negatively supercoiled regions [16]. The crucial finding regarding supercoil separation is that gyrase binding alone produces positive supercoils, mostly in the absence of ATP, which are located close to the enzyme because they are resistant to relaxing enzymes. Because the number of connections must remain constant, the other parts of the molecule compensate for the negative supercoils. In addition to causing a specific supercoiling stoichiometric well with the quantity of gyrase molecules, the addition of (AppNHp)comprises a stable, non-hydrolyzablephosphodiester bond formed by adenosine monophosphate (AMP) and pyrophosphate (PPP). β, γ-Non--hydrolyzable Adenosine Nucleotides (NHPN) also result in a corresponding decrease in protected positive supercoils [17]. Two antiparallel strands of DNA wound around each other to form a well-known double-helical pattern [18]. The classic topoisomerase paradigm of cutting one strand, rotating well about the helix axis to release positive tension, and resealing the nick to complete the next step in supercoiling has typically been used to describe this process.

Table 1
Overview of DNA topoisomerases.

Type	Description	Examples
Type I	Allows DNA strands to rotate to reduce supercoiling by introducing transient single-strand breaks.	Topoisomerase I
Type II	Causes transient double-strand breaks and is important in chromosomal segregation and DNA replication.	DNA Gyrase, Topoisomerase IV (prokaryotic), Topoisomerase II (eukaryotic)

Vinograd et al. [19] discovered that DNA secondary structure and superhelicity are tightly coupled, leading to the first understanding of the biological importance of DNA superhelicity. and a superhelical structure. As a result, DNA superhelicity affects the ability of the DNA duplex to unwind and rewind and vice versa. Owing to its direct linkage, it provides a unique opportunity. Molecular biology provides the opportunity to develop studies to gain knowledge of DNA structure and investigate the workings of enzymes that modify DNA structure [20]. *Salmonella enterica, Serovar typhimurium (S. typhimurium)* supercoil their DNA less than *E. coli*. This is surprising given the close relationship between these two bacterial species and the high degree of amino acid identity between their topA (topoisomerase I), GyrA, and GyrB proteins, which constitute the two DNA gyrase subunits, with scores of 91 % and 97 %, respectively [21].

4. DNA gyrase structure

DNA gyrase is a heterotetramer composed of two gyrA and two gyrB subunits, organized in a two-fold symmetric three-domain structure. ATP-binding and hydrolysis sites of DNA gyrase are found in the B subunit, whereas the A subunit is responsible for binding and wrapping DNA. Active-site tyrosine residues are also present in the A subunits and are situated in a catabolite activator protein (CAP)-like domain within a helix turn helix (HTH) motif. Gyrase B has a TOPRIM domain, which is thought to be important for catalysis because it provides divalent cations required for DNA breakage and religious binding. The 5′ end of DNA is covalently connected to the enzyme via the active site tyrosine, creating a phosphodiester bond. Topoisomerase IV is a type II topoisomerase that exhibits structural and functional characteristics similar to DNA gyrase. These two subunits comprise the heterotetrameric structure of DNA gyrase, which is classified as A2B2. The DNA molecule undergoes double-strand breaks caused by the GyrA subunits, which then rejoin both strands when torsional tension caused by supercoiling is released [24]. GyrB has an ATP-binding site that allows energy-dependent ATP hydrolysis to power enzyme functions. It has been discovered that two different proteins expressed by genes that have already been connected to genetic loci make up gyrase that controls nalidixic acid or coumermycin resistance [22].

Sequencing of *E. coli*gyrA and gyrB revealed that they created 874 (M, 97,000) and 804 (M, 90,000) amino acids, respectively. Genes that have been previously described as genomic areas dictating either nalidixic acid tolerance or coumermycin tolerance encode one of two proteins that make up DNA Gyrase (nalA and cou). GyrA and GyrB are the proposed identities for these genes (molecular masses of 96 and 88 kDa, respectively) and have been grouped as A2B2 tetramers since the discovery of gyrase [23,24].

The enzyme DNA Gyrases comprises of GyrA and GyrB subunit are complex in nature. The structure of the enzyme comprises "T" & "G" element which helps in holding the supercoiled DNA molecule. The G element refers to Gate element which holds the Gyr A C-Terminal Domains and T element refers to the Transfer element which bounds with the Gyr B domain. The enzyme requires two ATP molecules to perform the supercoiling function of DNA molecules. The break in the DNA molecule will occur at the G segment and the amino acid tyrosine which is present in the active site of the GyrA mediates and creates the DNA-Protein intermediate [25]. Fig. 1 depicts the conformational changes of the enzyme occurs by hydrolysing the ATP molecule which helps the T

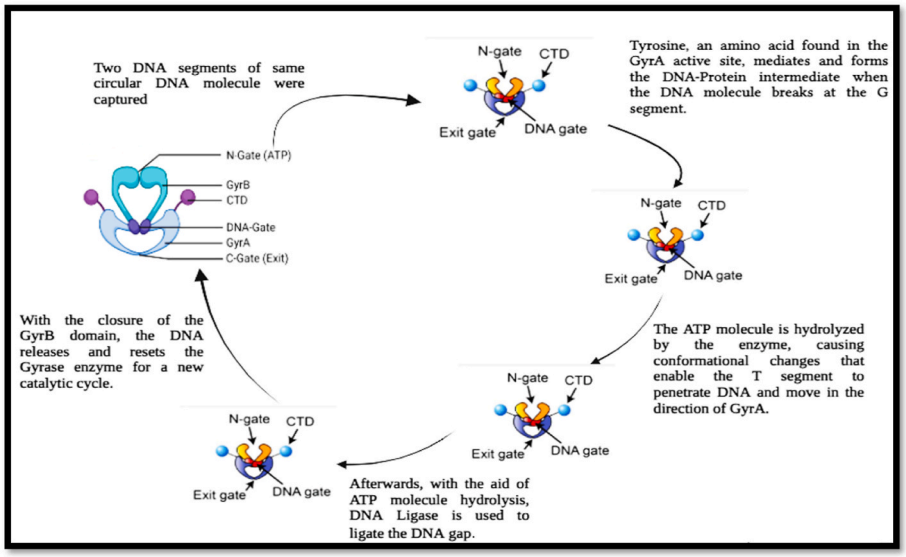

Fig. 1. Illustration of DNA gyrase's catalytic mechanism.

segment to break the DNA and makes it entry towards GyrA. Later the DNA gap is ligated using DNA Ligase with the help of ATP molecule hydrolysis. The DNA releases and resetting the Gyrase enzyme for new catalytic cycle with the reopening of the GyrB domain.

5. Functions of DNA gyrase

DNA gyrase as mentioned in Table 2, a member of the topoisomerase family, catalyzes negative supercoiling of DNA [24]. Although *Xenopus oocytes* exhibit negative supercoiling behavior, these claims are not supported by additional data. Mg2+, adenosine triphosphate (ATP), and spermidine, the most common polyamines in bacteria, are required by pure DNA gyrase from *Escherichia coli* to create negative supercoiled DNA. Although strand cleavage and strand transit require Mg^{2+} and ATP, respectively, the precise function of spermidine remains unclear [26].

At 30 °C, one molecule caused approximately 100 supertwists per minute. Negatively supercoiled DNA can be positively supercoiled or relaxed. These enzymes are likely similar to gyrase in their method of action, and all topoisomerase processes necessitate the interaction of the protein with DNA, DNA fragmentation, strand passing, DNA reunion, and, in some cases, ATP breakdown. The ability of DNA gyrase to actively supercoil DNA requires specific mechanistic properties in addition to conforming to the generic topoisomerase mechanism. The following is a list of documented DNA gyrase reactions: closed-circular double-stranded DNA negative supercoiling under the control of ATP, Relaxation of negatively supercoiled DNA without ATP, positively supercoiled DNA relaxation in a nucleotide-dependent manner, Catenated DNA construction and resolution, untangling of DNA strands, DNA double-strand breakage brought on by quinolone or calcium ions, and ATP hydrolysis, which is DNA-dependent.

Table 2
Functions of DNA gyrase.

FUNCTION	DESCRIPTION
Negative Supercoiling	Causes negative supercoils to be introduced into DNA, which affects gene expression and other cellular processes.
ATP-Dependent Processes	Depends on ATP binding in the GyrB subunit to provide the energy needed to break and rejoin strands.
Maintenance of DNA Super helicity	Essential for optimum DNA super helicity, guaranteeing that the transcription and replication processes run smoothly

6. Active sites of DNA gyrase for inhibition

Various bacterial species, including *Escherichia coli, Micrococcus luteus, Mycobacteria* sp., *Pseudomonas aeruginosa,* and *Staphylococcus aureus*, have been shown to be inhibited by 4-quinolone anti-bacterial, but with varying degrees of efficacy [27,28]. Potency is best determined by the balance between an agent's permeability through the bacterial cell wall and/or membrane(s) and its activities at the enzymatic level [29]. Activity is based on the discovery that 4-quinolone anti-bacterial targets the Gyr A subunit of the DNA gyrase holoenzyme, limiting supercoiling and promoting the "cleavable complex" by impairing DNA gyrase holoenzyme-mediated sequence-specific 4-base pair staggered cuts on the DNA duplex [30].

Any action that disrupts DNA-binding gyrase or catalytic activity in a non-mechanism-based manner may stop the development of "cleavable complexes." Flavones are novel DNA gyrase inhibitors that directly target the GyrA subunit. Unlabelled 4-quinolone compounds compete for [^{3}H] norfloxacin binding in a concentration- and potency-dependent manner. The new flavone inhibitors also engaged in concentration- and potency-dependent competition for [^{3}H] norfloxacin binding [30]. Interestingly, the results obtained from investigations with the novel DNA gyrase inhibitor flavones and DNA gyrase [^{3}H] norfloxacin were different from those of known 4-quinolones, indicating separate and/or more complicated binding of these novel DNA gyrase inhibitors. Compared to 4-quinolones, the new DNA gyrase inhibitors showed substantially less competition for [^{3}H] norfloxacin binding, indicating a distinct location for binding to the DNA gyrase complex [31,32].

7. DNA gyrase inhibitors

DNA gyrase inhibits DNA-enzyme interactions and prevents DNA strands from ligation. Alterations in DNA can lead to cell death. Nalidixic acid was identified to have such a mechanism of action, and the fluoroquinolone family of antibiotics was created using it as a reference for optimization [33]. Many gyrA inhibitors, including Ciprofloxacin, Gemifloxacin, Levofloxacin, Moxifloxacin, and Ofloxacin, have been used in the clinical setting. Unfortunately, this situation is complicated with the use of GyrB inhibitors.

The unique GyrB inhibitor to date, Novobiocin belongs to the class of aminocoumarins and belongs to that group. Extensive studies on antibacterial chemicals were conducted in the 1950s through microbial screening of natural materials. Soon after, its analogs clorobiocin and

coumermycin A1 were discovered. Penicillin was diminishing its power to combat the principal infections at the time, namely *Staphylococcus aureus*, and effective substitutes were needed. Novobiocin was promoted as AlbamycinR and used to manage infections caused by penicillin-resistant *S. aureus* owing to its mostly gram-positive range of action [34].

Although human cells do not contain DNA gyrase, they continue to be important pharmacological targets, because they are crucial for bacteria. Because its ATP-binding site is largely conserved, there is little chance that it will spontaneously evolve resistance to ATP-competitive inhibitors. This serves as the motivation for the current intensity of studies on ATP-competitive GyrB inhibitors. The 43-kDa N-terminal GyrB domain of DNA gyrase complexed with 5-adenylyl—imidodiphosphate, a nonhydrolyzableanalog of ATP, was solved to reveal the structural and mechanistic characteristics of the enzyme. The microstructures of the interactions between the 24-kDa N-terminal domain of *E. coli* GyrB and novobiocin, 5-adenylyl—-imidodiphosphate, and GR122222X were used to characterize the binding mechanisms of aminocoumarins (a representative of the cyclothialidine family) [35]. Similar investigations revealed that these binding spaces in the structures partially overlap, which accounts for their ATP-competitive inhibition despite having diverse structural makeups. Cyclothialidines, which are also synthesized by *Streptomyces* sp., are a different class of natural compounds with gyrase-inhibitory effects. Amycolamicin and kibdelomycin are two novel natural topoisomerase inhibitors that were discovered in 2010 and 2011. Amycolamicin was discovered in an actinomycete found in the *Amycolatopsis*sp. It has strong antibacterial properties that can kill both Gram-positive and Gram-negative bacteria. Kibdelomycin, a broad-spectrum gram-positive antibacterial agent, has been isolated from bacteria belonging to the genus *Kibdelosporangium*.

Chatterji et al. [36] investigated the cyclothialidines Ro 09–1437 and Ro 48–2865 were investigated for their ability to inhibit enzyme activity. When compared to *E. coli*gyrase, neither cyclothialidineanalog was active against *M. smegmatis* gyrase (Table 3). In contrast, mycobacterial gyrases showed no differential vulnerabilities. Novobiocin can inhibit mycobacterial gyrase at concentrations greater than 0.0625 mg/L [36]. Experiments were also conducted to determine the inhibitory activities of ciprofloxacin, etoposide, and CcdB on the gyrases of *E. coli* and *M. Smegmatis* (Table 3). Testing the impact of ciprofloxacin on the supercoiling response revealed that 1.3 M of the medication was necessary to achieve detectable inhibition (MIC), which is consistent with previous studies [30]. However, at concentrations (CC2) as low as 0.17 M, cleavage was doubled (Table 4). It should be noted that in both supercoiling and cleavage experiments, *M. smegmatis* gyrase was less sensitive than *E. coli* gyrase. This was most likely caused by several changes in the quinolone resistance-determining regions (QRDRs) of the *M. smegmatis* enzymes GyrA and GyrB, including S83A in GyrA (an amino acid position based on *E. coli*) and Y447R and S464N in GyrB [34]. This theory is supported by evidence that these amino acid changes in *E. coli* cause the affinity of the enzyme for quinolones and fluoroquinolones to decrease, and topoisomerase IV homologues have not been found in the genomes of *M. tuberculosis* or *Mycobacterium leprae*, suggesting that DNA gyrase is the exclusive aim of the inhibitor

Table 3
Inhibitory activities of novobiocin and cyclothialidines on the gyrases of *E. coli* and *M. smegmatis* [31].

Inhibitors	MNEC (mg/L)		IC_{100} (mg/L)	
	Gyrase of*E. coli*	Gyrase of *M. smegmatis* gyrase	Gyrase of *E. coli*	Gyrase of *M. smegmatis* gyrase
Ro 09-1437	0.0125 ± 0.0011	0.025 ± 0.002	0.4 ± 0.02	0.4 ± 0.03
Ro 48-2865	0.00156 ± 0.00012	0.0125 ± 0.0008	0.05 ± 0.003	0.2 ± 0.02
Novobiocin	0.125 ± 0.006	0.0625 ± 0.0034	1.0 ± 0.012	0.5 ± 0.04

Table 4
Inhibitory activities of ciprofloxacin, etoposide and CcdB on the gyrases of *E. coli* and *M. smegmatis* [30].

Inhibitors	CC_2 (μm)		Maximum cleavage	
	Gyrase of *E. coli*	Gyrase of *M. smegmatis* gyrase	Gyrase of *E. coli*	Gyrase of *M. smegmatis* gyrase
Ciprofloxacin	0.015 ± 0.001	0.17 ± 0.02	NS	NS
Etoposide	11.58 ± 0.62	34.59 ± 1.91	4.0 ± 0.3	13.3 ± 0.6
CcdB	0.483 ± 0.050	NDC	15.6 ± 1.1	NDC

quinolone class in these microbes [36]. Etoposide is effective against gyrase in *M. smegmatis* and *E. coli*. *M. smegmatis* DNA gyrase exhibited greater cleavage when exposed to etoposide at saturating doses while having a decreased affinity for the drug. Therefore, it does not seem that *M. smegmatis* fluoroquinolone resistance in *M. smegmatis* directly correlates with a reduction in etoposide sensitivity. These findings show that etoposide and fluoroquinolones interact differently with *M. smegmatis* DNA gyrase than with *S. aureus* [36].

Recently, many new overviews of GyrB inhibitors developed using synthetic methods have been published. They include Benzimidazoles, Pyrrolamides, Pyrrolopyrimidines, Pyridylureas, and Pyrazolopyridones. As shown, all these chemical classes can link with Asp73 of *E. coli*GyrB to form an H-bond donor-acceptor network. They also contain an aromatic component that can interact with arginine residues via π-cation interactions [37].

8. Natural precursors against DNA gyrase

Quinolones are the most effective antibacterial drugs that target DNA gyrase. Extensive research has been conducted to enhance the potency, activity spectrum, and ability of these chemicals to withstand microorganisms. New chemical entities and scaffolds that counteract the emergence of resistance in the field of microbial diseases have been discovered by screening chemical libraries. The possible plant-based compounds like alkaloids, flavonoids, terpenoids, and glycosides were thought to have a high potential for targeting against multidrug resistance bacteria *Staphylococcus aureus* [38].

8.1. Quinolones as a potential inhibitor

Over 5000 antibacterial substances based on the 4-oxo-1dihydroquinoline skeleton are currently known as “4-quinolones” or simply “quinolones.” It is important to remember that quinolone medications are synthetic rather than natural compounds. There are currently many quinolones, especially those of the fluoroquinolone class, with antibacterial activity up to ten times greater than that of nalidixic acid. Many germs that are resistant to the effects of earlier quinolones are now treatable owing to the development of new medications [39].

In contrast to other classes of antibiotics, quinolones do not appear to be susceptible to plasmid-mediated transferable resistance. When resistance occurs, the GyrA gene, the structural gene for the DNA gyrase A protein, is frequently found to have undergone chromosomal mutations. Several quinolone-resistant mutations in the *E. coli* GyrA gene have been discovered at the DNA sequence level. The gyrase A protein contains all of them within a tiny region known as the “quinolone resistance-determining region,” which is situated within amino acids 67 and 106 of a protein [40].

8.2. Coumarins as an effective inhibitor of DNA gyrase

Drugs known as Coumarins are antibacterial agents that were first identified in *Streptomyces* sp. Coumarins have been shown to be more

effective against gram-positive bacteria than gram-negative bacteria. The synthesis of bacterial DNA, RNA, and proteins has been shown to be inhibited by coumarins, with the synthesis of DNA as the main target. Early research identified coumermycin and chlorobiocin resistance in *E. coli* near the DNA gene [41]. This study offers a strong basis for the design and synthesis of coumarin-based DNA gyrase inhibitors, which may be useful for studying antibacterial resistance.

8.3. Flavonoids as an inhibitor

Flavonoids are promising precursors of DNA gyrases. In *E. coli* DNA activity is suppressed by flavonoid supercoiling. The minimum inhibitory concentrations (MIC) of kaempferol and nobiletin were 25 and 177 μg/mL, respectively. Methoxyl compounds exhibited minimal suppression activity compared to hydroxyl group flavonoids [42]. Quercetin is a flavonoid that has antioxidant properties. A previous study showed that the association of DNA and topoisomerase II leads to the cleavage of DNA when flavonoids bind [43].

8.4. Polyphenols inhibiting DNA gyrase

Catechins are tannins with various biological functions. A previous study showed that catechin retards DNA gyrase by binding to the gyrB subunit. The most active catechin from the list evaluated was epigallocatechin gallate (EGCG). Molecular docking studies revealed that the maximum activity of EGCG was due to the deep penetration of its benzopyran ring into the active site of DNA gyrase [44]. Alfonso et al. [45] continued their study to demonstrate that digallic acid is an inhibitor of DNA gyrase. These studies demonstrate that polyphenol precursors may be used as effective DNA gyrase inhibitors.

9. Synthetic precursors against DNA gyrase

In recent years, numerous novel structural families of GyrB inhibitors, including benzimidazoles, pyrrolamides, pyrrolopyrimidines, pyridylureas, and pyrazolopyridones, have been synthesized. When combined with Asp73 of *E. coli*GyrB, these chemicals can create an H-bond donor-acceptor network.

9.1. Pyrrolamides

Because DNA gyrase is a crucial enzyme for bacterial DNA synthesis, its inhibition causes cell death by interfering with DNA synthesis. Pyrrolamides represent a new class of antibacterial compounds that specifically target DNA gyrase [46]. Some carboxamides have been shown to have enhanced antibacterial abilities, possibly due to greater cell wall penetration. A few of the substances displayed IC50 <1 μM. Both *E. coli* and *S. aureus* strains of *S. aureus* gyrase have been tested with a few strong chemicals; however, they showed less activity than *E. coli* GyrB [47].

The inhibitory action of GyrB was tested on a vast group of substances inspired by the organic marine molecule oroidin, and the highest hit for the inhibitory activity against *E. coli* was the cyclized derivative (IC50, 12 μm) [48]. The pyrrolamide series binds to the ATP pocket of gyrB and inhibits its activity. A first-generation lead compound of the pyrrolamide series as shown in Fig. 2a, N-bromosuccinimide, has poor antibacterial activity. The following molecule in the pyrrolamide series as shown in Fig. 2b, compound 3-fluoropiperidines, showed invivo effectiveness against *S. pneumoniae* in a mouse pneumonia model and potential antibacterial activity [49].

Pyrroles are frequently present in GyrB inhibitors, as the 5-methylpyrrole moiety is present in chlorobiocin, the 5-methyl-3,4-dichloropyrrole moiety is present in amycolamicin and kibdelomycin, and a series of synthetic 5-methyl-3,4-dichloropyrrole derivatives from Astra Zeneca have demonstrated GyrB inhibition [50,51]. These substances had only weak antibacterial effects against gram-positive bacterial strains, and

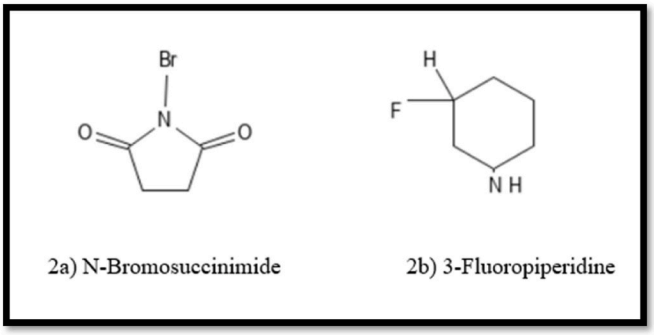

Fig. 2. 2a) Structure of compound N-bromosuccinimide and 2 b) Structure of compound 3-fluoropiperidine.

tests on wild-type, impA, and tolC*E. coli* strains indicated that they were efflux pump substrates lacking the physicochemical qualities required for cell wall penetration [52]. Some carboxamides have been shown to have enhanced antibacterial abilities, possibly due to greater cell wall penetration. A few of the substances displayed IC50 <1 μM. Both *E. coli* and *S. aureus* strains of *S. aureus* gyrase were tested with a few strong chemicals; however, they showed less activity than *E. coli* GyrB [52].

9.2. Azole based compounds

S. aureus MIC tests with 50 % human serum were performed to assess the relative serum-binding potential. When tested on all examined species, including *S. aureus, S. pneumoniae, H. influenzae*. Benzimidazole carbamate is a micromolar GyrB inhibitor with MIC values greater than 16 g/mL [53]. Two different types of structural modifications were instantly proposed by docking 1 into the *S. aureus* X-ray structure after withdrawal of novobiocin [54].

The first design, Asp-73, Thr-165 (*E. coli* gyrase numbering), and a structural water that is strongly preserved seemed to form a vast hydrogen bond network that could be furthered by changing the carbamate oxygen of benzimidazole carbamate to nitrogen (urea). It is believed that the scaffold of the benzimidazole component also contributes to the hydrogen bond network, which would further open a path for optimization of highly effective, low-MW inhibitors. The second design principle that emerged from this preliminary analysis suggested that a heterocyclic hydrogen bond acceptor affixed directly to the benzimidazole urea core might replicate the interaction of hydrogen bonds between Arg136 and the exocyclic carbonyl oxygen of the novobiocin coumarin ring [55].

Pharmacia and Upjohn found that 2′,4′-dimethyl-8′-nitrospiro [1,3-diazinane-5,5′-2,4,4a,6-tetrahydro-1H-[1,4] oxazino [4,3-a]quinoline]-2,4,6-trione (Fig. 3) has a DNA gyrase inhibitory effect using high-throughput screening [55]. Zoliflodacin (Fig. 4), which underwent additional structural alteration, had an oxazolidinone substituent on the benzisoxazole ring, entirely negating prior cytotoxic effects and enhancing antibacterial activity. In addition, a phase II clinical investigation of zoliflodacin as an anti-gonorrhea treatment was completed. However, the relatively low antibacterial activity and suboptimal PK characteristics of the molecule indicate that substantial dosages are necessary to achieve effectiveness, which leads to idiosyncratic drug toxicity [56,57]. The scientists found that altering the spiropyrimidinetrione or benzisoxazole scaffolds eliminated their antibacterial activity during SARs experiments. At the benzisoxazole 3-position, a few substituents were tolerated; however, replacing the fluoro group with a cyano group had a negative impact on bactericidal activity [56].

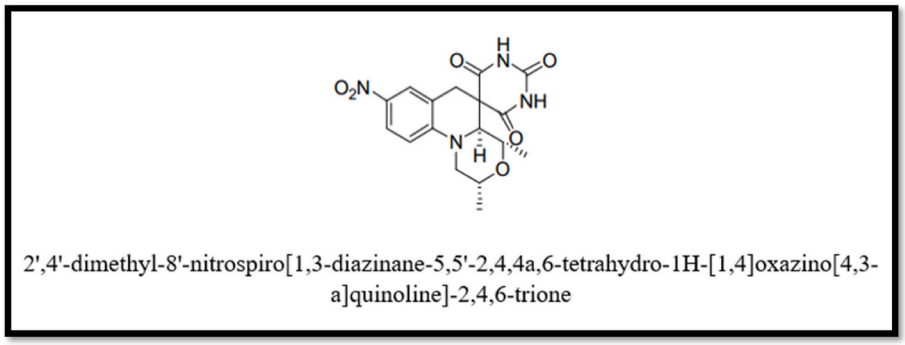

Fig. 3. Structure of compound 2′,4′-dimethyl-8′-nitrospiro [1,3-diazinane-5,5′-2,4,4a,6-tetrahydro-1H- [1,4] oxazino [4,3-a] quinoline]-2,4,6-trione.

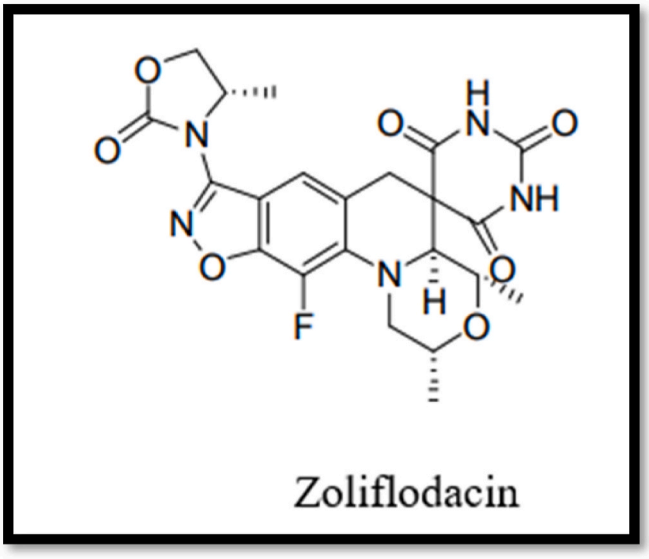

Fig. 4. Structure of compound Zoliflodacin.

10. Novel bacterial type II topoisomerase inhibitors (NBTIs)

10.1. Simocyclinone

Simocyclinone D8 exhibits a unique mode of action in which it can interact with two sections of DNA gyrase at once, since it has been shown to bind to a region of gyrB. Simocyclinone D8 did not stimulate the formation of cleavage complexes or competitively limit ATPase activity in GyrB. As a result, the mode of action is distinct in relation to the binding sites. Simocyclinone D8 primarily functions by preventing the development of cleavage complexes, whereas fluoroquinolones first permit the production of cleavage complexes before stabilizing them [58].

To convert 7-oxo-SD8 to SD8, an NAD(P)H-dependent ketoreductase, SimC7, is required. In Fig. 5, as a DNA gyrase inhibitor, 7-oxo-SD8 was largely inactive, and it was demonstrated that SimC7 reduction of the keto group was needed for high-affinity binding of the compound to the enzyme. Therefore, the biological action of simocyclinone depends on SimC7, an angucyclinoneketoreductase [59].

10.2. Thiophenes

With their distinct mode of action, the antibacterial thiophene class targets DNA gyrase and exhibits efficacy against various bacterial infections, including strains that are resistant to fluoroquinolones [60]. While antibacterial thiophenes maintain gyrase-mediated DNA cleavage complexes in either one or both DNA strands, fluoroquinolones stabilize double-stranded DNA breaks.

According to the-ray crystallography of DNA gyrase-DNA complexes, the chemicals bind to a protein pocket located between the winged helix domain and topoisomerase-primase domain [61]. Fig. 6 depicts the activity of thiophene inhibitors is affected by mutations in conserved residues surrounding this pocket, which is consistent with the allosteric inhibition of DNA gyrase. This drug-gable pocket offers options for targeting bacterial topoisomerases for the development of complementary antibiotics.

10.3. Gepotidacin

Novel and promising antimicrobials for the treatment of bacterial

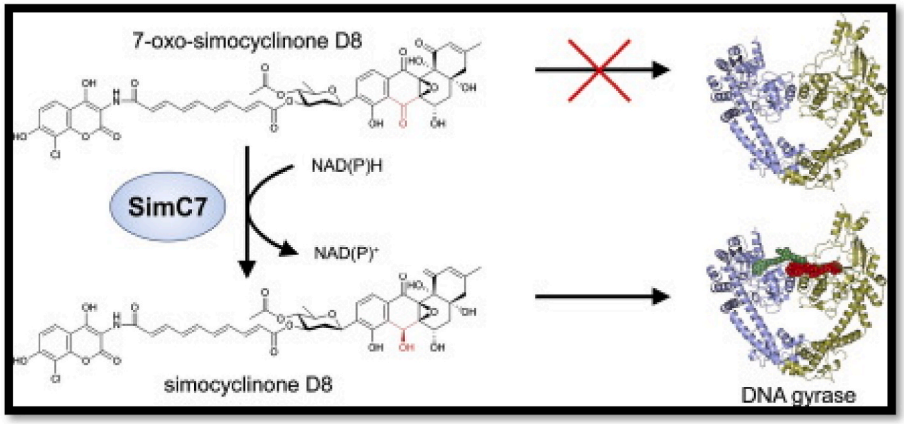

Fig. 5. Mechanism of simocyclinone in the inhibition of DNA Gyrase.

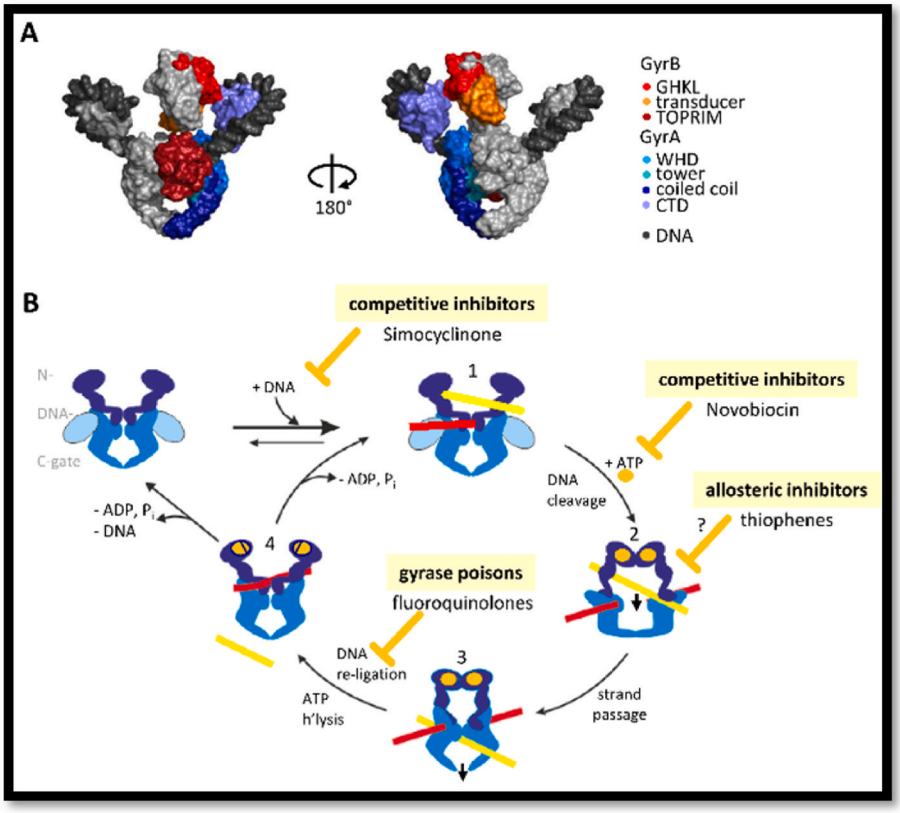

Fig. 6. Mechanism of thiophenes in the inhibition of DNA Gyrase.

infections resistant to several drugs are called novel bacterial topoisomerase inhibitors (NBTIs). Approximately 20 years ago, a promising family of antibacterial drugs known as "novel bacterial topoisomerase inhibitors" (NBTIs) was launched [62]. Their unique chemical structure, such as gepotidacin, an alternative binding site in bacterial topoisomerases, and their notably different mechanism of inhibition set them apart from fluoroquinolones and other non-quinolone antimicrobials, making them extremely intriguing and promising future antibacterials in the fight against resistant bacteria shown in Fig. 7.

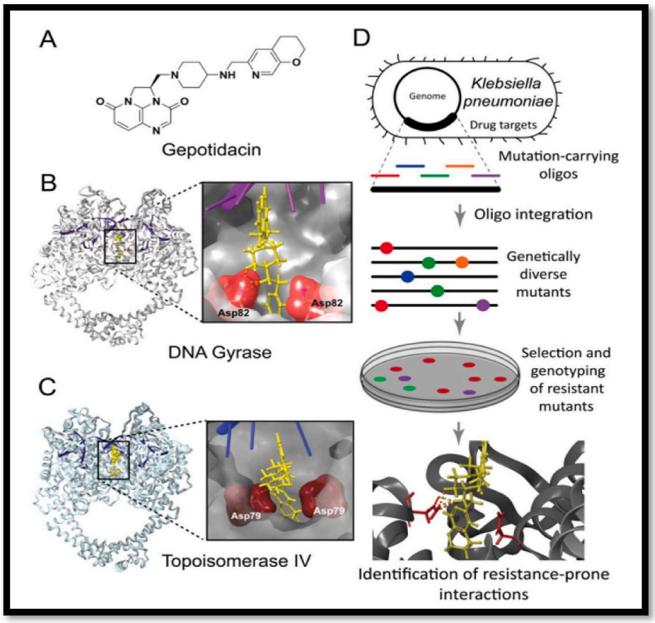

Fig. 7. Mechanism of gepotidacin in the inhibition of DNA Gyrase.

A significant factor contributing to the small number of NBTIs in clinical trials is their class-related suppression of the heart's human α-go-go-related gene (hERG) potassium channels, which prolongs the QT interval and therefore increases the risk of life-threatening arrhythmias.

10.4. Halogen atom in the para position of the phenyl right hand side (RHS) moiety

In terms of structure, NBTIs are composed of three parts: the right side (RHS) binds to the gyrA/ParC subunit of DNA gyrase/topoIV, while the left side (LHS) intercalates between base pairs of the DNA molecule [63]. The third part is a linker moiety that connects the two parts and is crucial for the proper spatial arrangement of the LHS and RHS fragments, as well as the best physicochemical characteristics of NBTIs. Although NBTIs have shown good therapeutic efficacy, this class of antibacterial agents is not yet available for purchase. The first member of the NBTI class of antibacterials to enter phase I clinical trials was quinidine; however, it was withdrawn because of its human Ether-a-go-go: Cardiotoxic problems associated with the potassium channel (hERG)gene.

Similar to DNA gyrase, the LHS moiety of NBTIs intercalates between core DNA base pairs. Different substituted bicyclic heteroaromatic systems, such as quinolines, quinoxa-lines, and naphthyridines, are the most widely employed LHS fragments. The linker itself makes only one crucial contact with the enzyme and not with the DNA because of the spaciousness of the NBTI binding pocket, especially at the entrance surrounding the NBTI's linker moiety shown in Fig. 8. Despite this, the linker is still crucial to NBTI's function. The binding of the RHS to the hydrophobic binding pocket of the topo IV ParC subunit is analogous to that of DNA gyrase GyrA subunit targeting.

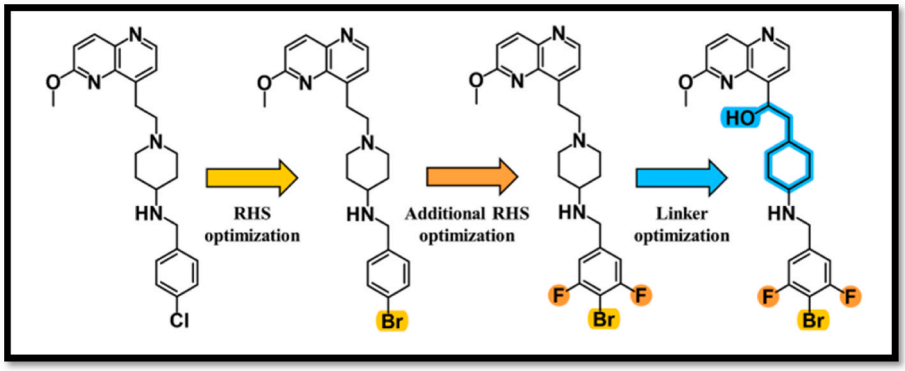

Fig. 8. Mechanism of Halogen atom in the para position of the phenyl right hand side (RHS) moiety in the inhibition of DNA Gyrase.

11. Other inhibitors of DNA gyrase

Gyrase inhibitors other than the quinolone and coumarin families are rare. Some anticancer agents that block eukaryotic topoisomerase II appear to affect gyrase activity. For instance, m-AMSA (4'-[9-aridinylamino] methane-suifon-m-aniside) can only moderately promote gyrase cleavage of DNA. It is intriguing to observe that both oxolinic acid and m-AMSA promoted DNA breakage by T4 topoisomerase II. These findings imply possible shared modes of action between quinolones and eukaryotic type II topoisomerase inhibitors.

A glycine-rich peptide with a molecular mass of approximately 3.2 kDa, microcin B17, is synthesized by enterobacteria that have pMccB 17 or similar plasmids. Antibiotics are produced by these plasmids using six structural genes, with the seventh gene providing immunity to antibiotics. As a DNA replication inhibitor that has been shown to be effective against a range of enterobacteria, microcin B17 rapidly stops DNA synthesis, triggers the SOS mechanism, breaks down DNA, and causes cell death. An overview of DNA gyrase inhibitors is provided in Table 5, along with information on their unique properties and mechanisms of action.

Topoisomerases are enzymes conserved across a wide range of bacterial species and are ideal targets for drug discovery [64]. However, it is important to remember that creating novel medicinal medicines is a time-consuming and expensive process. In contrast, bacteria can amaze their ability to rapidly evolve resistance determinants [65]. The abundance of topoisomerase II inhibitor studies in the most recent literature is indicative of the great interest in these compounds as potential therapeutic targets. The structural connection among topo IIα and topo IIβ has been validated by multiple investigations. Since the majority of these binding sites are obscure, allosteric inhibition—which has been shown by substances like evybactin and the thiophene class—likely plays a significant role in controlling the timing of gyrase inhibition during catalysis [66]. On the other hand, a variety of compounds specifically contend with gyrase and associated topoisomerases to inhibit the binding of the DNA substrates, hence preventing abnormal ATP turnover and DNA breaks by efficiently keeping gyrase activity confined. Lead compounds such as benzoindole, indole derivatives, and carbazole derivatives, which offer a novel framework for inhibiting the activity of DNA gyrase ATPase. The ATP binding site of DNA gyraseGyrB subunit was modeled by MD simulations for complexes of carbazole, benzoindole, and indole derivatives. These simulations demonstrated the crucial role that hydrogen bond interactions with Asp79 play in binding. The hydroxyalkyl amine alternatives of the benzoindole and indole derivatives, which our models associate with hydrogen bonding to Asp79, may play a significant role in the suppression of DNA gyrase ATPase activity, according to SAR analysis [67].

A class of DNA gyrase allosteric inhibitors that exhibit antibacterial efficacy against clinical isolates of *Escherichia coli* that are resistant to fluoroquinolones. An early isoquinoline sulphonamide hit with resistance to isoquinoline sulphonamides was found using small-molecule library screening, and all signs pointed to the DNA gyrase complex, a crucial bacterial topoisomerase and known antibacterial target. The drug differs from all other gyrase

Inhibitors that have been described to date and the clinically utilized fluoroquinolones in its mode of action. It occupies an allosteric, hydrophobic pocket in the GyrA subunit [68].

Natural products using the DNA supercoiling assay kit, the biological efficacy of daidzein and khellin was assessed in vitro for their capacity to suppress *E. coli* DNA gyrase activity. Furthermore, daidzein and khellin showed IC50 values of 0.042 and 0.822 μg/mL, respectively, in their inhibition of the DNA gyrase enzyme. With IC50 values of 300.23 μg/mL for khellin and 160.81 μg/mL for daindelzin, it was discovered that they were less hazardous to the vero cell line. Additional research using MD simulation and molecular docking analysis of daidzein showed that it stayed stable for 100 ns inside the DNA GyrB domain cavity [69].

Much work has been done in the quest to find new bioactive compounds that can block bacterial topoisomerases at locations other than the fluoroquinolone binding site. Notably, scientists have searched for anti-infective drugs that can block topoisomerases via different pathways. Study on topoisomerase inhibitors that work differently from fluoroquinolones, especially those that act on an allosteric site in the GyrA/ParC subunit that was just found, and on GyrB/ParE inhibitors that bind to the ATPase site. The study stated above demonstrates the wide range of potential applications for novel topoisomerase inhibitors. Some of the research may already be in clinical trials, and future studies could provide new antibiotics that could save lives [70].

Table 5
Inhibitors of DNA gyrase.

Inhibitor	Description
m-AMSA	Significantly increases DNA gyrase cleavage while blocking eukaryotic topoisomerase II.
Oxolinic Acid	Encourages T4 topoisomerase II to break DNA; quinolones and eukaryotic type II topoisomerase inhibitors may have similar mechanisms of action.
Microcin B17	Enterobacteria harbouring pMccB17 plasmids produce a 3.2 kDa glycine-rich peptide, which is known to hinder DNA replication, trigger the SOS mechanism, break down DNA, and cause cell death.

12. Conclusions

Since the discovery of DNA gyrase over 15 years ago, many studies have been conducted on this enzyme. As this review attests to the vast range of professionals interested in this enzyme, there are numerous scientific journals in which papers on gyrase can currently be found. These professionals include physicists, biochemists, microbiologists, medicinal chemists, and clinicians. Workers in this field have long been fascinated by the process of DNA supercoiling by gyrase, but crucially

significant aspects of this reaction, such as the strand passage step and mechanism of energy coupling, remain completely unresolved. Through the investigation of antibacterial substances, notably those in the quinolone class, many researchers have become interested in gyrase. To combat the significant global health issue of multidrug-resistant bacterial infections, bacterial topoisomerases remain valuable targets for the development of innovative antibacterial medications that might prevent cross-resistance with existing antibiotics. Clarifying the mode of action of well-known medications will aid in this effort. DNA gyrase is a therapeutic target for the development of novel antibacterial medicines is DNA gyrase. Although selective antibacterial gyrase inhibitors are available, their development of resistance is difficult. Thus, new gyrase inhibitors that function through new pathways are required.

13. Future perspectives

It is likely that in-depth studies on gyrase inhibitors will lead to the development of new drug candidates. Because bacterial resistance is less likely to arise, a potential area is the development of new Gyrase B ATP-competitive inhibitors. DNA Gyrase is a validated and well-explored target from a medicinal perspective, and current efforts are concentrated on the final stages of clinical development, with improved clinical candidates acquired more recently. A variety of projects link industrial, governmental, and academic endeavors; these actions also represent potential routes for new medications to enter the market.

CRediT authorship contribution statement

K. Rajakumari: Writing – original draft, Conceptualization. **K. Aravind:** Investigation, Data curation. **M. Balamugundhan:** Resources, Methodology. **Manjunathan Jagadeesan:** Validation, Resources. **Ambiga Somasundaram:** Visualization, Data curation. **Parthiban Brindha Devi:** Supervision, Conceptualization. **Pasiyappazham Ramasamy:** Writing – review & editing, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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