Drug-resistant *Escherichia coli* (*E. coli*) has overburdened the healthcare facilities in recent years and is getting hard to combat, mandating search for novel therapeutics with a broad antibacterial spectrum and high chemotherapeutic index. The 24 kDa domain of DNA gyrase B that is involved in the ATPase activity has been reported to be a promising target for inhibitors. A PDB structure (1KZN) of the 24kD domain of gyrase B with the co-crystallized ligand clorobiocin was used for the docking studies to explore a library of 2924 FDA approved drugs from www.zinc.docking.org. FlexX docking module from Biosolve IT was used for receptor preparation and *in silico* docking experiments. Docking studies on the pocket created around the reference ligand clorobiocin revealed the best score with diminazene aceturate and it also demonstrated interactions with the crucial amino acids present within the pocket. Diminazene aceturate has been conventionally been used as an antiparasitic molecule in animals and it has also been demonstrated to exhibit repurposing potential in the treatment of disorders triggered due to overproduction of inflammatory cytokines, pulmonary hypertension, ischemia-induced cardiac pathophysiology, etc. among others. Findings from this study indicate the possibility of repurposing the age-old molecule diminazene aceturate into a DNA gyrase B antagonist to combat not just the drug-resistant *E. coli* but also other gram-negative ESKAPE pathogens. It may also aid in alleviating the inflammatory response induced in the body of the patients suffering from septicemia caused by a variety of Gram-negative bacterial pathogens.
it include gastroenteritis, Urinary Tract Infections (UTI), bacterial meningitis, post-operative abscesses and neonatal sepsis. There are at least seven known pathotypes for enteric E. coli namely enteropathogenic, enterotoxigenic, enterohaemorrhagic, enteroaggregative, diffusely adherent, enteroinvasive and adherent invasive E. coli. Besides, there have been reported three pathotypes of E. coli which are extraintestinal in their effect, namely, uropathogenic, neonatal meningitis E. coli and avian pathogenic E. coli. Indiscriminate and improper usage of antibiotics has already resulted in an alarming multiple drug resistance among the pathogenic bacteria [2-5] and has become a global public health concern. Among many mechanisms of MDR, the predominant one is the plasmid-mediated synthesis of Extended–Spectrum Beta Lactamases (ESBLs), which breakdown the beta lactam antibiotics, including all the three generations of cephalosporins, penicillins and aztreonam particularly witnessed in E. coli and other Gram–ve EKAPE pathogens Enterococcus faecium, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species [6,7].

Considering the newly emerging and life threatening AMR E. coli, there is an urgent requirement of newer strategies, such as repurposing of older drugs, re-evaluation of many abandoned compounds using modern chemical synthesis methods, tools and technologies [5]. Another potential approach could be to explore unusual targets within the pathogen system, such as topoisomerases. DNA gyrase or topoisomerase type 2 is an important enzyme which controls DNA supercoiling as needed for processes like replication and transcription, and it is reported to be the target of some successful antibiotics [8-11]. A functional DNA gyrase is heterotetramer (A2B2), consisting of 2 subunits each of gyr A and gyr B, with 875 and 804 amino acids respectively [8]. GyrA creates single stranded nicks in the DNA and reseseals them, whereas GyrB serves to provide energy for this process through ATP hydrolysis [9,10], which is essential for the unwinding of the DNA. Hence, DNA gyrases serve as the nanomachines that maintain the DNA in its appropriate topology throughout its replication and transcription [11]. Aminoumarin antibiotics, quinolines and floquinolones are the well-known catalytic inhibitors of the DNA gyrase [12–19]. An elegant evidence was provided by Gilbert and Maxwell [12] that a 24 kDa sub-domain located near the N-terminal of DNA gyrase B enzyme possessed the binding site towards coumarin antibiotics. They also suggested that the interaction of coumarins with the protein was predominantly hydrophobic in nature [14].

For carrying out meaningful and productive research on these lines, it is of paramount importance to explore the specific residues of Gyrase B which play a crucial role in maintaining its functionality and stability, and also to know which compounds disturb the same. This kind of approach would make it possible to target DNA gyrase of the drug resistant, pathogenic E. coli, that has become resistant to older antibiotics targeting their cell wall, nucleic acid or protein synthesis etc.

Compounds belonging to three families namely quinolones such as norfloxac, clyclofluralines and coumarins (eg. Clorobiocin and novobiocin) have been reported to inhibit gyrB (14). A number of other compounds are also reported in the literature, which inhibit Gyrase B. These include Pyridoxal 5’-diphospho–5’-adenosine (PLP-AMP) [9], tyrosine-based diamides [20], 1H-pyrrole–2-carboxamide moieties [21], N-phenylpyrrolamides [22], N-phenyl 4,5-dibromo–pyrrolimides [23], and (1,2,4) triazole (4,3-a)quinoxaline derivatives of different heteroaomatization members [24], cinodine, clerodin, albicidin, GSK299423, a series of tetrahydroindazoles, quinazoline–2,4-diones PDE305970 and PDE326448, QPT-1, quinolone based drug NXL-101 [25] etc. In addition to gyrB, even gyrA possesses a pocket which is capable of binding to coumarin or aminocoumarin, suggesting that any inhibitor like simocyclinone binding to gyrB may bind to gyrA. This is expected to enhance the effectiveness of the drug used [25].

Important amino acids for the ATP hydrolysis in E. coli gyrB as reported in the literature include Tyr5, Ile10, His38, GLu42, Asn46, Glu50, Asp73, Arg76, Gly77, Ile78, Phe79, Phe79, K903, Arg136, Thr165, Asp426 and Lys447 [26-28]. Tyr5 and Ile10 help the process of dimerization in gyrB so to increase binding affinity and also to double the length of DNA site bound protein as well as to increase binding specificity. Some dimer interface residues are important and mutations of these residues result in loss of enzyme catalysis [27]. Glu42 helps in hydrolysis process and His38 in ATPase reaction, acting to orient and polarize glutamate residue in gyrase B [10]. Substitution with Alanine at Glu42, Asn46, Glu50, Asp73, Arg76, Gly77, Ile78 resulted in decreased or not demonstrable ATPase activity, signifying their importance in ATP hydrolysis [26]. Pro79 and Lys103 are reported to be important in the coupling of ATP hydrolysis and DNA unwinding and mutations in Asp73, Gly77, Ile78, and Thr165 lead to resistance to novobiocin [28]. Lastly, Asp426 and Lys447 are described to be the part of a quinolone-binding pocket of gyrB [26].

Diminazene aceturate / diminazene / dize / 4,4’-(1-Triazene-1,3-diy) bis (benzenecarboximidamide) sold with several trade names such as Pirocide, Berenil, Azidin, Ganasag, etc. is an anti-parasitic drug. Though it primarily targets pathogenic protozoans including Trypanosoma, Babesia, and Cytauxzoon, it is also reported to be active against some bacteria namely Brucella and Streptococcus. The potential of dize in the treatment of inflammatory cytokine triggered disorders [29], pulmonary hypertension, ischemia–induced cardiac pathophysiology.
possibly through the activation of angiotensin converting enzymes 1–7 as studied in the rats appears promising [30,31]. It upturns ACE2 and AT1 receptor expression in the kidney cells in the type 1 diabetes rat model and inhibits nephropathy [32]. It has also been reported to completely inhibit the topoisomerase I in Caenorhabditis elegans, though at high concentration of 125 μM [33] and was reported to dock successfully with Chikungunya Virus RNA polymerase [34]. To further support its therapeutic potential in different conditions Oliveira, et al. [35] reported improved activity of diminazene aceturate on Trypanosoma evansi when encapsulated in the liposomes.

Drug repurposing is now viewed as a faster and efficient solution to discover novel therapeutics with a goal to address a number of health issues concerning infectious diseases, cancers etc. This could equip healthcare industry to serve the society better in obtaining a viable solution in fighting the dreaded problem of MDR posed by pathogenic bacteria. Computer–Aided Drug Discovery (CADD) is one of the most powerful approach to investigate drugs that would act upon the novel targets of pathogenic bacteria [36–38]. Correctly termed “Drug Repurposing”, this strategy is indeed proving to be very productive, quicker, and more practical towards fighting the menace of multiple drug resistant pathogenic bacteria in general [39]. This in silico study was undertaken to analyse the interactions of the GyrB subunit towards a number of inhibitors, in an attempt to find a potential lead against MDR E. coli.

METHODS

Receptor preparation

Target protein and important residues were described through the thorough literature search. The conservation of these residues in other gram negative ESKAPE bacteria was determined using Clustal omega. The appropriate 3D structures of the E. coli GyrB were downloaded in PDB format from the database: www.rcsb.org. 1KZN was selected from the available 3D structures of E. coli gyrB [14]. It is a 24 kD fragment of gyrB (Gly15–Glu219) with the co-crystallized antibiotic Clorobiocin (CBN). Docking pocket was created around the reference ligand CBN (referred to as pocket 1) with a diameter of 6.5 angstrom that included 30 residues namely Val43A, Val44A, Asp45A, Asn46A, Ala47A, Ile48A, Asp49A, Glu50A, Ile59A, Val71A, Gln72A, Asp73A, Gly75A, Arg76A, Gly77A, Ile78A, Pro79A, Thr80A, Gly81A, Ala86A, Ala87A, Ile90A, Met91A, Ala96A, Val120A, Arg136A, Gly164A, Thr165A, Met166A, and Val167A (Figure 1a). An ‘A’ here with the residues signifies the chain A recognized in the PDB structure. Three water molecules (HOH1001, HOH1066, HOH1160) with at least 2 interactions were included in the receptor and were made freely rotatable.

Ligand selection, docking and lead selection

Docking protocol as per Ghildiyals, et al. [34] was followed for the initial screening of molecules with some modifications. Docking was performed with 500 solutions per iteration and 500 solutions per fragmentation for each molecule of the ZDD subset from zinc small molecule database that consists of a library of 2924 FDA approved drug molecules. Top 14 lead compounds were selected based on the FlexX docking score. The selected Zinc Ids were explored for their chemical nature, name and current therapeutic indications.

Refining docking

The autodetected docking pocket was altered by removing certain non-conserved residues and to take account of some more of the crucial conserved residues, without upsetting the core pocket’s integrity. We used PyMOL for visualizing the pocket created automatically by the lead IT software and all the changes in residues were made after confirming the position of each residue in the surface view using PyMOL. The modified pocket (pocket 2) included 24 residues i.e. Glu42, Val43A, Asp45A, Asn46A, Ala47A, Ile48A, Asp49A, Glu50A, Ile59A, Val71A, Glu72A, Asp73A, Gly75A, Arg76A, Gly77A, Ile78A, Pro79A, Thr80A, Gly81A, Ala86A, Ala87A, Ile90A, Met91A, Ala96A, Val120A, Arg136A, Gly164A, Thr165A, Met166A, and Val167A (Figure 1b). Further shortlisted top leads were docked for 2000 solutions per iteration and 2000 solutions per fragmentation in the auto-detected pocket as well as in the modified pocket. The details...
of the contacts between the leads and the residues of interest were examined using PyMOL and the Discovery Studio Visualizer platforms.

RESULT AND DISCUSSION

Among the available PDB structures 1KZN was considered suitable for carrying out in silico screening of drug molecules as this 24 kD C-terminal subdomain of the N-terminal of the DNA gyrase B encompasses the coumarin (gyrB inhibiting antibiotic) binding site. Bacteria overproducing this sub-domain exhibit resistance to coumarins, suggesting its role in in vivo interaction with the drug [12]. 1KZN is an X-ray diffracted determined structure with a resolution of 2.30Å along with a co-crystallized ligand clorobiocin an antibiotic belonging to the coumarin family [14]. Clorobiocin is used as a reference point in our docking studies. In silico studies revealed that the receptor prepared using clorobiocin as the reference ligand shaped a very deep seated and narrow pocket, exhibiting good binding scores (as low as -33) with the reference ligand shaped a very deep seated and narrow pocket, exhibiting good binding scores (as low as -33) with the ligands. This pocket is referred to as pocket 1. Among the 30 residues present in the pocket 1 namely Val43, Val44, Asp45, Asn46, Ala47, Ile48, Asp49, Gly50, Ile59, Val71, Gly75, Arg76, Gly77, Ile78, Thr80, Gly81, Ala86, Ala87, Ile90, Met91, Ala96, Val120, Arg136, Gly164, Thr165, Met166, Val167, nine residues as depicted in口袋图 (Figure 3) revealed a noteworthy similarity in occupying the position within the coumarin binding pocket as well as interactions with the strategic residues.

A comparative study of the binding of co-crystallized clorobiocin (Figure 2) and docked diminazene aceturate (Figure 3) revealed a noteworthy similarity in occupying the position within the coumarin binding pocket as well as interactions with the strategic residues.

Diminazene aceturate was found to interact with almost all the key residues with which the co-crystallized clorobiocin interacted, with a few exception (Arg76, Arg136, Pro79 and Ile90). Further details of these interactions with respect to the types and bond length are given in the supplementary table 1. Our results advocates the repurposing potential of this compound to target E. coli gyrB. Diminazene aceturate or simply dize is available in the market with several trade

| S.no | COMPOUNDS | CURRENT INDICATION | SCORE |
|------|-----------|--------------------|-------|
| 1    | zinc03830706 Diminazene | Antiparasitic trypanocidal drug | -33.79 |
| 2    | zinc03830435 Ceforanide | Antibacterial; Second generation cephalosporin | -33.30 |
| 3    | zinc00125031 Niflumic acid | Anti-inflammatory and analgesic agent used in the treatment of rheumatoid arthritis | -33.24 |
| 4    | zinc00373877, Ketanserin | Treat Severe Sepsis, Diabetic Foot Ulcer, Septic Shock | -31.96 |
| 5    | zinc35342789, Tolcapone | Antiparkinson drug and catechol O-methyltransferase inhibitor | -31.19 |
| 6    | zinc18456289, Folic acid | Treat and prevent folate deficiencies and megaloblastic anaemia | -30.76 |
| 7    | zinc01530747, Oxacillin | Antibacterial; Broad spectrum beta lactam antibiotic | -30.42 |
| 8    | zinc04676376, Cefmenoxime | Antibacterial; Third generation cephalosporin | -30.04 |
| 9    | zinc01481815, Exjade | Treat chronic iron overload due to blood transfusion | -29.40 |
| 10   | zinc18456286, Leucal | Treatment of overdose of methotrexate | -29.08 |
| 11   | zinc03830430, Ceftriaxone | Antibacterial; Cephalosporin | -29.01 |
| 12   | zinc03830399, Cefamandole nafate | Antibacterial; Cephalosporin | -28.79 |
| 13   | zinc03812994, Flunitrazepam | Treat anxiety and sleep disorders | -28.49 |
| 14   | zinc03918453, Ertapenem | Treat wide variety of bacterial infections (bactericidal) | -28.41 |
names such as pirocide, berenil, azidin, ganasag, etc. It is an anti-trypanosomal drug with the molecular formula C_{14}H_{15}N_{7} and chemical structure as shown in figure 4. Though the repurposing potential of this compound for various conditions has been explored widely [29–35], but still it is approved for use only in the animals due to the potential toxicity and low therapeutic index of the molecule[40]. More work is required in optimization of Dize to reduce its toxicity using fragment building and/or fragment replacement keeping the core structure of the molecule same.

Alignment of the sequence of E. coli 24 kD domain with other Gram negative pathogens Enterococcus faecium, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species belonging to the ESKAPE group of nosocomial pathogens described by Infectious Diseases Society of America revealed highly
Figure 4 Structure of Diminazene aceturate (Pubchem).

Figure 5 Sequence alignment of 24 kDa domain of E. coli gyraseB (1KZN) with representative EKAPE pathogens [Enterococcus faecium (ENTFA), Klebsiella pneumonia (KLEPN), Acinetobacter baumannii (ACIBS), Pseudomonas aeruginosa (PSEAE) and Enterobacter species (ENTR)] depicting conservation of the key residues at 46, 50, 73, 76, 77, 78, 79, 136 and 165 positions.

| Accession no. | 268 |
|---------------|-----|
| sp|Q8392J|GYRB_ENTFA |---------------------|MIRERAQYDASIQVLEGLEAVRKPQMGIVGSST-SGEGHLHHLVVEIPE
| sp|Q917C2|GYRB_PSEA |-------------MSENNTYDSSSISKVGLDAVRKPGYIGTDGDTGGLHHWF EW
| tr|B0VMK3|B0VMK3_ACIBS |-------------------NSSESOSQASQETQTNKAVDSSSTIKVRGLDAVRKPGYIGTDGDTGGLHHWF EW
| tr|J2E065|J2E065_KLEPN |-------------------GLDAVRKPGYIGTDGDTGGLHHWF EW
| tr|G8LE52|G8LE52_9ENTR |-------------------MPSNSDSSSSKGVGLDAVRKPGYIGTDGDTGGLHHWF EW
| sp|Q8392J|GYRB_ENTFA |-------------SIDNLAGFKSIWETDPDSITVTGKRGQSHVQAKTRPAVTFTVHLHAGKFGG
| sp|Q917C2|GYRB_PSEA |-------------SIDNLAGQCEFISITHTDESITVRKGAGPGIVTHHEEGSAAEVMTLHLAGKFGDD
| tr|B0VMK3|B0VMK3_ACIBS |-------------AIDE-LAGHDECIIIVTIEEESVSIVSGIFITDIHPEEVSAAEVILITLHLAGKFGDD
| tr|J2E065|J2E065_KLEPN |-------------AIDE-LAGHCDIVTENYSDSIVSGIFITDIHPEEVSAAEVILITLHLAGKFGDD
| tr|G8LE52|G8LE52_9ENTR |-------------AIDE-LAGHCEIVTENYSDSIVSGIFITDIHFPPEVSAAEVILITLHLAGKFGDD
| sp|Q8392J|GYRB_ENTFA |-------------SGGYKVSGGLHGVSWSVLASSTDLVWEGKYOYGQGYGRRGVDOLKLV1ETTEDKKEIT
| sp|Q917C2|GYRB_PSEA |-------------NTYGKVSGGLHGVSWSVLASSTDLVWEGKYOYGQGYGRRGVDOLKLV1ETTEDKKEIT
| tr|B0VMK3|B0VMK3_ACIBS |-------------NSYSVSGGLHGVSWSVLASSTDLVWEGKYOYGQGYGRRGVDOLKLV1ETTEDKKEIT
| tr|J2E065|J2E065_KLEPN |-------------NSYSVSGGLHGVSWSVLASSTDLVWEGKYOYGQGYGRRGVDOLKLV1ETTEDKKEIT
| tr|G8LE52|G8LE52_9ENTR |-------------NSYSVSGGLHGVSWSVLASSTDLVWEGKYOYGQGYGRRGVDOLKLV1ETTEDKKEIT
| sp|Q8392J|GYRB_ENTFA |-------------VHFIPDPIFTETTYDDFQKALTTRVRELAFNLGHLISDEKROEEDGQDEKKYHYYEGGGK
| sp|Q917C2|GYRB_PSEA |-------------VHFIPDPIFTETTYDDFQKALTTRVRELAFNLGHLISDEKROEEDGQDEKKYHYYEGGGK
| tr|B0VMK3|B0VMK3_ACIBS |-------------VRFPWSAATETLQSTFFIVNVIARRLSLNSNGVIIILLREDTGEE---LFLYEYGGLKA
| tr|J2E065|J2E065_KLEPN |-------------VRFPWSAATETLQSTFFIVNVIARRLSLNSNGVIIILLREDTGEE---LFLYEYGGLKA
| tr|G8LE52|G8LE52_9ENTR |-------------VRFPWSAATETLQSTFFIVNVIARRLSLNSNGVIIILLREDTGEE---LFLYEYGGLKA

CONCLUSION AND FUTURE ENHANCEMENT

Outcomes from our study specify the repurposing potential of a very simple molecule like diminazene aceturate as a DNA gyraseB antagonist. Our results indicate that conserved nature of the selected important residues and also the other interacting residues (Figure 5). This signifies our results as diminazene aceturate is predicted to have broad spectrum activity against gyrB including that of EKAPE gram negatives pathogens that are posing utmost challenges in the healthcare facilities because of their multidrug resistance.
dize may be developed into a broad spectrum antibacterial compound through the optimization process using scaffold hopping and pharmacokinetic analyses. It may also be helpful in easing the inflammatory responses in the patients suffering from Gram negative bacteria induced septicemia. Precise in vitro experiments could be deliberated in future, based on our CADD studies and their outcome is expected to be fruitful to mankind in terms of coping with MDR E. coli and the related EKAPE pathogens.

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