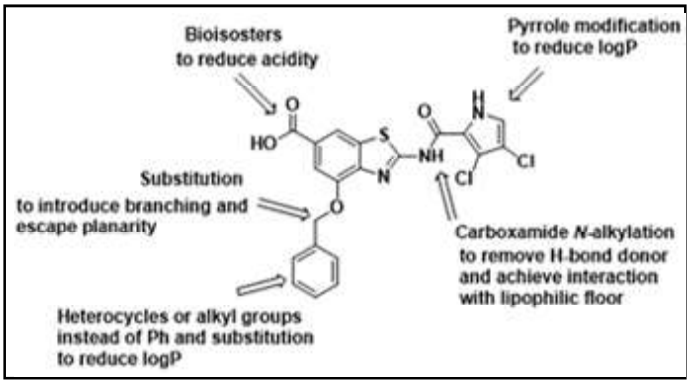
CRIPS Digest

Design and synthesis of Benzthiazole analogs as DNA gyrase inhibitors with potent activity against Pseudomonas aeruginosa and Acinetobacter baumannii

Bacterial topoisomerase IV (topo IV) and DNA gyrase are one of the most important targets for antibiotics as they are responsible for generation of negative



Prototype structure of Benzothiazole analogs targeting bacterial DNA gyrase

supercoils compelled by ATP and followed by Supercoil relaxation driven by ATP. Gyrase is composed of two subunits, GyrA and GyrB, while topo IV is composed of two subunits, ParC and ParE. There is currently no promising therapeutic application for antibiotics that target the ATP binding to the ParB/GyrB subunits. To achieve this, a novel class of DNA gyrase inhibitors based on benzothiazoles was designed, synthesized, and tested for antibacterial efficacy against six nosocomial pathogens that are members of the ESKAPE group.

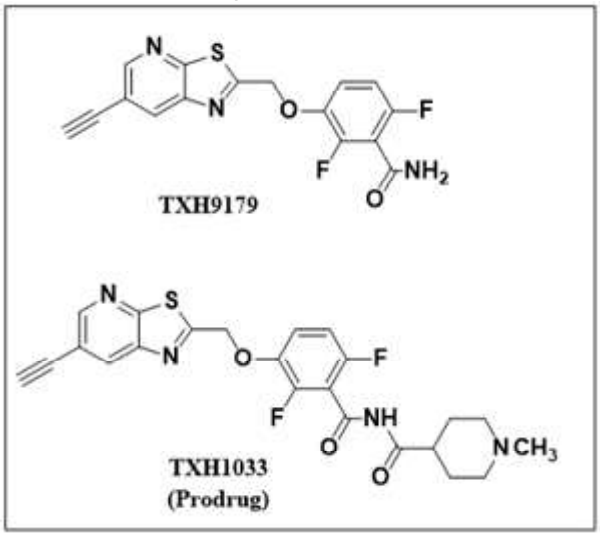
In order to increase the potency and pharmacokinetic properties of the benzthiazole analogs several modifications on the basic structure were carried out. In total 70 compounds were designed and synthesized and among them one of the compounds showed most potent antibacterial activity against the multidrug resistant class of bacteria.

In this lead compound, the benzylic proton was substituted with a methyl group. Due to this that benzylic carbon centre becomes chiral and it's found out that (S)-enantiomer of the lead compound showed more potent antibacterial activity than the other enantiomer. This lead compound showed remarkable inhibitory action against the DNA gyrase of P. aeruginosa (IC_{50} < 10 nm), *E. coli* (IC_{50} < 10 nm), and also showed excellent inhibitory action against topo IV of *P. aeruginosa* (IC_{50} = 29 nm) and *A. baumanni* (< 10 nm). To observe the binding of both enantiomers (S and R) to the ATP binding domain of the gyrase in *A. baumanni* and *P. aeruginosa*, a molecular docking study was carried out. The results of the docking experiment indicated that the (S)-enantiomer has a higher binding affinity for the various amino acids found in the Gyrase cavity than the (R)-enantiomer.

J. Med. Chem, 2023, 66, 1380-1425.

Structural and Antibacterial Characterization of a New Benzamide FtsZ Inhibitor with Superior Bactericidal Activity and *In Vivo* Efficacy Against Multidrug-Resistant Staphylococcus aureus

A strain of *S. aureus* resistant to methicillin (MRSA) stands as a clinically significant pathogenic bacterium. The resistance of clinical isolates of MRSA obtained from hospitals and communities to contemporary standard-of-care antibiotics such as linezolid and vancomycin is on the rise.



This article reported studies on TXH9179, a novel thiazolopyridine-benzamide FtsZ inhibitor of the next generation, which has a 6-acetylene substituent attached to the thiazolopyridine nucleus. The authors compared TXH9179's antistaphylococcal potency to that of two previously discovered thiazolopyridinebenzamide FtsZ inhibitors, each of which had a 6-Cl substituent (PC190723) or a 6-CF_3 substituent (TXA707).

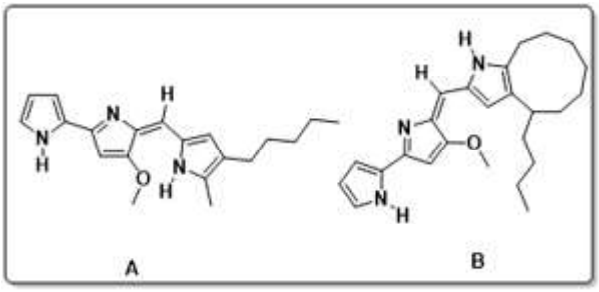
Against a library of 55 clinical strains of MRSA and methicillin-sensitive *S. aureus* (MSSA), including MRSA isolates resistant to vancomycin and linezolid, TXH9179 was found to be four times more potent than TXA707. TXH9179 was also associated with a reduced incidence of resistance compared to TXA707 in all except one of the MRSA and MSSA isolates investigated; the observed resistance was caused by mutations in the FtsZ gene. The alterations observed in MRSA's cytoskeleton, cell division process, and FtsZ localization, induced by TXH9179, align perfectly with its inhibitory effects on FtsZ.

Crystallographic investigations reveal the crucial molecular connections that facilitate complex formation while demonstrating the direct binding of TXH9179 with S. aureus FtsZ (SaFtsZ). Even at 10 times the concentration that exhibited antistaphylococcal action, TXH9179 did not exhibit any mammalian cytotoxicity. Serum acetylcholinesterases rapidly catalyze the hydrolysis of the carboxamide prodrug TXH1033 into TXH9179. Remarkably, the intravenous and oral administration of TXH1033 demonstrated improved in vivo effectiveness in addressing a mouse model of systemic MRSA infection (specifically, peritonitis) when contrasted with the carboxamide prodrug of TXA707, namely TXA709. When taken as a whole, this work identifies TXH9179 as a novel benzamide FtsZ inhibitor that shows promise and should be investigated further.

ACS Chem. Biol. 2023, 18, 629-642

Exploiting Differences in Heme Biosynthesis between Bacterial Species to screen for Novel Antimicrobials

Porphyrins are condensed heterocyclic molecules playing a crucial role of stabilizing and reducing the toxicity of free metal cations (Fe, Ni and Co) biologically by covalently binding with them. However, Heme, an iron containing porphyrin, is biologically abundant complex amongst other metal cations serving as a cofactor in many enzymes catalytic processes involving protein syntheses which makes it as a potential target for the antibacterial strategy.



Potential inhibitors A) BCHP B) prodigiosin

Recently, a bacterial classification was made based on the cell wall framework to understand the bacterial pathogenesis and they are of two types I) monoderm bacteria which is characterized by the presence of thick peptidoglycan only, II) diderm bacteria which is characterized by the presence of -peptidoglycan between cytoplasmic membrane and outer membrane. They follow two different pathways former involved with the coproporphyrin-dependent pathway (CPD) latter with protoporphyrin-dependent pathway (PPD) for Heme biosynthesis. CPD pathway follows last three steps: I) decarboxylation II) Oxidation III) insertion of Iron with different orders as that obtained in PPD pathway mediated by diderm bacteria. Monoderm bacterial pathogenesis can be controlled due to its distinct pathway, unlike that obtained in Host, of Iron-porphyrin complex formation. A set of library compounds was screened onto the recombineering *E. coli* strain *Sa*-CPD-YFP where diderm *E. coli* genes associated with PPD pathway were replaced with the genes involved in CPD pathway from monoderm staphylococcus aureus (SA) to realize which pathway is potentially inhibited. Nitrogen containing heterocycles were put into competitive bacterial viability assay and it was found out that tripyrrole class of drugs showed selective inhibition of CPD pathway of that hybrid *E. coli* strain due to structural planarity, electrostatic interaction and sufficient H-bond donor and acceptor properties. The observation became more conclusive, after control experiment performed with diderm E. coli strain (WT-CFP) and hybrid E. coli strain (sa-CPD-YFP), that it selectively inhibit CPD pathway to greater extent.

BCHP was able to reduce the bacterial growth by 4% of the sa-CPD-YFP strain and showed z factor of 0.76 which is considered as excellent value (Z > 0.5). The mechanism indicated significant dissimilarity in the order of the following three steps and the monoderm bacteria induced infection can be prevented with higher degree of selectivity and specificity by targeting and inhibiting monoderm mediated CPD pathway. In silico study was also carried out and it came with the agreement that

specific inhibition of ChdC enzyme occurred preventing the Heme formation.

Biomolecules **2023**, *13* (10), 1485.

Conformational Restriction: Requisite for Antibiotic drug discovery

Conformational restriction is well-precedented strategy involving the scaffold modification to develop new potential drug candidates. This rigidification minimise the entropy loss, improves the binding of drug to the target site, increases the selectivity of the lead compounds and also decreases the metabolic degradation. Quinolone antibiotics constitute a large group of broad-spectrum bacteriocidals that share a bicyclic core structure. Although offering excellent potency and wide spectrum action, the problem of resistance has encouraged the scientists to search for novel antibiotics, structurally related to quinolones. Increasing the restriction in the free movement of the bonds while moving from one generation to the other is one of the perspectives of having better activity and low resistance. Similiarly, in case of isoquinoline class of antibiotics, increasing the conformational restriction by replacing the double bonds with that of spiro ring improves the antibacterial activity against the strains of *Escherichia coli*, *Pseudomonas aeroginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *S. aureus*, *Enterococcus faecalis* and *Bacillus subtilis.* Spiro indoles forms the another class of compounds that are potent against *Klepsilae pneumonia*, *Bacillus cereus*, and *Salmonella typhi*.

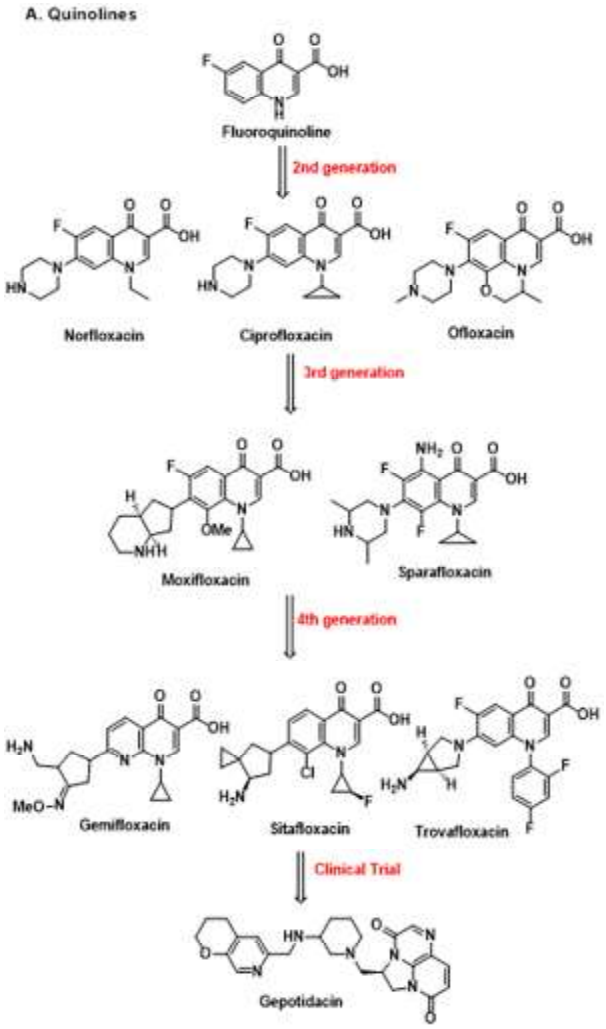


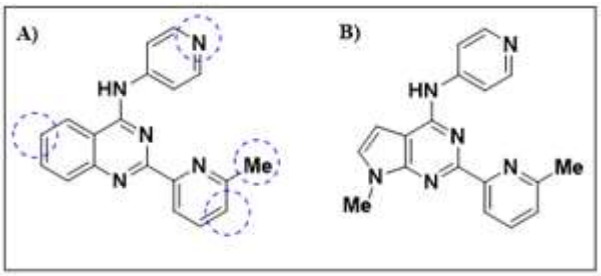
Fig. Conformational restriction leading to increased antibacterial activity.

These conformationally restricted molecular architectures help introducing specific molecular constraints in the lead candidate, allowing it to adopt the particular bioactive conformation and hence easy recognition and binding of ligand to the target.

Expert Opinion on Drug Discovery. **2020**, 3;15(5):603-25.
J. Antimicrob. Chemother., **2023**, 3;78(5):1137-42.

Dual Inhibitory activity of Anilinoquinazoline against *Mycobacterium tuberculosis* and the Host TGFBR1

The control on tuberculosis is slowed down due to increasing drug resistance in *Mycobacterium tuberculosis* (Mtb), thus strategies are required that specifically target drug resistance mechanism. A recent report showed that the responsiveness of T-cell is suppressed due to activation of TGFBR in the lungs. This report suggests that an approach to inhibit TGFBR in TB to restore CD4 T-cell function might be beneficial. Recently, a reported molecule, Anilinoquinazoline (AQA), found to be active against replicating, non-replicating, and drug-tolerant Mtb persisters along with inhibition of TGFBR1.



A) Metabolically susceptible sites of AQA. B) AQA analogue with metabolic stability

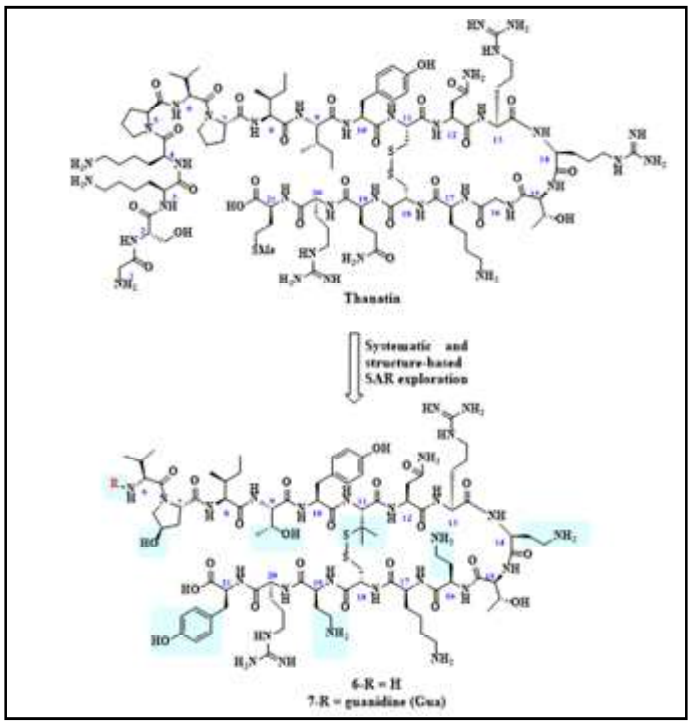
The SAR was established for this dual inhibitor and it was found that the pyridyl-6-methyl group is necessary for potent inhibition however it is also susceptible to metabolism by CYP450. By maintaining dual activity, pyrrolopyrimidine demonstrated balance of all three requirements. Additionally, metabolic stability and pharmacokinetic profiles were improved. Although it did not show the most potent inhibition, it demonstrated a balanced profile across these key

parameters. AQA is a highly intriguing compound with possibly many beneficial anti-Mtb properties in a single molecule. However, further testing is necessary to determine whether AQA has a dual activity against the host TGFBR1 and Mtb that is bactericidal to Mtb, including persisters.

J. med. Chem. **2023** DOI: doi.org/10.1021/acs.jmedchem.3c01273

Peptidomimetic Antibiotics Disrupt the Lipopolysaccharide Transport Bridge of Drug-Resistant Enterobacteriaceae

Antimicrobial resistance due to carbapenem-resistant Enterobacteriaceae (CRE) needs the attention of drug discovery scientists. Hence there is an urgent need to develop novel targets by replenishing antibiotics of standard-of-care (SoC). Lipopolysaccharide (LPS) transport (Lpt) machinery consists of seven Lpt A to G proteins in bacteria. This can serve as a target and can be interfered by naturally occuring peptide thanatin. It is a defense peptide consisting of 21–amino acid and exhibit broad-spectrum antimicrobial activity. Thanatin inhibit LPS transport across the periplasm having high affinity with proteins LptD and LptA. However due to shortcomings like rapid emergence of resistance and poor drug-like properties associated with it, we introduced thanatin-based synthetic macrocyclic peptides by tBu /Fmoc strategy using solid-phase peptide synthesis (SPPS). Disruption of the N-terminal helix in LptAm could be the reason for thanatin lessen binding to mutant LptAm^{Q62L}.



Structure of thanatin and modified antibiotic peptides 6 and 7 obtained through systamic and structure-activity relationship study. Structural modifications are shown in blue.

Screening contrary to a panel of LptA^{Q62L} clones combined with structure-based molecular design aided in recognizing lead compounds 5, 6, and 7 with enhanced antimicrobial potency. *In vitro* activity against Enterobacteriaceae revealed MIC90 values of 1, 0.5, and 8 mg/liter for compounds 6, 7, and thanatin respectively. Compound 7 was bactericidal against XDR and MDR Enterobacteriaceae including colistin and carbapenemase resistant strains with good drug-like pharmacokinetic and ADME properties, *in vitro* activity further translating into potent in vivo antimicrobial activity in infective mouse models. The approach of combining these attractive new antibiotics with standard of care antibiotics or alone could serve as treatment options to combat AMR.

Sci. Adv. **2023**, *9*(21),3683.

NSRS2023 – a report

NIPER-SAS Nagar organized a three days Symposium "NIPER Students Research Symposium-2023 (NSRS-2023)" from 10th to 12th August, 2023. This event was organized on the occassion of the silver jubilee of NIPER Act.

The National Institute of Pharmaceutical Education and Research (NIPER) SAS Nagar is governed by the Parliament act known as NIPER Act. This was enacted in 1998. This act changed many aspects of pharmaceutical sciences and education in India. The contribution of this pioneering decision by Govt. of India is responsible for the transformation of India into "Pharmacy of the world".

- Total number delegates (7 NIPERs): 220
- Research Scholar oral presentations : 26
- Research scholar poster presentation : 88
- Industrial expert presentations : 10
- Academic expert presentations : 28
- Alumini atteded : 25

Panel Discussion on The Role of NIPERs in shaping Pharmaceutical Sciences and Technology in India, deleberated by following panelists. The key suggestions that came out of this Session are:

1. NIPER system has done excellent groundwork in the field of pharmaceutical education -- but it is time for country to reap the benefits of the excellent training given by NIPERs.
2. The research at NIPERs should be able to touch the local problems and should provide possible answers for the local challenges being faced by our country. For example, in Punjab, Cancer is at troublesome levels. Hence, the NIPER, SAS Nagar should shift its focus to anti-cancer drug discovery. Similarly, the antibiotic drug resistance and avirus spread are recent troubles in India.
3. NIPERs should also form a strong relations with medical institutes as well as pharmaceutical industries which can foster the research and innovation in pharmaceutical areas.
4. Considering that the pharmaceutical sciences is addressing the societal issues, the Government agencies may be impressed to promote this applied field in the big way. DoP is rightly positioned to do this pioneering effort, as it has gained extensive experience and it is mentoring a network of NIPERs.
5. The Panel suggetsed for putting efforts on newer and upcomng raeserach area like QbD, AI/ML, bipharmaceticals/biosimilars etc.
6. NIPER should also put efforts in tranining skilled manpower to meet ever increasing demand of pharma industries.
7. During the discussion, it was recongnized that in the past four years, DoP became quite positively active and progressing in the direction. They expressed confidence, when these efforts progress in the direction, DoP may become a larger organization on par with DST and DBT; thus it can prositively impact the healthcare research in India.
8. India is already being considered as a pharmacy of the world, because it is catering to the medical needs of the third world countries. The sincere efforts of the Ministry of Chemicals and Fertilizers are going to provide further impetus and make India a true leader and a pioneer in Pharmacetucal Sciences.
9. NIPER should also improve its interactions with other meritorius organizations in India – the IITs, the autonomous institutions under DST, DBT, CSIR and ICMR.
10. NIPER-S should actively engage industry researchers in their teaching, such that the students get first hand information for the persons who solve the current problems.
11. NIPER-S should also improve international relations with the topmost pharmacy schools of the world.

On Day 2 there was a panel discussion on Current challenges in Pharmaceutical Industries and expectations from Academia.

- Dr Sudhir Sharma, Quantoom Biosciences, Belgium
- Dr C Subbarao, CRIUS life, Solan,
- Dr M Bommagani, Cadila, Ahemdabad,
- Dr Manoj Karwa, Aurigalife Sciences, New Delhi
- Dr Naveen jain, Panacea Biotech.
- Dr Srinivas Lanka, Chairman, APDC-NIPER Mohali

Virtual networking of chemical society to mitigate the chemical problem related to API synthesis, process safety, and process redesigning for cost-effectiveness and optimization, impurity troubleshooting

1. Better affordability of the government-funded Instrument at NIPERSs by reducing the price for measurements for the better affordability of MSME.
2. Establishment of NABL and GMP accelerated labs to help MSME.
3. Establishment of single point support kiosk for industrial cells to troubleshoot the problem of the pharma industry.
4. Showcasing the best research data on the institute's website to attract the industry.
5. Industrial houses spend time on currently active problems and academia focuses on fundamentals. To bring out synergy between these two, it is important to realize the strengths and weaknesses of both the parties.
6. A few MS / PhD research projects at NIPER may be based on current industrial needs in consultation with industrial scientists as co-supervisors.

Faculty members from NIPER Mohali presented their work on process and product development.

During the valedictory function of the NSRS2023 Prof. Panda appreciated the excellent presentations by the research scholars. Prof. Panda advised the researchers have real duty to return to society. The importance of AI tools in Pharmaceutical Sciences was emphasised by many speakers during NSRS2023. Lastly the awards were given to the winners.