Details of the Project sanctioned under the Human Resource Development scheme of Department of Health Research

1. Project Title: "Identification of novel *E.coli* Mur A inhibitors and its application in the clinical isolates"

2. Category of fellowship: DHR/HRD Women Scientist

3. PI (Name & Address): Dr. Farrah Gul Khan
Clinical Microbiology Division Indian Institute of Integrative Medicine (IIIM-CSIR) (Formerly Regional Research Laboratory)
Jammu Tawi – 180 001
Email: fairygk@rediffmail.com

4. Qualifications: PhD

5. Mentor or Co.PI (Name & Address):

Dr. Ram A. Vishwakarma
Director
Indian Institute of Integrative Medicine (IIIM-CSIR)
(Formerly Regional Research Laboratory)
Jammu Tawi – 180 001

6. Duration of the project: 3 years

7. Broad area of Research: Communicable diseases

7.1 Sub Area : Bacterial infections

8. Summary of the Project: (Give in about 300 words)

Both Gram-positive and Gram-negative bacteria are surrounded by a cell wall which protects the cell from destruction by osmotic pressure. It is well established that interference with cell wall biosynthesis is an excellent mechanism for bacterial killing; for example, penicillin and vancomycin specifically interact with the cell wall at different stages of its formation. A major component of the cell wall is a layer of peptidoglycan (murein), which is a polymer of the sugars *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid, and various amino acids.

The bacterial MurA enzyme (UDP-NAG enolpyruvyl transferase), in the first committed step of peptidoglycan biosynthesis, catalyzes the transfer of enolpyruvate from phosphoenolpyruvate (PEP) to UDP-NAG (UNAG), releasing inorganic phosphate.
MurA is conserved across both gram-positive and gram negative bacterial species; gram-negative bacteria have one copy of the *murA* gene, and gram-positive bacteria have two copies . MurA is an essential enzyme in that its deletion from *Escherichia coli* and *Streptococcus pneumoniae* (both copies) is lethal and it has no mammalian homolog. One marketed antibacterial drug, fosfomycin, is a natural product that is a specific inhibitor of the MurA enzyme. MurA is thus an attractive target for antibiotic discovery.
The institute has a library of ~50,000 compounds (both acquired as well in house) These compounds are carefully selected from large number of compounds library and possess drug like properties.. Herein, we propose to screen the library first through in-silico approach such as docking and similarity search through the known inhibitors published in the literature. The MIC of the identified inhibitors will be evaluated in the MDR clinical isolates acquired from various hospitals and characterized in this study. *In-vitro* ADME and cytotoxicity profiling of identified inhibitors will be done to identify the hit compounds.

9. Objectives of the Proposal:

- Cloning of MurA gene in an appropriate vector.
- Standardization of enzyme assay and kinetics of the purified Mur A protein.
- Screening of inhibitors of MurA against clinical isolates.
- *In-vitro* ADME and cytotoxicity profiling of identified inhibitors to select the hit compounds.

10. Innovations in the project:(Give in about 100 words)

We in our endeavour wish to create target based assays so that a chemical library consisting of drug like molecules can be evaluated.
The present study is designed to seek potentially exploitable inhibitors which show the preferences of *E.coli* Mur A. The in-silico screening of 50,000 compounds library will result in the identification of probable inhibitors which may lead to the identification of novel inhibitors in the Mur A enzyme assay. Screening of natural compounds library will give us an opportunity to identify to identify novel the Mur A inhibitor/s from microbial or plant source. The identified inhibitors will profiled for in-vitro ADME and cytotoxicity to select metabolically stable and safe chemical structures for lead optimization.

Identification and characterization of novel scaffolds having Mur A inhibition activity will be further tested against clinical isolates.

11. Significance of the outcome of the project:(Give in about 150 words)

Mur A is an enzyme involved in cell wall synthesis pathway. Such enzymes are considered to be good drug target because integrity of cell wall structure closely correlates to the survival

of bacteria. Mur A is an essential for bacterial survival. The gene knock out mutants of MurA had defective cell wall composition leading to cell death. There is no eukaryotic equivalent of Mur A.

Novel inhibitors of MurA will address the issue of MDR bacterial strains. MurA inhibitors of *E.coli* have shown synergistic activity with other anti-bacterial drugs.

The institute has a compound library of ~50,000 compounds. These compounds are carefully selected from large number of compounds library and possess drug like properties. In-silico studies and similarity search studies may give us the opportunity of selecting novel MurA inhibitors.

12. Relevance in Public Health:

This study is designed to develop a strategy to selectively screen for inhibitors of MurA enzyme in *E.coli* which will give us a library/pool of inhibitors which can be exploited with comprehensive and elegant studies in future as a starting point to develop Mur A enzyme inhibition based anti-infectives.

The rationale behind this study is that we know that as serious infectious diseases and multidrug resistance are emerging repeatedly, new anti-biotics are needed badly to combat these bacterial pathogens. Till date fosfomycin is the only broad-spectrum antibiotic which is the known MurA inhibitors available in market. So we wish to create a pool for the inhibitors of the enzyme which may serve as an antimicrobial drug in future.

Signature of the Fellow

(Dr. Farrah Gul Khan)