P-1

Prime boost immunization strategies using experimental animal (mice) to improve the efficacy of BCG vaccine against Tuberculosis

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Bacillus Calmette Guerin (BCG) vaccine is currently most widely used vaccine against Tuberculosis (TB), however protection afforded by it are insufficient and new vaccination strategies have been investigated since past several years. In our preliminary work carried out in children of different age groups, we found the BCG vaccine provides short term protection which last for period of 10 years. Similarly, in earlier studies we have shown that the repeat BCG stimulation of peripheral blood mononuclear cells (PBMC's) obtained from BCG vaccinated and unvaccinated individuals showed enhanced immune activation compared to single dose. Keeping this in mind the present study was undertaken to evaluate the effect of homologous (BCG/BCG) and Heterologous (BCG/Ag85B, BCG/Ag85B peptide) immunization strategies to improve the immunogenicity of BCG in mice model. Groups of mice were immunized with BCG vaccine and later on boosted with BCG, Ag85B and Ag85 peptide based on analysis of antimycobacterial immune resposne (Anti-BCG, Total IgG, Anti-PPD, IFN-γ, IL-12). Blood was collected every week for analysis of immune markers and also for proteomic analysis. Groups of mice boosted with BCG, Ag85B and Ag85 peptide showed enhanced immune activation as compared to single dose of BCG. Moreover Ag85B peptide induced better boosting potential compared to its booster counterparts Ag85B and BCG. Similarly, from proteomic studies we found number of biomarkers expressed after booster doses with respective candidate molecules that were absent after administration of single dose. Prime boost strategies are effective ways to increase immunogenicity of BCG. Further, peptides in heterologous combination with BCG can act as promising booster candidates. However, further work is needed using experimental model of TB infection to justify the result.

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P-2

A study on Oxidative stress level and Antioxidant status in patients on Anti tubercular treatment (ATT) developing hepatotoxicity in a tertiary care hospital.

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Background: Antitubercular regimen has its own domain of deleterious effect on normal body functions of which liver is hampered most. There are studies suggesting a role of free radicals in development of this hepatotoxicity.

Aim: To compare the Oxidative stress level and Antioxidant status in patients on Category I & II ATT developing hepatotoxicity and those on the same regimen not developing it.

Material and Methods: 30 patients on ATT developing hepatotoxicity were considered as subjects for the study. The oxidative stress level marker, Malondialdehyde and Antioxidants like Glutathione peroxidase, Superoxide Dismutase, Vitamin C were estimated and compared with same age and sex matched controls, after eliminating the history of confounding factors like Acute alcoholic hepatitis, Acute viral hepatitis, Cirrhosis of liver before start of the study.

Results: Hepatotoxicity due to Category I & II ATT were found both amongst male and females. Clinical symptoms ranged from early satiety, nausea to frank jaundice, and biochemically shown many fold rise in Liver enzymes with incidence of Alanine Transaminase (ALT) more than that of Aspartate Transaminase (AST). The results showed a significant increase in the level of oxidative stress and reduced level of antioxidants which may be accounted for the molecular mechanism of hepatotoxicity.

Conclusion: ATT induced liver damage was found considerably amongst the patients on treatment. It leads to rise of Liver enzymes ALT in most of the cases. Hepatotoxicity due to ATT may be attributed due to imbalance of oxidants and antioxidants leading to free radical mediated damage to liver.

Key Words: Oxidative Stress, Antioxidant status, ATT induced Hepatitis.

■ ■ ■

P-3

In silico studies, synthesis and evaluation of DprE1 and AHAS inhibitors for control of Mycobacterium tuberculosis

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Background: Recently decaprenylphosphoryl-β-D-ribose 2´-epimerase (DprE1) and acetohydroxyacid synthase (AHAS) has been identified as promising drug target for development of antitubercular agents.
Aim: This study aims to develop potent benzthiazolylphenylhydrazine carbothiamide derivatives that will act on both the targets decaprenylphosphoryl-β-D-ribose2´-epimerase (DprE1) and acetohydroxyacid synthase (AHAS), thus acting as dual inhibitors.

Material and Methods: The methodology involved in silico studies and synthesis followed by in vitro evaluation for inhibition of M. tuberculosis. In silico studies involved protein preparation for DprE1, AHAS, docking and analysis of docking results. Sixty two substituted (thiazolidine-2-yl amino) benzthiazolylphenylhydrazine carbothiamide derivatives were studied. In vitro evaluation was carried out by modified agar diffusion method.

Results: About 62 compounds were analyzed and synthesized based on molecular docking studies. In case of DprE1 maximum interactions were found with His 132, Asn 385, Gly 133, Leu 134, Leu 363, Val 365, whereas in case of AHAS maximum interactions were shown between Arg 318, Gly 138, Lys 197 and few interactions were shown with Trp 516, Phe 147. All compounds were synthesized in satisfactory yield and structurally elucidated. The range of MIC was found between 40-80 mg/L with percentage inhibition in range of 80-95%.

Conclusion: Our experimental results revealed that newly developed compounds exhibited promising antitubercular activity which can be further explored for development of potent drugs.

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P-4

Ag 85 complex and R_v2623 *Mycobacterium tuberculosis* antigens: Promising marker(s) for the diagnosis of Tuberculous meningitis

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The early diagnosis of Tuberculous meningitis (TBM) is very crucial, since delayed diagnosis can lead to various neurological manifestations. We developed an in-house Indirect Enzyme Linked Immunosorbant Assay (ELISA) for TBM diagnosis using Antigen 85 (Ag-85 B) complex and Dormancy regulon protein (R_v2623 protein). In the present study, we described a prospective evaluation of Ag 85 complex and R_v2623 antigen in the cerebrospinal fluid(CSF) of TBM patients using polyclonal antibodies against Ag 85 complex and R_v2623 antigens. Using the indirect ELISA method, we demonstrated sensitivity and specificity of 84% and 86%, respectively for Ag85 complex and 80% sensitivity and 83% specificity in R_v2623 protein. The detection of Ag85 complex and R_v2623 antigens in the CSF samples of TBM by indirect ELISA is a promising method and can be used to develop an immunodiagnostic assay with increased sensitivity and specificity.

Source of Support- All authors would like to acknowledge Department of Biotechnology, Govt. of India and *Central India Institute of Medical Sciences, Nagpur* for funding the study.

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P-5

Bedaquiiline: A New Approach and Challenge to Multidrug Resistant Tuberculosis

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Objectives: Tuberculosis is a chronic necrotizing granulomatous disease caused by acid fast bacillus. Mycobacterium tuberculosis. In 2012 as per World Health Organization, an estimated 8.6 million people and 450000 people developed TB and MDR- TB respectively and estimated 170000 deaths from MDR-TB worldwide. Current treatment for MDR-TB is highly toxic, costly with low cure rate and long therapy time leading to failure of treatment. Thus, it is a major public health problem and alarms need for better treatment options. In December 2012, the Food and Drug Administration approved Bedaquiline (TMC-207) for MDR tuberculosis.

Mechanism of action: Bedaquiline is adiarylquinoline containing a quinolinic central heterocyclic nucleus with side chains. The chemical name of bedaquiline is 1-(6-bromo-2-methoxy-3-quinoly1)-4-dimethylamino-2-(1-naphthyl)-1 -phenyl-butan-2-ol. Its molecular formula is C32H31 BrN202.Unlike Quinolones which target DNA gyrase; Bedaquiline's unique action inhibits the proton pump activity of mycobacterial adenosine triphosphate (ATP) synthase; an essential enzyme in the generation of energy for M. tuberculosis. It is highly active against both replicating and dormant mycobacteria. Its bioavailability is affected by food and metabolized by cytochrome P-450.

Conclusion: Thus it is crucial to treat MDR- TB with newer advanced, safer and shorter treatment regimens with drugs particularly like bedaquilline being easily accessible and affordable to the community. There is also an immense need for interventional studies to confirm its safety and efficacy.

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P-6

Synthesis and antimycobacterial Screening of Benzimidazoles and new series of benzimidazole derivatives bearing thiophene moiety for various biological activities.

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Various substituted Benzimidazole and new series of benzimidazole derivatives bearing thiophene moiety were synthesized and investigate their biological activities.
The increasing global tuberculosis burden due to the curse of HIV, MDR and XRD TB has led to the search of newer therapeutic agents to tackle the menance.Substituted aryl cinnamic acid derivatives were reacted with O-phenylene diamine to get corresponding Benzimidazole substitutes and further reacted with chloroacetic acid to form 1-carboxy-benzimidazole substitutes then with Thioglycolic acid to form new series benzimidazole derivatives bearing Thiophene moiety were synthesized.
In the present study the structure of compound has been established on the basis of their elemental analysis and spectral data {IR, 1HNMR}. Biological activities (Antitubercular, Antifungal and Antibacterial) were carried out.
Antimycobacterial screening was carried out by using Almar blue assay method,against Mycobacterium tuberculosis $H_{37}RV$ maintained in Middle brook 7H9 medium. Most of the compounds exhibited significant antitubercular activity compared with standard marked drug streptomycin.

Keywords: Antimycobacterial, Benzimidazole, Simple-Fast-Screening.

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P-7

Identification of Inhibitors Against Mycobacterium tuberculosis Thiamin Phosphate Synthase, an Important Target for the Development of Anti-TB Drugs

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Due to increasing resistance against the existing tuberculosis drugs, the importance of developing new, more effective drugs cannot be over emphasized. Thiamin biosynthesis, represents an important metabolic pathway of M.tuberculosis. Thiamin pyrophosphate (TPP), the active form of thiamine (vitamin B1), is a coenzyme for several enzymes and is essential to all known cellular life forms. However, while most microorganisms can synthesize thiamin de novo, animals and many fungi require thiamin or its immediate precursors as a supplement in their diet. Thiamin phosphate synthase (TPS) is involved in the biosynthesis of TPP. *M.tuberculosis* TPS (MtTPS), the product of the last gene (thiE, rv0414c) of thiamin biosynthetic pathway, has been identified as an in vivo essential enzyme for the pathogen and the absence of thiamin transporters in *M.tuberculosis* emphasizes the importance of thiamin biosynthesis for the pathogen's survival. Thus, MtTPS is an attractive target for the development of anti-tubercular

drugs. In this work, we generated a three-dimensional homology model for MtTPS and carried out virtual screening with a library of diverse compounds against the substrate cavity of the enzyme. Further, the shortlisted compounds were evaluated for the inhibition of MtTPS activity as well as the growth of *M.tuberculosis* in broth culture. This study establishes MtTPS as a novel drug target against *M.tuberculosis* leading to the identification of new lead molecules for the development of anti-tubercular drugs. Further optimization of these lead compounds could result in more potent therapeutic molecules against tuberculosis.

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P-8

Whole cell screening based identification of potent inhibitors against the intraphagosomal survival of *Mycobacterium tuberculosis*

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Concerted efforts are required to combine the current knowledge and technology to leverage advantage for the identification of new antitubercular molecules. In an effort to identify lead molecules against *Mycobacterium tuberculosis* H37Rv, we employed whole cell based screening of several libraries leading to the selection of ~40 hits with MIC_{90} 10 µg/ml. 15 compounds further selected on the basis of cytotoxicity in mammalian cells were then subjected to a fluorescence based intracellular assay. Four potent hits exhibited MIC_{99} values of 0.5 µg/ml or less against the growth of *M. tuberculosis* H37Rv and M. tuberculosis Erdman in broth culture and also showed potent inhibition of the intraphagosomal survival of M. tuberculosis. These compounds also inhibited the growth of multidrug resistant strains of *M. tuberculosis* but showed no inhibitory effect on the saprophytic non-pathogenic mycobacteria or other bacterial species such as *E.coli* and their inhibitory effect on *M. tuberculosis* was bactericidal in nature.

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P-9

Repurposing drugs: vROCS based screening & identification of selective antitubercular agents from drug database

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Background: Drug repurposing helps reduce financial burden and time required for approval as it needs only shorter and effective clinical trials as most of the clinical and toxicity profiles of the existing drugs are available. Phenothiazine based antipsychotics were reported to possess antimycobacterial activity. Recently, thioridazine was used for treatment of patients infected with extremely drug resistant strains of *Mycobacterium tuberculosis* on 'compassionate basis' and found to be very effective. This drug was found to act via killing the microbe by arresting its oxidative metabolism and also by activating host macrophaes, a very interesting mechanism. The only problem with this therapy is the unwanted CNS activity, neuronal and cardiac toxicity.

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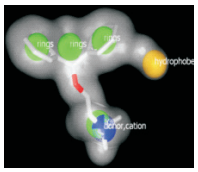
Materials and Methods: An online database of drugs (~ 6000 molecules) were obtained and prepared for virtual screening. A software tool vROCS (Rapid Optimization of Chemical Structures) was used for preparing pharmacophore of thioridazine and screening drug database. A total of 12 chemically and therapeutically diverse drug molecules were selected based on their scorers. All these compounds were submitted for antimicrobial and antitubercular activities.

Results and Discussion: Among the screened drugs paroxetine and triprolidine emerged as potent antitubercular agents with MIC 6.25µg/ml and 12.5µg/ml respectively. Out of the eight active molecules none showed antimicrobial activity at 100µg/ml. Our study identified several approved drugs as selective antitubercular agents.



Diphenhydramine (anti Mtb MIC 25 µg/ml)

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P-10

Evaluation and comparison of IS6110 and duplex PCR assay for rapid diagnosis and simultaneous identification of tuberculous and bacterial meningitis

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Background: Tuberculous meningitis (TBM) and Bacterial Meningitis (BM) are serious complications of central nervous system and continues to result in significant morbidity and mortality worldwide. The etiological diagnosis of Meningitis remains a problem in clinical practice as cerebrospinal fluid (CSF) biochemical analysis findings, cellular responses and clinical manifestation of patients often overlap for different organisms. It is difficult to diagnose these cases due to a lack of rapid, sensitive and specific tests.

Objective: Our aim was to evaluate and compare the polymerase chain reaction (PCR) techniques using primers directed against the IS6110 gene of *Mycobacterium tuberculosis (M. tuberculosis)* for the diagnosis of TBM and a duplex polymerase chain reaction (D-PCR) with primers amplifying portions of the *M. tuberculosis* IS6110 and the eubacteria 16SrDNA gene for diagnosis and simultaneous identification of TBM and BM in a single reaction.

Methods: A total of 150 CSF samples from different groups of patients were selected for the study. The IS6110 and D-PCR was developed and tested on DNA extracted in clinical samples from different categories (TBM n=39, BM n=26, control infectious and non-infectious category n=85).

Results: IS6110 PCR gave a sensitivity of 91.4% and specificity of 75.9% for the diagnosis of TBM. When evaluated in the same set of clinical samples, D-PCR overall diagnosed 100% confirmed TBM and 100% confirmed BM cases with overall specificity of 96.5%.

Conclusions: We conclude that the performance of an in-house IS6110 PCR assay is valuable in the rapid diagnosis of TBM. D-PCR can be an effective tool for diagnosis and simultaneous identification of TBM or BM in a single PCR reaction. It saves time, cost, labour, sample amount and help in administration of appropriate antimicrobial therapy. The proposed diagnostic assays would be helpful in correct and rapid management of TBM and BM patients.

Source of Support- All authors would like to acknowledge Central India Institute of Medical Sciences, Nagpur for funding the study.

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P-11

Molecular modelling and docking study of 5-substituted phenyl-1, 3-dihydro-2*H*-1, 4-benzodiazepines against *Mycobacterium tuberculosis* ribonucleotide reductase (RNR) activity.

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Background: Benzodiazepines, besides their CNS, CVS, anticancer, antimicrobial and other activities is found to control mycobacterial infection. Literature review reveals that benzodiazepines inhibit *Mycobacterium tuberculosis* (M. tb) ribonucleotide reductase (RNR) activity, responsible for the reduction of ribonucleotides to the corresponding deoxyribonucleotides. This RNR activity is required by the bacteria for DNA synthesis and repair at every stage of infection. It is also reported that molecularly diverse coumarins clubbed with benzothiazepines as well as its aza-analogues-benzodiazepines are also active against *Mycobacterium tuberculosis*-ribonucleotide reductase (M. tb-RNR).
Aim: The major goal is to develop and synthesize potential benzodiazepines derivative of proposed nucleus 5-Substituted Phenyl-1, 3-dihydro-2*H*-1, 4-benzodiazepine **(I)**, to act against M. tb-RNR.
Material and Methods: The M. tb-RNR was studied using various *in silico* studies and homology module was developed and evaluated. Derivatives of the proposed nucleus **(I)** were evaluated by carrying out molecular docking at various active sites of the receptors. On the basis of data obtained from these studies the derivatives which have shown valuable results were proposed for further evaluation.
Results: About four out of thirty five proposed derivatives were found to show promising results on the basis of *in silico* studies. These compounds are proposed for synthesis and subsequent *in vitro* studies.
Conclusion: We have successfully applied *in silico* method of drug design and screening for development of novel inhibitors of M. tb-RNR.

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P-12

Multiplex ELISA Kit for Tuberculosis diagnosis: Towards a better tomorrow

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Tuberculosis (TB) remains a major threat to global health from last one decade along with increased incidences of pulmonary TB and extrapulmonary TB (EPTB). AmongEPTB, Tuberculosis meningitis (TBM) is one of the commonest and most serious forms of EPTB. Despite the magnitude of the problem, the general diagnostic outlook is discouraging. In the absence of reliable and cost effective diagnostic methods, a large number of TB patients remain undiagnosed, which may lead to the development of secondary complications and consequently death of the patients. Our study was intended to develop a reliable and rapid diagnostic methodology for detection of *M.tuberculosis*(MTB) antibodies in cerebrospinal fluid (CSF). In our laboratory, we have developed a MULTIPLEX ELISA system for MTB antibodies detection using a panel of MTB $H_{37}R_v$antigens namely Ag 85B, ESAT-6(Early secreted antigenic target 6-kD protein), GroES (Heat shock protein 10kDa), CFP-10 (Culture filtrate protein 10), 45

kD a glycoprotein, Hsp16(Heat shock protein alpha –crystalline). Using this system, we have evaluated CSF, Serum and Pleural fluid from TBM, pulmonary TB and pleural TB patientsrespectively, using suitable controls with an average sensitivity of 85% and specificity of 90%.The Multiplex ELISA Kit we developed is rapid, cost effective and showed acceptable sensitivity and specificity for all kinds of TB and can be adapted as a method of choice in combination with other clinical criteria for the screening of TB infection cases where suspicion is high.

Source of Support- All authors would like to acknowledge Department of Biotechnology, Govt. of India and *Central India Institute of Medical Sciences, Nagpur* for funding the study.

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P-13

Sequence analysis, Homology modeling and binding site identification of Acetyl -CoA acetyltransferase (fadA5) of *Mycobacterium tuberculosis*

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Background: Tuberculosis, a consistent cause of death in developing countries, is emerged up with drug resistance and latency. Cholesterol metabolism in pathogen *Mycobacterium tuberculosis* (Mtb) has taken much attention in last several year as it is proven as essential during Mtb infection and virulence. Therefore, the enzymes, involved in this pathway can be served as new target for Mtb.

Objective: To resolve Tertiary structure of mycobacterium acetyl Co-A acetyltransferase FadA5 (Rv3546 gene), involved in cholesterol catabolic process, by means of Insilco homology modeling and further structural study and probable binding site identification.

Materials and Method: Here, the Modeller program and Swiss-Model server has been employed for comparative modeling of 3D structure of protein. Among the multiple model generated by both tools, the most reliable model was selected on the basis of structural assessment by PROCHECK, QMEAN, Verify3D and Ramachandran Plot. Concurrently, refinement of loops and energy minimization is done by Prime module of Schrodinger suite. Furthermore probable binding sites were identified by SiteMap module.

Results: The selection of template protein is done by analysis of Discrete Optimized protein energy (DOPE) score, Crystallographic R-factor and sequence similarity. The values of E-score, Z-score and verify 3D indicate the superiority of homology model. Furthermore, better steriochemical qualities have been exposed after verified by PROCHECK. Subsequently, feasible binding site on protein surface was identified by Site score, Drug ability score and Hydrophobic-hydrophilic balance.

Conclusion: The necessity of novel therapeutic agents also underlines the need of novel targets. The results of present study suggest that this protein can serve as possible drug target for treating tuberculosis. Further exploration on this protein may lead to the establishment of novel target and design potential inhibitors.

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P-14

Antimycobacterial potential, antioxidant profile and cytotoxic profile of selected Four Indian medicinal plants.

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Background: Indian medicinal plants were studied for their anti mycobacterial, antioxidant, cytotoxicity etc.
Aim: To study Antimycobacterial potential, antioxidant profile and cytotoxic profile of selected Four Indian medicinal plants
Materials and Methods: Antimycobacterial activities were carried out with the nonvirulent strain of *M.tuberculosis* H_{37} Ra. Four medicinal plants with the three solvent extracts of each were screened for their antimycobacterial potential using agar cup well method and MIC were done by the Alamer blue plate assay. Also all the extracts were tested for their antioxidant potential using DPPH and SOR assay and cytotoxicity were done by MTT assay.
Results: From selected plant extracts ethanolic extracts of *Martynia annua* and ethanolic extract of *Cajanus scarabaeoides* shows significant activity up to 35-39 mg/ml against mycobacteria. Chloroform extract of *ziziphus xylopyrus* shows highest radical scavenging activity upto 85%. And there is 2-5% cytotoxic effect of few plant extracts.
Conclusion: Use of the medicinal plants in the therapy of tuberculosis may help to invade the growth of the *M. tuberculosis* and also in other diseases.

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P-15

Phenothiazine – new scaffold for selective antitubercular drug design

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Background: Tuberculosis (TB) is the seventh leading cause of death worldwide. Developemnt of resistance and ability to hibernate in the host made this microbe very tough to eradicate, thus new drugs are needed with novel mechanisms. Thioridazine, an antipsychotic drug, was recently found to have excellent clinical efficacy in the treatment of MDR and XDRTB. But the problem with this drug is its unwanted CNS effects and toxic side reactions. Inspired by these results we made an effort to synthesize phenothiazine analogues to remove CNS activity and retain antitubercular activity using classical QSAR established for the phenothiazine antipsychotic agents.
Materials and Methods: We have synthesized several chalcones and heterocycles possessing phenothiazine and screened them for CNS activity using behavioral studies (actophotometer). The CNS-inactive compounds were screened against a panel of bacteria, fungi (disc diffusion method) and Mycobacterium tuberculosis H37Rv (MABA method). The compounds were also screened for antioxidant activity.
Results: Out of a total 54, seven were found to have antitubercular activity (MIC 12.5µg/ml) and devoid of any CNS activity. These compounds did not show any antimicrobial activity. Chalcones were also found to have antioxidant activity.
Conclusion: Classical structure based design using QSAR resulted in separation of unwanted pharmacological activities and increased the required antitubercular activity. The chalcones also has antioxidant activity, which helps protect liver.

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P-16

Mycobacterium tuberculosis bacterioferritins- Structural and biochemical characterization to facilitate rational drug design

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Bacterioferritin A (BfrA) and Bacterioferritin B (BfrB) are crucial for *Mycobacterium tuberculosis* (*M.tb*) as they store iron in iron rich conditions and make it available to the cell during iron deficiency. Previously, we have shown that these proteins are essential for the growth of *M.tb* and disease progression in guinea pigs making them attractive targets for structure determination and inhibitor design. Here, we report the crystal structures of BfrA and BfrB and show that the characteristic fold of ferroxidase centre responsible for ferroxidase catalytic activity is similar to other ferritins and is represented by a four-helical bundle. However, unlike most other homologs, both mycobacterial proteins have an unique C-terminus which, as verified in the case of BfrB, is involved in ferroxidase activity, iron release and in providing stability to the protein. Site-directed mutational studies identified key residues involved in iron oxidation, metal binding and thermal stability. The analysis of mutants also led to the identification of "interface hot-spot residues" (R69, L129, and F159) that act as "switch points" for BfrB oligomerization and our observations show the importance of 4- fold axis residues in assembly formation. We believe that this study provides a greater understanding of the structure-function relationship of bacterioferritins which would facilitate rational drug design based on their crucial and unique structural features. Besides, the use of ferritin nano-cages as drug delivery vehicles is recently gaining importance and alterations in the properties of these protein by single point mutations, as shown in this study, could prove very useful for the engineering and design of new nanomaterials.

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P-17

Rapid and sensitive loop mediated isothermal amplification assay targeting *sdaA* gene for the detection of *Mycobacterium tuberculosis*

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Background : Tuberculosis (TB) is a public health problem worldwide. The major challenge in fight against TB is lack of rapid, reliable and inexpensive diagnostic test for detection of *M. tuberculosis*.
Material and Methods : In the present study, in-house *sdaA* loop mediated isothermal amplification (LAMP) assay was developed and evaluated with clinical specimens. DNA was extracted from sputum specimens using universal sample processing (USP) method and used as template for nucleic acid amplification. The analytical sensitivity was determined using purified genomic DNA of *M. tuberculosis* and specificity was evaluated using reference strains of *M. tuberculosis* and species of non-tuberculous mycobacteria (NTM).
Results : The in-house developed LAMP assay was highly sensitive and could detect upto 5fg of purified genomic DNA of *M. tuberculosis* and the detection speed of this assay was higher than that of any other isothermal methods reported so far. The *sdaA* LAMP assay showed high specificity (97.2%) and sensitivity (100%) in comparison to culture as gold standard.
Conclusion : Owing to its speed, simplicity, sensitivity and specificity, *sdaA* LAMP assay is a potential diagnostic test for diagnosis of tuberculosis especially in resource limited settings.

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P-18

Integrase-LEDGF interactions inhibitor: Designing and computational docking analysis of novel allosteric inhibitor targeting HIV integrase

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Background: Despite development in Anti-Retroviral Therapy (ART), report of HIV infection remains in continuous momentum and cure to infection seems to be imaginary. Raltegravir, an Integrase (IN) inhibitor provides some life expectancy to patients on salvage therapy. Nowadays, IN inhibitors reported with resistant cases and shows cross resistance to other drugs in this class. Human Lens Epithelium-Derived Growth Factor (LEDGF)/p75 plays vital role in the HIV life cycle.
Aim: The major goal is to approach IN-LEDGF interactions site as a novel target in anti- viral therapy.
Material And Methods: The computational studies involved protein preparation, ligand preparation and energy minimization, grid generation, docking and analysis of results by using Maestro, Discovery studio and VLife Sciences suits. Library of 396 molecules were prepared considering pyrimidine ring as core.
Results: It is known that Ile365 establishes a hydrogen bond with backbone carbonyl group of IN Gln168 whereas Asp366 of LEDGF/p75 forms a hydrogen bond with Glu170, on similar basis it was found that AMP_1071 exhibits hydrogen bonding with Gln95 of one monomer and Gln168, His171 and Thr174 of another monomer of IN.
Conclusion: The designed molecule AMP_1071 shows topological similarity to LEDGF/p75 binding surface. Further antiviral activity, pharmacokinetic and tolerability studies are ongoing. The LEDGF binding inhibitors lacks the cross resistance to any class of ART, possibly making this class as add on to Highly Active Anti-Retroviral Therapy.

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P-19

Isolation of Mycobacteria from Sputum Specimens Using Modified Lowenstein-Jensen (LJ) Media Compared with the Conventional LJ Media

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Background: Processing of sputum for culture of mycobacteria should be done as early as possible for to provide rapid turn around time for reporting growth and identification of MTB Complex. To accomplish these goal modifications of the existing conventional media is one of the important methods to reduce the turn around time for culture reporting.
Aim: To compare the time required for primary isolation of *Mycobacterium tuberculosis*(MTB) on routine Lowenstein-Jensen media and modified Lowenstein-Jensen media (LJ).
Materials and Methods: The LJ medium was prepared according to the WHO guidelines. The DE-LJ *was prepared by adding duck egg solution instead of hen egg in the LJ medium. The DEB-LJ by adding* anti-coagulated human blood in DE-LJ and DET – LJ by adding *tender coconut water instead of mineral salt solution.* Smear positive sputum samples of AFB (+++), (++), (+) were decontaminated using modified Petroff's method and inoculated on to all the media.
Results: A total of seventy specimens were cultured on three modified Lowenstein-Jensen Media and the control. Mean numbers of weeks taken for the mycobacteria to grow on three experimental slants were compared with mean number of weeks on control slants using paired test. The difference in mean number of weeks taken on

supplemented medium was significantly less than that on un-supplemented medium. The P value (<0.001) obtained was significant for DE-LJ and DEB-LJ.

Conclusion: Improved isolation and growth rate is possible if duck egg culture techniques are used in place of conventional egg based culture medium for the recovery of *Mycobacteria*.

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P-20

Evaluation of human & *Mycobacterium tuberculosis* Heat Shock Proteins and toll like receptors induced cytokines in CSF of Tuberculous meningitis patients.

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Among of all extrapulmonary forms of Tuberculosis (TB) , Tuberculous meningitis (TBM) is the commonest form of neurotuberculosis ,caused by *Mycobacterium tuberculosis* (MTB).In the present study, using one dimensional electrophoresis , immunoblotting and LC-MS/MS analysis we have identified MTB Heat Shock Proteins(Hsp) 65 in the cerebrospinal fluid (CSF) of TBM patient. Along with the identified Hsp, host Hsp(s) which were evaluated in the study are Hsp 25 ,Hsp 60, Hsp 70 and Hsp90 whereas MTB Hsp(s) are Hsp16(alpha crystalline R_v2031c) and Hsp 71(R_v0350) derived from MTB strain $H_{37}R_v$. All the Hsp(s) were evaluated in the CSF sample of TBM (n=50), Non TBM infectious meningitis group [Pyogenic meningitis (n=13, Viral meningitis (n=07)] and non infectious control group (n=80) using indirect ELISA. It was observed that the host and MTB Hsp(s) were up regulated in the CSF of TBM patients as compared to other studied group. In addition to that we also investigate the relationship of MTB Hsp(s) with human toll like receptor (TLR) induced cytokines (panel of 12 cytokines) using conventional ELISA in selected TBM patients. Our preliminary studies suggest that MTB Hsp(s) (16, 65 and 71) use diverse TLR to activate pro-inflammatory cytokines such as IL-6 and IL-12. In addition to that it was observed that MTB Hsp 71 activates eotaxin , an eosinophil-selective chemokine in the TBM patients. Further, all the Hsp(s) and cytokines were evaluated in the cell lysate/supernatant of monocytes infected with MTB isolated from different clinical isolates to study profile of Hsp(s) in different strains of MTB. The findings are extremely important for future study with respect to Hsp(s), cytokines and TLR in TBM patients. Overall our findings suggest that Hsp which we have identified in the present study can be evaluated for the diagnosis and also in understanding the pathogenesis of the TBM infection.

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