3. ANTI-TUBERCULAR ACTIVITY

Besides the multi drug resistant Gram-positive bacterial pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VRE), the most infectious *Mycobacterium tuberculosis* H37Rv, a slow-growing Gram-positive bacterium is also a life threatening pathogen and is the etiologic agent of contagious tuberculosis (TB). According to WHO service, multiple-medicine resistant tuberculosis is diagnosed today in an average of 7% patients which can rapidly seriously destabilize to a worldwide epidemiological situation and develop into a global human threat. Therefore, the development of new drugs with activity against multi drug resistant (MDR) TB, extensively drug-resistant (XDR) TB, and latent TB is a priority task, which can shorten the current chemotherapy. The synergy between tuberculosis and the AIDS epidemic as well as the surge of multidrug-resistant isolates of *M. tuberculosis* has reaffirmed tuberculosis as a primary public health threat. The current threat in TB treatment lies in the emergence of strains resistant to two of the best anti-tubercular drugs, isoniazid (INH) and rifampicin (RIF). The current TB-treatment comprises of 3-4 drugs for a period of 6-9 months. Novel drugs are urgently required which can shorten this long-treatment period and target multidrug resistant strains of TB.

*Chemotherapy*

Chemotherapy is the keystone of the management of all forms of tuberculosis in man, ever since Koch announced the discovery of the tubercule bacilli, many attempts have been made to find suitable therapeutic agents for this
disease. The modern chemotherapeutic era began when rich and Follis demonstrated in 1938 that large doses of sulfanilamides produced a beneficial effect on the development of experimental tuberculosis. To be effective in therapy, a drug must interfere with a vital function of the tubercule bacillus without harming the host. The determined efforts of numerous investigations have led to the discovery of streptomycin, INH, Rifamycin and PASA etc. The choice of therapy should be guided by several well-established principles.

- Bacilli must be susceptible to the drug chosen.
- Even in a generally susceptible population of bacilli, a naturally resistant mutant occurs about one in $10^5$ to $10^6$ organism. For this reason at least two effective drugs should always be given to patients with clinical tuberculosis to avoid multiplication of drug resistant mutants.
- When treatment appears to be failing the addition of a single drug in an invitation to disaster. Therapy should always be changed to an entirely new regime of at least two new drugs and great care should be taken to ensure that the patient takes the medication regularly.
- Therapy must be continued long enough to eradicate the bacilli from the body.
- All the medications should be given in a single dose before breakfast to achieve peak concentration of the drug for maximum effect on the bacilli.
- Bacterial drugs should be preferred.

Anti-tubercular drugs are broadly classified as follows:

I) Standard drugs used in initial treatment
   Ex: Streptomycin, INH, Rifamycin, Ethambutol, PAS and Thiacetazome.

II) Reserve drugs used in resistant cases.
   Ex: Pyrazinamide, Ethionamide, Capriopmycin, Viomycin, Kanamycin and Cycloserine.
The development in chemotherapy of synthetic drugs is totally depending upon the biological results of the chemotherapeutic agents, which ultimately give the guidelines to the research worker in the selection of agents for further clinical studies and set the pattern of future drug evaluation.

Considering the above facts, in the present investigations, different benzimidazole derivatives were synthesized and tested for their *in vitro* anti-tuberculosis activity by Alamar Blue Assay method against H$_{37}$Rv strain of *Mycobacterium tuberculosis*.

**Material and methods**

**Anti-TB activity using Alamar Blue Dye**

1) The anti mycobacterial activity of compounds were assessed against *M. tuberculosis* using microplate Alamar Blue assay (MABA).

2) This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.

3) Briefly, 200µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.

4) The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate.

5) The final drug concentrations tested were 100 to 0.2 µg/ml.

6) Plates were covered and sealed with parafilm and incubated at 37°C for five days.
7) After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs.

8) A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.

9) The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

Results and discussion

The benzimidazole derivatives (Iaa'-Icd') were screened for their anti-tubercular efficacy against virulent strain *M. tuberculosis* H37Rv at different concentrations ranging from 0.2 to 100 %. As evident from Table-2 & Fig.2, most of the compounds displayed anti-tubercular activity with good percentage of inhibition amongst (Iaa'-Icd'), was potent showing better inhibition at concentrations 25, 50 and 100%. However, compound Iac' has shown significant activity even at 12.5% concentration. Compounds (Iab'-Icd') were found to be promising against the activity at 25% concentration. All the compounds showed significant activity when comparable with standard Streptomycin at 50% and 100% concentration. Henceforth, this sample offers a prototype lead for further optimization and development.

Conclusions

The data of the anti-tubercular activity screening reveals that the compounds Iac' showed potent activity against *M. tuberculosis* strain, whereas (Iab'-Icd') compounds showed moderate activity at 25 µg/ml.
Table-2

Anti-tubercular activity of synthesized compounds (Iaa'-Icd')

<table>
<thead>
<tr>
<th>Compd.</th>
<th>100</th>
<th>50</th>
<th>25</th>
<th>12.5</th>
<th>6.25</th>
<th>3.125</th>
<th>1.6</th>
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Standard: Streptomycin (100% inhibition);
All compounds tested at concentration of 25 μg/ml
S= Sensitive, R= Resistance
Fig. 2: Anti-TB activity using Alamar Blue Dye