A systematic search for antimycobacterial and antinocardial agents was carried out among non-conventional chemotherapeutics and antibiotics against major pathogenic mycobacteria and nocardioform bacteria. The inhibitory spectrum in in vitro and in some cases in in vivo was determined and their interaction to produce synergism, indifference or antagonism was studied to shortlist new non-conventional chemotherapeutics agents as addition to the arsenal to fight mycobacterial and nocardial agents in clinical medicine.

6.1 A study was undertaken to find out the existence of antimycobacterial activity of methyl-DOPA already well known for its antimicrobial activity against Gram positive and Gram negative bacteria. For this purpose a total of 55 strains of Gram positive and Gram negative bacteria (as control) and 14 strains of mycobacteria were screened using Lowenstein-Jensen medium and Kirchner's silicate medium as the test media. The MIC of m-DOPA with respect to 4 strains of mycobacteria e.g. M. intracellularae, M. flavescens, M. scrofulaceum, M. fortuitum was 100 µg/ml, whereas that of M. smegmatis 1546, 798 was 200 µg/ml on Kirchner's silicate medium. In contrast the minimum inhibitory concentration with respect to the rest of the mycobacteria on Kirchner's silicate medium, and all the 14 strains on Lowenstein-Jensen medium was 200 µg/ml. As far as Gram positive and Gram negative bacteria were concerned, 3 strains of S. aureus, Shigella spp and V. cholerae each, 1 of E. coli and Salmonella, were inhibited at 50 µg/ml; 1 of E. coli, V. cholerae and Salmonella each were inhibited at 100 µg/ml; 6 strains of S. aureus, 1 of P. aeruginosa each were inhibited at 200 µg/ml.

6.2 An interaction of effects between methyl-DOPA (200 µg/disc) and trimethoprim (2 µg/disc for non-mycobacteria and 20 µg/disc in case of M. smegmatis) was studied with respect to a few sensitive (against methyl-DOPA) strains of Gram positive and Gram negative bacteria (as control), as well as with respect to M. smegmatis 1546, by disc diffusion technique of lamanna and Shapiro or by that of Waterworth on Mueller Hinton agar in case of Gram positive and Gram negative bacteria, and on Lowenstein-Jensen medium in case of M. smegmatis 1546.
The result showed that with respect to *V. cholerae* N 17, 540, *Sh. boydii* 5, 7; *S. typhi* 2, *B. subtilis* and *M. smegmatis* 1546 there was an increase (%) of the surface area of the zones of inhibition ranging from 15.6% to 189%, thus suggesting an in vitro synergism.

6.3 Studies on the sensitivity of *Mycobacterium* spp (14) and *Nocardia* spp (3) with respect to augmentin (i.e. amoxycillin : clavulanic acid = 2:1 weight/weight) showed that all strains of mycobacteria except *M. smegmatis* 1546, 798 and *N. caviae* were inhibited at 6.25 µg/ml of augmentin (i.e. breakpoint concentration of amoxycillin) in Kirchner's liquid medium and Emmon's modification of Sabouraud's glucose broth respectively.

6.4 The study of combination of augmentin (3.12, 6.25 µg/ml) with others (in concentrations µg/ml) like cloxacillin (10), cephalexin, carbenicillin (50) with respect to *Mycobacterium* spp. showed that minimum inhibitory concentration of cloxacillin, cephalexin, carbenicillin was not remarkably changed in any of the combinations compared with augmentin alone with respect to any of the strains.

Further, confirmation by disc diffusion technique showed no significant variation of the above findings with respect to augmentin (10 µg/disc) and its combination with other β-lactam antibiotics. It also revealed that amoxycillin, cloxacillin and carbenicillin discs alone do not produce any zone of inhibition, suggesting resistance to them individually.

6.5 The combination study of augmentin with antitubercular agents in their critical concentrations: (µg/ml) INH (0.2), streptomycin (2), rifampicin (1), ethambutol (8) was done by colony counting method on Lowenstein-Jensen medium slants. The combinations were
found to slow down the appearance of resistant colonies by 1 to 2 weeks in almost all the strains of mycobacteria.

6.6 The study of augmentin (10 µg/disc) in combination with antinocardial agents like trimethoprim-sulfamethoxazole (2-40 µg/disc), trimethoprim (2 µg/disc), dapsone (5 µg/disc) was done in Emmon's modification of Sabouraud's glucose broth. *N. caviae* was found to be the most resistant organism (only sensitive to trimethoprim-sulfamethoxazole). The combination did not produce any increase in the zones of inhibition compared with that of single drugs, suggesting an in vitro indifference.

6.7 To explore the antimycobacterial effects of methdilazine 14 strains of mycobacteria were tested against methdilazine in Kirchner's liquid medium. The concentrations (µg/ml) used were 0, 0.12, 0.25, 5.0, 7.5, 10.0, 12.5 and 15.0. All the strains were inhibited at 12.5 µg/ml except *M. smegmatis* 1546, 798. To study the combined effect of methdilazine with streptomycin and methdilazine with rifampicin the minimum inhibitory concentration with respect to different mycobacteria was first determined against methdilazine, streptomycin and rifampicin by tube dilution technique in Kirchner's liquid medium. On the basis of their sensitivity pattern *M. gordonae*, *M. tuberculosis* H$_37$ Rv, K$_1$ and K$_2$ were selected for studying the combined effect of methdilazine and streptomycin; methdilazine and rifampicin by the "checkerboard" dilution technique.

The results reveal that methdilazine and streptomycin produce indifference (Fractional inhibitory concentration index = 1) whereas methdilazine and rifampicin produce antagonistic effects (Fractional inhibitory concentration index = 2).
6.8 Fifteen strains of nocardioform bacteria isolated from leprosy patients and 6 strains from different freeze-dried and animal passaged strains of leprosy bacilli supplied from abroad, were screened for their sensitivity pattern against a battery of commonly used antimicrobials in the following concentrations (µg/ml); streptomycin (50), kanamycin (25), erythromycin (4), trimethoprim-sulfamethoxazole (2-40), doxycycline (4), rifampicin (14), augmentin (10), metronidazole (25), dapsone (5) and against the antitumour agents like cytosine arabinoside (1), 6 mercaptopurine (1), vincristine (2), methotrexate (3), mitomycin C (2), dauxorubicin (2) and antihistaminic, methdilazine (20) on solid gelatin minimal agar and liquid aniline minimal medium. All the strains showed remarkable resistance to all the drugs used so far except against mitomycin C (minimum inhibitory concentration 10 µg/ml). The combination of polymyxin B (16 µg/ml), a cationic detergent, with mitomycin does not change the results whatsoever.

6.9 To determine the in vivo antimycobacterial activity of augmentin, mouse footpad infection was produced by M. marinum (3 x 10^4 colony forming unit) in 20 animals. Twelve animals were treated with augmentin (amoxycillin: clavulanic = 100: 50 µg/gm of body weight x 10 days, per os). Eight animals remained untreated. The mouse footpad thickness following inflammation was measured. The untreated group showed increase (%) of mean thickness 60.26 (standard deviation 15.28) compared with 6.53 (standard deviation 5.48) in the treated group. The result was statistically significant.

Another parameter, that was looked for, was the number of bacteria (colony forming unit/ml) recovered from inflamed footpad of treated and untreated groups in a batch of 6 and 3 (in last 2 weeks) after every week up to 5 weeks. The result was significant at 5%
To study the in vivo antimycobacterial activity of methdilazine, M. tuberculosis H$_{37}$ Rv strain was used to produce systemic infection in mice (20), of which ten animals were treated with methdilazine by intraperitoneal route in a dose of 10 µg/gm of body weight/day x 42 days. The other 10 animals were not given any drug (i.e. control). Macroscopic lesions like tubercles and caseations on viscera, smear examination and recovery of M. tuberculosis H$_{37}$ Rv strain after subculture on Lowenstein-Jensen medium slants from tissue homogenates were looked for and the results showed that in the untreated group of 10 animals, macroscopic (i.e. tubercles/caseation) or microscopic (smear positivity) lesions were detected in each of them and in 5 of them, bacilli of M. tuberculosis H$_{37}$ Rv strain were recovered on subculture. Contrarily, in 10 animals of the treated group, only 2 showed either macroscopic or microscopic lesions, and only from 1 animal, M. tuberculosis H$_{37}$ Rv bacilli were recovered on subculture.

These studies appeared to establish that these drugs (methdilazine and methyl-DOPA) which are not conventionally used antimicrobics can modify the activity of other chemotherapeutic agents in the form of synergism, antagonism, indifference or by producing drug-resistant mutants with respect to some commonly used antibiotics and these themselves have significant antimicrobial properties with scopes for development as powerful antimicrobial derivatives by structural modifications.

The present study shows the importance of β-lactamases of mycobacteria in accounting their resistance to β-lactamas which has
so far been explained on the basis of peculiar cell-wall structure and high mycolic acid with lipid content. The successful combination of clavulanic acid in amoxycillin (i.e. augmentin) with other anti-tubercular agents is a finding of clinical significance which gives scopes to include the large number of β-lactams for treating different type of mycobacteriosis. In this respect its action against other members of nocardioforms is also encouraging.

6.12 A comprehensive review of the literature has been presented in this thesis and the results summerised here have also been discussed in details.