PART V

ANTIMICROBIAL ACTIVITY OF COMPOUNDS PREPARED IN

PART I, II, III AND IV

CHAPTER I

INTRODUCTION

Chemical agents not only provide the structural basis and energy supply of living organisms but also regulate their functional activities. The interaction between potent chemicals and living systems contribute to the understanding of life processes and provide effective methods for the treatment, prevention and diagnosis of many diseases. Chemical compounds used for this purpose are called 'drugs' and their actions on living systems are referred to as 'drug effect'. The subject of drug is as old as disease. Sickness has been man's heritage from the beginning of his existence and search for remedies to combat is perhaps equally old. Fighting disease with drug is the timeless struggle. Its beginning echoed out of premodern jungle. Man's survival on this planet is dependent upon its success.

Antibacterial agents have been used in folk remedies from early times. The earliest source of medicine comes from 'Egypt' and two kingdoms of 'Assyria' and 'Babylonia'. The 'Papyri' were the first written account of medical experiences from Egypt. The papyrus discovered by Eder in 1872 was prepared in 1500 B.C. and mentions about 700 herbal
medicines. A Babylonian clay-tablet (700 B.C.) has been discovered, which mentions about 300 drugs.

Modern medicine is considered to date from 'Hippocrates', a Greek physician (450 B.C.), who for the first time introduced the concept of disease as a pathologic process and tried to organise the science of medicine on the basis of observation, analysis and deduction.

Till the beginning of 19th century the treatment of disease consisted of obnoxious remedies such as flesh, excreta, and metallic and plant preparations. James Gregory (1733-1821) was responsible for popularising heroic symptomatic treatment consisting of blood letting, large doses of emetics and drastic purgatives often with disastrous results. Such treatment without any rational basis was called 'Allopathy' (meaning the other suffering).

The concept of 'Homeopathy' was first introduced in the early 19th century of 'Hanneman' who thought that 'like cure like' and dilution potentiates the action of drugs. Homeopathy outlines the therapy for various ailments with drugs in very high dilution.

Various animal experiments have been designed to study the effects of drugs on living organisms and isolated tissues and these give an insight into where and how a drug acts. Knowledge of the mode of action of drug, its effect on
various body systems and the probable adverse effect is important.

Pasteur and Koch established that, microorganisms were the cause of infectious diseases. Paul Ehrlik was the first to propose that infectious disease might be curable by using chemicals that inhibit or kill the infectious agent but do not harm the host at the concentration employed. He discovered the famous organoarsenical compound 'Salvarsan' which was active against the causative organism of 'Syphilis'. It was he who used the term 'chemotherapy'. According to his theory of drug action, cell possess chemical receptors to which the drug binds. He recognises the importance of quantitative measurement to determine the drug dose, that would be effective against the causative agent and not have toxic effect on the host. He also pioneered methods for screening a large number of compounds for biological activity in relation to chemical structure. Chemical variants of effective compounds were then synthesised and tested to see whether they have improved antimicrobial activity and reduced toxicity.

REVIEW OF THE LITERATURE

The world's oldest pharmacological writing come from 'India' and 'China'. The great herbal or chinese 'materia-medica', 'Pan Tsaen' was probably written in 2735 B.C. The earliest Indian records are the 'Vedas'. Although there are medical discriptions in 'Rigveda' (2500-3000 B.C.), it was
'Charaka' renowned ancient Indian physician and later 'Sushutra' and 'Vagbhat' who discovered various medical preparations included in 'Ayurveda', 'the science of life'. Initially these consisted mostly nonpoisonous vegetable drugs and minerals. Thus, Charaka described about 300 vegetable drugs and classified them according to their effects, mostly on symptoms into fifty groups.

Development of modern pharmacology as a science is fairly recent and probably started taking shape following the introduction of experimental procedure by Francois Magendie (1783-1855) and Claude Bernard (1813-1878).

Spectacular developments in physiology, biochemistry and organic chemistry during the recent years have greatly accelerated the advance in pharmacology.

Literature shows that much work has been done over many heterocyclic compounds of their antibacterial activities including both gram positive and gram negative bacteria. Chalcones and their derivatives are reported to have antibacterial\(^4\), antifungal, antiparasitic, antitubercular, anti-inflammatory and insect repellent properties\(^2,3\). Chalcones were known to have bactericidal and fungicidal properties\(^4,5\). Bhatt et al.\(^6\) synthesised quinoline derivatives of chalcones and screened the products for antibacterial activities. Ahluwaliya and coworkers\(^7\) screened in vitro dihydrochalcones and their derivatives against some microbial organisms.
Pyrazolines were known to have bactericidal, fungicidal and insecticidal properties. Some pyrazolines are also reported for antiinflammatory, antidiabetic, anaesthetic and analgesic properties. Ozawa and coworkers worked on pyrazolines and found them effective in killing house flies on contact. The insecticidal properties of pyrazolines was studied in 1982 by Von Hees and coworkers. Pyrazolines and derivatives of pyrazoline also possess antibacterial activity. Roda and coworkers synthesised some new pyrazoline derivatives and tested them for antimicrobial activity. Recently, pyrazolines and its derivatives shows biological activity. Isoxazolines are also reported to have antibacterial and antifungal activity.

Like Pyrazolines and Isoxazolines, Pyrazoles and Isoxazoles also synthesised and tested for antibacterial activity. Sharma et al., reported that hydroxy arylpyrazoles were found to be effective antimicrobials. The antifungal nature of 1-substituted 3-(2-hydroxyphenyl)-5-(4-nitrophenyl) pyrazole was studied by Giri, while trifluoromethyl-1-arylpyrazole was reported to be analgesic, antipyretic and antiinflammatory agents. Anderson and Paolella showed that 1-phenylpyrazole derivatives are effective antidiabetic while Faucher reported that (phosphonadiathioacetamido)phenylpyrazoles are good insecticides. Some pyrazoles have found to possess antidiuretic, antifungal, antibacterial and antiauxthalminic properties.
Chlorosubstituted isoxazoles\textsuperscript{35-37}, also possess antibacterial activity. Sharman et al.\textsuperscript{38} synthesised chlorosubstituted isoxazole derivatives as very effective antibacterial agents.

Thiourea and related compounds exhibits antithyroid activity. The more useful drugs have been found to be derivative of 2-thiouracil and 2-thioimidazole. Propyl-thiouracil (6-propyl-2-thiouracil) and methimazole (1-methyl imdazole-2-thiol) are known to be important antithyroid drugs, 1,3-Thiazines act as potential fungicidal\textsuperscript{39}.

**ORIGIN OF THE PROBLEM AND PROBLEM**

From the review of literature it is quite evident that chlorosubstituted heterocycles have antibacterial, antifungal, antiparasitic and insecticidal activity. From the literature it was also expected that chlorine substituted heterocyclic compounds must have really a high antibacterial power as compared to it's iodo and bromo-substituted heterocyclic compounds.

Literature survey indicates that a very few work has been done for determining antimicrobial activities in case of nitrosubstituted heterocyclic compounds. Hence, it was thought interesting to study antimicrobial activity of nitrosubstituted heterocyclic compounds: like nitrochalcone, nitropyrazoline, nitroisoxazoline, nitroisoxazole, nitropyrazole and nitrothiazine.
The work presented in this part deals with the study of antimicrobial activity of nitrosubstituted heterocycles synthesised by us against different test organisms namely, *Salmonella typhi*, *Salmonella paratyphi*, *Proteus vulgaris*, *Xanthomonas* spp., *Fusarium solani*, and *Botrytis cinerea* to cover antibacterial and antifungal class.

The following compounds were tested.

1. 2'-Hydroxy-3'-nitro-5'-methyl-3-nitrochalcone
2. 2'-Hydroxy-5'-methyl-3-nitrochalcone
3. 2'-Hydroxy-5'-chloro-3-nitrochalcone
4. 1-H-3-(2-hydroxy-3-nitro-5-methylphenyl)-5-(3-nitrophenyl)-2-pyrazoline
5. 1-H-3-(2-hydroxy-5-methylphenyl)-5-(3-nitrophenyl)-2-pyrazoline
6. 1-H-3-(2-hydroxy-5-chlorophenyl)-5-(3-nitrophenyl)-2-pyrazoline
7. 1-Acetyl-3-(2-hydroxy-3-nitro-5-methylphenyl)-5-(3-nitrophenyl)-2-pyrazoline
8. 1-Acetyl-3-(2-hydroxy-5-methylphenyl)-5-(3-nitrophenyl)-2-pyrazoline
9. 1-Acetyl-3-(2-hydroxy-5-chlorophenyl)-5-(3-nitrophenyl)-2-pyrazoline
10. 1-Benzoyl-3-(2-hydroxy-3-nitro-5-methylphenyl)-5-(3-nitrophenyl)-2-pyrazoline.
11. 1-Benzoyl-3-(2-hydroxy-5-methylphenyl)-5-(3-nitrophenyl)-2-pyrazoline.
12. 1-Benzoyl-3-(2-hydroxy-5-chlorophenyl)-5-(3-nitrophenyl)-2-pyrazoline.
14. 1-Phenyl-3-(2-hydroxy-5-methylphenyl)-5-3-nitrophenyl)-2-pyrazoline.
15. 1-Phenyl-3-(2-hydroxy-5-chlorophenyl)-5-(3-nitrophenyl)-2-pyrazoline.
16. 3-(2-Hydroxy-3-nitro-5-methylphenyl)-5-(3-nitrophenyl)-isoxazole.
17. 3-(2-Hydroxy-5-methylphenyl)-5-(3-nitrophenyl)-isoxazole.
18. 3-(2-Hydroxy-5-chlorophenyl)-5-(3-nitrophenyl)-isoxazole.
19. 3-(2-Hydroxy-3-nitro-5-methylphenyl)-5-(3-nitrophenyl)-isoxazole.
20. 3-(2-Hydroxy-5-methylphenyl)-5-(3-nitrophenyl)-isoxazole.
21. 3-(2-Hydroxy-5-chlorophenyl)-5-(3-nitrophenyl)-isoxazole.
25. 4-(2-Hydroxy-3-nitro-5-methylphenyl)-6-(3-nitrophenyl)-2-imino-6H-2,3-dihydro-1,3-thiazine.
26. 4-(2-Hydroxy-5-methylphenyl)-6-(3-nitrophenyl)-2-imino-6H-2,3-dihydro-1,3-thiazine.
27. 4-(2-Hydroxy-5-chlorophenyl)-6-(3-nitrophenyl)-2-imino-6H-2,3-dihydro-1,3-thiazine.
28. 4-(2-Hydroxy-3-nitro-5-methylphenyl)-6-(3-nitrophenyl)-2-iminophenyl-6H-3-phenyl-1,3-thiazine.
29. 4-(2-Hydroxy-5-methylphenyl)-6-(3-nitrophenyl)-2-iminophenyl-6H-3-phenyl-1,3-thiazine.
30. 4-(2-Hydroxy-5-chlorophenyl)-6-(3-nitrophenyl)-2-iminophenyl-6H-3-phenyl-1,3-thiazine.
31. 4-(2-Hydroxy-3-nitro-5-methylphenyl)-6-(3-nitrophenyl)-2-iminophenyl-3,6-dihydro-1,3-thiazine.
32. 4-(2-Hydroxy-5-methylphenyl)-6-(3-nitrophenyl)-2-iminophenyl-3,6-dihydro-1,3-thiazine.
33. 4-(2-Hydroxy-5-chlorophenyl)-6-(3-nitrophenyl)-2-iminophenyl-3,6-dihydro-1,3-thiazine.

The organisms selected are-

2. *Salmonella paratyphi* - Causative agent of paratyphoid
3. *Proteus vulgaris* - Commensal in intestine and present on skin.

4. *Xanthomonas spp.* - Plant Pathogen

5. *Fusarium solani* - Plant Pathogen causing wilt disease.


**SUMMARY OF THE WORK**

The biological activities of nitrosubstituted heterocycles were studied on different test organisms namely *Salmonella typhi, Salmonella paratyphi, Proteus vulgaris, Xanthomonas spp., Fusarium solani*, *Botrytis cinerea* to cover antibacterial and antifungal class.

From the results, it has been observed that thiazines containing nitrogen and sulphur are most active and also nitrogen-oxygen heterocycles are more active than nitrogen-nitrogen heterocycles. The activity order was found to be

\[ N, S > N, O > N, N \]

The presence of nitrogroup increases the activity and increase in activity is also related to number of nitrogroup. However, if chlorogroup is introduced in the structure, the increase in activity is more. The order was found to be

\[ \text{NO}_2, \text{Cl} > \text{NO}_2, \text{NO}_2 > \text{NO}_2 \]

It has been observed that aromatic rings are more active than non-aromatic ring. This is possibly, due to more
stability and hence, prolonged activity of the compounds.

Thus, the relationship between the structure and activity of the compounds are established and plausible explanation suggested.