

CHEMOTHERAPY OF TUBERCULOSIS
SYNTHESIS AND STUDY OF ORGANIC COMPOUNDS AS POSSIBLE
ANTITUBERCULAR AGENTS

THESIS SUBMITTED
UNIVERSITY OF BOMBAY

FOR THE DEGREE OF
MASTER OF PHARMACY
IN
PHARMACEUTICAL CHEMISTRY

SUBMITTED BY
CHETANA N. DESAI

UNDER THE GUIDANCE OF
DR. B. G. KHADSE

HAFFKINE INSTITUTE FOR TRAINING, RESEARCH & TESTING
BOMBAY

340

1987

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CHEMOTHERAPY OF EXPERIMENTAL MALARIA

**Synthesis and Evaluation of
Dihydro - s - Triazines as Possible Antimalarial
Agents**

A THESIS

Submitted to

The University of Bombay

For

The Degree of

MASTER OF PHARMACY

FACULTY OF TECHNOLOGY

in

(Pharmaceutical Chemistry)

by

Zarine N. Khety

B. Pharm.

Department of Chemotherapy

HAFFKINE INSTITUTE

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THIS THESIS IS DEDICATED TO.....

..... the Circumstances which inspired
me to aspire.....

..... My Teacher who helped realize
these aspirations.....

..... My Parents, sisters and a
friend who sustained these
aspirations during the period.....

..... MISS ZARINE

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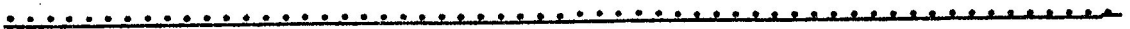
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PART - I

I N T R O D U C T I O N



INTRODUCTION

From times immemorial malaria has been one of the most prevalent of human diseases, affecting particularly the populations of the tropical regions but also in the recent past those of temperate climates. It is also one of the oldest infections mentioned in the early writings in Egypt, India and China. Its clinical symptoms were fully described by Hippocrates 400 years, before the Christian era.

The social, economic and political impact of malaria over the centuries has been severe. (There was no effective treatment for the disease until quinine the active constituent of the cinchona bark was discovered in the seventeenth century). The concept of malaria eradication which has been developing since the early 1950's when residual insecticides were introduced in many fields of public health, was endorsed by W.H.O. and given an integrated plan in 1956. But just as it seemed to be showing good progress, a new and menacing event in the history of chemotherapy of malaria occurred in 1960 with the observation of resistance of P. falciparum to chloroquine the most widely used drug in chemotherapy. (Fig. 1 gives the current data on distribution of malaria and chloroquine resistance). Simultaneously the frequency of

The present geographical distribution of chloroquine and multiple resistant P. falciparum in relation to the overall areas of endemicity is summarized in following figure (Fig. 1)

(Ref:- W.H.O. Report; Problems with presently used antimalarial drugs, 1983).

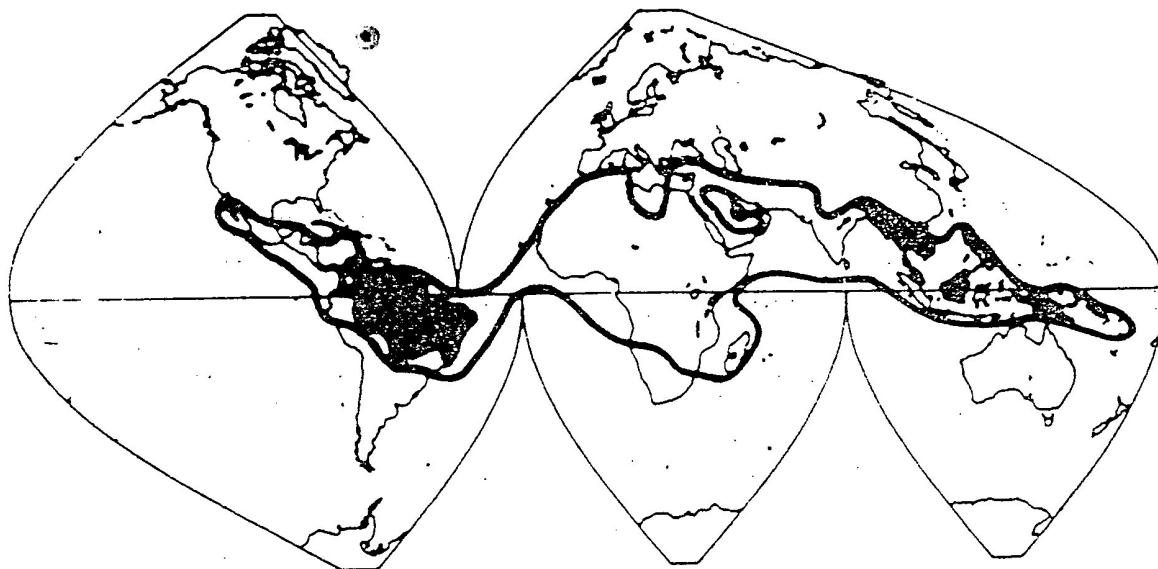


Fig. 1.1

Distribution of malaria — (solid line) and chloroquine-resistant P.falciparum (black shading). (Note foci in East Africa, Madagascar and India).

reports on the resistance of human plasmodia to proguanil and pyrimethamine also increased. The Scientific Group was thus concerned about maintaining the efficacy of the antimalarial drugs.

The global malaria situation throughout the world causes increasing concern today. This raises a major question i.e. "Do the currently available drugs fulfil all the needs of individual or community malaria prevention and control. If not, why not ?" The reasons are both technical and logistic. Since, solely antimalarial drugs make it possible to pursue the eradication steps, in areas where other antimalarial measures cannot be applied for technical, operational or financial reasons; it is most important to rationalize the use of current antimalarial drugs and search for newer agents. A series of reports from the W.H.O. discuss the many facets of the global problem.^{1a,b.}

With the realization that the world-wide eradication of malaria is not an attainable goal in the near future given existing resources, many countries have tried to develop antimalarial programmes adopted to their local conditions.

REVIEW OF LITERATURE

World Malaria Situation in 1984 :

In 1984, 5.3 million cases of malaria (provisional figures) were reported, compared to 5.6 million in 1983 and 6.5 million in 1982. Though the figures indicate a decrease in world malaria situation, the global figures are strongly influenced by the number of cases reported from China and India, together comprising about 56 % of the world population, where malaria cases have increased substantially.²

The S.E. Asian region has shown a two fold increase in the last 3 years.

Of a total world population of 4,51 million, about 1311 million (28 %) live in areas where malaria either never existed or disappeared without specific antimalaria measures. Some 776 million people (16 %) live in areas where the disease has been eliminated during recent decades by the general improvement of health services. Nearly half the world's population, 2266 million people 48 % live in areas where antimalaria measures are carried out.² Some 398 million (81 %) inhabit areas where no specific measures are taken to control malaria transmission and where the prevalence of malaria remains virtually unchanged.

Malaria in India :

India has always been in the forefront of the battle against malaria. The Indian subcontinent reported 6,00,000 cases of malaria during 1984-85. The eradication programme in 1958 paid rich dividends and by 1965 the incidence of the disease had declined to 0.1 million cases with no deaths against the background of 75 million cases with 0.8 million deaths, annually in the pre-independence era. In Bengal alone almost 3 lakh abortions of mothers afflicted with malaria used to occur every year.³

However, due to technical, economical and administrative reasons and near cessation of research in malariology reversal of good results started and by 1976 there were over 5.8 million cases with 57 deaths due to malaria. Nearly 75 % of the central health budget was spent on malaria control in 1976-77. This work out to be Rs. 33 crores a year which is being doubled presently to nearly Rs.66 crores per annum.⁴

Mainly the money is used to provide insecticides and antimalarials in areas which are badly affected. The emphasis is on antimalarials which are used for mass chemoprophylaxis. For a developing country like India this is an enormous investment in fighting a single communicable disease, indicating the seriousness of the problem.⁴

BIOLOGICAL PRINCIPLES :

The Vector of Human Malaria-Anopheles :

Approximately 3000 species of mosquitoes are known and some 400 belong to the genus *Anopheles*. Of these only about 100 transmit malaria throughout the world in the primates including man. The life cycle of human malaria parasites is confined to man and the female anopheline mosquito.⁵ In India only 10 species are regarded as vectors.⁶

The Malaria Parasites and the course of infection :

Malaria parasites are intracellular protozoans whose life cycle involves. 1) An asexual development in the vertebrate host, and 2) sexual development in mosquitoes. (Fig. 2).

Human malaria is generally caused by four species of parasites. *P. vivax*, *P. falciparum*, *P. malariae* and *P. ovale*. In India it is caused by the first three species. Of all the four species causing human malaria *P. falciparum* and *P. vivax* account for almost 95 % of the prevalent cases. Amongst the malaria positive cases in India in 1975; 85.69 % were *P. vivax*; 14.12 % *P. falciparum*; 0.2 % *P. malariae* and 0.17 % mixed infections.⁴

The antimalarial drugs are commonly classified according to the stages of the parasite cycle upon which they act as follows (Fig.2):-

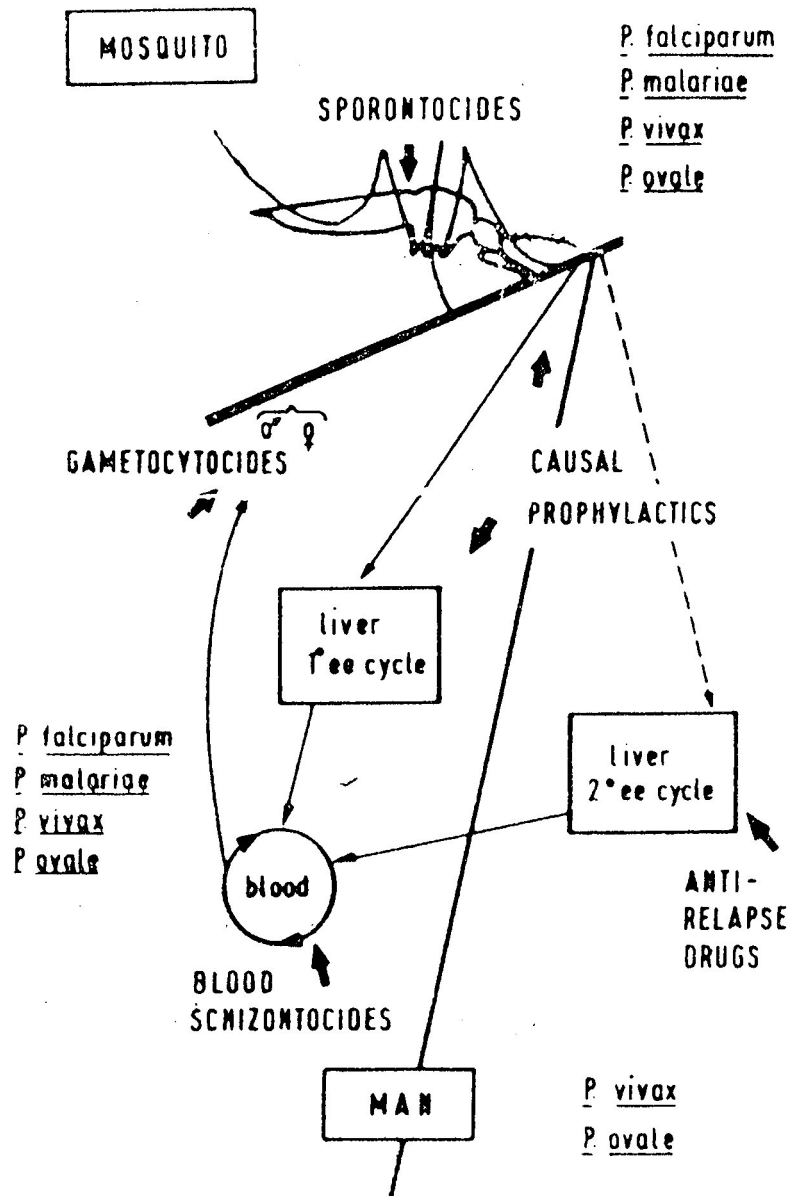


Fig.2 Sites of action in life cycle of malaria parasites.

P. falciparum is the most virulent and is often called malignant tertian malaria or pernicious malaria. If not promptly treated it proves fatal usually to non-immune persons. A small percentage of these patients suffer from cerebral malaria. Vivax infected malaria is also called benign tertian malaria, it evokes milder clinical attacks and has a low mortality rate. Quartan malaria caused by P. malariae and that caused by P. ovale are geographically unevenly distributed and are milder forms.

In addition to the four species infecting humans, there are plasmodia that infect apes, monkeys, rodents, birds, lizards and frogs. The development of malaria parasite in other vertebrates, such as birds and rodents, is similar but not identical to that of man.⁷

HURDLES IN MALARIA ERADICATION :

(A) Resistance to Plasmodial Drugs :

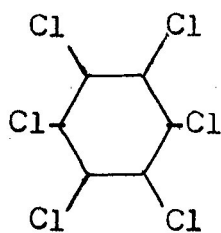
Drug resistance has been defined as the ability of a parasite strain to multiply or to survive in the presence of concentrations of a drug that normally destroy parasites of the same species or prevent their

multiplication. Such resistance may be relative (yielding to increased doses of the drug tolerated by the host) or complete (withstanding maximum doses tolerated by host).⁸

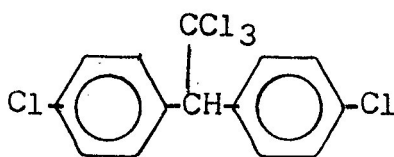
This definition applies for all species and all development stages of malaria parasites specially the blood schizonts. The resistance of P. falciparum to quinine was reported at the beginning of this century from Brazil, long before the advent of synthetic anti-malarials. Resistance to Proguanil and Pyrimethamine first occurred in the early 1950's shortly after their wider use for suppressive effects. However, the almost simultaneous occurrence of chloroquine resistance in 1960 threatened to invalidate the most useful drug. Recently resistance to the second line of defence i.e. sulfadoxine. pyrimethamine combination and the recent introduction, Mefloquine have spurred drug research to a large extent.⁸

(B) Resistance of the Vector to Insecticides :

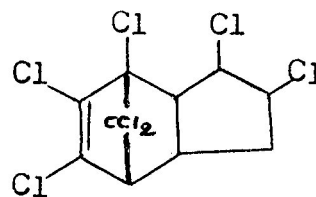
Resistance to insecticides has been defined as the development of an ability in a strain of insects to tolerate doses of toxicants which are lethal to the majority of individuals in a normal population of the same species.



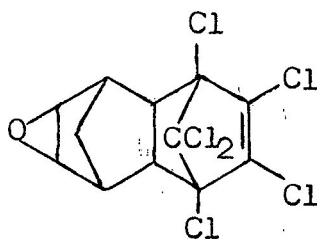
γ -HCH



DDT



Chlordane



Dieldrin

Resistance shown by mosquitoes carrying malarial parasites to DDT and allied insecticides has become a potent factor contributing to the spread of malaria.

(C) The genetics of drug resistance :

This topic was investigated by Bishop⁹ in Cambridge and Beale¹⁰ and his colleagues in Edinburgh who made extensive use of avian and rodent malaria models.

All investigations have shown that resistance to sulfonamides, pyrimethamine and chloroquine is transferred by multiple genetic mutations. It is assumed that under

multiple drug pressure, once able to survive the presence of each compound, the parasites compensate for metabolic deficiencies created by the drugs by stimulating their host cells to increase the production or transport of the missing metabolites. Thus the continuing growth of the parasites under multiple drugs may be dependent upon a common factor in the new host-parasite relationship.¹¹

EVALUATION OF COMPOUNDS FOR ANTIMALARIAL ACTION :

Advancing chemotherapy depends to a large extent on adequate measures for the evaluation of candidate compounds.

A variety of procedures are available for evaluating drugs in experimental animals, depending on the phase of life cycle and the type of infection being investigated.

The experimental infections used most in chemotherapeutic research have progressively shifted. In the 1920's P. relictum or P. cathemerium which cause plasmodial infections in birds were chiefly used. The works of Roehl¹² using P. relictum demonstrated the successful use of pamaquine on avian malaria. P. lophurae in ducklings or

P. gallinaceum in chicks became available in the late 1930's. An excellent discussion of the evaluation of antimalarials is given by Davey¹² and more recent work by Thompson and Werbel¹⁴ and Peters.¹⁵ Test systems are still being refined and developed.^{16,17}

During the late 1940's the rodent parasite P. berghei became available for experimental use. The primary test system employed by the U.S. Army Antimalarial Drug Development Programme is the Rane's method. It utilizes P. berghei in mice and is described by Osdene et al.¹⁸ The most recent development is the current work on the infection of lower primates with plasmodia of man.

Thus the more promising antimalarial drugs in use today have evolved from evaluations using avian, monkey and rodent malarias.

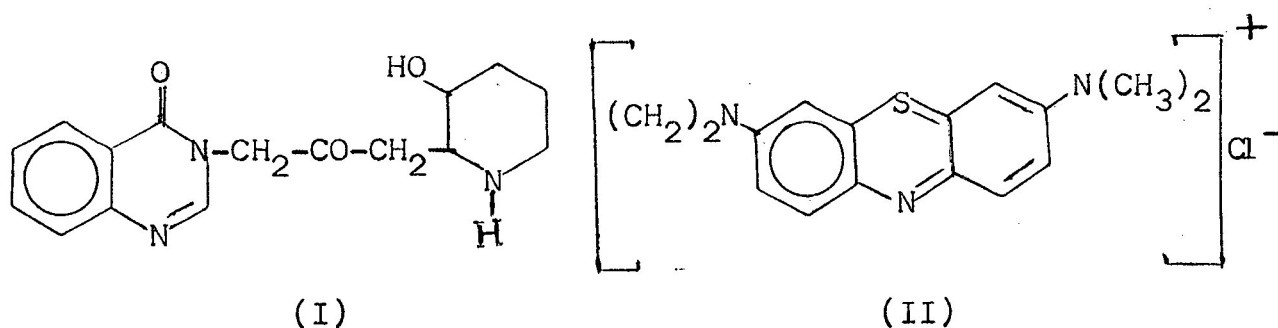
FUNDAMENTAL ASPECTS OF THE CHEMOTHERAPY OF MALARIA :

Historical development :

The story of malaria is as old as man but not until 1880 did Alphonso Laveran a french army surgeon definately establish a Plasmodium as the causative agent.

The oldest remedy for malaria known is the chinese drug Ch'ang Shan which was prepared from powdered roots of *Dichroa febrifuga*. The most active principle febrifugine (I) was isolated by Jang and co-workers in 1946,¹⁹ which was found to be highly active, but also highly toxic.²⁰

In 1891 Guttman²¹ discovered the dye methylene blue (II) which had slight antimalarial activity. Quinine the most valuable drug was isolated from the bark of the *Cinchona* trees and its structure was elucidated about a hundred years later.²² The extensive use of the alkaloids quinine and cinchonine from these barks led to the near extinction of the trees in the midnineteenth century. This led to the development of synthetic antimalarials.



The main concepts that guided Ehrlich in his search for potent chemotherapeutic compounds were -

1. that the drug in a single dose should destroy all parasites.
2. that of chemotherapeutic index.

Based on this during the Second World War extensive antimalarial programmes were started.

The efficacy of an antimalarial drug depends not only on its specific action on a given species and strain of malarial parasite but also on such host-related factors as the speed of absorption, degree of concentration in plasma and the erythrocytes, the localization in the tissues and the rate of degradation and excretion. Thus the triad of the drug, the parasite and the host must be considered.

Depending on the biological spectrum of activity and chemical structure antimalarials can be broadly classified as :

(1) Fast Acting Blood Schizontocides :

- i) Amino Alcohols : Cinchona alkaloids.
- ii) 9-Amino acridines : Mepacrine.
- iii) 4-Aminoquinolines : Chloroquine.

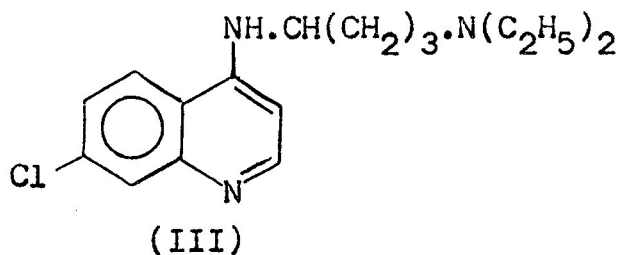
i) Amino Alcohols :-

Cinchona trees contain a mixture of about 10 alkaloids. The most important being quinine, quinoline, cinchonidine and cinchonine.

Quinine has the greatest antimalarial action and is the oldest of all the therapeutic agents used against malaria. Its total synthesis was accomplished almost a 100 years after its isolation by Woodward et al.²³

ii) 9-Amino Acridines :-

The first synthetic antimalarial drug with blood schizontocidal activity was discovered in Germany in 1932 and was called Atebrin (Mepacrine)(III). This drug was found very active, against P. berghei in mice.

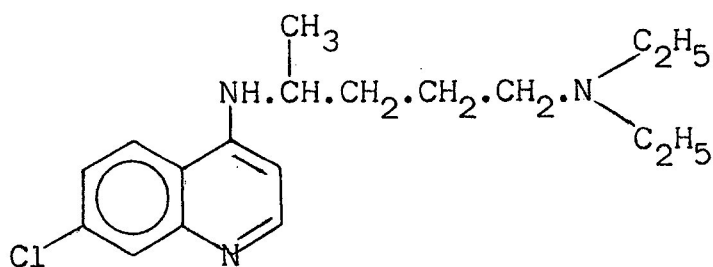


Structurally this drug encompasses the 6-methoxy-4-quinolyl moiety and the basic dialkylaminoalkylamino side chain of Pamaquine. A number of 9-alkyl (or aryl) amino acridines having different substituents in the quinoline moiety were found to be devoid of activity.²⁴ Quinacrine is highly active against asexual blood stages of plasmodia and against the gametocytes of P. vivax and P. malariae.²⁵

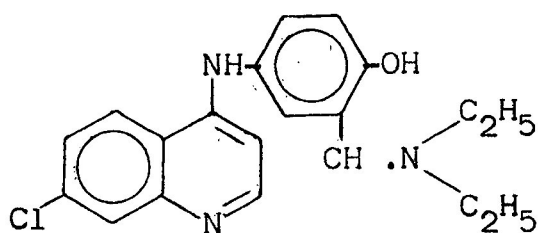
iii) 4-Aminoquinolines :-

The presence of the quinoline ring in the structure of quinine and mepacrine and therapeutic properties of the 8-aminoquinolines proved as precursors of further studies on antimalarials and led to the discovery of 4-aminoquinolines. Important and widely used drugs of this class includes chloroquine (IV), amodiaquine (V), and amopyraquine (VI).

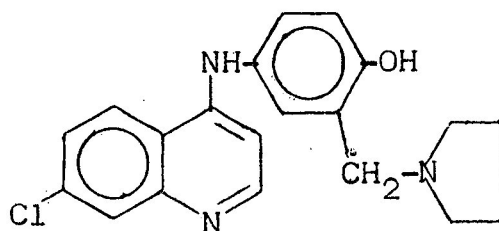




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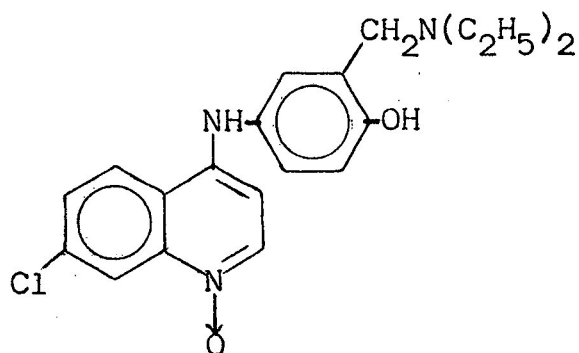
(V)



(VI)

The 4-amino quinolines chiefly chloroquine are drugs of choice for the treatment of acute attacks of plasmodia. They achieve a radical cure in non-resistant strains of P. falciparum.

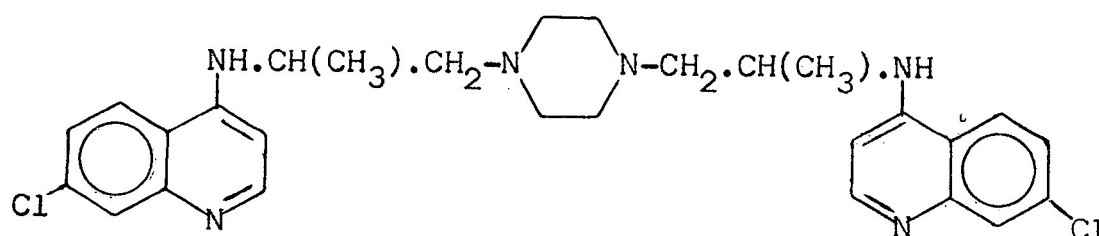
Elslager and co-workers²⁶ have reported the synthesis and activity of amodiaquine-1-oxide (VII) related N-oxides.



(VII)

Preliminary testing shows them to be approximately 5 times more active than amodiaquine against P. berghei in mice.

A respository form VIII in the 4-aminoquinoline class was synthesized in France,²⁷ which at a single dose of 500 mg/kg gave complete protection against P. berghei upto 60 days.

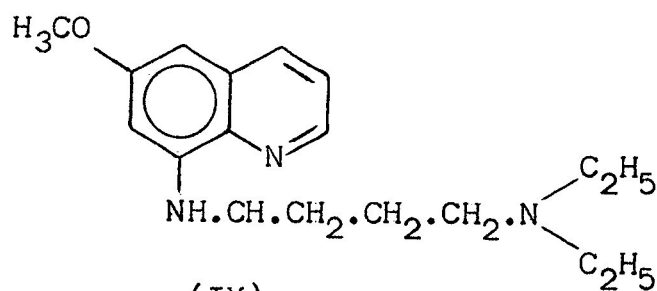


(VIII)

(2) Tissue Schizontocidal drugs :

i) 8-Aminoquinolines :

The search for synthetic antimalarials led to the discovery by Schulemann et al.²⁸ of Pamaquine (IX).

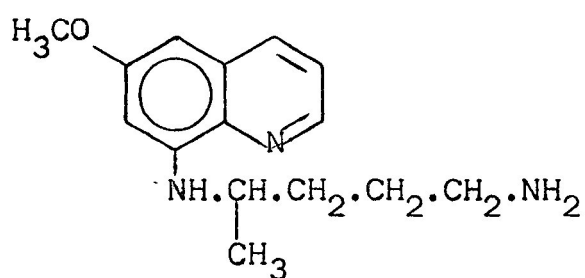


(IX)

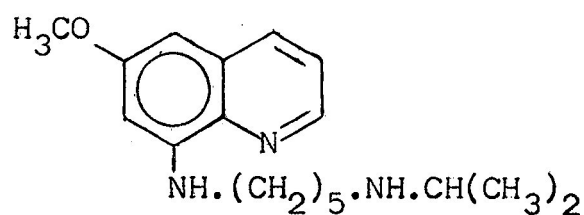
Other drugs of this series are Primaquine (X), Pentaquine and (XI), Quinocide (XII).

Primaquine is widely used clinically as a Tissue schizontocide in combination with chloroquine.²⁹

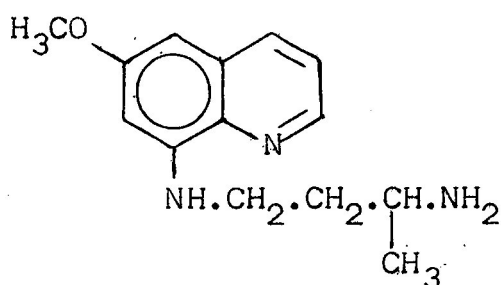
It is active on all stages of malarial plasmodia in combination with amodiaquine. It has a high prophylactic and curative ratio when given with or without any suppressive antimalarials like proguanil or quinine.³⁰ It is used in the prevention of relapses caused by P. vivax, P. malariae and P. ovale.



(X)



(XI)



(XII)



(3) Antifolates :

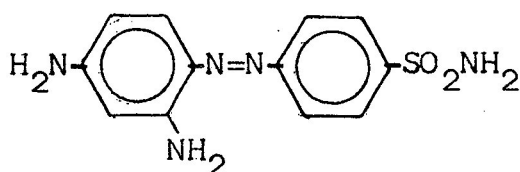
- i) Sulfonamides : Sulfadiazine.
- ii) Sulfones : Acedapsone/Dapsone.
- iii) 2,4-Diamino-pyrimidines : Pyrimethamine.
- iv) Biguanides : Proguanil.
- v) Dihydro-s-triazine : Cycloguanil.

Malaria parasites, like many bacteria, are unable to utilize preformed folic acid and require para-aminobenzoic acid as a substrate in order to synthesize folic acid. Sulfonamides and sulfones act as competitive antagonists of this substrate. Pyrimethamine and proguanil (and related compounds like the dihydro-s-triazines) inhibit a later step in the folate synthetic pathway, that mediated by the enzyme dihydro folate reductase.

i) Sulfonamides :-

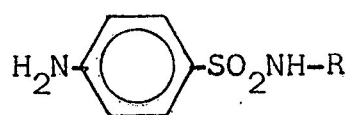
It was shown that Prontosil (XIII) was converted in animals to sulfanilamide (XIV) and the entire structure was not necessary for antibacterial action. The antimalarial activity of Prontosil was demonstrated against P. falciparum and P. knowlesi³¹ and P. vivax.³² The metabolite sulfanilamide was shown to have an appreciable antimalarial activity.³³ This ushered in a succession of studies on the synthesis and testing of sulfonamides.

The presence of a free aromatic amino group was apparently important to activity. Movement of the aromatic group to the m-position decreased or abolished activity. Metachloridine (XV) a metanilamide³⁴ is an exception and in contrast to the p-amino benzenesulfonamides, it is more effective against avian than mammalian malaria.



Prontosil

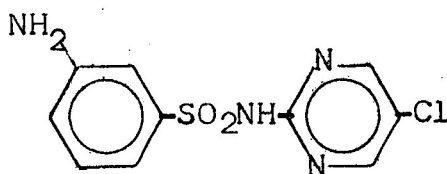
(XIII)



R = H

Sulfanilamide

(XIV)



Metachloridine

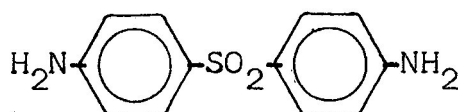
(XV)

Investigations confirm that sulfonamides combined with other drugs like pyrimethamine are useful against drug resistant malaria.³⁵



ii) Sulfones :-

The recognition of the antileprotic diaminodiphenyl sulfone (Dapsone) (XVI) as an antimalarial drug was revealed from the work of Coggeshall et al.³³



(XVI)

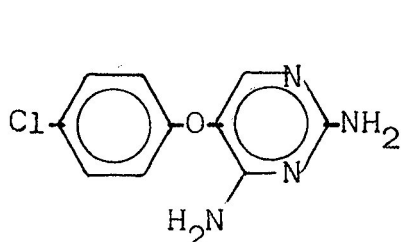
Recently many sulfone derivatives have been prepared in an effort to develop repository agents against P. berghei in mice.³⁶

Several derivatives of dapsone have been developed including a repository form acedapsone which has shown promising activity.

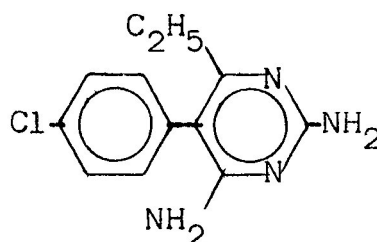
iii) 2,4-Diamino Pyrimidines :-

Hitchings and his colleagues in the U.S.A. discovered potent group of pyrimidines which proved to have a pronounced antagonistic effect on folic acid in cultures of L. casei. The close analogy of 2,4-diamino-5-para-chloro phenoxy pyrimidine (XVII) and the biguanides led to the proliferation of a variety of allied compounds.³⁷

Thus a lot of pyrimidines were tested for antimalarial activity which led to the development of the most potent compound pyrimethamine (XVIII).³⁸



(XVII)

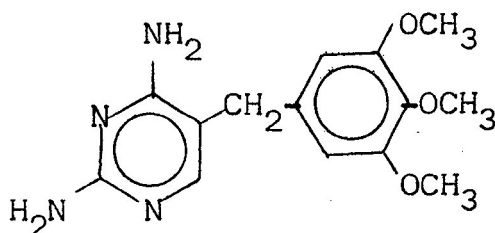


(XVIII)

Pyrimethamine is the most potent suppressive anti-malarial in clinical use today.

Though it is slow acting and must be used alongwith a fast acting blood schizontocide against fulminating infections, it is the most widely used drug in combination with sulfadoxine as a second line of defence where chloro-quine fails.

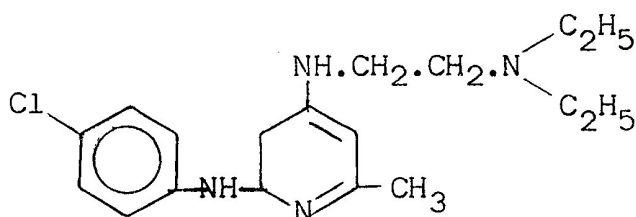
Recently emphasis has been placed on the evaluation of the closely related trimethoprim which has shown activity against *P. falciparum* resistant to pyrimethamine, chloro-quine and quinine (XIX).³⁹



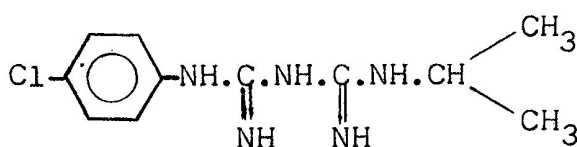
(XIX)

iv) Biguanides :-

Concurrent with the American Research on Antimalarials, that was carried out during World War II, British workers at I.C.I. Ltd. discovered a new type of antimalarial drug as acyclic analog of anilino (XX) pyrimidine system.⁴⁰ This acyclic analog was an aryl biguanide. Structural modifications around the biguanide moiety eventually led to the antimalarial drug Chlorguanide (XXI) (Proguanil, Paludrine).



(XX)

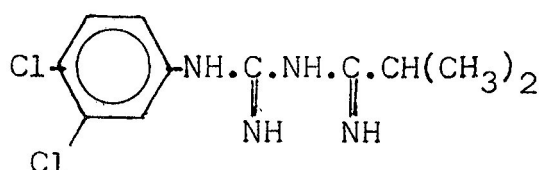


(XXI)

This drug represented an important chemotherapeutic advance because of its extremely low toxicity. Chlorguanide is not only a potent schizontocide but is also effective against actively developing pre-erythrocytic tissue forms



of certain plasmodia. However, it must be given daily because of its rapid metabolism and excretion. The other biguanide used as an antimalarial is chlorproguanil (Lapudrine) (XXII).



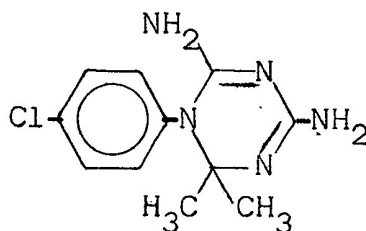
(XXII)

Chlorproguanil has almost the same activity as pyrimethamine and more potent and persistent than chlorguanide.⁴¹

v) Dihydro-s-triazines :-

It was shown that Proguanil had no activity against malarial parasites in vitro,⁴² suggesting that a metabolite or metabolites are responsible for the antimalarial activity. Carrington et al.⁴³ found the active metabolite as 4,6-diamino-1-(p-chlorophenyl)-1,2-dihydro-2,2-dimethyl-s-triazine (cycloguanil) (XXIII). This metabolite has also been demonstrated in man,⁴⁴ and the analogous metabolites have been isolated from 3,4-dichloro and 4-bromo analogues of chlorguanide.^{45,46} Cycloguanil has been administered in a poorly soluble salt, the embonate as a repository drug.

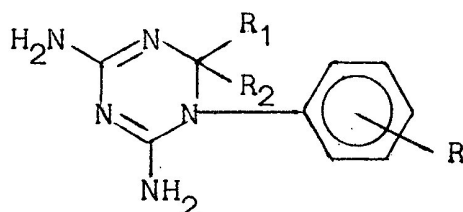
In this form it produces very prolonged suppression and probably causal prophylaxis of sporozoite induced vivax and falciparum malaria.



(XXIII)

The structure activity pattern of the dihydro-s-triazines follows the corresponding biguanides with the 4-chloro and 3,4-dichloro phenyl derivatives showing high activity, with (substitutions at 2-position) R_1 and R_2 being optimal as in ethyl groups. The 4-bromo analogue also received considerable attention during the early work on the series.^{46,47}

The 1-aryl 4,6-diamino-1,2-dihydro-s-triazines (XXIV) were synthesized independently by Modest⁴⁸ and co-workers in the course of the work on aryl biguanides.

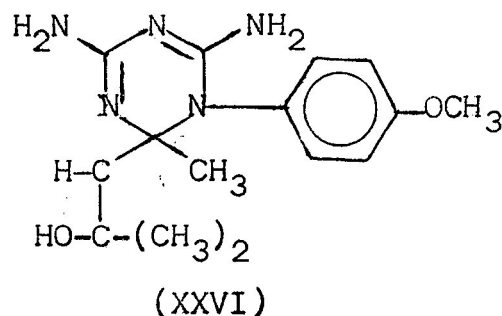
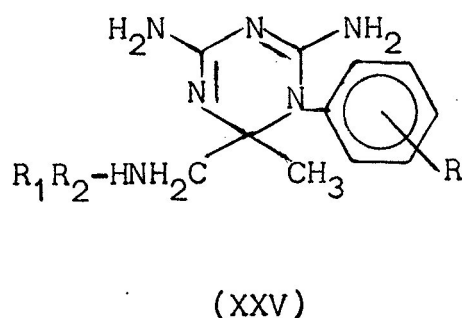


(XXIV)

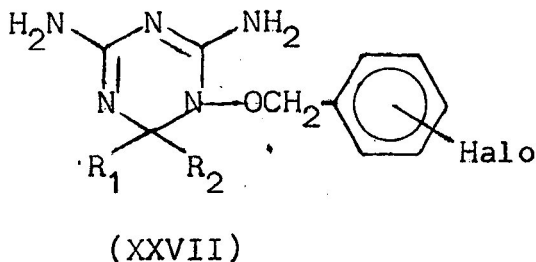
Variety of analogues were prepared based on substituents on the phenyl ring and the nature of R_1 and R_2 .^{49,50}

Many other analogues were prepared subsequently and shown to be active against many other organisms, these include pathogenic^{51,52} and non-pathogenic bacteria,^{48,52} coccidia,⁵³ toxoplasma,⁵⁴ intestinal helminths⁵⁵ and experimental tumours.^{56,57}

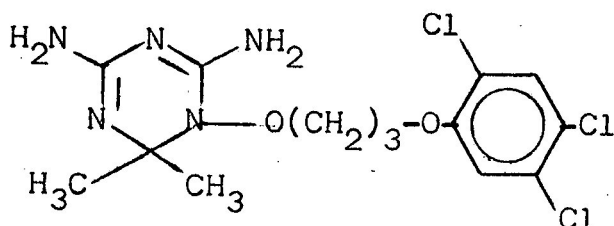
Though a few major modifications have been made on the 2,2-alkyl substituent of the dihydro-s-triazines, two of interest are (XXV)⁵⁸ and (XXVI)⁵⁹ both of which retained good activity.



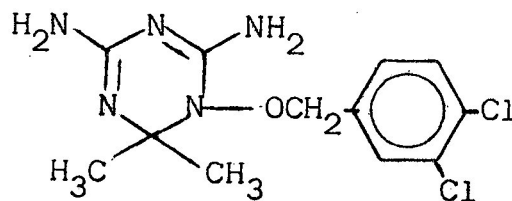
The benzyloxy analogues (XXVII) have also been reported to possess antimalarial activity.⁶⁰



Further two similar types and (XXVIII) and (XXIX) were found active against both cycloguanil sensitive and resistant P. berghei in mice.^{61a,b}



WR 99210
(XXVIII)



WR 38839
(XXIX)

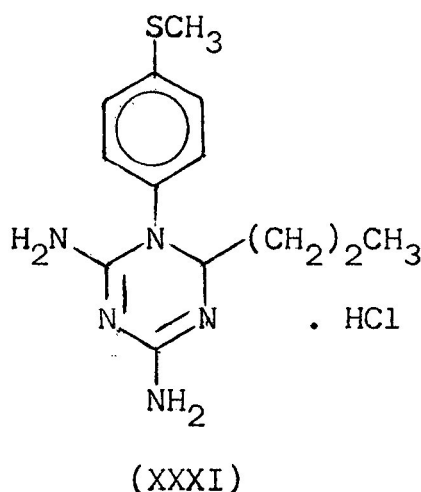
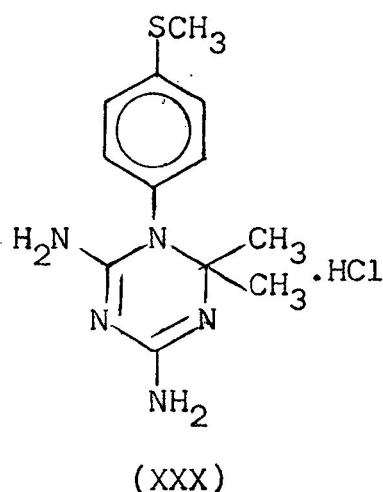
Dihydro-s-triazines with a
Broad Spectrum of Activity :

1-Aryl-4,6-diamino-1,2-dihydro-s-triazines have been investigated for their use against helminthiasis, bacterial infections, trypanosomal and leishmanial infections.

Capps et al.⁶² synthesized many dihydro-s-triazines and tested them against various pathogens. The inhibitory effects of these compounds on S. fecalis; L. casei, and dihydrofolate reductase enzyme were studied. Many of the triazines were active in a variety of these biological systems.

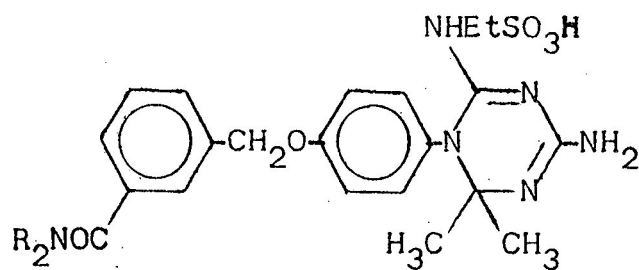


Antihelminthic activity was first reported by Schalit and Cutler⁵² who reported that both 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-[p-(methylthio) phenyl]-s-triazine hydrochloride (XXX) and 4,6-diamino-1,2-dihydro-1-[p-(methylthio)-phenyl]-2-propyl-s-triazine hydrochloride (XXXI) exhibited curative effects against Syphacia obvelata in mice at oral doses of 200 mgs/kg/day.



Dihydro-s-triazines as irreversible
enzyme inhibitors :

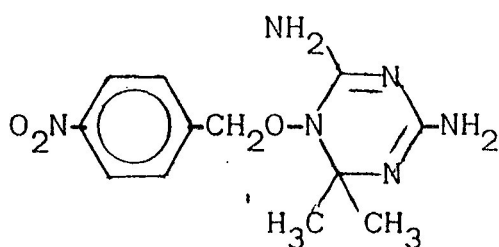
Many derivatives of 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-(phenyl)-s-triazine (XXXII) were synthesized in search of water soluble inhibitors of DHFR for use as antitumor agents by Baker et al.⁶³ Some of these antifols showed excellent activity against Walker 256 ascites, Intramuscular 256 and Dunning Leukemia ascites in the rat.



R = Et, Me

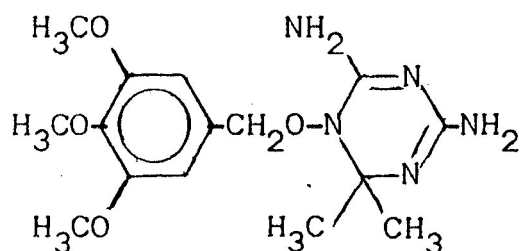
(XXXII)

Triazines have also been evaluated for leishmaniasis. Mattock and Peters⁶⁴ have shown that some of the antimalarial antifols in clinical use have a leishmanicidal action in tissue culture and some have reached clinical use for cutaneous leishmaniasis in man, e.g. (XXXIII) and (XXXIV).



BRL 50242

(XXXIII)

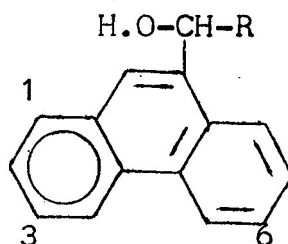


BRL 50435

(XXXIV)

New Antimalarial drugs under development :

i) 9-Phenanthrenemethanols :

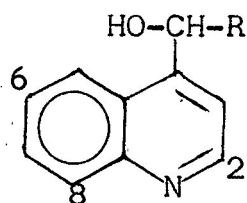


(XXXV)

Where, $R = CH_2N \left[(CH_2)_8CH_3 \right]_2$
 $6 = Cl; R = CH_2N \left[(CH_2)_6CH_3 \right]_2$
 $6 = Br; R = CH_2N \left[(CH_2)_6CH_3 \right]$
 $3 = CF_3; 6 = CF_3; R =$  WR-122455
H

The above type of phenanthrenemethanols (XXXV) have been tested for antimalarial activity. They are effective against the erythrocytic stages of all species of malaria tested, but ineffective against the exoerythrocytic forms. Generally well tolerated in man, from animal studies they appear to be poorly absorbed or largely excreted in the bile, the greatest amount appearing in faeces. These compounds which are undergoing clinical trials are effective against multidrug resistant strains. Synthesis of WR-122455 was reported by Nodiff et al.⁶⁵ It cured all P. berghei infected mice when given subcutaneously in peanut oil at 80 mg/kg and above.

ii) 4-Quinolinethenols :



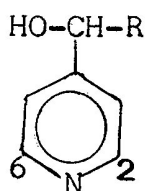
(XXXVI)

Mefloquine : 2 = CF₃; 8 = CF₃; R =

WR-184806 : 2 = CF₃; 8 = CF₃; R = (CH₂)₂-NHC-(CH₃)₃

SN 10275 : 6 = Cl; 8 = Cl; 2 = C₆H₅; R =

Almost 14 quinoline methanols (XXXVI) have been evaluated for activity in man. Effective against erythrocytic stages of all species of malaria. Mefloquine was first reported by Ohnmacht et al.⁶⁶ in 1971. This drug is a mixture of d and l erythro forms. It is effective at 20 mg/kg and above, when administered subcutaneously in peanut oil to P. berghei infected mice.⁶⁶ The oral dose of 8.5 mg/kg day was effective in producing 90 % suppression in parasitaemia of P. berghei in mice.⁶⁷ A single dose of 1.5 g. was found uniformly effective in curing volunteers with multidrug resistant strains of falciparum malaria. It is currently used as a suppressive prophylactic.⁶⁸

iii) 4-Pyridinemethanols :

(XXXVII)

WR-172435 : 2 and 6 = -CF₃; R = (CH₂)₂N(C₄H₉)₂

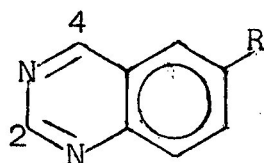
WR-180409 : 2 = CH₃; 6 = -CF₃; R =

Synthesis of WR-180409 (XXXVII) was first reported by LaMontagne et al.⁶⁹ This drug was curative against P. berghei in mice, but no pyridinemethanols have been tested for antimalarial activity in man. However many are active in animal models and two mentioned above have been selected for clinical trials.

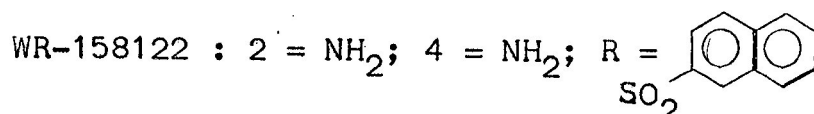
Good evidence of activity was observed against the blood stage of the parasite in all species including P. falciparum in owl monkeys.⁶⁸ Larger single doses (>1 g.) produce nausea, vomiting and dizziness. These compounds appear to persist in tissues for some time and require only single oral doses to cure monkeys with multi-drug resistant falciparum malaria.



iv) (Aryl) Thio quinazolines :

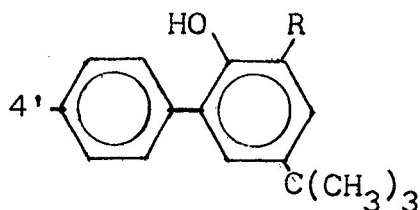


(XXXVIII)

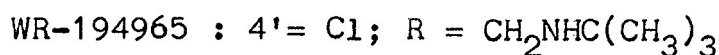
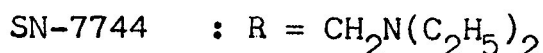


Only a single (arylthio) quinazoline (XXXVIII) has been tested^{68,69} for antimalarial activity in man. This drug was screened and detailed by Elslager and Werbel.⁷⁰ These drugs are effective blood schizontocides with a marked potentiation with the sulfonamides. Mechanism of action is by inhibition of dihydrofolic acid reductase. The above compound has been tested in man and found well tolerated at the greatest dose administered, 1-3 g. daily for 3 days. Well absorbed orally, it is bound to plasma proteins, but cleared rapidly from the blood. If given by parenteral injection, it can protect mice for as long as 3 weeks.

v) Phenylphenols :

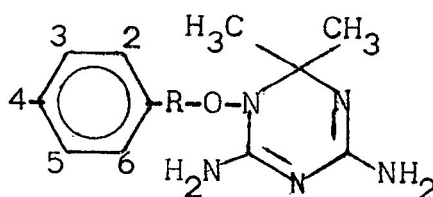


(XXXIX)



The above two phenylphenols have been tested for antimalarial activity in man. These compounds have been evaluated only for blood schizontocidal effects. Well tolerated and moderately well absorbed and single oral doses are as effective as total dose divided into 7 parts.

vi) Dihydro-s-triazines :



(XL)

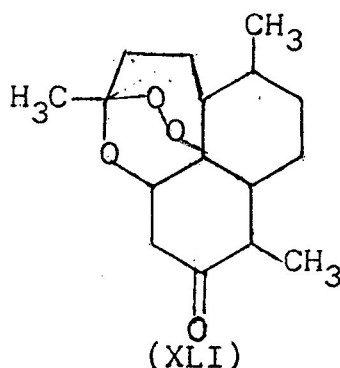
WR-38839 : 3 = Cl; 4 = Cl; R = CH₂

WR-99210 : 2 = Cl; 4 = Cl; 5 = Cl; R = O(CH₂)₃

Only one dihydro-s-triazine (XL) has been tested for antimalarial activity in man. These dihydro-s-triazines are effective against blood schizonts of both falciparum and vivax malaria. When combined with a sulfonamide they exert prophylactic activity, against P. falciparum.

WR-38839 is cross resistant with pyrimethamine, and is well tolerated. The dose limiting factors being gastro-intestinal symptoms. The compounds have been found effective against drug resistant falciparum malaria. Hence of great interest.

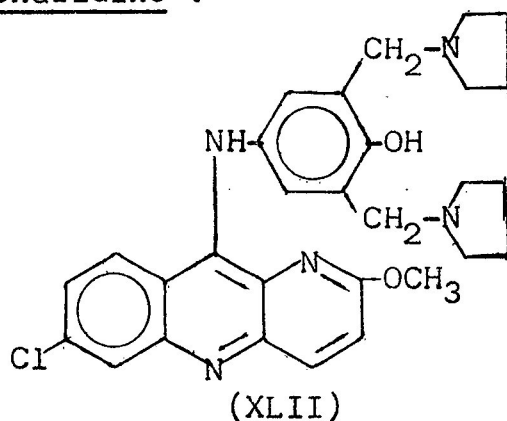
vii) Sesquiterpene lectones : (Quinghao su)



Quing hao su (XLI) is the antimalarial constituent extracted from the plant *Artemisia annua* L in 1972 at the Chinese Institute of Materia Medica. Quing hao su is highly effective against asexual blood forms. Rapidly absorbed in animals and extremely well tolerated. For clinical trials the drug was formulated in the form of tablets, oil suspension, etc. and the total dose administered per adult was 2.5-3.2 g. per tablet and 0.8-1.2 g. oil suspension.⁷¹

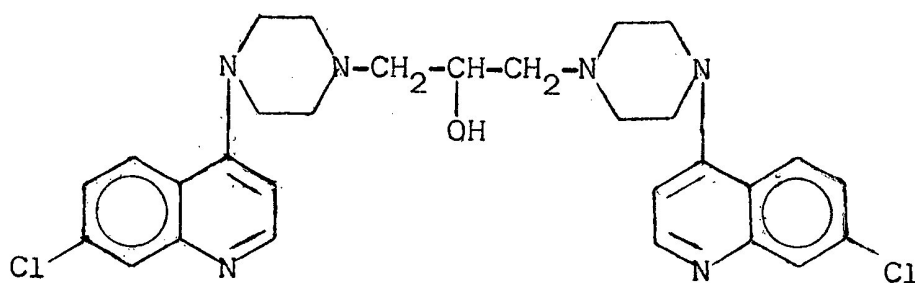
The rate of recrudescence (upto 30 %) occurring 15-30 days after the initial treatment is reduced when the drug is given as an oil suspension.⁷¹

viii) Pyronaridine :



Compounds of this series have been shown to be active blood schizontocides, effective against chloroquine resistant strains in animals and man. More information should be available with future investigation.

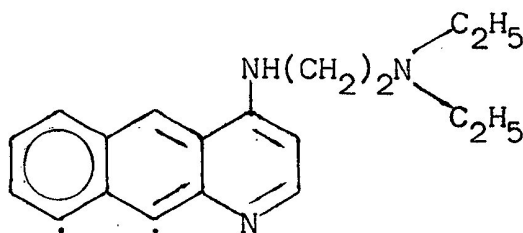
ix) Piperaquine :



(XLIII)

Piperaquine (XLIII) a symmetrical compound in which two molecules of 7-(Chloro)-4-(1-piperazinyl)-quinidine are linked through a propyl group, has shown blood schizontocidal activity similar to chloroquine. Hydroxypiperaquine has shown a high suppressive and therapeutic activity.⁸

x) Dabequine :



(XLIV)

Benzo (g)-4-(diethylaminoethylamino) quinoline (XLIV) is a 4-aminoquinoline derivative in which the quinoline

nucleus has been expanded by a benzene ring. Active against blood schizonts of P. berghei. However clinical trials indicate cross-resistance with chloroquine.⁸

xi) Naphthoquinones :

Research reveals that these drugs may have tissue as well as blood schizontocidal activity.⁸

Chemotherapy being an important element of malaria control, new drugs and a constant research with various chemical modifications is inevitable and thus continues.

MECHANISM OF ACTION :

Antimalarial agents act by various mechanisms. At the molecular level they act primarily by either inhibiting enzymes involved in the biosynthesis of precursors of DNA or forming molecular complexes with the DNA itself, thus blocking the synthesis of DNA and RNA of the plasmodia. Quinine, 8-aminoquinolines and 4-aminoquinolines, owing to their flat ring system, intercalate in vitro between pairs in double helical DNA by their side chains, and get electrostatically attached to phosphate groups. Recent evidence from Burckhalter's⁷² laboratories suggests that the aromatic side chain of amodiaquine might be involved in the process of intercalation alongwith the quinoline ring.

Pyrimethamine, trimethoprim, biguanides and cyclo-guanil have their antimalarial activity due to selective inhibition of dihydrofolate reductase which converts dihydrofolic acid to tetrahydrofolic acid of the parasite. This inhibition interferes with plasmodial biosynthesis leading to the formation of purine and pyrimidine bases and eventually of DNA. These agents bind to the dihydrofolate reductase of plasmodia much more tightly than to the enzyme of the host. Further, the host is not affected by this blocking reaction, because the folinic acid required by host is obtained from food.

Drugs that act by binding to a specific enzyme logically could be expected to act on all stages of an organism such as malaria parasites in which that enzyme functions, provided that the compound is able to reach the actual binding site by penetrating the membranes and is retained at the site for an adequate period of time.⁷³

Sulfonamides and sulfones act by inhibiting dihydropteroate synthesis, thus competing with para-amino benzoic acid.⁷³

Biological Classification of Antimalarials :

Since various stages in the life cycle of malaria parasites show different susceptibility to antimalarial

drugs, the latter may be classified into the following groups, as shown in Fig. 2 and Table A.

(1) Tissue schizontocides (used for causal prophylaxis) acting on the pre-erythrocytic stages of the parasite (primary tissue forms or primary exo-erythrocytic forms) and thus completely preventing invasion of the red blood cells.

(2) Tissue schizontocides (used as antirelapse drugs) acting on the exoerythrocytic stages or tissue forms of P. vivax and P. ovale and thus able to achieve radical cure of these infections.

(3) Schizontocides (blood schizontocides or schizontocidal drugs) acting on the erythrocytic stages of parasites commonly associated with acute disease, though these stages may be present in some infections accompanied by few clinical symptoms. Schizontocides may achieve clinical cure or suppression to a subpatent level of infection with all four species of malaria parasite susceptible to their action. They also act on the sexual erythrocytic forms of P. vivax, P. ovale and P. malariae but not directly on the mature gametocytes of P. falciparum.

(4) Gametocytocides (gametocytocidal drugs) destroying all sexual forms including those of P. falciparum; they also act on the developmental stages of malaria parasites in the anophelines and some of them form the next group of drugs.

(5) Sporontocides (sporontocidal drugs) preventing or inhibiting the formation of oocysts and sporozoites in anophelines that have fed on carriers of gametocytes. They interfere with the transmission of malaria, though they may have no direct action of gametocytes in the human host. These drugs have also been called antisorogonic drugs.

(6)(a) Clinical Cure- Relief of symptoms of a malaria attack without complete elimination of the infection.

(b) Radical Cure- Complete elimination of the malaria parasite from the body so that relapses cannot occur.

(c) Suppressive Cure- Complete elimination of the parasite from the body by means of continuous suppressive treatment.



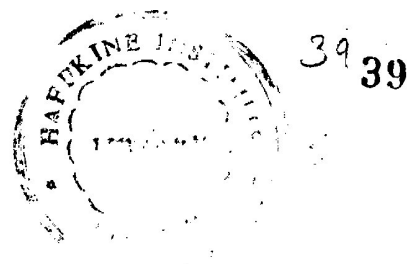
TABLE A. ACTION OF COMMONLY USED DRUGS ON THE CYCLE OF DEVELOPMENT OF MALARIA PARASITES

Drug	Sporozoites	Tissue phase during the incubation period	Erythrocytic phase		Latent tissue phase (responsible for relapses)	Development of gametocytes in the mosquito (sporontocidal action)	Chemical class of the relevant antimalarial compound
			Asexual parasites	Sexual forms (gametocytes)			
Quinine	No action	No action	Fast action	Active against <i>P. vivax</i> and <i>P. malariae</i> . No direct action on <i>P. falciparum</i>	No action	No action	Cinchona alkaloids
Mepacrine	No action	No action	Fast action	As quinine	No action	No action	9-aminoacridines
Chloroquine } Amodiaquine }	No action	No action	Fast action	As quinine	No action	No action	4-aminoquinolines
Primaquine	No action	Active but not used for prophylaxis	Active but only in toxic doses	Direct and fast action on all species but particularly <i>P. falciparum</i>	Highly active	Highly active	8-aminoquinolines
Proguanil	No action	Active, particularly on <i>P. falciparum</i>	Active, but relatively slow	No direct action	No action	Highly active	Biguanides
Pyrimethamine	No action	Probably as proguanil	As proguanil	No evidence of direct action	Some action on <i>P. vivax</i>	Little evidence	Diaminopyrimidines
Sulfones and Sulfonamides	No action	Possible action	Moderate action when given alone	As pyrimethamine	Little evidence		Sulfones and sulfonamides comprise a large number of short-acting and long-acting compounds
Mefloquine	Probably no action	Probably no action	Marked action	As quinine	Probably no action	Probably no action	Quinolinemethanols

-o-

P L A N O F W O R K

-o-



PLAN OF WORK

Malaria remains the greatest single infectious disease threat to the health of the world. According to statistical reports of W.H.O.⁷⁴ one in every one hundred Indians is found to suffer from malaria.

Overshadowing all other limitations to the development of current, limited spectrum of antimalarials is the problem of drug resistance. Resistance to chloroquine has been restricted to P. falciparum, but many strains of P. vivax are resistant to pyrimethamine/sulfadoxine combination and proguanil. Since a country like India has predominantly P. vivax infection, this poses a serious threat. Drugs with high safety, long duration of action, antirelapse activity and gametocytocidal effects need to be developed to add to the present armamentarium of anti-malarials.⁷⁵

Chemotherapy is one of the best approaches which is financially and administratively feasible to control malaria. Though it must be stressed that no single approach including the use of vaccines is likely to produce a significant reduction in the prevalence of malaria in highly endemic areas.⁷⁶ Quinine was the first true chemotherapeutic agent to be discovered. Its potency against malarial fevers and

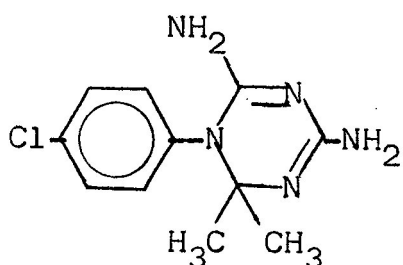
excessive use led to the depletion of the cinchona barks from which it was obtained. During the Ist and IInd World Wars, this triggered the intensive search to find synthetic drugs to replace quinine. Schulemann²⁸ discovered the first synthetic antimalarial "Pamaquine" and Andersag⁷⁷ gave quinacrine and chloroquine. Following its release in 1946, chloroquine became the drug of choice but in 1959 the first case of chloroquine resistance was reported. Since then interest in the research of new chemotherapeutic agents has been on the increase. Old drugs like Paludrine, its active metabolites cycloguanil and such other DHFR inhibitors like various dihydro-s-triazines have been studied.

Dihydro-s-triazines :

4,6-Diamino-1,2-dihydro-1-aryl-s-triazines :-

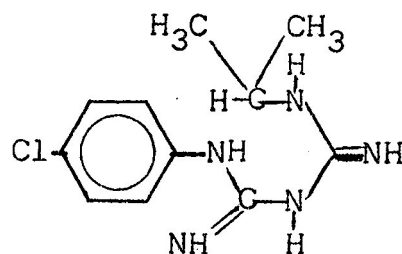
After the introduction of Proguanil evidence was presented that the drug had essentially no biological action⁴² but an active metabolite was isolated from the urine of Proguanil treated rabbits and humans. This metabolite was highly active against P. gallinaceum in vitro and in vivo.⁴⁴ This metabolite was chemically identified as 4,6-diamino-1-(p-chlorophenyl)-1,2-dihydro-2,2-dimethyl-s-triazine termed as cycloguanil.

The^o structural relationship of cycloguanil(XLV)to proguanil(XLVI)can be observed as follows when the latter is drawn in the curved form.



Cycloguanil

(XLV)

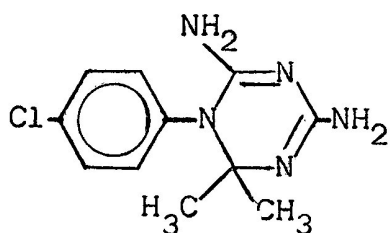


Proguanil

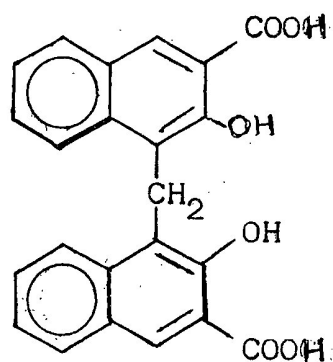
(XLVI)

The structure of cycloguanil and its biological activity stimulated the synthesis of many dihydro-s-triazine.^{49,50}

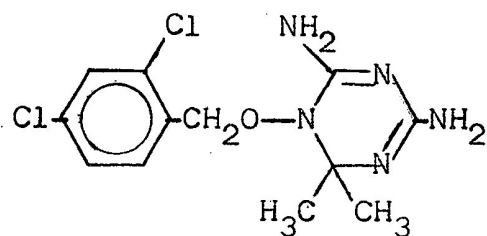
These compounds showed greater activity than the corresponding biguanides against avian malaria⁷⁸ but not against simian infections or human malaria.⁷⁹ Enzymatic studies⁸⁰ show that the mode of action of s-triazines is due to the interference with the folic acid pathways of the parasite. The well known dihydro-s-triazines recently proved as antimalarials are cycloguanil pamoate (XLVII), clociguanil (XLVIII) and WR-99210 (XLIX) which contain the halogen substituted phenyl, benzyloxy and phenoxy alkoxy moieties respectively at 1-position of the s-triazine ring.⁸¹



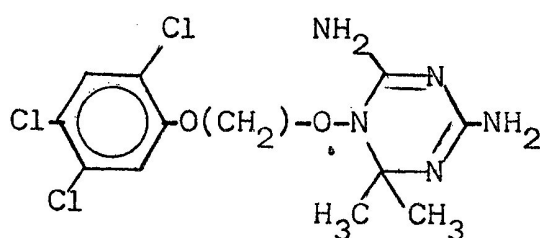
y2



(XLVII)



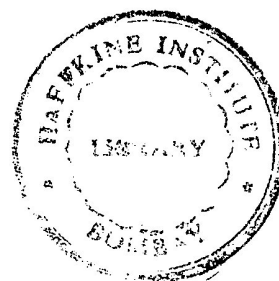
(XLVIII)



(XLIV)

These compounds have been found effective in human in combination with sulfadiazine.

Thus it was planned to synthesize and evaluate the structural modifications in a series of dihydro-s-triazines. The compounds synthesized are briefly summarized under various sections as follows.



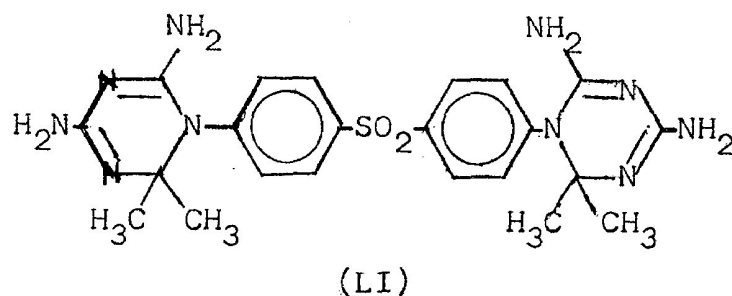
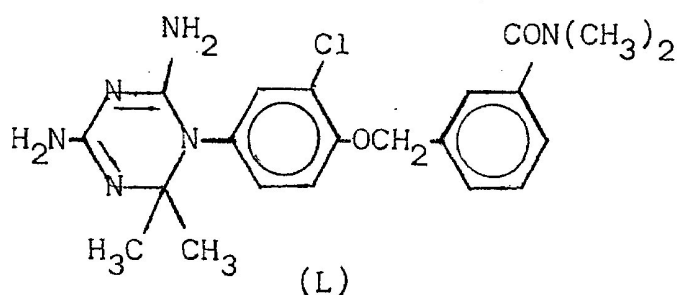
o

SECTION - A :

Synthesis of 4,6-diamino-1,2-dihydro
1-(substituted phenyl) s-triazine
hydrochlorides :

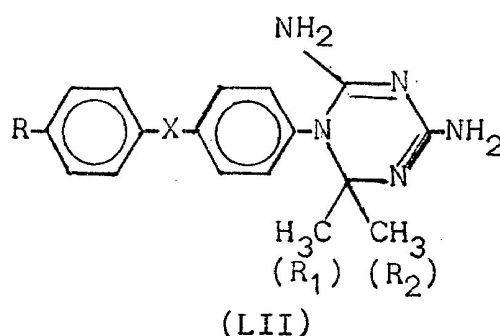
Literature shows that s-triazines have been widely exploited and are still being screened for anti-helminthic,⁶² antitumor,⁸³ antitrypanosomal⁸⁴ and also the historical antimalarial activity.

Dihydro-s-triazines which are analogues of cyclo-guanil are dihydrofolate reductase inhibitors (DHFR) and get rapidly excreted from the body. Many triazines attached to bis-(arylamine) salts are reported viz; Baker's antifols⁶³ (L) and Capp's sulfonamidotriazines (LI)⁶².



Evidence that, the above type of molecules bind well with the enzyme receptor surface and antagonize dihydrofolate reductase enzyme are also available from Quantitative Structure Activity Relationships.⁸⁰

Assuming that introduction and variations of a spacer between two aryl groups may increase lipophilic character of the entire moiety which may consequently decrease the rate of excretion, the compounds of the following general structure (LII) were proposed.



Where, $R = \text{Cl}, \text{Br}, \text{NO}_2, \text{NHCOCH}_3, \text{NHCOOCH}_3$

$X = -\text{O}-, -\text{S}-, -\text{CH}_2\text{O}-$

$R_1 = \text{CH}_3; R_2 = \text{C}_2\text{H}_5$

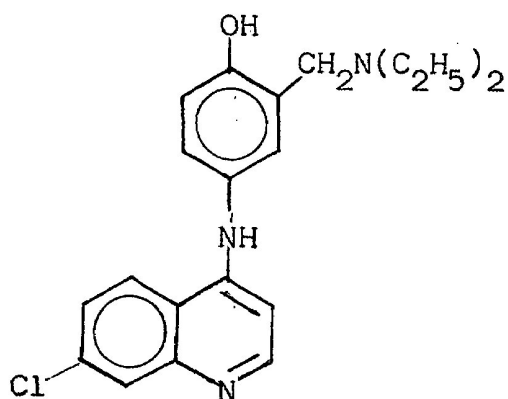
The arylamines required were prepared by the known methods in the literature.

SECTION - B :

Synthesis of o-cresol, 4-(4,6-diamino-2,2-dialkyl or aryl-1,2-dihydro-s-triazine)- α -substituted amino methyl hydrochlorides :

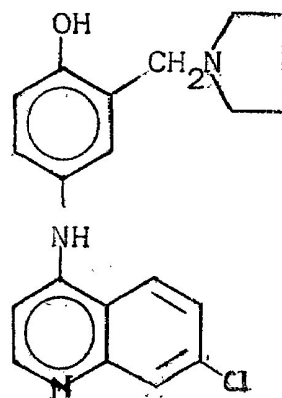
It is known that the quinoline compounds having basic side chain at 4,6 and 8-position give rise to active antimalarials. Burckhalter⁸⁴ and co-workers prepared a large number of compounds based on the known activity of certain α -dialkylamino o-cresols.

The 3-diethylamino methyl-4-hydroxy anilino side chain was found to be most essential part of the antimalarial drug like amodiaquine. The side chain of amopyraquine containing pyrrolidine, nucleus is nothing but the side chain of Amodiaquine in disguise.



Amodiaquine

(LIII)



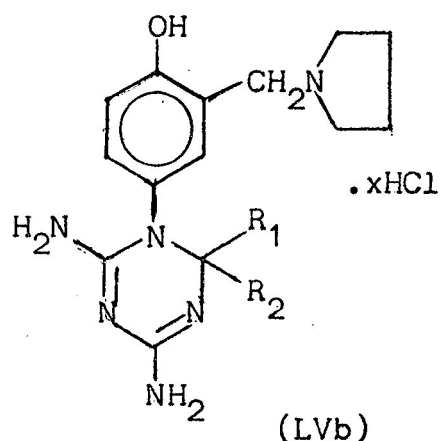
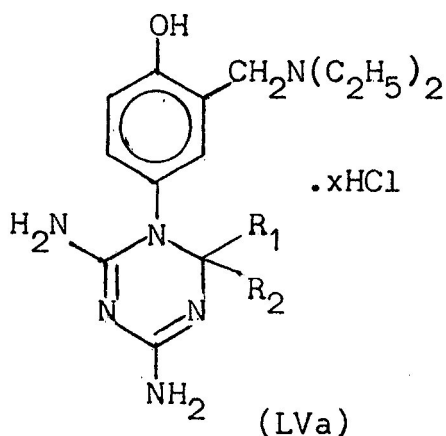
Amopyraquine

(LIV)

Both these compounds (LIII) and (LIV) possessed high antimalarial activity. When one studies the structure activity relations of the 4-amino quinolines it is observed that those containing a hydroxyl group in the side chain are very active, besides the halogen substitution in the nuclear ring.

Hence from the history and SAR of the 4-amino quinolines it was thought appropriate to use the 3-diethyl-amino methyl-4-hydroxy aniline and 3-pyrrolidinomethyl-4-hydroxy aniline side chain of amodiaquine and amopyraquine respectively as basic arylamines to build the s-triazine moiety. The arylamines were synthesized as per the Mannich Condensation Reaction described in the literature.

Thus the synthesis of the following types of compounds (LV) was planned for testing their antimalarial activity.

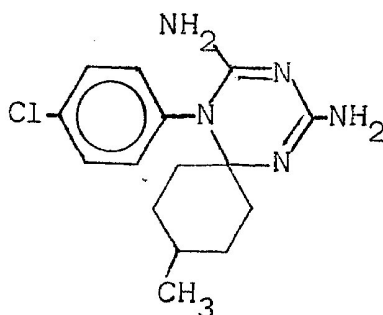


Where, $R_1 = \text{H}, \text{CH}_3$; $R_2 = -\text{C}_6\text{H}_5, -\text{C}_6\text{H}_5-\text{N}(\text{CH}_3)_2, -\text{CH}_3, -\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_3, -\text{C}_2\text{H}_5, -\text{C}_6\text{H}_3(\text{Cl})(\text{OCH}_3)(\text{OH})$

SECTION - C :

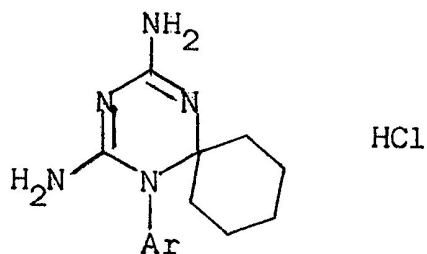
Synthesis of 2,4-diamino-1-[(4'-hydroxy, 3'-substituted amino-methyl) phenyl] 1,3,5-triazaspiro (5-5) undeca 2,4-diene :

Roth et al.⁸⁵ in their course of studies prepared a compound analogous to cycloguanil with a slight variation in the triazine ring having a bulky substituent at 2-position (LVI).



(LVI)

This compound was found to be active as an anti-helminthic. The activity was attributed to a hypothesis that a bulky group at 2-position of the triazine ring may enhance activity. Following a similar pattern attempts to synthesize the following s-triazines (LVII) with arylamines of biologically important nature was decided.



(LVII)

Where, Ar = Substituted arylamines, viz; 3-diethylamino-methyl, 4-hydroxy aniline; 3-pyrrolidino amino methyl-4-hydroxy aniline; 4'-substituted diphenyl sulfide.



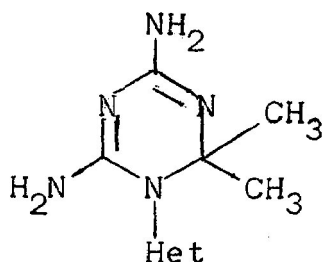


SECTION - D :

Synthesis of 4,6-diamino-1,2-dihydro-
2,2-dimethyl s-triazines with heterocyclic
substituents at 1-position of the triazine
ring :

Literature studies show that dihydro-s-triazines attached to aromatic amines have been extensively worked on as aliphatic amines do not cyclize with dicyandiamide to form a s-triazine.⁴⁹

Utilization of amino groups on heterocyclic moieties which are biologically active for triazine formation is relatively unexplored. Hence in this series amino functions of quinazoline, quinoline and adamantane are to be utilized to synthesize the following types of s-triazines (LVIII).

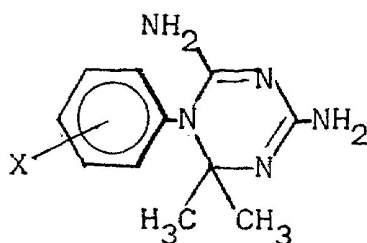


(LVIII)

Where, Het = Quinoline, adamantine, pyridine, etc.

SECTION - Eⁿ :Synthesis of 4,6-diamino-1-(p-substituted phenyl)-1,2-dihydro, 2,2-dimethyl s-triazine hydrochlorides :

Literature gives enough evidence to prove that pyrrolidine, N-methyl piperazine, morpholine and piperidine type of compounds confer antimicrobial activity on their parent compounds.⁸⁶ Besides it has been postulated that in cycloguanil the p-chloro phenyl nucleus acts as a carrier moiety for the triazine ring. Assuming that an aryl amine having heterocyclic substituent can play a similar role, different amines of the above type were to be synthesized and utilized to built up triazine compounds (LIX) of the following nature.



(LIX)

Where, X = morpholine; pyrrolidine; N-Methyl piperazine etc.

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Pharmacological Testing :

It was proposed to follow Rane's preliminary screen method¹⁸ and the Peter's 4- Day Suppressive Test⁸⁷ to evaluate the compounds for antimalarial activity in vivo against P. berghei in mice.

On the basis of the compounds prepared an attempt to correlate structure activity relationship will be made.

The resume of the Thesis will summarize the salient features of the research work in detail.

PART - II

C H E M I S T R Y

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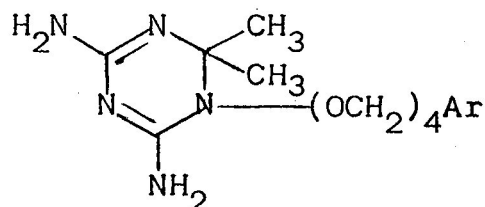


1-ARYL-DIHYDRO-s-TRIAZINES :

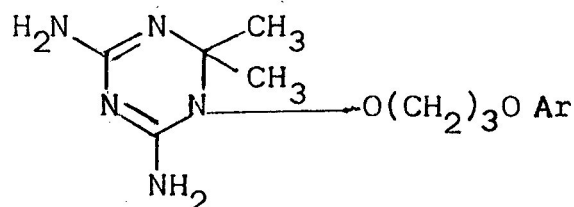
The realization of the potent antimalarial activity present in a wide range of 1-aryl-2,4-diamino-1,2-dihydro-2,2-dimethyl-1,3,5-triazine came with the finding that the prophylactic diguanide proguanil was in fact a prodrug of the active dihydro-s-triazine later known as cycloguanil.⁴⁴

The antimalarial activity of the triazines was demonstrated by Modest et al.⁴⁸ (1952), many of which as well as showing activity against simian, avian and rodent malaria, showed activity against various microbiological systems, against chicken coccidiosis and against experimental tumours. Despite the promising activity of this series of antifols their clinical use and further research was limited because of the ready emergence of resistant strains of P. falciparum. Recently several compounds describing the 1-arylalkoxy and 1-aryloxyalkoxy, diamino-dihydro-s-triazines I and II have appeared showing activity against both, pyrimethamine and cycloguanil sensitive and resistant strains of parasite,^{49,88} and not showing cross-resistance with cycloguanil against triazine resistant

strains of P. berghei.^{89,90} One of them has reached the stage of clinical trials.⁹¹ With this, the search for antimalarial activity in the triazine class has been renewed with vigour and also recommended by W.H.O.

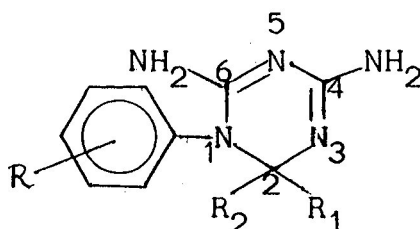


(I)



(II)

In the present work our objective was to investigate the effects of molecular modification upon antimalarial activity. A few compounds of this class were found to show encouraging results in our laboratory as mentioned in previous publication.⁹² Attention centered upon biological action, thus a programme of structural variations at 1, and 2-position of the 1,3,5-triazine (III) was undertaken with a view to develop compounds with greater efficacy against malarial parasites.



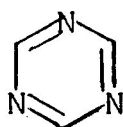
(III)

General Chemical Structure of Antimalarials :

- (1) All antimalarials contain a single or fused benzene rings (polycyclic).
- (2) Nitrogen is the most essential heteroatom e.g. pyridine with one nitrogen, pyrimidine with two nitrogens and cycloguanil with three nitrogen atoms are the basic nuclei of a number of antimalarial compounds.
- (3) Free amino groups as in 2,4-diamino pyrimidine or the 4,6-diamino-s-triazine enhance activity.
- (4) Lastly it is hypothesized that most antimalarials are electron rich species and electron donors to a large extent.

s-Triazine :

This is a six membered heterocycle consisting of 3-nitrogen atoms and 3-carbon atoms alternating each other. It is abbreviated as s-triazine (IV)⁹³ although the designation 1,3,5-triazine is also common.



(IV)

the earliest organic compounds reported in the literature.

Interest in triazine derivatives for industrial application and as chemotherapeutic agents has stimulated a great deal of activity in this field.

Synthesis of 4,6-diamino-
1,2-dihydro-s-triazines :

Various methods for the synthesis of s-triazine derivatives have been reviewed by Smolin and Rapoport,⁹⁴ Modest⁹⁵ and Hoggarth.⁹⁶ 4,6-Diamino-s-triazines have been obtained by reacting dicyandiamide with nitriles,⁹⁷ amidines,⁹⁸ cyanamides,⁹⁹ guanidines,¹⁰⁰ urea,^{101,102} etc. Acylguanidines¹⁰³⁻¹⁰⁵ on heating at high temperatures also yield 4,6-diamino-s-triazines.

Biguanides can be condensed with acids,¹⁰⁶ acid chlorides,¹⁰⁷ acid anhydrides,^{108,109} lactones,¹¹⁰ and esters,^{111,112} to obtain the above s-triazine. Later publications give more complete details of the preparation of these compound.^{112,50}

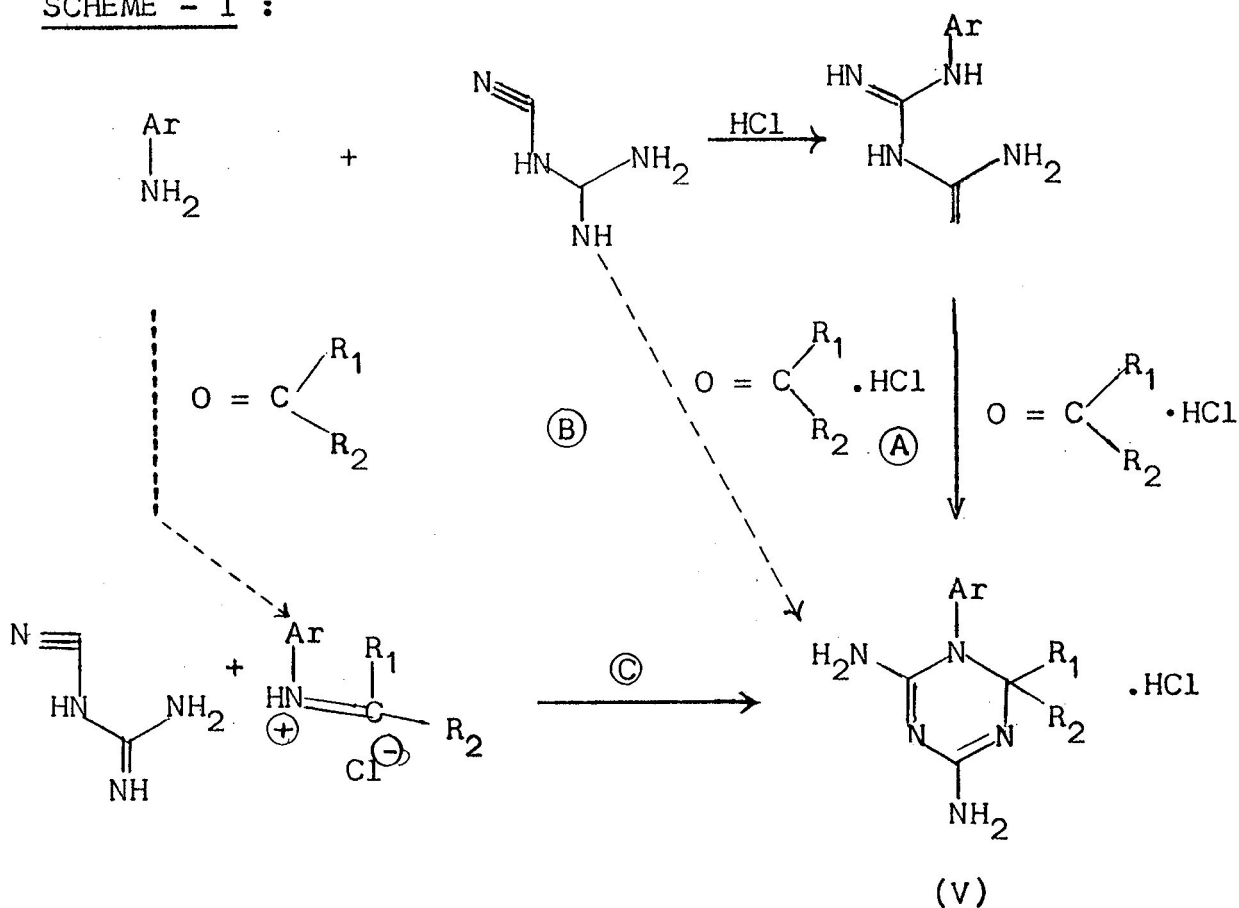
General : The ease of synthesis of diamino dihydro-s-triazines depends very much on the nature of the substituents R, R¹ and R² in structure (V)

Synthesis of 4,6-diamino-1,2-
dihydro-1-substituted-2,2-
dialkyl (or aryl)-s-triazine
hydrochloride :



Scheme I indicates the most useful methods available for obtaining dihydro-s-triazines in which a C-atom of group R is directly attached to the N¹ of the dihydro-s-triazine ring.

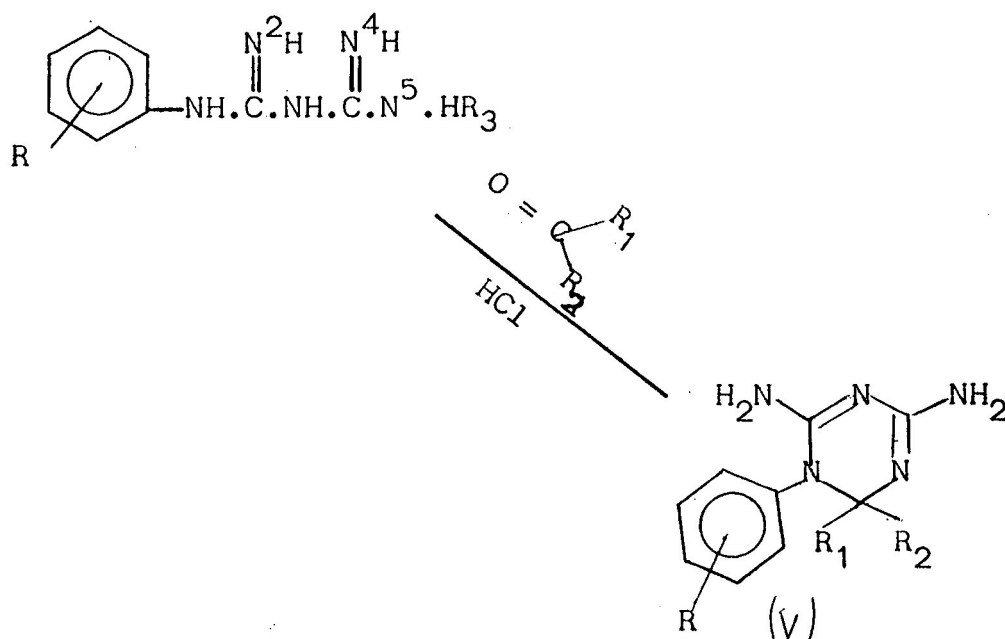
SCHEME - I :



The two component synthesis (Route A) :

This involves the acid catalysed reaction of an arylbiguanide with a ketone or aldehyde. It requires more than one equivalent of a strong acid per molecule of arylbiguanide and can be effected at a wide range of temperatures. The excess amount of acid acts as a catalyst for the reaction. The arylbiguanide may be unsubstituted or may have substitution in the ring, monoalkyl substitution at N² or N⁵ or dialkyl substitution at N⁵. The reaction succeeds with lower dialkyl ketones and all but extremely reactive aldehydes such as formaldehyde or chloral.⁹⁵

Route - A :



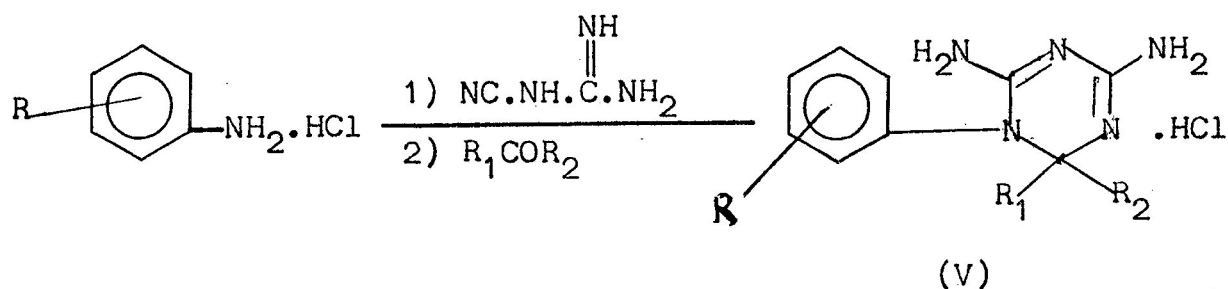
Where, $\text{R} = \text{p-Cl}$; $\text{R}_1 = \text{R}_2 = \text{CH}_3$; $\text{R}_3 = \text{H}$

The Three Component Synthesis (Route B) :

Succeeds with less than one equivalent of a strong acid per molecule of arylamine and again at varying temperatures. Various substituted arylamines may be used.

The reaction requires an unsubstituted amine function and fails with aliphatic amines, N-methylamine or NH_4Cl . Short chain dialkyl ketones and aromatic aldehydes can be used but synthesis fails with aliphatic aldehydes.

(Route-B) :



The acid used may be any strong inorganic or organic such as HCl , HNO_3 , picric. HCl being preferable. In the present study all compounds have been prepared as hydrochloride salts.

The reaction proceeds best with sterically unhindered ketones. Moreover only those ketones react which form bisulfite addition products. Aromatic aldehydes can

be used successfully but aliphatic aldehydes because their high reactivity tend to form schiff bases rather than s-triazines.⁹⁵

In the present work we utilized ketones such as acetone, cyclohexanone and methylethyl ketone. It was observed that reactions proceeded easily with acetone as the carbonyl reagent and in most cases, the same was also used as a solvent. Sodium dry methanol or ethanol was used in very small quantities to make the reaction mixture homogenous. Excess of alcohol resulted in poorer yields of the final product.

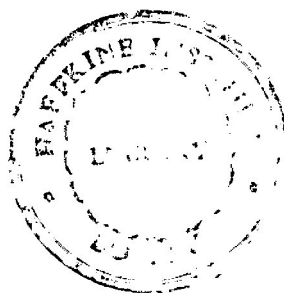
In case of cyclohexanone utilized as a carbonyl reagent the reaction temperature and time had to be modified several times with trial and error to avoid getting a resinous product which was observed to be the general tendency with slightly bulky carbonyl reagents other than acetone. Similar difficulty was faced with ethyl methyl ketone and cyclopentanone where in the latter case usually a resinous mass was obtained despite various modifications in the reaction procedures. The net result of the 3-component synthesis was the condensation of molar equivalents of the arylamine acid salt, dicyandiamide and ketone or aldehyde with loss of one molecule of water.

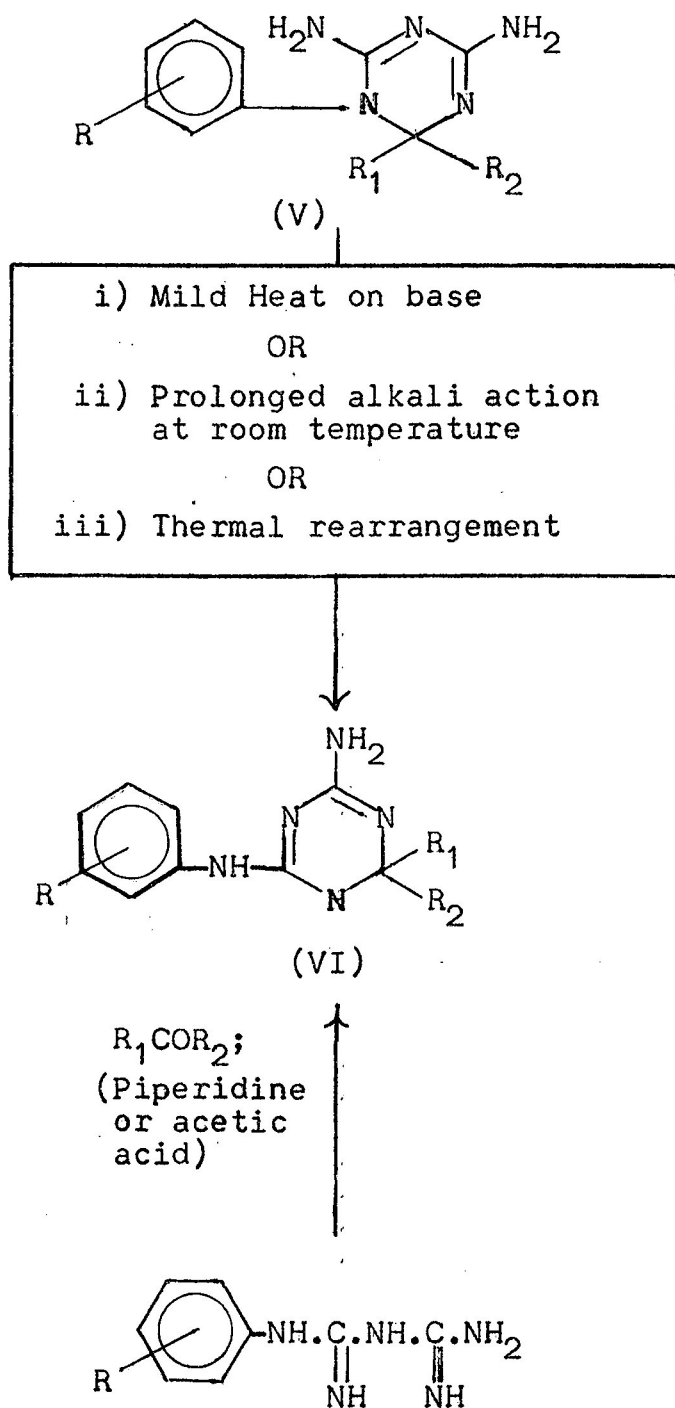
Synthesis through a Schiff Base (Route C) :

In this method the carbonyl component is present insitu as a Schiff base of the amine. Reaction fails to take place in absence of acid which suggests that cleavage of the anil occurs before the desired condensation and cyclization.^{50,113}

Reaction of 4,6-diamino
dihydro-s-triazines :

When compounds with the general structure V are treated with an excess of alkali at elevated temperature, they readily undergo an irreversible, intramolecular, rearrangement to isomeric 4-amino-6-anilino-1,2-dihydro-s-triazines (VI) which are relatively inactive biologically. Other methods for the isomerization of V to VI include (i) the action of mild heat on the free base of V; (ii) the prolonged action of alkali on V in aqueous solution at room temperature and (iii) the thermal rearrangement of the hydrochloride salt of V to that of VI in the dry salt at the melting point.⁵⁰

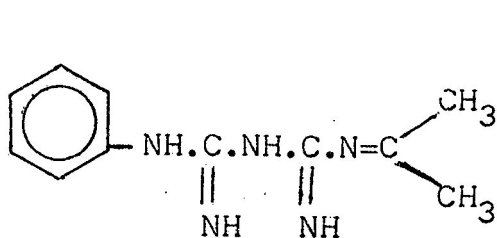




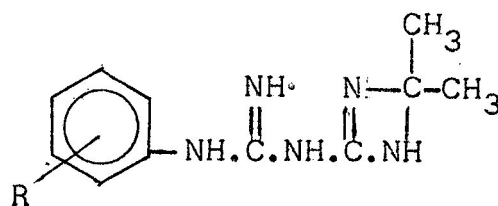
The anilino dihydro-s-triazines VI can also be prepared by the condensation of arylbiguanide with ketones or aldehydes in the presence of a base such as piperidine^{114,115} or a weak acid^{115,116,117} or without catalyst.

Both the isomeric structures V and VI can be characterized by a distinctive ultraviolet absorption spectrum differing from that of the arylbiguanide.

Open chain aryl biguanide structure such as IX or four membered ring structures such as X are unlikely on the basis of the U.V. absorption spectra.



(VII)

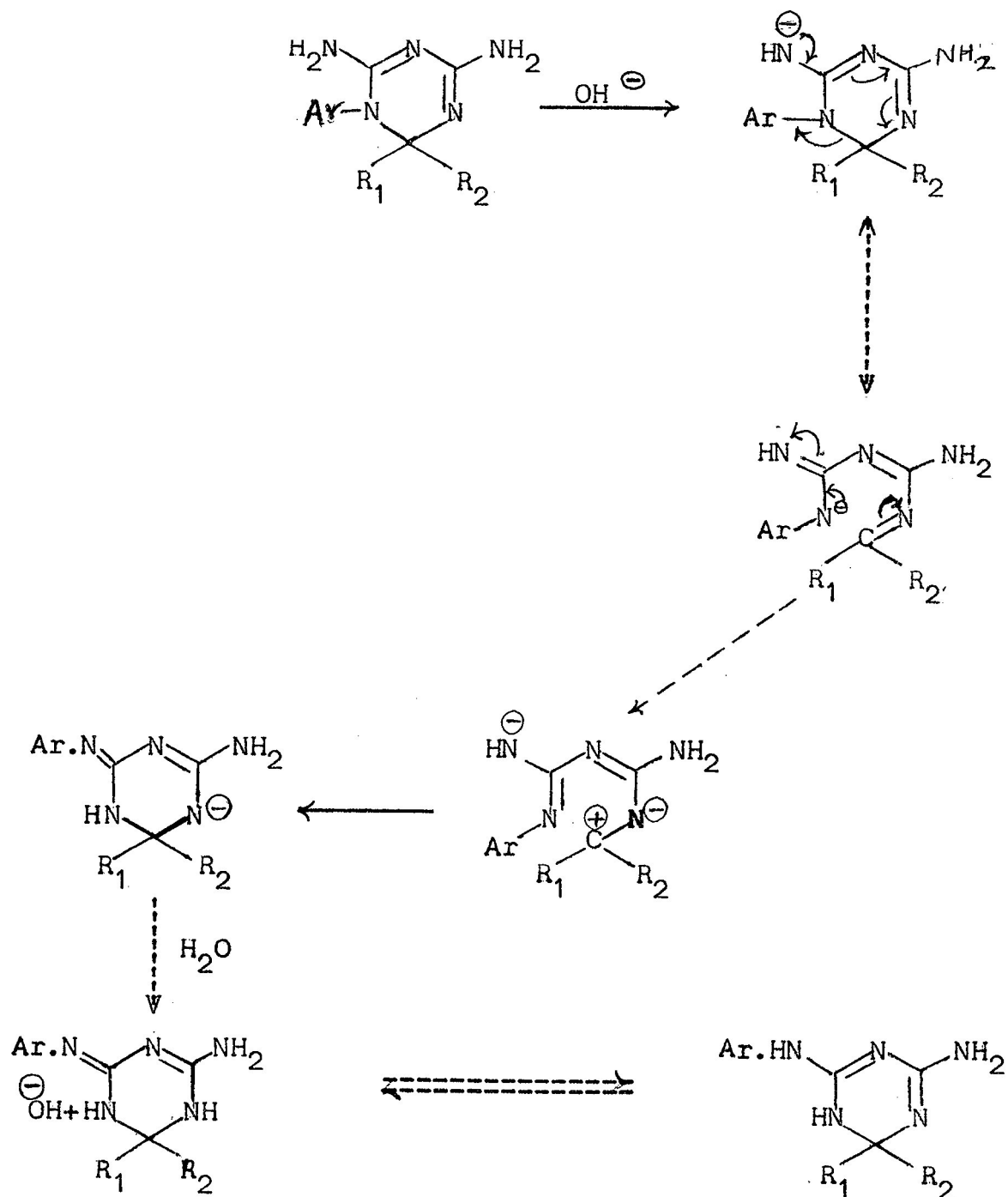


(VIII)

A proposed mechanism for the rearrangement of V to VI in alkali involves anionic attack at the 6-amino group followed by rupture of the bond between N¹ and C². The resulting carbonium ion C², then reccyclizes at the 6-amino group to form a new ring.⁴⁹

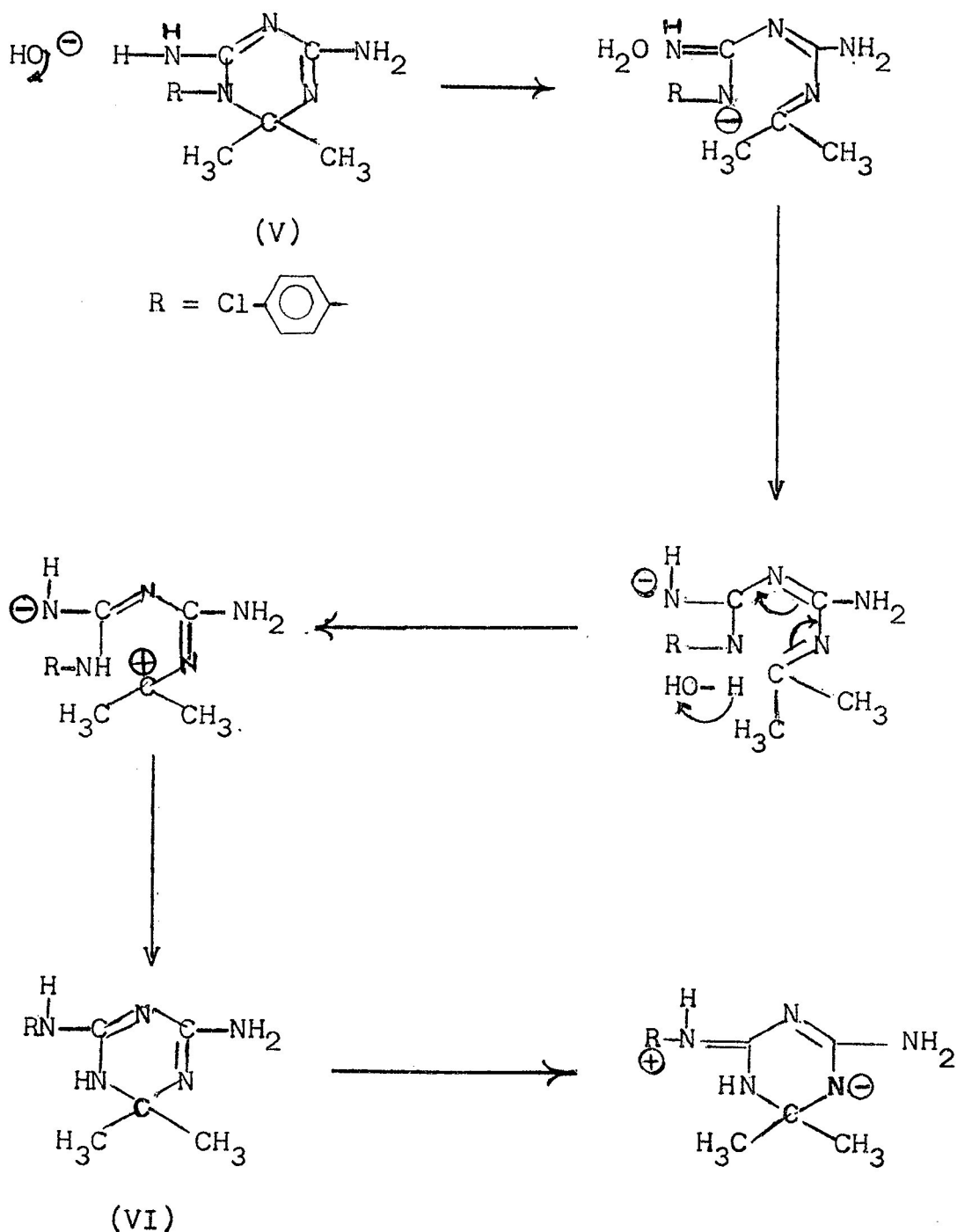


I) Mechanism :



Another speculation is that the rearrangement proceeds through the following steps, the OH^- being the catalyst.¹¹⁸

II)



The driving force for such a rearrangement would be the achievement of the coplanarity of the benzene ring with the dihydrotriazine ring.

The presence of s-triazine was confirmed by U.V. and the absence of biguanide formation observed by the colour complex test during the course of each reaction.

Colour Complex Test of

Cuprammonium Sulphate Test :

The colouration of precipitate (usually pink) formed by treatment of an aqueous solution of the reaction sample with a drop of 5 % CuSO_4 in excess ammonium hydroxide indicates a positive biguanide test.⁴⁹



-o-

SPECTRAL STUDIES

-o-

SPECTRAL STUDIES :

Spectroscopic properties :

In the present work some of the compounds were treated with an excess of alkali at elevated temperature to confirm the isomerization. It was observed that they underwent intramolecular rearrangement to their isomers. The rearrangement from a phenyl dihydrotriazine to an anilino dihydro-s-triazine was accompanied by characteristic changes in U.V. absorbance.

Most of the compounds in our work had λ_{max} between 210-245 m μ in agreement with the U.V. spectra of diamino-s-triazines.^{50,53} Four of the compounds were converted by treatment with 0.5 N NaOH to their respective anilino dihydro-s-triazines and a (bathochromic shift) to higher wavelength was observed. Table B indicates the λ_{max} and physical data of the isomers of four such compounds.

Spectra were determined at varied concentrations by trial and error using CH₃OH as solvent on a Perkin-Elmer Model 402 spectrophotometer.

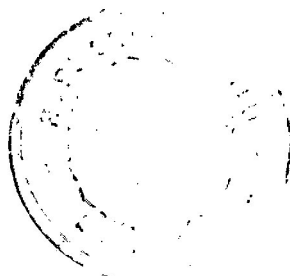
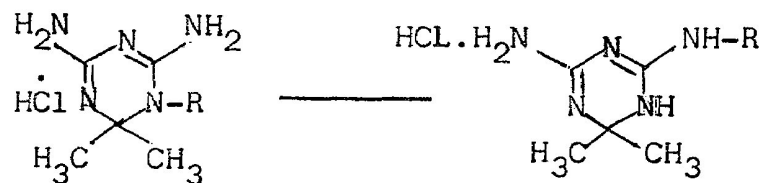


TABLE-B: U.V. SPECTRA DATA



No.	R	max mu	M.P. °C	max mu	M.P. °C
1.		232	215	257	121
2.		237	174	259	201
3.		245	212	262	300
4.		210	249	310	197

Spectra were determined at a concentration of 2 mg/100 ml.
on a Perkin-Elmer Model 402 Ultra Violet visible spectrophotometer

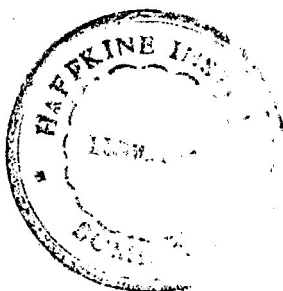
IR Spectral Studies of 1-aryl-
4,6-diamino-s-triazines :

Some representative members were studied for their I.R. spectra, and the graphs were found to be compatible with the assigned structures. Perkin-Elmer Model 257 spectrophotometer-KBR phase was used.

The common features of all triazine compounds as observed are strong peaks near 6.4 u and 6.6 u supporting the assignment of Degraw¹¹⁹ of absorption of the triazine ring near 6.40 u and 6.60 u.

s-Triazines have been studied by Lancaster et al.¹²⁰ All symmetrically substituted derivatives show only two ring stretching bands near 1560 and 1410 cm^{-1} , which is observed in the graphs taken for the compounds in the present work.

All s-triazines absorb in the 800 cm^{-1} region. The position of the bands depends upon the response characteristics of the substituents and the overall range is 817-736 cm^{-1} .



Analysis of I.R. Spectra of Fig. 1 :

4,6-Diamino-1,2-dihydro 2,2-dimethyl-
1-[(p-chloro benzyloxy)phenyl] 1,2-
dihydro-s-triazine :

$-\text{C}(\text{CH}_3)_2$ 1365 cm^{-1} ; $\text{C} = \text{C} + \text{C} = \text{N}$ (Str.) $1540\text{--}1580 \text{ cm}^{-1}$;
 $\text{NH}(\text{Str.})$ 3300 cm^{-1} ; C-Cl 690 cm^{-1} , amino substituted
s-triazine absorption at 1500 , 1240 and 810 cm^{-1} .

$\text{C} = \text{C}(\text{Str.})$ 1590 cm^{-1} ; 1,4-disubstituted benzene =
 $\text{C-H}(\text{Str.})$ weak band at 2975 cm^{-1} ; N-H def. 1650 cm^{-1} ;
 $\text{C} = \text{N}(\text{Str.})$ 1290 cm^{-1} ..

Analysis of I.R. Spectrum of Fig. 2 :

4,6-Diamino 1,2-dihydro 2,2'-dimethyl-
1-(1-adamantyl)-s-triazine hydrochloride :

$\text{N-H}(\text{Str.})$ 3400 , 3300 , 3210 cm^{-1} ; C-H Str. (asym.)
 2900 cm^{-1} ; $\text{C-H}(\text{Str.})$ (Sym.) 2810 cm^{-1} ; C-H scissoring
 1405 cm^{-1} ; ring str. bands 1560 ; 1410 cm^{-1} ; s-triazine
absorption $770\text{--}800 \text{ cm}^{-1}$; $\text{C=NH}^+(\text{Str.})$ 2210 cm^{-1} , 1610 cm^{-1}
 C-H twisting and wagging 1310 cm^{-1} ; $-\text{C}(\text{CH}_3)_2$ 1365 cm^{-1}

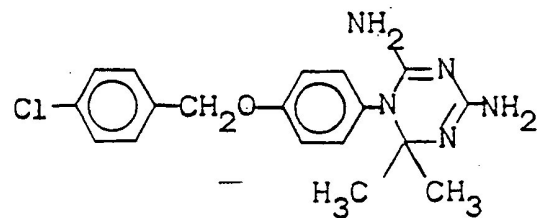
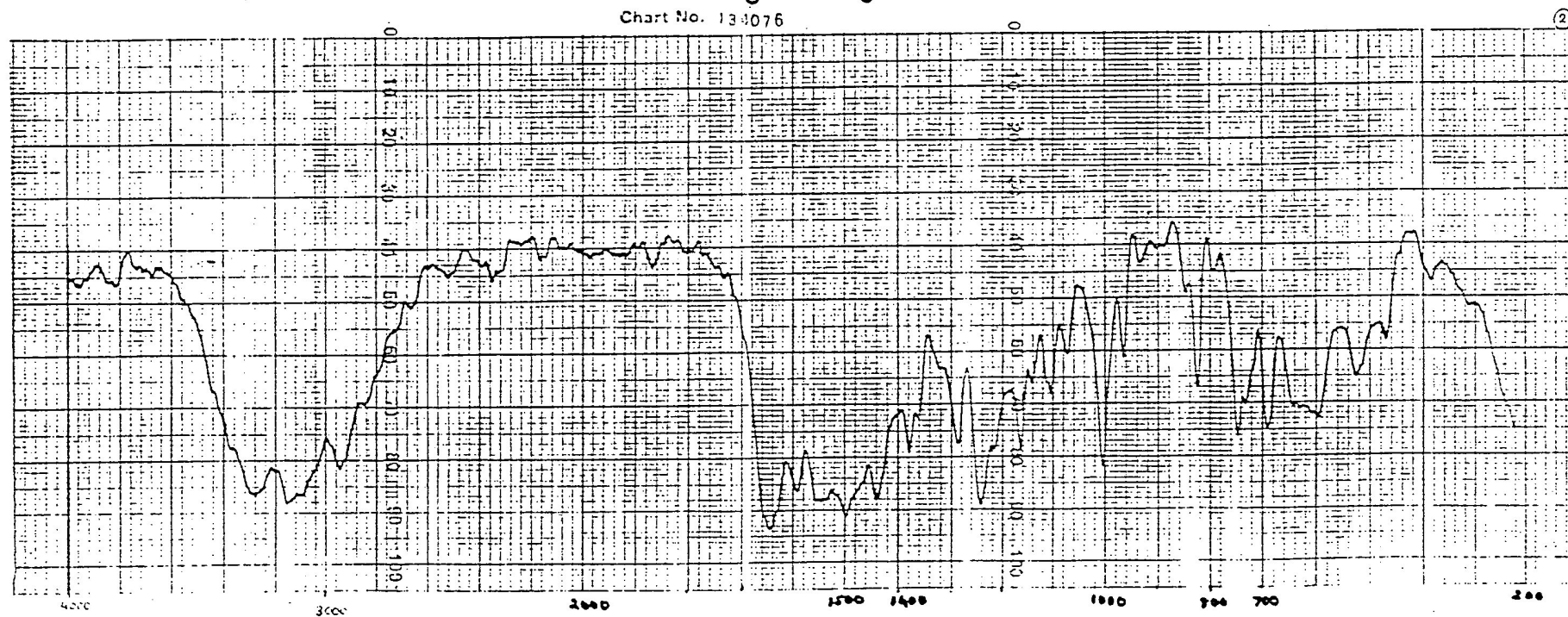
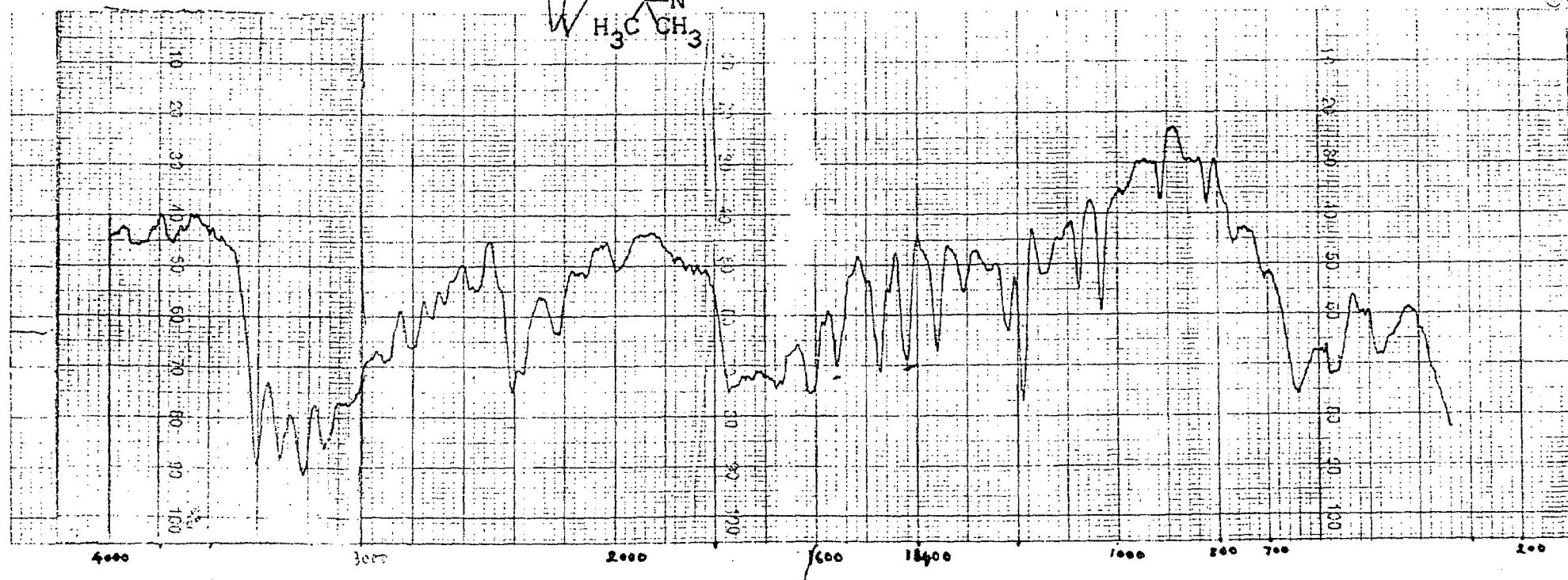
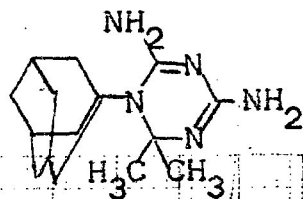


Chart No. 134076

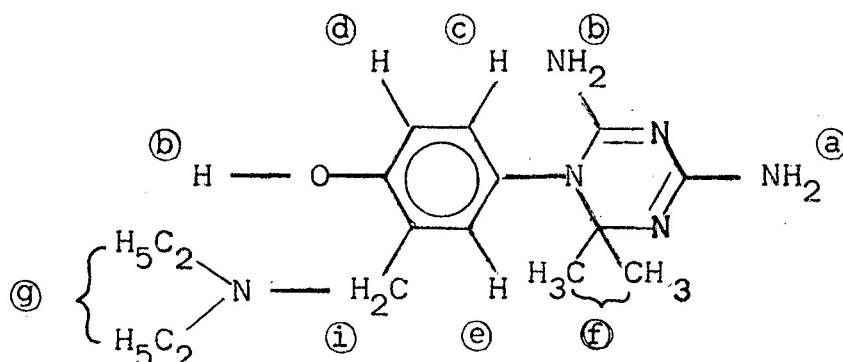


(Fig. 1)



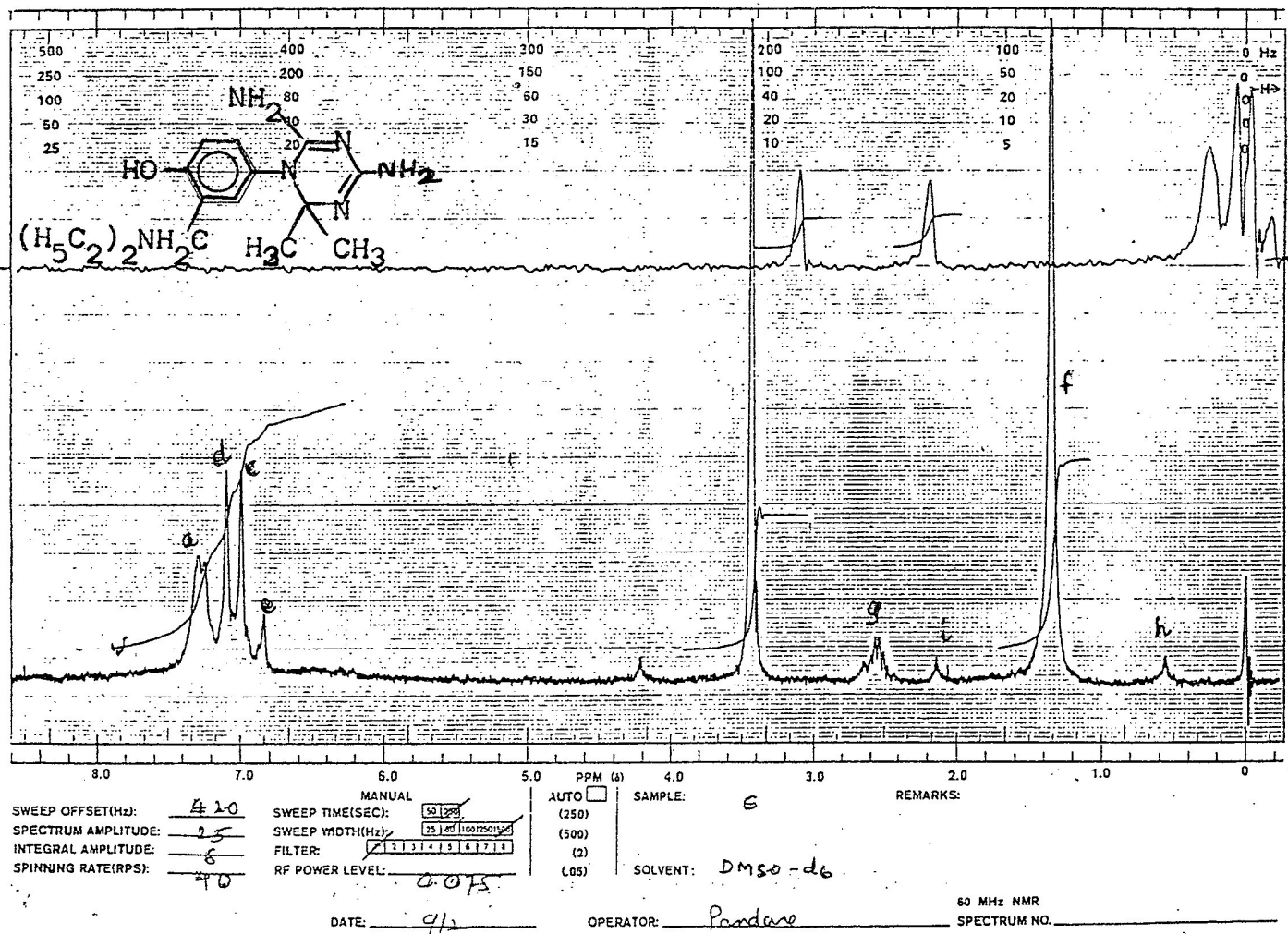
(Fig. 2)

Analysis of N.M.R. Spectrum :

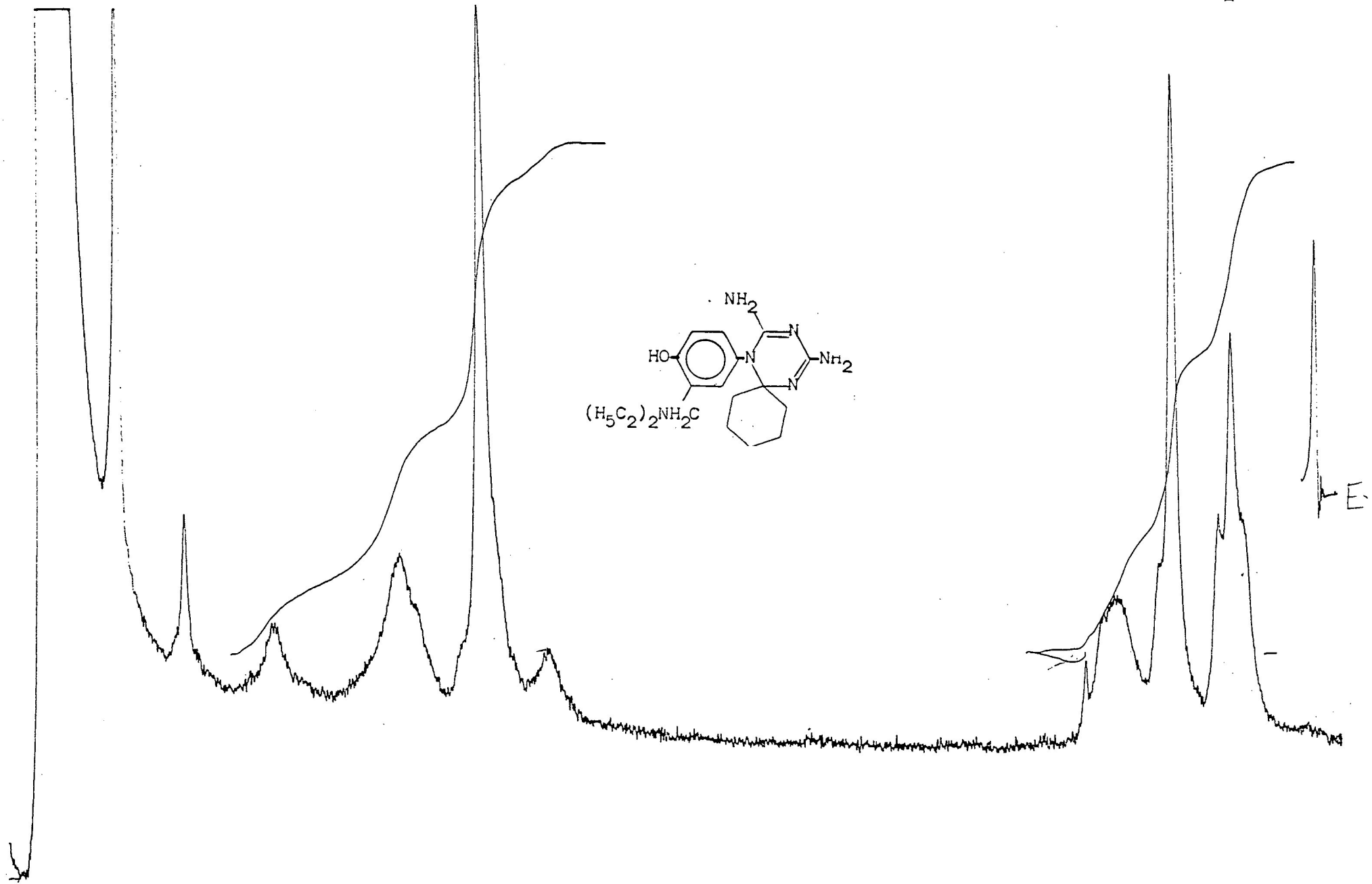


The peaks at δ 7.3 (a) and 7.4 (b) are due to the amino protons on triazine ring. Gem-dimethyl protons show a strong singlet at δ 1.4 (f). N-N-Diethyl protons (g) show a multiplet at δ 2.4-2.6. Methylene protons (i) shows a weak singlet at δ 2.2. Proton of -OH group (h) shows singlet at δ 0.5. Aromatic protons of phenyl ring shows peaks at δ 7.0 (c), δ 7.1 (d) and 6.8 (e).





(Fig. 3)



MASS SPECTRA :

Fig. 4 :-

The mass spectra of the chlorobenzyloxy analog shows an intense base peak at 125 m/e of the chloro benzyl cation.

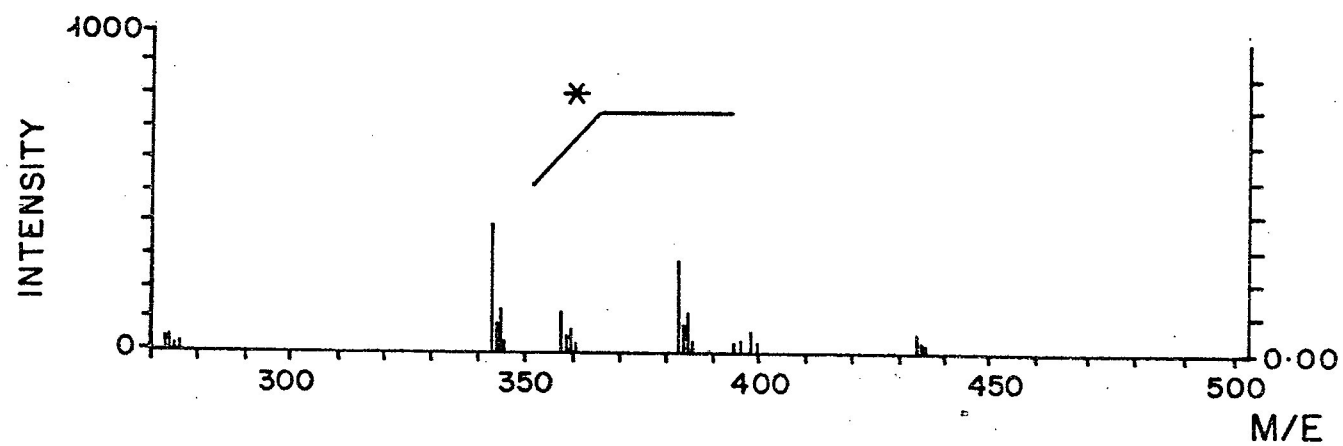
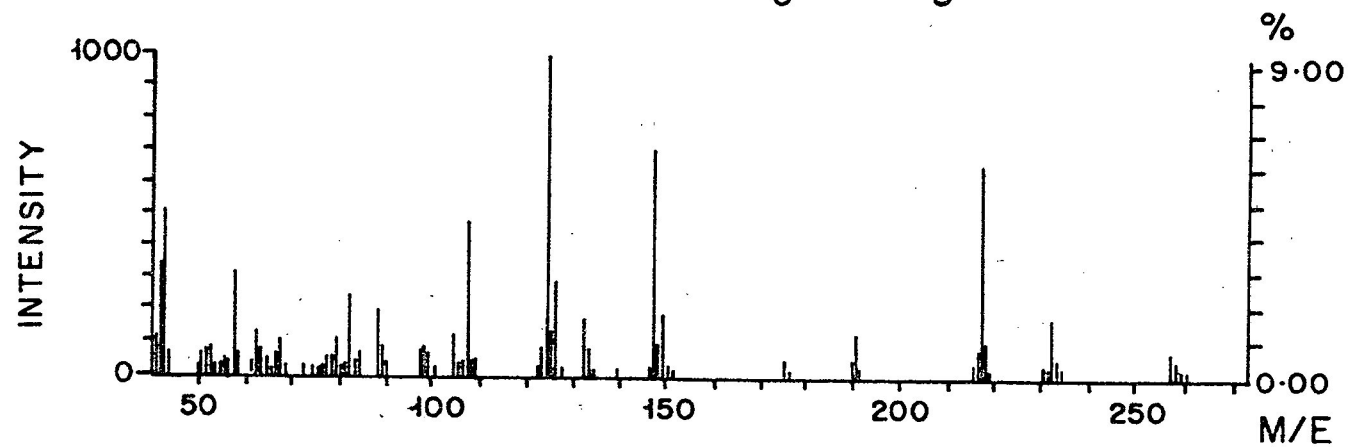
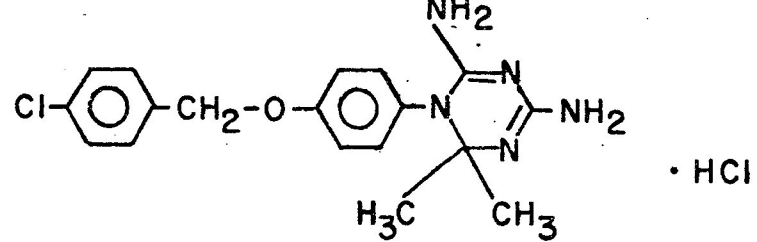
A peak of slightly lower intensity is observed at 149 m/e corresponding to the fragment $(C_9H_{11}NO)^+$

Molecular ion peak can be observed at 357.5 (M), 358.5 (M+1), 359.5 (M+2) and weak peaks at 396, 398 and 399 of the hydrochloride salt

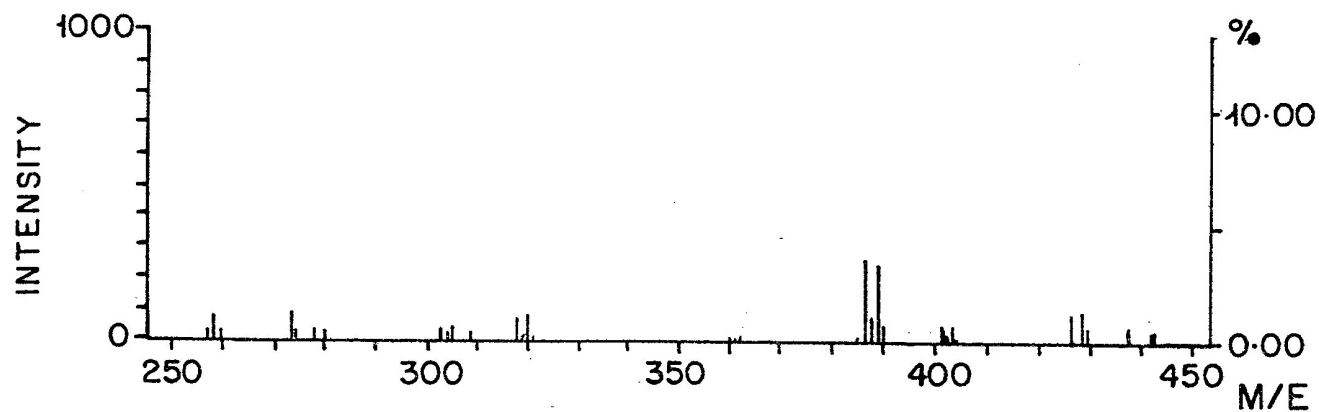
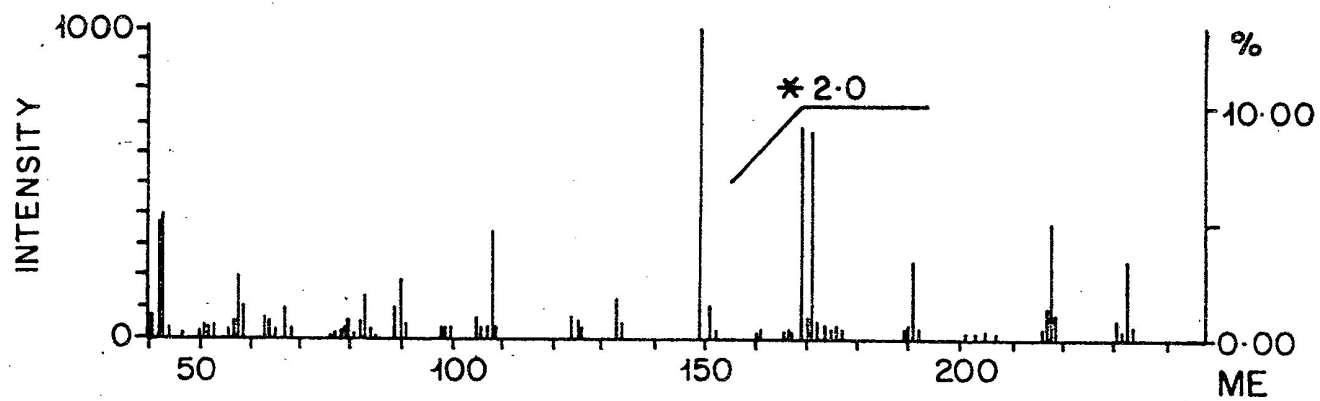
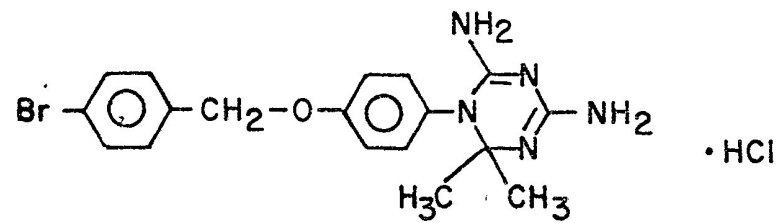
Fig. 5 :-

The mass spectra of the bromo benzyloxy analog show a typical fragmentation pattern of the substituted triazine. It shows an intense base peak at 149 m/e of $(C_9H_{11}NO)^+$ fragment.

A molecular ion peak at 402 (M), 403 (M+1), 404 (M+2) and weak peaks at 438, 439, 442, 443 as the hydrochloride salt.



(Fig. 4)



(Fig. 5)

Section - A

.....

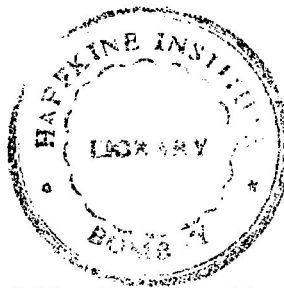
SYNTHESIS OF 4,6-DIAMINO-1,2-DIHYDRO-
2,2-DIMETHYL-1-(SUBSTITUTED) PHENYL
s-TRIAZINES.

.....

-O-

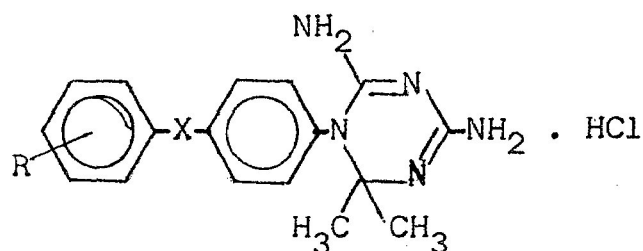
C H E M I S T R Y

-O-



CHEMISTRY :

This section deals with the synthesis of compounds having general structure.



.(IX)

Where, R = Cl, Br, etc.

X = -O-, -S-, -CH₂O-

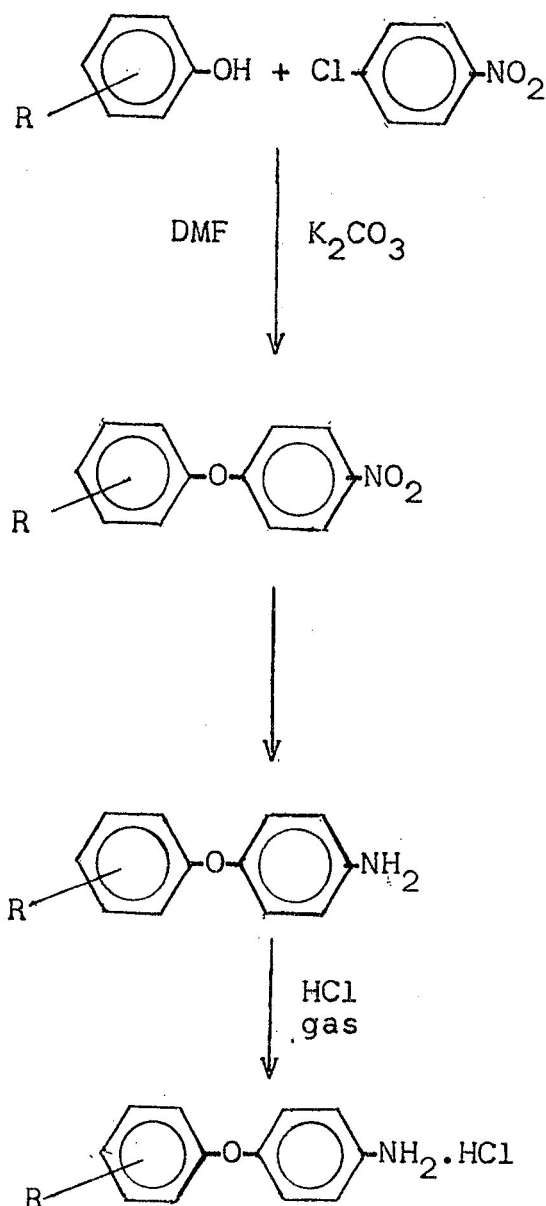
The various bisarylamines utilized for triazine formation were prepared as per the method of Williamson¹²¹ which involves direct nucleophilic displacement of halogen in an alkyl or aryl halide by a phenoxide ion. The following methods were used.

(a) Synthesis of 4'-substituted 4-amino diphenyl ethers :

Substituted amino diphenyl ethers were prepared by condensing different substituted phenols with p-chloro nitro benzene in dimethylformamide, using potassium carbonate as catalyst. Temperature of the reaction mixture was kept at 130-140°C. Nitroethers thus obtained were further

subjected to catalytic reduction using Raney Nickel. The amino ethers were converted to their amine hydrochloride salts by passing dry hydrogen chloride gas in cold conditions. The schematic view of the synthetic pathway is as below :

Scheme - II :



(b) Synthesis of 4'-substituted
4-amino diphenyl benzyl ethers :

Benzyl ethers of phenols were synthesized by coupling an alkali phenolate or phenol and benzyl halide in an appropriate solvent.¹²² These benzyl ethers of phenols can also be prepared by the method of Boehme¹²³ in which case a sodium salt of acetamidophenol is refluxed with benzyl halide in alcohol and the product hydrolysed to give the corresponding amines.

In our work the p-halogeno benzyl halides were prepared from the corresponding p-halogeno toluenes.

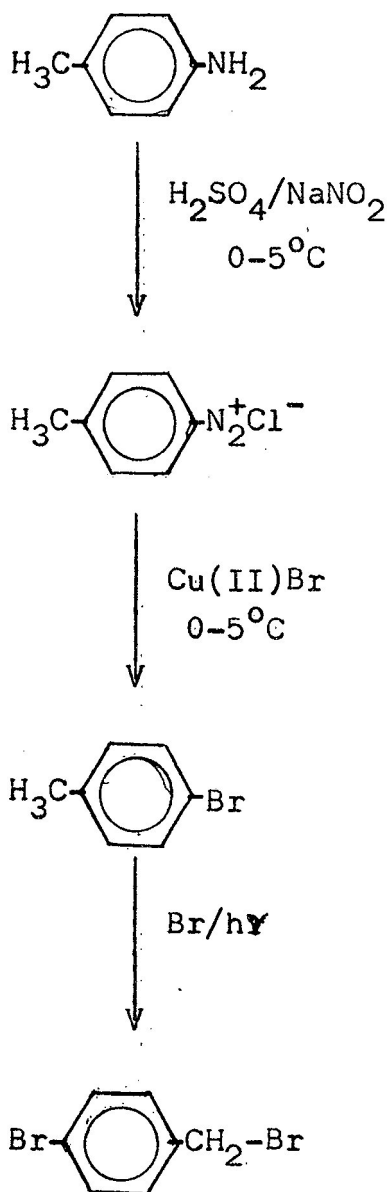
The required p-bromo toluene and p-chloro toluene were prepared from p-toluidine with diazotization followed by the Sandmeyer's reaction. p-Halogeno benzyl bromides were prepared by bromination at 120-140°C in the presence of light.

The p-halogeno benzyloxy anilines were prepared by reacting p-halogeno benzyl halides with p-acetamidophenol in the presence of anhydrous potassium carbonate in dimethyl formamide followed by the hydrolysis of the acetamido derivatives.

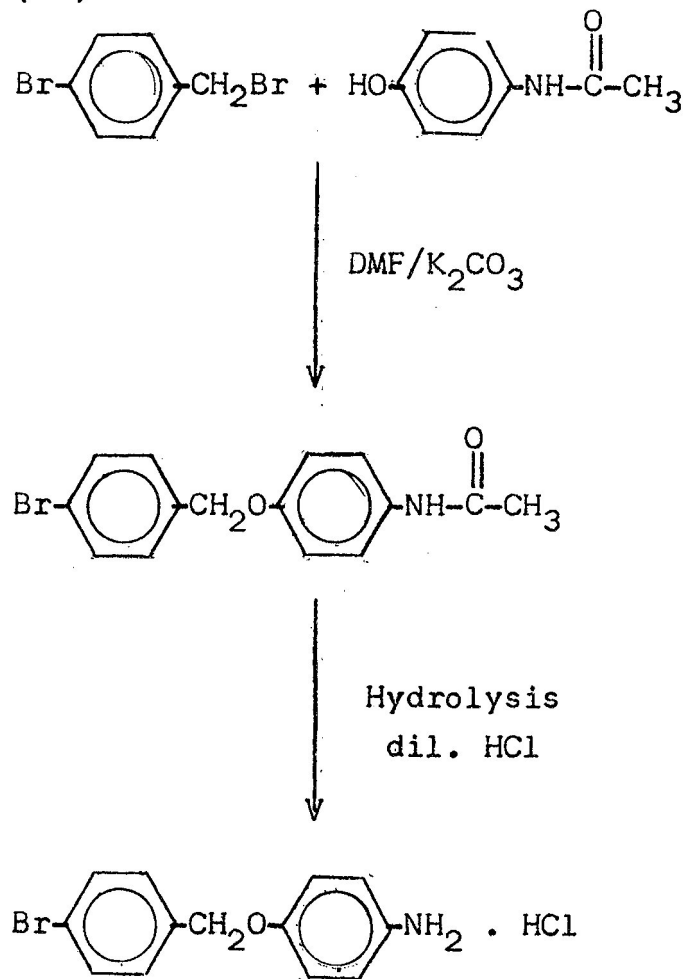
The sequence of reactions involved are as follows:

Scheme - III :

(i)

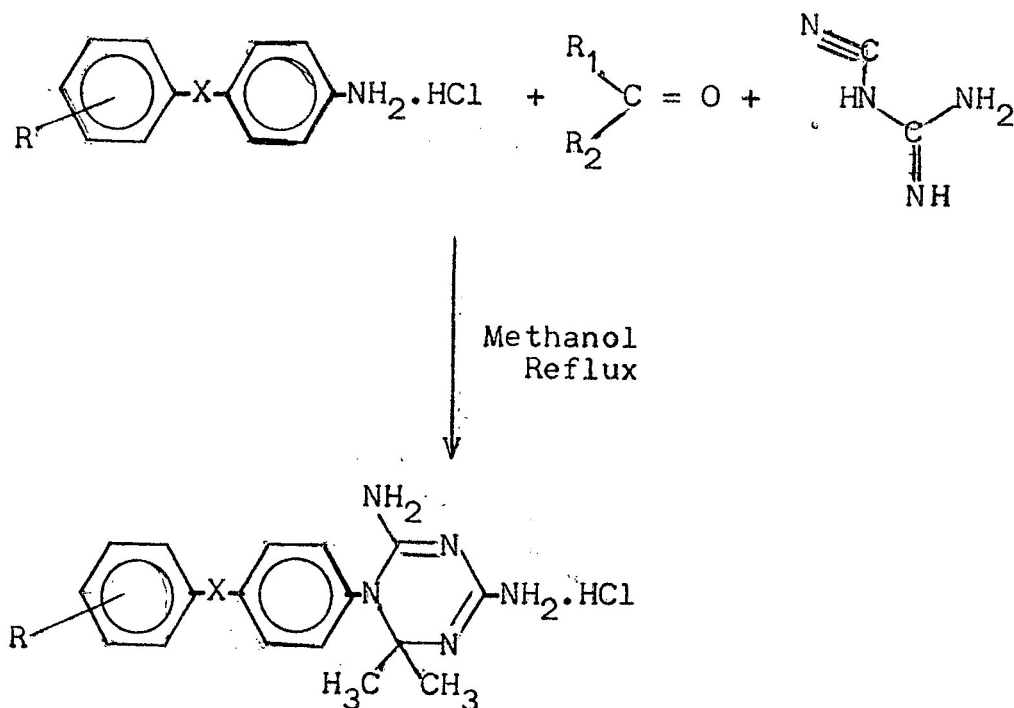


(ii)



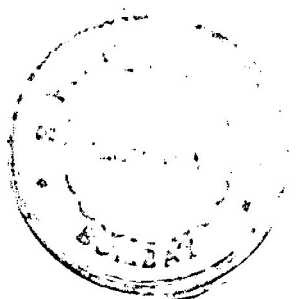
Scheme - IV :Synthesis of s-triazine :

All the bis-arylamine hydrochlorides were utilized for the preparation of s-triazines as shown in Table-II.



Where, $X = -O-, -S, -CH_2O-$

$R = Cl, Br, NO_2, NHCOCH_3, NHCOOCH_3$



-O-

EXPERIMENTAL

-O-

EXPERIMENTAL ^o :

Synthesis of 4'-Chloro,4-Nitro
diphenyl ether :

In a 3 necked flask was placed p-chlorophenol 12.85 g. (0.1 M) in 15 ml. of dry DMF. Fine powder of anhydrous K_2CO_3 (15.0 g.) was added to this reaction mixture. This was then heated to 100-110°C in an oil bath and treated dropwise with a hot solution of p-chloro nitro benzene (prepared by dissolving 15.75 g. (0.1 M) of p-nitro chlorobenzene in 20 ml of DMF. The addition took 2-3 hours. After addition, the reaction mixture was refluxed for 6-8 hours, at 140-150°C. Excess solvent was removed under reduced pressure and the residue was poured on crushed ice-pieces to precipitate the 4-chloro, 4-nitro diphenyl ether. The crude product was purified from ethyl alcohol.

Yield : 17.47 g. (70.0 %); m.p. 69-70°C.

(Lit.¹²⁴ m.p. 67-69°C).

Table-I lists the compounds similarly prepared.

Reduction of 4-nitro-4'
substituted diphenyl ethers :

4-Nitrodiphenyl ether (21.5 g; 0.1 M) or (4'-substituted 4-nitro diphenyl ether) was dissolved in 50 ml of hot EtOH and this solution was placed in hydrogenation bottle. Then 3.5-4.0 g. of Raney Nickel catalyst was added to it, and the resultant reaction mixture was hydrogenated in parr hydrogenator. After complete absorption of hydrogen, the reaction mixture was filtered and the catalyst was decomposed with 10 % HCl. The filtrate was concentrated under reduced pressure to give 4-amino diphenyl ether recrystallized from alcohol or any suitable solvent.

Yield : 17.0 g. (92.0 %); m.p. 85°C.

Lit.¹²⁵ m.p. 84°C.)

Synthesis of p-Bromo toluene :

(A) Preparation of Cu(II)Br solution :

A mixture of 31.5 g. (0.125 M) of crystalline CuSO_4 : 10 g. (0.155 atoms) of copper turnings; 77 g. (0.55 M) of $\text{NaBr} \cdot 2\text{H}_2\text{O}$; 15 g. (8.15 cc; 0.14 M) of concentrated H_2SO_4 (Sp.gr 1.84) and 1 litre of water was refluxed over a flame for 3-4 hours, until the colour becomes yellowish.

(B) Preparation of diazonium chloride solution :

When the hydrobromic acid cuprous bromide solution was ready for use the diazonium solution was prepared.

A solution of 53.5 g. (0.5 M) of p-toludine and 98 g. (53.5 cc; 0.95 M) of concd. H_2SO_4 (Sp.gr 1.84) in 500 ml water was cooled below 20°C and diazotised with a solution of 35 g. (0.5 M) of NaNO_2 in 62.5 ml of water. This required 20-30 minutes, the temperature was maintained between $15-20^\circ\text{C}$.

(C) Preparation of p-bromo toluene :

A 2 litre round bottom flask containing the hydrobromic acid-cuprous bromide solution (A) was arranged for steam distillation. After the copper solution was heated to boiling the diazonium solution (B) was slowly added and a vigorous current of steam was passed through the reaction mixture in about 2 hours time. The aqueous distillate was made alkaline with NaOH solution and the p-bromo toluene was separated from the water layer. The crude product was purified by washing once with concentrated H_2SO_4 and then with water. It was dried over CaCl_2 , filtered and distilled.

Yield : 49.60 g. (58.0 %); b.p. 183°C ; m.p. $24-25^\circ\text{C}$.

(Lit.¹²⁶ b.p. $183-185^\circ\text{C}$; m.p. $25-26^\circ\text{C}$.)

Synthesis of p-bromo benzyl bromide :

p-Bromo toluene 20.4 g. (0.12 M) was taken in a 250 ml three necked round bottomed flask, equipped with a mercury sealed stirrer, a dropping funnel and a reflux condenser with a gas trap and heated in an oil-bath at 120°C (bath temperature). Illuminated with a lamp of 100 watts. Then bromine 13.6 g. (0.17 M) was added dropwise during a period of 2 hours and stirring was continued for another 2 hours. By this time the evolution of hydrogen bromide ceases. The product p-bromo benzyl bromide solidified upon cooling to a brownish crystalline mass and was filtered and washed three times with 10 ml of hexane or petroleum ether.

Yield : 27.0 g. (75.0 %); m.p. 60°C.

(Lit.¹²⁷ m.p. 61°C.)

Similarly was prepared p-nitrobenzyl bromide and p-chlorobenzyl bromide.

Synthesis of 4'-bromo-4-acetamido diphenyl Benzyl ether :

In a 250 ml round bottomed was placed 10 g. (0.04 M) of p-bromo benzyl bromide in 30 ml of DMF. To this was added 6.191 g. (0.041 M) of acetamido phenol and 6.9 g.

(0.05 M) of potassium carbonate. The flask was then fitted with a condenser and guard tube and placed in an oil-bath. The reaction mixture was heated for 10-12 hrs maintaining the bath temperature between 130-140°C. Excess solvent was removed under reduced pressure and the residue was poured on crushed ice-pieces with vigorous stirring to precipitate the 4-bromo, 4-acetamido diphenyl benzyl ether.

Yield : 7.3 g.; m.p. 175°C. (82.0 %)

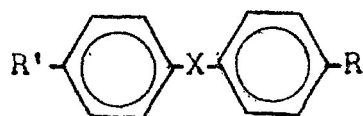
(Lit.¹²³ m.p. 180°C.)

Similarly were prepared 4-chloro, 4-acetamido diphenyl benzyl ether.

The above obtained acetanilide compounds were converted to their respective amines by hydrolysis with 10 % hydrochloric acid at reflux temperature till the reaction mixture became homogenous. The hydrolysed solution was then treated with NaOH in cold conditions to obtain neutral pH and the free base isolated as white solid which was pure enough to be used further.



TABLE - I : Bis-arylamines utilized for triazine
formation in Section-A indicating
physical data :



No.	R'	X	R	m.p. °C	
				uncor. obtained	Reported
1.	Cl	O	NH ₂	110	101
2.	2,4-dichloro	O	NH ₂ .HCl	233-34	236-37
3.	NHCOCH ₃	O	NH ₂ .HCl	282	280
4.	Cl	-CH ₂ O-	NH ₂	102-105	102
5.	Br	-CH ₂ O-	NH ₂	145	148
6.	NHCOOCH ₃	-S-	NH ₂	80-81	82
7.	NO ₂	-O-	NH ₂	125-130	131
			NH ₂ .HCl	123	220

Synthesis of 4,6-diamino-1,2-dihydro-
2,2'-dimethyl-1-[(p-chlorophenyl) ether]
phenyl s-triazine hydrochloride :

0.5 g. (0.0019 M) of 4'-chloro-4'-amino diphenyl ether hydrochloride; 0.21 g. (0.0025 M) of dicyandiamide and 20 cc. of acetone were refluxed on a water bath for 6 hrs. About 5 cc. of dry methanol was added to make the reaction mixture homogenous. A dark violet coloured solution was obtained, which gave a negative biguanide test with cuprammonium sulfate solution. The homogenous solution was refluxed for another 4-5 hrs, given Norit treatment, the filtrate concentrated and refrigerated for two days. Off white crystals of the final product were obtained, which were filtered, washed with acetone and dried in vacuo at 100°C for 2 hrs. Crystallized once from methanol.

Yield : 0.497 g. (57.5 %); m.p. 221°C.

Analysis for : $C_{17}H_{18}N_5ClO.HCl.0.5H_2O$

	<u>% C</u>	<u>% H</u>	<u>% N</u>
Calcd.	52.44	5.14	17.19
Found.	53.06	5.86	17.44

4,6-Diamino-1,2-dihydro-2,2-dimethyl
1-[p-(p-chloro benzyl) oxy] phenyl
s-triazine hydrochloride :

A mixture of 4'-chloro-4-amino diphenyl benzylether hydrochloride 0.500 g. (0.00101 M); dicyandiamide 0.21 g. (0.0025 M); 1.5 ml of dry methanol and 10-15 cc. of acetone was stirred and heated under reflux for 6 hours under anhydrous conditions. The reaction mixture became a clear, brownish solution within 30 minutes. Crystalline material started depositing after about 3 hrs. of reflux. The reaction was completed after 17 hrs, cooled, the product collected by filtration and washed with acetone and ether. It was recrystallized from aqueous CH_3OH to obtain colourless to pale pink shining prisms of the title compound.

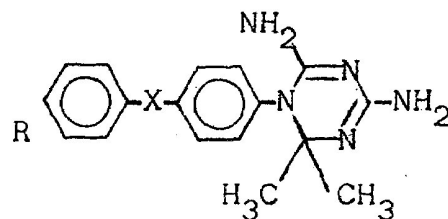
Yield : 0.270 g. (85.61 %); m.p. 234°C .

Analysis for : $\text{C}_{18}\text{H}_{20}\text{ClN}_5\text{O}.\text{HCl}$

	<u>% C</u>	<u>% H</u>	<u>% N</u>
Calcd.	54.82	5.07	17.77
Found.	54.24	5.58	18.03

Similarly were prepared the other triazines of the diphenyl ether series as shown in Table-II.

TABLE - II : Synthesis of 4,6-diamino-1,2-dihydro 2,2-dimethyl-1-(substituted) phenyl-s-triazine hydrochlorides.



No.	R	X	R'	M.p. °C (Uncorr.)	Yield %	Molecular Formula	Elemental Analysis					
							Calculated			Formula		
							C	H	N	C	H	N
1.	Cl	-CH ₂ O-	CH ₃	234	65.6	C ₁₈ H ₂₀ N ₅ ClO.HCl	54.82	5.076	17.17	54.24	5.58	18.03
2.	Br	-CH ₂ O-	CH ₃	225	15.2	C ₁₈ H ₂₀ N ₅ BrO.HCl	49.25	4.56	15.96	49.15	4.73	14.62
3.	Cl	-O-	CH ₃	215	57.0	C ₁₇ H ₁₈ N ₅ ClO.HCl	52.44	4.88	17.99	53.06	5.86	17.44
4.	3,4-dichloro	-O-	CH ₃	221	57.5	C ₁₇ H ₁₇ N ₅ Cl ₂ O.HCl	47.16	4.16	16.18	46.75	4.46	16.52
5.	NO ₂	-O-	CH ₃	212	22.3	C ₁₇ H ₁₈ N ₆ O ₃ .HCl	52.24	4.16	21.52	52.48	5.13	21.82
6.	NHCOCH ₃	-O-	CH ₃	245	65.0	C ₁₉ H ₂₂ N ₆ O ₂ .HCl	56.71	5.47	20.89	55.9	5.69	19.23
7.	NHCOOCH ₃	-S-	C ₃ H ₅	Oily	29.3	C ₂₀ H ₂₄ N ₆ O ₂ S.HCl	53.51	5.35	18.72	-	-	-
8.	NHCOOCH ₃	-S-	CH ₃	215	75.6	C ₁₉ H ₂₂ N ₆ O ₂ S.HCl	52.46	5.29	19.32	51.72	5.08	19.74

Section - B

.....

SYNTHESIS OF o-CRESOL, 4-(4,6-DIAMINO-
2,2-DIALKYL OR ARYL-1,2-DIHYDRO-s-TRIAZINE)
-1-SUBSTITUTED AMINO METHYL HYDROCHLORIDES.

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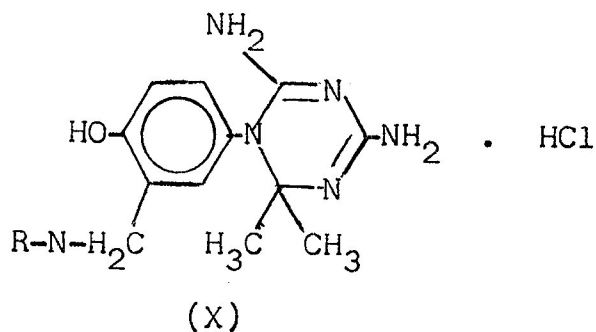
-O-

C H E M I S T R Y

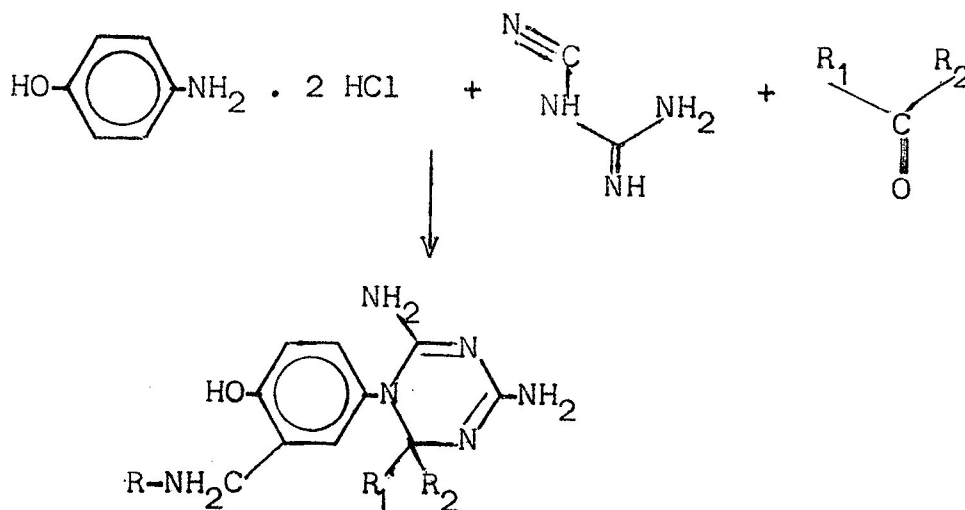
-O-

CHEMISTRY :

This section comprises of compounds of the following general structure (X).



The above type of compounds were synthesized by condensing 3-dialkyl amino methyl 4-hydroxy aniline with dicyandiamide in various substituted aromatic aldehydes and ketones.

Synthesis of s-triazines :

Where, $R = -(C_2H_5)_2-$

The 3-diethylaminomethyl-4-hydroxy aniline side chain was synthesized by the Mannich Condensation Reaction.¹²⁸ The Mannich Reaction consists in the condensation of a secondary amine hydrochloride, with formaldehyde and a compound containing at least one hydrogen atom of pronounced activity. The essential feature of the reaction is the replacement of the active H-atom by an aminomethyl or substituted aminomethyl group.

Use of Secondary Amines with Phenols :

The o- and p-hydrogens in phenols are sufficiently active to enter into the Mannich Reaction. Thus phenol,¹²⁹⁻³¹ 4-acetamidophenol,¹³¹ o and p-cresol etc. react¹²⁹ with formaldehyde and any secondary amine.

Amongst the secondary amines dimethylamine is very reactive and gives excellent yields. Diethylamine is comparatively less reactive. Cyclic secondary amines like pyrrolidine, morpholine, etc. react to give good yields.

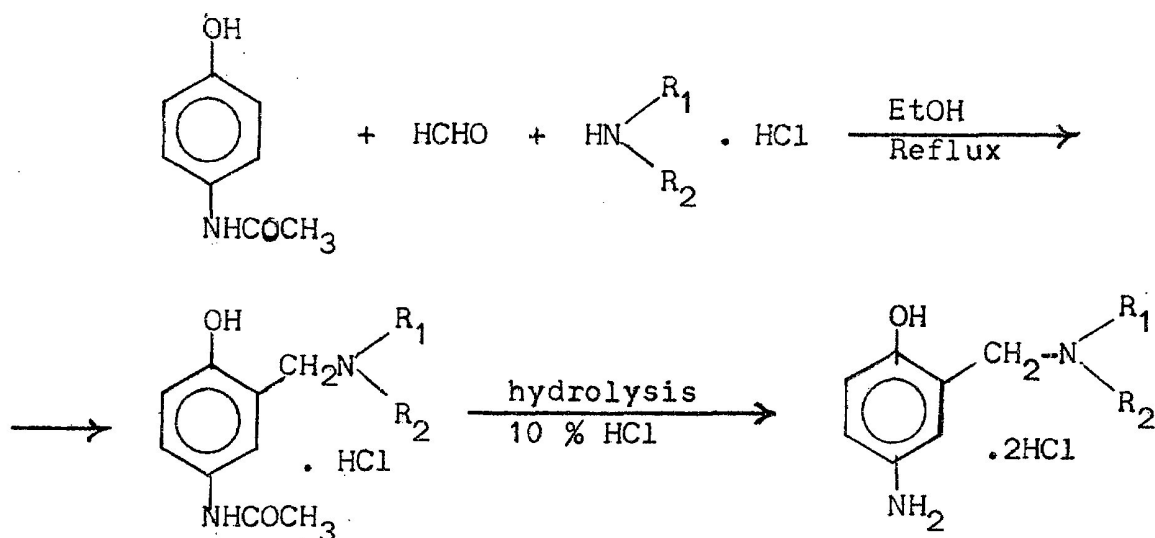
Use of formaldehyde and hydrochloric acid :

Formaldehyde is used in the form of a 20-40 % aqueous solution or as paraformaldehyde. In some cases enough concentrated hydrochloric acid is added at the

beginning^c of the reaction. In some instances the mixture is acidified at the end of the reaction in order to depolymerize unchanged paraformaldehyde and bring it into solution.

Preparation of triazines of this section was done as per the Scheme given below :

Scheme - V :



The reactions of 3-diethylamino methyl-4-hydroxy aniline and 3-pyrrolidinyl methyl-4-hydroxy aniline were carried out using equimolar quantities of 4-acetamidophenol dissolved in Ethanol (95 %), HCHO and diethylamine or pyrrolidine hydrochloride.

Isolation of the Product :

The salt of the product precipitated when cooled or triturated with ether.

The arylamines were checked during the reaction by T.L.C. and final compound confirmed by elemental analysis of C, H, N.

The amino group of these products was further employed to build the symmetrical triazine with various aromatic aldehydes to give aryl substituents at 2-position of the triazine ring. Those s-triazines which were oily in nature were purified by column chemotherapy.



-O-

E X P E R I M E N T A L

-O-

EXPERIMENTAL :Synthesis of 4-amino- α -diethylamino-o-cresol dihydrochloride :

This was prepared both by (A) acid deacetylation of 4-acetamido- α -diethylamino-o-cresol and by (B) reduction of the corresponding nitro compound.

A mixture of equivalent amounts of the acetamidophenol, formaldehyde, and the hydrochloride salt of diethylamine suspended in about 250 cc. of alcohol per mole of the phenol, was heated on a steam-bath for 1 to 3 hrs. Upon cooling no crystalline mass precipitated out. But it was a clear amber coloured solution which when concentrated left behind a sticky residue. This sticky residue on trituration with acetone or ether and refrigeration crystallized as a white coloured solid (monohydrochloride).

A mixture of 10 gms of 4-acetamidophenol- α -diethylamino o-cresol and 100 ml of 20 % HCl was heated at refluxing temperature for an hour. The solution was evaporated under reducing pressure to a thick syrup and it was purified from benzene to remove the unreacted impurities. The

desired salt was then precipitated by the addition of 200 ml ether and trituration. A total of 9.6 g. of off white product was obtained.

Yield : 9.6 g. (86 %); m.p. 218-220°C.

(Lit.¹³² 82 %, m.p. 225°C.)

o-Cresol 4-(4,6-diamino-2-
methyl, 2-ethyl 1,2-dihydro-
s-triazine)- α -diethyl amino
methyl dihydrochloride :

A mixture of 3-diethyl amino methyl 4-hydroxy aniline dihydrochloride 0.5 g. (0.0018 M); dicyandiamide 0.160 g. (0.002 M), a few drops of alcohol and 6-7 ml of ethyl methyl ketone was stirred with a magnetic stirrer at reflux temperature for a period of one hour, when it became a clear brown solution. The reaction mixture was cooled and stirred vigorously for 12 hrs at room temperature. A crystalline material was deposited on the walls of the flask. This material was filtered and gave a positive biguanide test. The filtrate was once again refluxed with 1-2 drops of concentrated HCl

and dicyandiamide 0.084 g. (0.01 M) and stirred further. The filtrate purified from ether was concentrated and refrigerated for 1 week in an ethereal solution and afforded a buff coloured solid which was collected by filtration.

Yield : 0.32 g. (55.0 %); m.p. 240°C.

Analysis for : $C_{17}H_{26}N_6O \cdot 2HCl$

	<u>% C</u>	<u>% H</u>	<u>% N</u>
Calcd.	50.62	6.45	20.84
Found	50.45	6.32	21.20

o-Cresol, 4-(4,6-diamino,2-phenyl 1,2-dihydro
s-triazine)- α -diethyl amino
methyl dihydrochloride :

0.89 g. (0.003 M) of o-cresol, 4-(4,6-diamino methyl
4-hydroxy aniline hydrochloride was placed in a 100 ml round bottomed flask. Equivalent quantities of dicyandiamide 0.280 g. (0.0033 M), benzaldehyde 0.53 g. (0.005 M) and sodium dry ethanol 1-2 ml was added. The reaction mixture was refluxed at 60-70°C for 5-6 hrs under anhydrous

conditions. During the course of the reaction absence of biguanide formation was checked by cuprammonium sulfate colour test. The dark amber coloured solution was then treated with charcoal, filtered and concentrated. The oily residue was triturated with ether, and purified by chromatography.

Yield : 0.420 g. (28 %); m.p. 165°C.

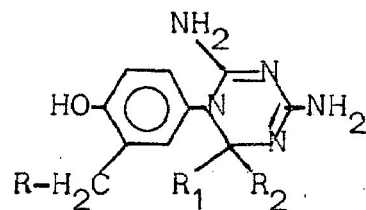
Analysis for : $C_{20}H_{26}N_6O \cdot 2HCl \cdot H_2O$

	<u>% C</u>	<u>% H</u>	<u>% N</u>
Calcd.	52.52	6.13	18.38
Found.	52.24	5.36	19.69

Similarly were prepared the other 2-aryl substituted compounds of Section-B, as shown alongwith their physical data in Table-III.



TABLE - III : Synthesis of *o*-cresol, 4-(4,6-diamino-2,2-dialkyl or aryl-1,2-dihydro
s-triazine)- α -substituted amino methyl.



No.	R	R ₁	R ₂	m.p. °C (Uncor.)	Yield %	Molecular Formula	Elemental Analysis					
							Calculated			Found		
							C	H	N	C	H	N
1.	N(C ₂ H ₅) ₂	CH ₃	CH ₃	235	56.5	C ₁₆ H ₂₆ N ₆ O.2HCl	49.35	7.16	21.59	48.92	6.66	20.80
2.	"	CH ₃	C ₂ H ₅	245	43.2	C ₁₇ H ₂₈ N ₆ O.2HCl	50.37	7.40	20.74	51.11	7.44	-
3.	"	CH ₃	CH ₃ (CH ₂) ₄ -CH ₃	Oily	-	C ₂₁ H ₃₆ N ₆ O.2HCl	54.66	7.809	18.22	-	-	17.47
4.	"	H	C ₆ H ₅	165	52.3	C ₂₀ H ₂₈ N ₆ O.2HCl H ₂ O	52.52	5.92	18.38	52.24	5.36	19.69
5.	"	H	p-N(CH ₃) ₂ - C ₆ H ₅	-	26.0	C ₂₂ H ₃₁ N ₇ O.2HCl H ₂ O	52.80	6.60	19.6	49.66	6.02	19.66
6.	"	H	(Cl)(OH)(OCH ₃) C ₆ H ₅	-	19.9	C ₂₁ H ₂₇ N ₆ ClO ₃ . 2HCl.H ₂ O	46.88	5.39	15.63	46.61	5.36	17.90
7.	-N	CH ₃	C ₂ H ₅	240	26.5	C ₁₇ H ₂₆ N ₆ O.2HCl	50.62	6.45	20.84	50.45	6.32	21.20
8.	-N	CH ₃	C ₆ H ₅	264	48.0	C ₂₀ H ₂₄ N ₆ O.2HCl	55.05	5.50	19.26	54.76	6.1	-

Section - C

.....

SYNTHESIS OF 2,4-DIAMINO-1-[(4'-HYDROXY,
3'-SUBSTITUTED AMINO-METHYL) PHENYL]-1,3,5-
TRIAZASPIRO (5-5) UNDECA 2,4-DIENE.

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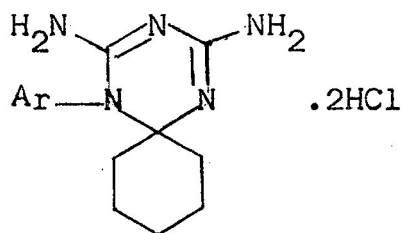
-O-

C H E M I S T R Y

-O-

CHEMISTRY

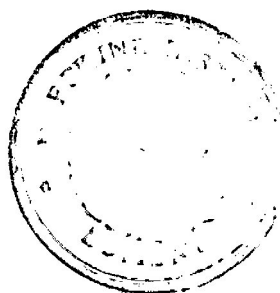
The substituted anilines utilized in Section-B are used here again except that instead of aliphatic ketones a cyclic ketone cyclohexanone is used and compounds of the following type are prepared.



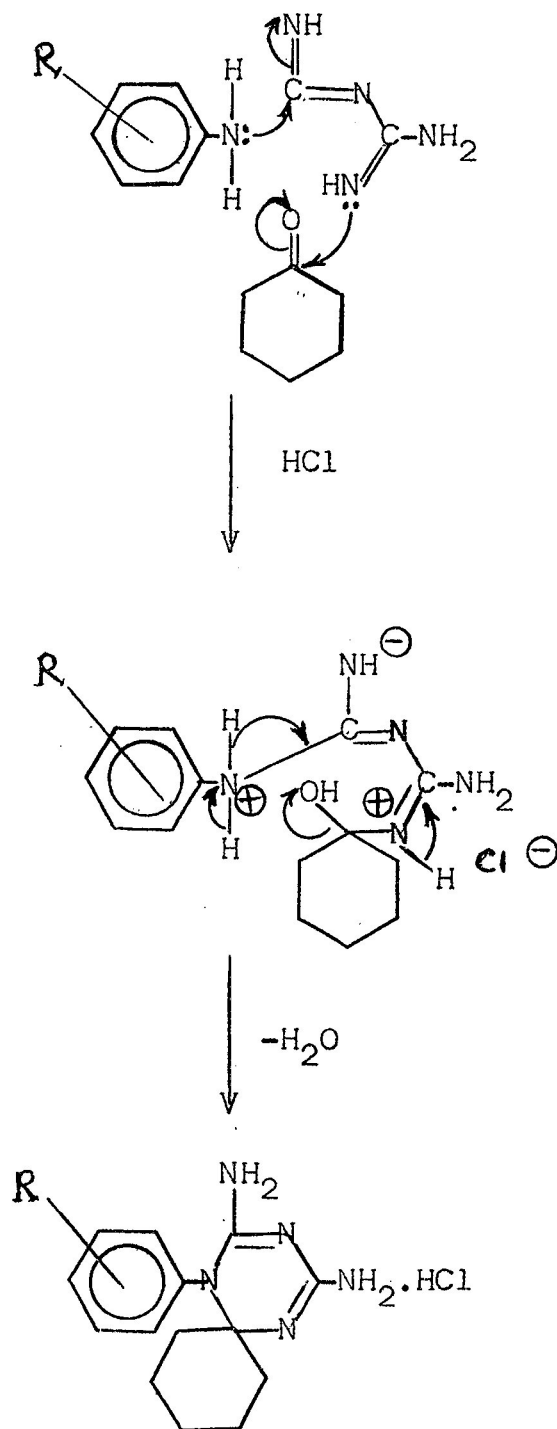
(XI)

Considering the biological importance of sulfide linkage and N-carbomethoxy linkage 4-amino-4-N-carbmethoxy diphenyl sulfide was also taken to build-up the triazine derivatives at reported in Table IV.

The effort to incorporate cyclopentanone instead of cyclohexanone failed to give the appropriate compound.



The probable reaction mechanism for the following type of compounds is as follow :-



-O-

EXPERIMENTAL

-O-

EXPERIMENTAL :

2,4-Diamino-1-[(4'-hydroxy, 3'-substituted amino methyl) phenyl] 1,3,5-triazaspiro (5,5') undeca 2,4-diene :

A mixture of diethyl amino methyl, 4-hydroxy aniline dihydrochloride (0.5 g.) (0.0018 M); 0.9 ml (0.002M) cyclohexanone, 0.168 g. (0.022 M) of dicyandiamide and 2 ml of sodium dry methanol was stirred and refluxed for 8 hrs. A clear solution was obtained which was stirred for another 3 hrs. at room temperature. The reaction mixture was treated with charcoal, filtered concentrated and kept overnight at room temperature. A crystalline solid was obtained, which was filtered washed with 2-propanol and ether. The title compound thus obtained weighed 0.37 g; m.p. 219°C. Two more crystallizations with methanol ether gave 0.3 g. of the title compound.

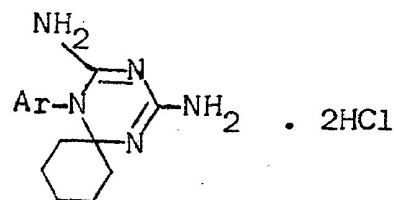
Yield : 0.300 g. (44.7 %); m.p. 220°C

Analysis for : $C_{19}H_{30}N_6O \cdot 2HCl$

	<u>% C</u>	<u>% H</u>	<u>% N</u>
Calcd.	52.90	6.96	19.48
Found.	52.65	7.24	19.56

Similarly were prepared other 4',6'-diamino 1'-aryl-spiro (cyclohexane)-1,2'-(1'-H)-s-triazine hydrochlorides as listed in Table IV alongwith their physical data.

TABLE - IV : Synthesis of 2,4-diamino-1- $\left[(4'\text{-hydroxy, } 3'\text{-substituted amino methyl) phenyl} \right]$
1,3,5-triazaspiro (5-5) undeca 2,4-diene.



No.	Ar	m.p. °C (Uncor.)	Yield %	Molecular Formula	Elemental Analysis					
					Calculated			Found		
					C	H	N	C	H	N
1.		222	46.7	$C_{19}H_{30}N_6O \cdot 2HCl$	52.90	7.42	19.48	52.65	7.24	19.56
2.		247	13.38	$C_{19}H_{28}N_6O \cdot 2HCl$	53.15	6.53	19.5	53.61	6.63	19.52
3.		78	8.65	$C_{19}H_{17}N_6O_2S \cdot HCl$	53.60	5.79	17.65	54.26	6.32	16.14

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C H E M I S T R Y

-O-

Section - D

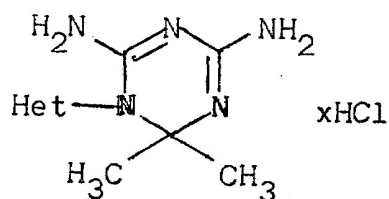
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SYNTHESIS OF 4,6-DIAMINO-1,2-DIHYDRO-
2,2-DIMETHYL s-TRIAZINES WITH HETERO-
CYCLIC SUBSTITUENTS AT 1-POSITION OF
THE TRIAZINE RING.

.....

CHEMISTRY :

As an empirical approach to the synthesis of triazine derivatives it was decided to utilize some alicyclic amines and heterocyclic amines to build up unknown triazines having the general structure as below.



Only those heterocyclic amines where the amino group was highly basic in character were utilized, viz; adamantamine, quinolines and quinazoline; 2,4,6-triamino quinazoline was prepared as per literature method starting from basic chemicals.

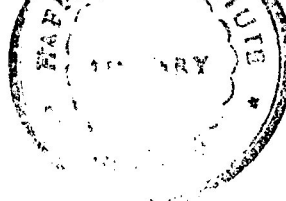
In case of 3 and 8 amino quinolines, the 3-amino quinoline-triazine was obtained with difficulty. This was probably due to the proximity of the amino function to the heteroatom nitrogen. Numerous attempts to construct the triazine ring on 2-amino pyridine, pyrimidine, etc. resulted in a failure to give the s-triazine compounds of this section are listed in Table V.

--O-

EXPERIMENTAL

--O-

EXPERIMENTAL :



4,6-Diamino-1,2-dihydro-2,2 -dimethyl
1(1-adamantyl)-s-triazine hydrochloride :

1.87 g. (0.01 M) of 1-adamantamine hydrochloride was dissolved in 5 ml of sodium dry ethanol and placed in a 3 necked flask fitted with a mechanical stirrer, a condenser with a calcium chloride guard tube and a stopper. 1.092 g. (0.013 M) of cyanoguanidine was added to the flask together with excess of acetone and refluxed on steam bath with stirring for 12 hrs. A solid white crop precipitated from the homogenous reaction mixture, which was found to be quite pure. One crystallization from aqueous methanol provided an analytically pure compound.

Yield : 1.15 g. (36.8 %); m.p. 174°C.

Analysis for : $C_{15}H_{26}N_5 \cdot HCl$

	<u>% C</u>	<u>% H</u>	<u>% N</u>
Calcd.	57.60	8.64	22.40
Found.	57.10	9.16	22.15

Similarly were prepared the remaining 4,6-diamino 1-(substituted)-1,2-dihydro, 2,2 -dimethyl-s-triazine hydrochlorides as shown with their physical data in Table V, implementing the above experimental procedure.

Quinoline 3-(4',6'-diamino, 1',2'-dihydro
2',2'-dimethyl s-triazine) hydrochloride :

The title compound was prepared by the two component synthesis. A mixture of 3-aminoquinoline hydrochloride 1.8 g. (0.01 M) was refluxed with 0.84 g. (0.01M) of dicyandiamide in aqueous ethanol for 5-6 hrs. Thereafter the homogenous solution was charcalized, filtered and concentrated.

On standing overnight a drop of shining* crystals was obtained weighing 1.3 g.

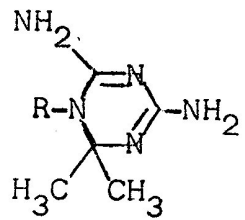
0.5 g. (0.002 M) of quinoline biguanide was further refluxed with 20 ml of acetone for 8 hrs and 0.07 g. (0.002 M) of concentrated hydrochloric was added as catalyst. The reaction mixture was charcoalized, filtered and concentrated. A sticky solid was obtained after 2 days of refrigeration. This was washed with 2-propanol and ether several times to give a yellow, solid of analytical purity.

Yield : 0.15 g. (26.04 %); m.p. 239°C.

Analysis for : $C_{14}H_{16}N_6 \cdot HCl$

	<u>% C</u>	<u>% H</u>	<u>% N</u>
Calcd.	55.26	5.26	27.63
Found.	54.5	6.1	-

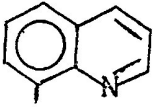
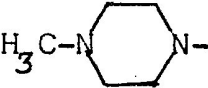
TABLE - V : Synthesis of 4,6-diamino-1,2-dihydro-2,2-dimethyl s-triazines with heterocyclic substituents at 1-position of the triazine ring.



No.	R	m.p. °C (Uncor.)	Yield %	Molecular Formula	Elemental Analysis					
					Calculated			Found		
					C	H	N	C	H	N
1.		174	66.1	C ₁₅ H ₂₆ N ₅ .HCl	57.60	8.64	22.40	57.10	9.16	22.15
2.		285	50.2	C ₁₃ H ₁₇ N ₉ .3HCl	58.19	4.13	30.84	57.59	5.0	27.19
3.		239	48.0	C ₁₄ H ₁₆ N ₆ .HCl	55.26	5.26	27.63	54.5	6.1	-

Contd.....

(Table-V.....Contd.)

No.	R	m.p. °C (Uncor.)	Yield %	Molecular Formula	Elemental Analysis					
					Calculated			Found		
					C	H	N	C	H	N
4.		216	62.1	$C_{14}H_{16}N_6 \cdot HCl$	55.26	5.26	27.63	-	-	28.2
5.		210	72.0	$C_{10}H_{21}N_6 \cdot 3HCl$ $\cdot 2H_2O$	28.77	6.23	23.50	29.21	5.41	23.93

Section - E

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SYNTHESIS OF 4,6-DIAMINO-1-(HETEROARYL OR
HETEROARYLOXY)-1,2-DIHYDRO, 2,2-DIMETHYL-
s-TRIAZINE HYDROCHLORIDE.

.....

-O-

C H E M I S T R Y

-O-U-U-U-U-U-U-O-O-

CHEMISTRY :

This section comprises of preparation of :

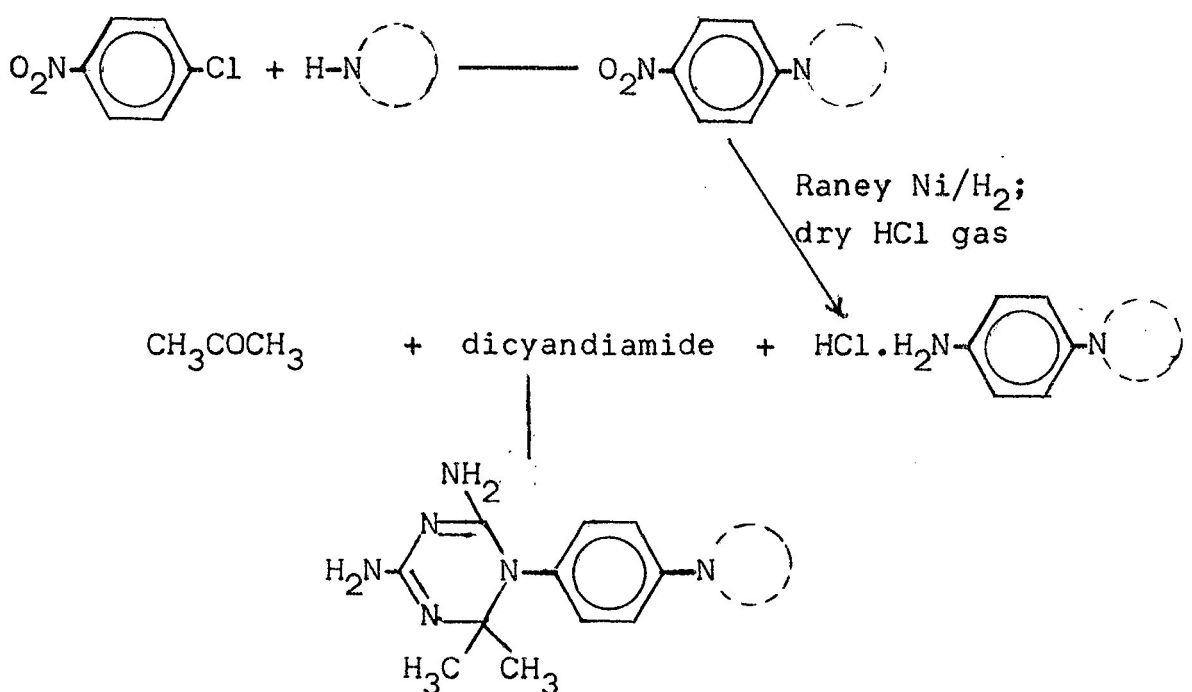
(A) 4-amino-phenyl-N-heterocyclic amines;

(B) 4-amino phenyl-1-(heteroaryloxy):

(A) 4-Amino phenyl-N-heterocyclic amines :

The secondary cyclic amines are quite reactive and could be easily reacted with p-nitrochloro benzene by using two moles of secondary amine, e.g. morpholine, pyrrolidine, piperazine are easily reacted to get the desired compounds. The additional mole of the secondary amines acts to remove the hydrochloric acid formed during the reaction as shown in Scheme VI below.

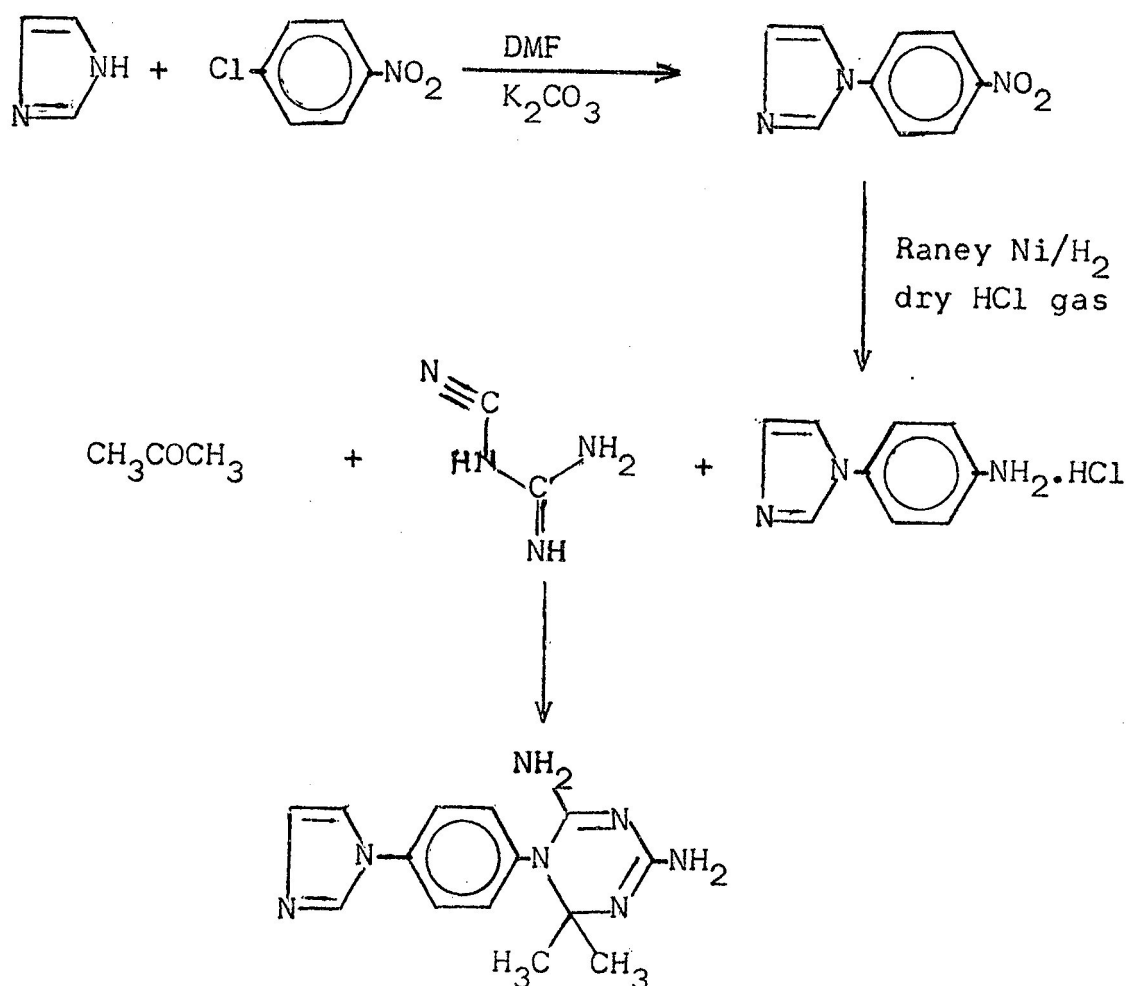
Scheme - VI :



(XIII)

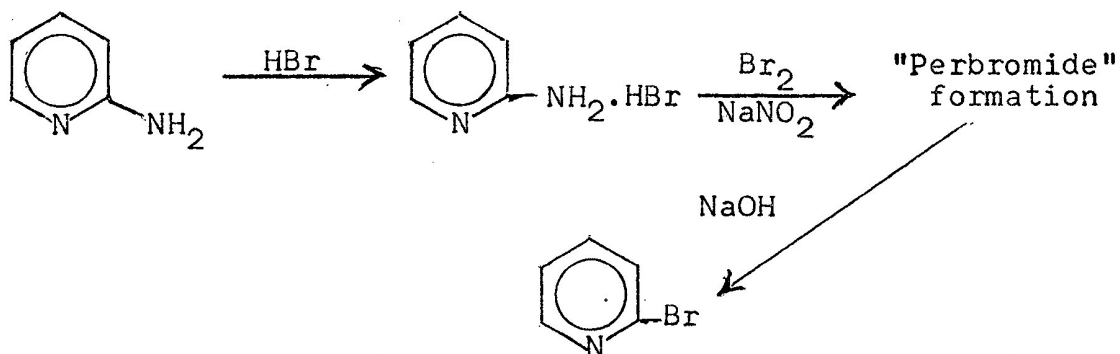
4-(Nitrophenyl) imidazole was synthesized by the method of Johnson et al.¹³³ and was found quite satisfactory by way of yield and isolation of the product. In this method a mixture of molar equivalents of chloronitrobenzene, imidazole and anhydrous potassium carbonate was refluxed in dimethyl formamide from 2-72 hrs. The sequence of reactions is shown in Scheme VII.

Scheme - VII :



Preparation of halogenated heterocycles :

2-Bromo pyridine was prepared as per literature methods.¹³⁷ The reaction was as follows :



2-Chloro pyrimidine was similarly prepared starting from 2-amino pyridine as basic chemical.



--O-

E X P E R I M E N T A L

--O-

EXPERIMENTAL :4-Imidazo nitro benzene :

A mixture of imidazole (0.1 M) 6.8 g. anhydrous sodium carbonate (0.103 M) 14.2 g. and p-nitro chloro-benzene (0.1 M) 15.75 g. and DMF 60 ml) was refluxed with stirring for several hrs. After the completion of the reaction period, the mixture was cooled and poured over ice-water (500 ml), allowing the tarry product to settle down for a few hours. The upper clear liquid was decanted and the tarry product washed with water. It was then treated with dil HCl, filtered to remove insoluble unreacted nitrohalobenzene or decomposed products. The filtrate after treatment with charcoal evaporated to dryness on a steam bath. The crude dry product obtained was extracted with benzene to remove final traces of impurities. The residue that remained after extraction was dissolved in minimum amount of water, treated with charcoal and neutralized by NH_4OH with cooling. The precipitated solid was filtered, washed with cold H_2O and dried at room temperature. It was dissolved in dry benzene and adsorbed on alumina (neutral 50 g.), eluted with dry benzene. The eluent treated with charcoal was concentrated to a small volume and precipitated with hexane, dried and crystallized from benzene-hexane mixture as pale yellow compound.

Yield : 15.68 g. (83.0 %); m.p. 204.5-205°C.

(Lit.¹³³ m.p. 205°C.)

4-Imidazo^o aniline :

The nitro compound obtained was reduced by means of Raney Nickel and hydrogen, m.p. 140°C.

1-(p-Nitrophenyl) morpholine

In a 250 ml round bottomed flask was taken a mixture of p-nitrochlorobenzene 39.4 g. (0.25 M), and morpholine 43.5 g. (0.5 M). Minimum quantity of DMF and potassium carbonate 34.5 g. (0.25 M) was added. The reaction mixture was heated at 120-130°C for 4 hrs. After completion, the reaction mixture was poured on ice cold water and the product was precipitated and filtered.

Yield : 32.0 g. (33.97 %); m.p. 150-152°C.

(Lit.¹³⁵ m.p. 150-151°C.)

4-(p-Aminophenyl) morpholine :

In a 150 ml Erlenmeyer flask was taken a mixture of N-(p-nitro phenyl morpholine) 2.0 g. (0.001 M), in 30 ml dil HCl (20 %). It was treated with Zn-dust and

gently warmed till yellow colour disappeared. The Zinc dust was filtered off and the filtrate when made strongly basic with 50 % NaOH in ice cold conditions gave 1.6 g. (94 %) of the crude amine.

Yield : 1.6 g. (94.0 %); m.p. 128°C .

(Lit.¹³⁶ m.p. 130°C .)

2-Bromopyridine :

Place 395 ml of 48 percent hydrobromic acid in a 3 litres three necked flask and fitted with a dropping funnel, mechanical stirrer and thermometer. Cool to $10-15^{\circ}\text{C}$ in an ice salt bath and add 75 g. of 2-amino pyridine over a period of about 10 minutes. Whilst maintaining the temperature at 0°C or lower add 375 g. (120 ml) of bromine dropwise with stirring. The reaction mixture thickens during the addition of the first half of the bromine (30 minutes) owing to the formation of a yellow orange "Perbromide"; the second half may then be introduced more rapidly (15 minutes).

Diazotisation :- Now add a solution of 140 g. of A.R. sodium nitrite in 200 ml of water dropwise over a period of 2 hrs whilst keeping the temperature at 0°C or lower continue the stirring for 30 minutes; then run a

solution of 300 g. of sodium hydroxide in 30 ml of water at such a rate that the temperature does not rise above 20-25°. Extract the reaction mixture with four 125 ml. portions of ether, dry the ethereal extracts for 1 hour over 50 g. of potassium hydroxide pellets, remove the ether on a steam bath and distil the residue from a claisen flask with fractionating side arm under reduced pressure. Collect the 2-Bromo pyridine at 74-75°/13 mm; the yield is 115 g. The b.p. at atmospheric pressure is 193-195°C.

Yield : 115 g. (91.22%) b.p. 193-195°C.

Lit.¹³⁷ b.p. 193°C.

Acetanilide-4'-(2-pyridinyloxy) :

In a 250 ml round bottomed flask was placed acetamidophenol 9.815 g. (0.065 M); 2-bromopyridine 10.0 g. (0.063 M), and DMF (7 ml) together with anhydrous potassium carbonate 9.66 g. (0.07 M). The reaction mixture was refluxed in an oil bath at 130-140°C. for 7-8 hrs. The solution poured into ice water and the heteroaryloxy acetanilide which precipitated out, filtered and dried at room temperature.

The product obtained above was taken in a round bottomed flask fitted with a condenser and 20 % HCl added to it. The acetanilide was hydrolysed by refluxing on a heating mantle, till the reaction mixture became homogeneous. Hydrolysis was continued for 6-7 hrs

The solution was then treated with ammonium hydroxide in cold conditions to obtain the free base as a white solid which was sufficiently pure to be used further.

Yield : 6.0 g. (41.58 %); 182°C.

Analysis for : $C_{13}H_{12}N_2O_2$

	<u>% C</u>	<u>% H</u>	<u>% N</u>
Calcd.	61.97	6.10	13.14
Found.	62.41	7.4	12.14

Similarly were prepared the other heteroaryloxy and heteroaryl amines.

4,6-Diamino-1,2-dihydro 2,2-dimethyl,
1-[p(pyrrolidinyl) phenyl] s-triazine
hydrochloride :

p-Pyrrolidinophenyl biguanide hydrochloride 1.5 g. (0.0076 M); concentrated hydrochloric acid 0.1368 g. (0.16 ml; 0.0038 M), 23.2 ml (0.4 M) of acetone and absolute ethanol 5-6 ml were stirred at reflux temperature for 6 hrs.

A solid product which settled at the bottom of the flask was filtered and was found to be the starting material i.e. the biguanide. The undiluted mother liquor was refluxed for a few hours again concentrated and refrigerated. After two weeks of refrigeration 0.980 g. of shining flaked crystals m.p. 159-62°C. were collected from the mother liquor. This product was analysed and U.V. absorption max 245 mu in $\text{CH}_3\text{OH}-\text{H}_2\text{O}$ was in accordance with the assigned structure. Recrystallized from ether methanol mixture.

Yield : 0.63 g. (36.08 %); m.p. 160°C.

Analysis for : $\text{C}_{15}\text{H}_{24}\text{N}_6 \cdot \text{HCl}$

	<u>% C</u>	<u>% H</u>	<u>% N</u>
Calcd.	55.81	6.82	26.04
Found.	54.2	5.9	-

The other triazines of this section are listed in Table - VI.

4,6-Diamino-1,2-dihydro, 1-[1-(phenoxy) adamantyl]
2,2-dimethyl s-triazine hydrochloride :

1.0 g. (0.0036 M) of 4-(adamantyloxy) aniline hydrochloride 0.37 g. (0.0044 M), of cyanoguanidine, concentrated hydrochloric acid 0.146 g. (0.004 M)(0.178 ml),

and dry acetone 20-25 cc. was refluxed on a water bath for 20 hrs. until a light purple coloured solution was obtained. The reaction solution was treated with charcoal, filtered and concentrated. On standing for two days pink flakes of the title compound were obtained. The flakes were collected, washed with ether and a very small quantity of alcohol to obtain a white product of analytically pure grade.

Yield : 0.82 g. (57.2 %); m.p. 236°C.

Analysis for : $C_{15}H_{22}N_6O \cdot HCl$

	<u>% C</u>	<u>% H</u>	<u>% N</u>
Calcd.	62.25	7.19	17.37
Found.	61.82	8.10	17.92

Other new heteroaryloxy triazines thus prepared as per the above experimental details are reported in Table - VII.

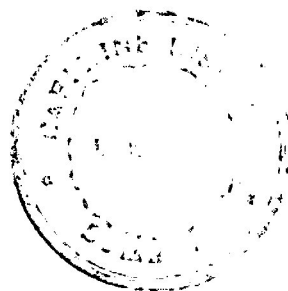
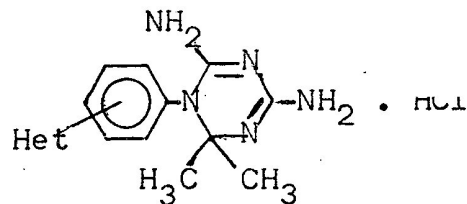
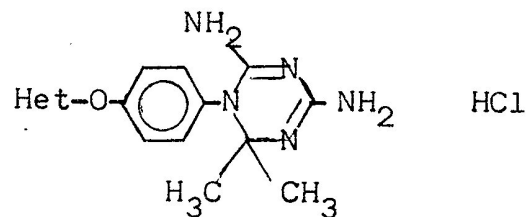


TABLE - VI : Synthesis of 4,6-diamino-1-(heteroaryl or heteroaryloxy)-1,2-dihydro
2,2-dimethyl s-triazine hydrochloride.



No.	Het	m.p. °C (Uncor.)	Yield %	Molecular Formula	Elemental Analysis					
					Calculated			Found		
					C	H	N	C	H	N
1		256	42.0	C ₁₅ H ₂₂ N ₆ O.HCl	53.18	6.5	24.82	52.84	7.03	25.07
2.		249	48.0	C ₁₆ H ₂₅ N ₇ .2HCl	47.52	8.18	24.25	47.58	8.46	23.80
3.		159-65	37.0	C ₁₅ H ₂₂ N ₆ .HCl	55.81	6.82	26.04	-	-	25.21
4.		197	--	C ₁₄ H ₁₇ N ₇ .HCl	52.58	5.32	30.67	51.28	6.4	-
5.		183	-	C ₁₆ H ₂₄ N ₆ .HCl	57.05	7.13	24.96	58.33	6.78	-

TABLE - VII :



No.	Het	m.p. °C (Uncor.)	Yield %	Molecular Formula	Elemental Analysis					
					Calculated			Found		
					C	H	N	C	H	N
1.		182	52	$C_{16}H_{18}N_6O \cdot 2HCl$	55.41	5.19	22.04	56.20	7.52	23.21
2.		245	41	$C_{15}H_{17}N_7O \cdot 2HCl \cdot H_2O$	49.18	5.46	24.24	48.84	5.72	26.64
3.		210	43	$C_{15}H_{15}N_7OCl_2 \cdot 2HCl$	43.12	3.32	23.60	42.76	4.12	23.38
4.		260	57.2	$C_{21}H_{29}N_5O \cdot HCl$	62.25	7.19	19.37	61.82	8.10	19.58
5.		284	-	$C_{20}H_{20}N_6O \cdot 2HCl \cdot H_2O$	57.90	5.3	20.20	57.81	5.55	20.0

PART - III

PHARMACOLOGICAL SCREENING

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PHARMACOLOGICAL SCREENING

Biological Evaluation for antimalarial activity :

Chemotherapeutic research is closely associated with the development of adequate screening methods which guide the workers for future investigations.

The compounds synthesized in the current work have all been screened for antimalarial activity in the rodent malarial mouse system.

The first isolation of the rodent malaria was reported by Vincke and Lips.¹³⁸ Thereafter many species and subspecies of rodent malarias have been isolated and divided into two groups the berghei group and the vinckei group. The group used for the majority of chemotherapy research is the berghei group.

Advantages of the rodent model :

The rodent plasmodium model is considered to be the most important tool in the primary phase of the search for better antimalarial drugs. Its unparralled simplicity and convenience helps in the screening of a large number of compounds.



P. berghei infections in the mouse can suggest ways of administering new agents most effectively at relatively low costs and can determine the value of a new agent in the face of established resistance to existing drugs.

To test the blood schizontocidal activity in the rodent malaria system, two tests were mentioned in a report by a WHO scientific group.⁸⁰ (W.H.O. 1973). The tests were :

- a) Evaluation of activity by assessment of survival time: Rane test for blood schizontocidal screening.¹⁸
- b) Evaluation of activity by means of parasite counts : The 4-Day Suppressive Test using P. berghei (in swiss mice) modified from Thurston.¹³⁹

Susceptibility of P. berghei to different hosts :

Satyaprakash et al.¹⁴⁰ investigated the susceptibility of various animals to blood induced infection with P. berghei. The animals like monkeys, rabbits, guinea pigs, cats etc. were investigated. The authors demonstrated that none of the animals inoculated with P. berghei developed the infection. The infectivity of P. berghei to several

other animals was studied by various workers during 1950's and the animals found susceptible were golden hamster, gerbil, bat and squirrel. The infectivity of P. berghei to the rodents like mice and rats which were found susceptible was a breakthrough for antimalarial screening.

The P. berghei infection in laboratory mouse is the most economical and feasible approach since it performs as a primary and secondary screen before monkey malaria screens. Hence the P. berghei strain maintained in Haffkine Institute was used for this study.

MATERIALS AND METHODS :

Strain :- Plasmodium berghei used for the study was obtained from Hindustan Ciba-Geigy, Bombay. The strain was maintained by regular passage in mice.

Malarial parasites :

Blood passage :- Blood was drawn by cardiac puncture from infected mice, collected in citrated saline and 1×10^6 parasitized erythrocytes were inoculated into healthy male mice weighing 22-25 g.



Blood Smears and Staining :

Thin blood smears were made by snipping of a small portion of the tail and taking a drop of blood from it on a microslide. The smears were then fixed in methanol, dried and stained by Geimsa's in phosphate buffered saline and washed with the same till the smears turned pink violet in colour.

Parasite Counts :

a) From the blood smears prepared, parasite counts per 1,000 red blood cells were made and the percentage of the infected cells calculated. This was the Percent Parasitaemia.

b) Red blood cells per cubic millimeter were estimated by using the Neubauer bright line haemocytometer.

From (a) and (b) infected red blood cells/ml of blood were calculated.

Survival Time : (Δ MST)

This period was determined from the day the mouse was inoculated to the day it was found dead. Mean survival time was calculated at each dose level by subtracting the

Average Mean Survival Time of control Mice (MSTC) from the Average Mean Survival Time of Treated Mice (MSTT).

$$\text{i.e. MSTT} - \text{MSTC} = \Delta \text{MST.}$$

Experimental Chemotherapy of
rodent malarial infection :

Susceptibility of *P. berghei* strain
to standard antimalarial agents :-

This was confirmed by the Rane's single dose test and Peters' 4-Day Suppressive Test. Pyrimethamine and cycloguanil hydrochloride were screened to study the pathogenicity of the *P. berghei* maintained in the laboratory.

Cycloguanil hydrochloride at 20 mg/kg and pyrimethamine at 3.5-4.5 mg/kg gave curative results in single subcutaneous dose. The compounds were synthesized and screened at the dose levels of 160, 80, 40, 20 and 10 mgs/kg. The cycloguanil HCl used as standard was prepared in the laboratory as per reported methods.¹¹²

Methodology :

- | | | |
|-------------------------|---|---|
| Animal Model | : | Rodent malaria; white male mice of Haffkine's strain weighing 22-25 g. average. |
| <u>Primary Screen</u> | : | Involved the recording of survival time. |
| <u>Secondary Screen</u> | : | Involved the evaluation of activity by means of parasite counts. |

RANE'S TEST FOR BLOOD SCHIZONTOCIDAL ACTIVITY :

Three to four week old male mice weighing (22-25 g.) were housed in plastic cages with metal lids, and given palletted food and water ad lib. The animals were infected with 0.1 ml of citrated heart blood, containing 1 million parasitized RBC's.

The test compounds were suspended in distilled water by the addition of 6-7 drops of 1 % Tween-80. A single dose was given subcutaneously 72 hours after infection. A control group of 5 mice which was not administered any drug was kept as untreated control. Dose range selected was 160, 80, 40 and 20 mg/kg. Cyclo-guanil HCl and pyrimethamine were taken as standard drugs for comparison.

Observations and Results :

1) Untreated infected animals died within 5.0 to 7.0 days average. The survival time of these controls varied with every batch of experiment.

2) Treated animals were observed till death. Those animals which survived twice the untreated controls were arbitrarily considered to be active (A).

3) Deaths within 48 hrs after administration of compounds were considered toxic (T).

4) The smallest dose at which double the survival time was obtained was considered the Minimum Effective Dose (MED).

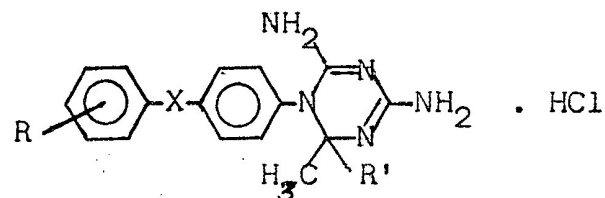
5) Thirty five compounds were synthesized and screened. Of these eight compounds showed activity at dose range varying from 160 mg/kg to 20 mg/kg. The activity could be categorized as Active and Slightly Active compounds. In case of those compounds which showed activity, the animals were found to be normal and healthy, unlike the infected untreated group. Four of the compounds with maximum activity were further taken for evaluation by Peters' 4-Day Suppressive Test.

Note:- Tables VIII, IX, X, XI, XII and XIII demonstrate the antimalarial action of the compounds screened by the Rane's Single Dose Test against P. berghei in mice.

The charts indicate the Mean Survival Time (Δ MST); where A = Active and T = Toxic. The dose given is in mg/kg; subcutaneously, 72 hrs. after infection.

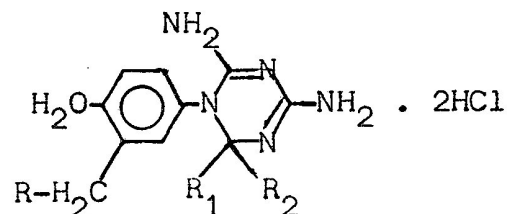


TABLE - VIII : Synthesis of 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-(substituted phenyl)-s-triazines.



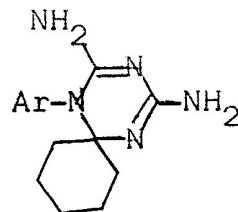
No.	R	X	R'	160	80	40	20	Control	LD ₅₀ g/kg
1.	Cl	CH ₂ O	CH ₃	18.21(A)	15.23(A)	12.3(A)	17.2(A)	5.0	
2.	Br	CH ₂ O	CH ₃	15.01(A)	13.3(A)	9.6(A)	10.3(A)	5.0	1.650
3.	NO ₂	-O-	CH ₃	3.8	3.3	3.8	3.4	5.0	
4.	Cl	-O-	CH ₃	2.33T ₃	2.6T ₃	0	0	6.0	
5.	2,4-Dichloro	-O-	CH ₃	1.0T ₄	T ₅	0	0	6.0	
6.	NHCOCH ₃	-O-	CH ₃	2.2	1.4	5.0	2.0	7.3	
7.	NHCOOCH ₃	-S-	CH ₃	2.67	1.6	1.5	1.3	6.3	
8.	NHCOOCH ₃	-S-	C ₂ H ₅	3.5	2.7	2.4	1.8	6.3	

TABLE - IX : Synthesis of o-cresol-4-[4,6-diamino-2,2-dialkyl or aryl-1,2-dihydro-s-triazine]-(substituted) amino methyl hydrochloride.



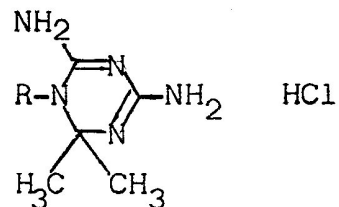
No.	R	R ₁	R ₂	160	80	40	20	Control
1.	N(C ₂ H ₅) ₂	CH ₃	C ₂ H ₅	0.3T ₃	1.3	0	1.3	6.1
2.	N(C ₂ H ₅) ₂	CH ₃	CH ₃	1.0T ₂	2.6	3.3	3.7	6.1
3.	N(C ₂ H ₅) ₂	CH ₃	CH ₃ (CH ₂) ₄	0	0	0	0	6.1
4.	N(C ₂ H ₅) ₂	H	C ₆ H ₅	7(A)	8.5(A)	7.7(A)	4.0	6.1
5.	N(C ₂ H ₅) ₂	H	p-N(CH ₃) ₂ -C ₆ H ₅	0.33T ₄	T ₅	0	0	6.1
6.	N(C ₂ H ₅) ₂	H	(Cl)(OH)-(OCH ₃)C ₆ H ₃	4T ₃	2.33T ₃	-	0	6.1
7.		CH ₃	C ₂ H ₅	0.3T ₃	1.3	0	1.3	6.1
8.		H	C ₆ H ₅	8.0	5.3	3.5	0	7.1

TABLE - X : Synthesis of 2,4-diamino-1-[(4'-hydroxy, 3'-substituted-amino-methyl) phenyl]
1,3,5-triazaspiro (5-5) undeca 2,4-diene.



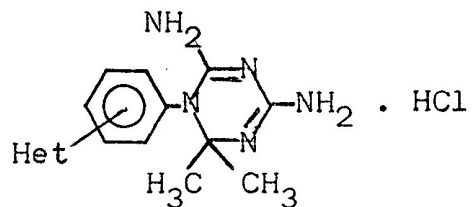
No.	Ar	160	80	40	20	Control
1.		0	2.1	0	0	7.1
2.		0.3	1.3	0	1.3	7.1
3.		0.25T ₃	2.4	4.4	6.3	7.1

TABLE - XI : Synthesis of 4,6-diamino-1,2-dihydro-2,2'-dimethyl-s-triazines with heterocyclic substituents at 1-position of the triazine ring.



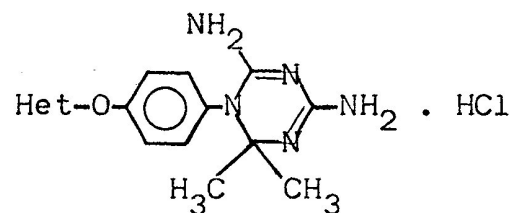
No.	R	160	80	40	20	Control	LD ₅₀
1.		20.1(A)T ₂	19.9(A)C ₁	17.0(A)C ₁	12.9(A)	6.3	190 mgs/kg
2.		12.2(A)	10.9(A)	13.9(A)	9.3(A)	6.3	
3.		4.66	4.5	2.33	2.45	7.0	
4.		16.0	15.0	14.0	13.2	7.5	
5.		4.1	6.1	3.9	0	7.0	

TABLE - XII : Synthesis of 4,6-diamino-1-(p-substituted-phenyl)-1,2-dihydro-2,2'-dimethyl-s-triazines



No.	Het	160	80	40	20	Control
1.		3.5	2.8	-	2.0	6.5
2.		3.7	3.7	0.3	1.0	6.3
3.		3.7T ₂	6.2	4.0	-	6.3
4.		1.2	0.2	2.3	1.7	-
5.		-	5.4	-	3.2	8.6

TABLE - XIII : Synthesis of 4,6-diamino-1-[(p-substituted) phenyl]-1,2-dihydro-2,2-dimethyl-s-triazine.



No.	Het	160	80	40	20	Control
1.		T5/5	T5/5	T1/5	1.8	8.4
2.		3.4	1.4	0	0	8.4
3.		T5/5	T3/5	3.8	3.4	8.4
4.		5.2	4.6	3.8	4.4	8.4
5.		T1/5	0	0	0	7.2

Suppressive Treatment :

It is generally observed that for prolonged effect of a compound (or drug) in vivo, it is much preferable to administer the compound in small and frequent doses rather than a single large dose.

The rate of clearance of drugs varies enormously, the range extending from less than an hour to well over a week. Most drugs are cleared in an exponential manner, so that when a single large dose is given the amount removed in unit time is a constant fraction of the amount still present. Thus it implies that it is impossible to produce prolonged action by giving a massive dose of a drug that is rapidly excreted. Prolonged action of such compounds can be obtained by delayed absorption or by frequent dosage, e.g. the quinine treatment of acute malaria when the drug is given every 6-8 hours.



Secondary Screen :

To observe the effect of the test compounds on antimalarial action and evaluate the suppressive blood schizontocidal activity a 4-day treatment of the compounds was given to the mice.

The compounds were administered both by the oral and subcutaneous routes. Survival time was observed and those animals which showed elimination of parasitaemia 40 days after infection were considered cured.

4-Day Suppressive Test for blood schizontocidal action :

A group of 5 albino male mice of Haffkine Institute strain weighing 22-25 g. were infected with 1×10^7 parasitized erythrocytes on the first day (D + 0). A suspension of the test compounds prepared in distilled water and 1 % Tween-80 was administered 4 hours after infection, and on day D + 1, D + 2, D + 3 consecutively.

The doses of 80, 40, 20 and 10 mg/kg were each divided over 4 days. Blood smears were prepared on the day following the last dose of treatment, i.e. D + 4, then on the sixth, seventh, ninth, thirteenth, sixteenth and twentieth days etc. parasitemia of mice surviving upto the

40th day post infection were thus monitored. Infected untreated controls and standard drug controls were also observed.

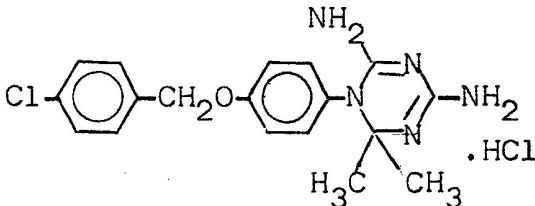
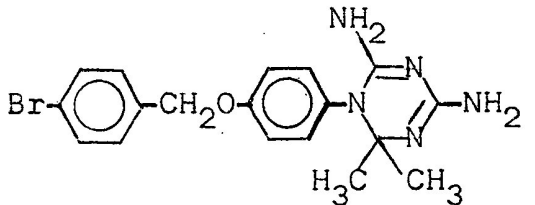
Four compounds were thus evaluated. A comparison of the oral and subcutaneous routes can be observed from Table XIV.

Observations and Results :

- (1) The mice were observed for 40 days after infection.
- (2) The effect of the compounds was prolonged and surviving mice were found to be cured of infection.
- (3) There was a slight increase in percentage parasitemia (2-6 %) on days 4 to 6. From the 7th day onwards the parasitemia gradually declined as was noticed from the blood smears until by the 20th day there was clearance or negligible parasitemia (less than 0.1%).
- (4) Table XIV shows the mean survival time of treated and infected untreated control mice. It can be clearly seen that the survival time in this test is longer compared to the survival time of mice in the single dose test, for the same compounds.

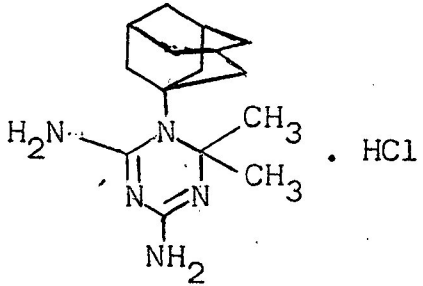
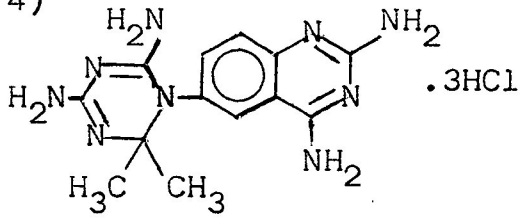
- (5) Compound 3 was toxic at 40 mg/kg x 4 orally. At 20 and 10 mg/kg x 4 both the routes showed 80 % survival of animals upto the 40th day and no detectable parasitemia.
- (6) For all compounds which showed antimalarial activity 60-100 % of the animals survived 40 days, after infection and parasitemia was nil in all the surviving mice, which may indicate that no reinfection has occurred.
- (7) Table XV reveals the reduction in percent parasitemia of the surviving mice which were cured of malarial infection one month after infection. This probably proves that the test compounds are good suppressive blood schizontocides. The blood smears of the test compounds were evaluated by comparison with the blood smears of standard drugs pyrimethamine and cycloguanil.
- (8) Antimalarial action was observed for all the four compounds at 2.5 mg/kg x 4 but none of the test compounds were curative and all mice died between the 10th to the 16th day after infection.
- (9) Cycloguanil hydrochloride was taken as a standard drug for comparison at dose levels of 10, 20, 40, 80 and 160 mg/kg. 100 % elimination of parasitemia i.e. curative results were obtained at 20,40,80 and 160mg/kg, 5 weeks after infection, in the surviving animals. The mice were healthy and devoid of parasites, in the blood.

TABLE - XIV : Evaluation of activity by Peters' 4-Day Suppressive Test on the 25th Day post infection.

C O M P O U N D	Dose mg/kgx4	Route of adminis- tration	Mean Survival Time		No. Dead		Remarks
			Treated	Control	No.Treated		
1) 	20	s.c	21.8	6.1	1/5		Active
	10	s.c.	17.8	6.1	2/5		Active
	25	s.c.	23.0	6.1	0/5		Active
	2.5	s.c.	13.2	6.1	4/5		Inactive
	40	p.o.	8.0	9	3/5		Toxic
	20	p.o.	16.0	9	0/5		Active
	10	p.o.	16.0	9	0/5		Active
	5	p.o.	9.14	9	2/5		Active
2) 	20	s.c.	22.6	6.1	1/5		Active
	10	s.c.	19.2	6.1	1/5		Active
	5	s.c.	17.8	6.1	1/5		Active
	2.5	s.c.	12.1	6.1	3/5		Slightly Active
	40	p.o.	16	9	0/5		Active
	20	p.o.	16	9	0/5		Active
	10	p.o.	16	9	0/5		Active
	5	p.o.	16	9	1/5		Active

Contd.....

(Table-XIV.....Contd.)

C O M P O U N D	Dose mg/kgx4	Route of adminis- tration	Mean Survival Time		No. Dead No.Treated	Remarks
			Treated	Control		
3) 	200.	s.c.	21.2	6.1	1/5	Active
	10	s.c.	18.3	6.1	1/5	Active
	5	s.c.	16.0	6.1	1/5	Active
	2.5	s.c.	13.5	6.1	4/5	Inactive
	40	p.o.	7.6	9.0	3/5	Toxic
	20	p.o.	17.8	9.0	0/5	Active
	10	p.o.	17.8	9.0	1/5	Active
	5	p.o.	12.7	9.0	2/5	Active
4) 	20	s.c.	19.2	6.1	1/5	Active
	10	s.c.	22.9	6.1	2/5	Active
	5	s.c.	20.6	6.1	2/5	Active
	2.5	s.c.	10.2	6.1	5/5	Inactive

Note :- A group of 5 animals was taken for each dose.

TABLE - XV : Antimalarial activity against P. berghei in mice.

Compound	Dose mg/kg x 4	% Reduction of Parasitemia on Days						
		4	6	7	9	13	16	25
Cycloguanil HCl	40	100	100	100	100	100	100	100
	20	100	100	100	100	100	100	100
	10	98	97	97	100	100	100	100
	5	98	97	97.8	98.2	98	100	100
	2.5	97.6	89.2	84.3	79.5	70.1	75.2	60
Pyrimethamine	20	T ₅						
	10	98T ₃	100	100	100	100	100	100
	5	96.3T ₁	99	98.2	100	100	100	100
	3.5	97	95	95	96	98	100	100
	2.5	99	99	98	95	98.5	98	98.2

(Table-XV.....Contd)

COMPOUND	Dose mg/kg x 4	Route	% Reduction of Parasitemia on Days						
			4	6	7	9	13	16	25
1)	20	s.c.	100T ₁	97	97.5	99	100	100	100
	10	s.c.	97	96	97.0	98	100	100	100
	5	s.c.	98	95	95	97	98.5	100	100
	2.5	s.c.	98	92	85	76	55	-	-
	40	p.o.	100T ₃	100	100	100	100	100	100
	20	p.o.	97	99	99	100	100	100	100
	10	p.o.	95	94	95	96	100	100	100
	5	p.o.	98	95	95	94	99	100	100
2)	20	s.c.	100	98	97	100	100	100	100
	10	s.c.	99	100	100	100	100	100	100
	5	s.c.	97	97	96	97.5	100	100	100
	2.5	s.c.	98	89	80	70	60.2	-	-
	40	p.o.	96	95	95	95	100	100	100
	20	p.o.	94	98.5	100	99	100	100	100
	10	p.o.	100	99	100	100	100	100	100
	5	p.o.	97	97	95	98	98	100	100

(Table XV.....Contd.)

COMPOUND	Dose mg/kg x 4	Route	% Reduction of Parasitemia on Days						
			4	6	7	9	13	16	25
3)	20	s.c.	100	100	100	100	100	100	100
	10	s.c.	98	100	100	100	100	100	100
	5	s.c.	98	97	97	98.5	100	100	100
	2.5	s.c.	98	96	96.5	96	45	-	-
	40	p.o.	95T ₃	96	-	-	-	-	-
	20	p.o.	100	100	100	100	100	100	100
	10	p.o.	100	100	100	100	100	100	100
	5	p.o.	100	100	100	100	100	100	100
4)	20	s.c.	96	94	96	98	100	100	100
	10	s.c.	98	98	95	95.5	99	100	100
	5	s.c.	96	96	95	92	86	95	85
	2.5	s.c.	94	95	95	79	-	-	-

PRELIMINARY ACUTE TOXICITY STUDY :

It is a known fact that any potential medicinally active compound has to be studied for its toxic effects before it reaches the stage of clinical trials. With this in view the preliminary acute toxicity study of two compounds showing potent antimalarial action was undertaken.

In order to assess the safety of a drug various toxicity studies are carried out in experimental animals like mice, rat, monkeys etc. by different routes of administration. The tests include :

- a) Acute Toxicity Tests.
- b) Chronic or long term Toxicity studies.
- c) Special tests for teratogenicity, mutagenicity, carcinogenicity, etc.

The main object of an acute toxicity test is to study the toxic effect that a compound produces when given in a single dose or in multiple doses over a period of 24 hrs. or less. The effect generally reported is mortality expressed as LD_{50} i.e. the dosage required to kill 50 % of the animals.



LD₅₀ value serves to provide a convenient way to classify tested compounds into toxicity classes and also serves as a guide for selecting subsequent doses for long term studies.

Compounds :- (1) 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-(1-adamantyl)-s-triazine hydrochloride and (2) 4,6-diamino-1,2-dihydro, 2,2-dimethyl, 1-|p-(p-bromo benzyl) oxyphenyl |s-triazine hydrochloride, were found to be active at 20 mgs/kg showing 80-100 % clearance of malarial parasites both by the oral and subcutaneous routes.

Hence both these compounds were taken for LD₅₀ determination.

Determination of LD₅₀ (Median Lethal Dose) :

The compounds (1) and (2) were suspended in distilled water with Tween-80. This emulsion was administered subcutaneously at various dose levels to groups of mice of both sexes equal in number. Before the actual LD₅₀ determination a pilot study was made, whereby test compounds were administered to pairs of mice in spaced doses. The injected mice were observed continuously and mortality was recorded.

On the basis of the pilot study a dose range was selected for further study. Mice weighing around 22-25 g. were selected and grouped into 10 each. The animals were given food and water ad lib. The test compounds were administered as mentioned above for the pilot study. A control group of mice was given only food and water and not the test compound. All the animals were observed for symptoms of toxicity and mortality for seven days.

Results :

The general symptoms of toxicity observed on subcutaneous administration of the compounds were excitation, convulsions, loss of righting reflex, respiratory disturbances and death. The LD_{50} was calculated as per the method of Litchfield and Wilcoxon.¹⁴¹

The LD_{50} obtained for compound 1 was 190 mg/kg with fiducial limit between 153.22 mg/kg and 235.6 mg/kg. The LD_{50} obtained for compound 2 was 1650.0 mg/kg with fiducial limits between 1204.38 mg/kg and 2260.5 mg/kg.

Initial study from the LD_{50} value suggests that compound 1 has more toxicity than compound 2.

-O-

STRUCTURE ACTIVITY RELATIONSHIP

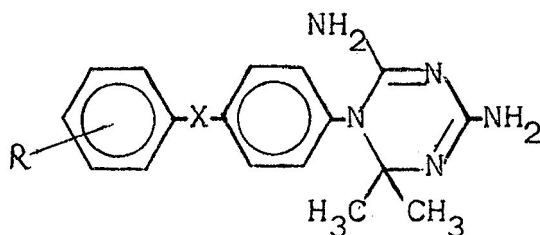
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STRUCTURE ACTIVITY RELATIONSHIP

A number of hypotheses have been laid down on the relationship between chemical constitution and activity of antimalarials. The possession of a ring structure that lent itself to the formation of tautomers and the release of electrons appears to be essential for activity in schizontocides of the quinine and 4-amino-quinoline class. Free amino groups as in sulfonamides, sulfones, diamino pyrimidines or dihydro-s-triazines are also essential for activity.

With this in view we have made an attempt to correlate aromatic substituents at 1-position and alkyl or aryl substituents at 2-position of a dihydro-s-triazine moiety with antimalarial activity. The structure activity correlation is discussed as per various sections.

Section - A :



Compound Nos. 1-8 in Table VIII of the above general structure.

Compounds in this section consist of s-triazines built up on various bis-arylamines consisting of :

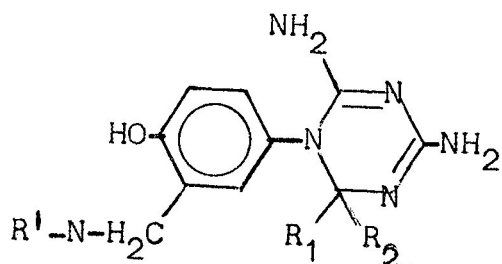
- i) Benzyloxy linkage i.e. $-\text{CH}_2\text{O}-$; ether linkage $-\text{O}-$; sulfide linkage $-\text{S}-$.
- ii) Substitutions at para position include electron withdrawing moieties such as Cl, Br, NO_2 , NHCOCH_3 and carbomethoxy amino functions.

Compound numbers (1) and (2) from Table VIII in this series showed prolonged extension in survival time, compared to infected untreated controls. These compounds showed activity at a minimum dose of 20 mg/kg. Those with ether or sulfide spacers were found to be toxic or inactive. Incorporation of a carbomethoxy (NHCOOCH_3) at 'R' in compounds (7) and (8) was not very encouraging.

Compounds (1) and (2) when studied for suppressive activity Tables (XIV; XV) by the 4-Day Test showed no parasitemia for a month after infection. Almost 80-100 % animals were cured at a dose of 5 mg/kg x 4, indicating the suppressive blood schizontocidal action of the compounds.

Thus we can probably conclude that $-\text{CH}_2\text{O}-$ spacer with substitutions such as halogens at p-position helped in imparting activity.

Section - B^o : (Table IX)



The early classic work of Burckhalter⁸⁴ and co-workers reports a large number of compounds based on the known activity of certain α -dialkyl-amino-o-cresols.

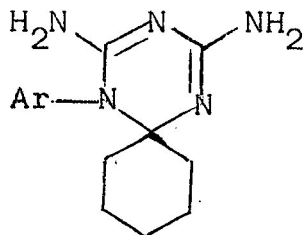
Besides Amodiaquine has been shown to be effective against certain chloroquine resistant strains of P.falciparum in vitro, in owl monkeys, and in man.¹⁴²

Hence we decided to utilize the Mannich bases viz; 3-diethylaminomethyl-4-hydroxy aniline, and 3-pyrrolidinyl methyl 4-hydroxy aniline side chains to be linked with the s-triazine ring and concomitant changes at 2-position were also observed. At the 2-position of the triazine moiety ethyl methyl, hexylmethyl and aryl groups were introduced.

Amongst the aromatic substitutions at 2-position of the triazine, compounds (4) and (8) with phenyl ring at 'R₂' and diethyl amino and pyrrolidino side chains at 'R₁' showed an extended survival time. However, substituted phenyl groups imparted toxicity or were devoid of activity.

It can be observed from the above results that introduction of higher alkyl or aryl groups at 2-position of 1,3,5-triazine ring system is not fruitful.

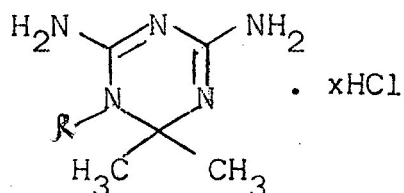
Section - C : (Table X)



Since in the previous series introduction of higher aliphatic or aryl groups at 2-position of triazine did not give encouraging results in a further attempt of structural modifications saturated cyclic moiety like cyclohexane was introduced and compounds (Table X) were thus prepared. The compounds though well tolerated did not exhibit antimalarial action. Thus the bulky substitution theory at 2-position of triazine does not hold in this case. A glance at Table X also indicates that the arylamines utilized in Section B and C are also not very helpful, contrary to our presumption that activity may be enhanced by increasing hydrophobicity of a molecule.



Section - D : (Table XI)

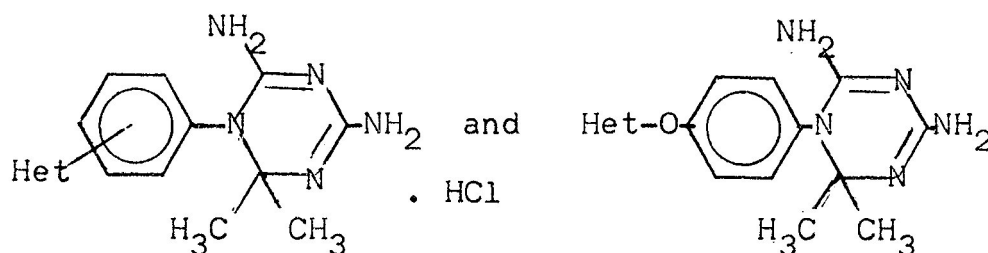


In the present series introduction of heterocyclic moieties like 2,4,6-triamino quinazoline, the 3 and 8-amino quinolines and N-methyl piperazine was tried. Similarly, considering the biological importance of adamantyl nucleus it was also incorporated in the triazine system. In this series more efficacious compounds were obtained in case of 2,4,6-triamino quinazoline, N-methyl piperazine and adamantyl derivatives. The adamantyl derivatives showed curative results in ~~most~~ of the animals at 5 mg/kg x 4 dose and no infection. The animals responded to the suppressive treatment even at 2.5 mg/kg x 4 dose level but parasitemia was observed from the 10th day onwards after infection and finally mortality.

The N-methyl piperazine-triazine compound showed activity at single subcutaneous doses of 160 mg/kg and lower.

Though quinoline derivatives are well known for their antimalarial activity, in the triazine series they could not prove their superiority.

SECTION - E :



On the basis of a hypothesis that *p*-chlorophenyl group in cycloguanil acts as a carrier moiety for the *s*-triazine nucleus, we synthesized various heteroaryl and heteroaryloxy triazines as above and evaluated for anti-malarial activity. The 4-position of the phenyl ring was substituted by morpholine, piperidine, pyrrolidine N-methyl piperazine and imidazole. The heteroaryl ethers prepared were 4-pyrimidinyl-oxy, pyridinyloxy, adamantyloxy derivatives. Substitution of heterocycles did not show any significant antimalarial activity. Infact pyrimidinyl-oxy and pyridinyloxy compounds showed acute toxicity.

Hence our efforts in this direction were not fruitful.



CONCLUSIONS OF STRUCTURE ACTIVITY RELATIONSHIP :

- (1) Comparison of Tables (VIII) to (XIII) reveals that the most active compounds lie in Tables (VIII) and (XI).
- (2) The active compounds 1 and 2 of Table VIII are probably due to the $-\text{CH}_2\text{O}-$ bridge. The bromo-analogue showed 100 % curative effects compared to the chloroanalogue and was more effective orally.
- (3) Compounds of Table (IX) show that introduction of higher alkyl or aryl homologues at 2-position of the triazine ring do not abide by the bulky substituent theory and are either inactive or toxic.
- (4) Attachment of the N^1 of triazine ring to the carbon of an aromatic phenyl group (substituted or not) is not always necessary. It can also be cyclized on basic amino groups of heterocyclic moieties like quinoline, quinazoline or alicyclic structures like adamantamine.
- (5) Conversion of the diamino triazines to anilino triazines demonstrated that both the amino functions are important for biological activity. The isomers are biologically inactive. This was indicated by testing the anilino triazines of some compounds against *P. berghei* in mice.

(6) The dimethyl groups at 2-position are relatively easy to introduce compared to higher alkyl or aryl group and are essential for good activity.

(7) Attaching Mannich bases to the s-triazine as in Table (IX) and (X) was not very useful.

(8) Incorporation of heteroaryl and heteroaryloxy substituents at N-1 of triazine ring did not produce the desired encouraging results.

Correlation of antimalarial activity
with Partition Coefficient values :

It was realized that change in substituent pattern in any part of an organic compound produces great variation in the activity. This encouraged research workers to relate the physico-chemical properties of the substituents with the biological activity. Attempts were made through the application of linear free energy related Hansch approach,^{143,144} additivity model of Free Wilson¹⁴⁵ and all valence molecular orbital methods¹⁴⁶ to get proper correlations.

In the present work a few compounds have been studied for their partition coefficient values. A modest endeavour to relate this physical parameter with biological activity is made in Table (XVI).

It is generally expected that in vivo for highly water soluble drugs (Low or negative log P) the probability of reaching some distant receptor site will be low because of rapid excretion. As log P becomes more positive transport will become favourable. Thus as the relative lipophilicity is increased activity rises to a maximum. Likewise a negative Π value indicates greater affinity for the aqueous phase and a positive value indicates greater affinity for the lipid phase.

Since all the compounds in Table XVI have a definite structure pattern, their partition coefficient has been related to activity. The partition coefficient values were obtained using the octanol-water system as per reported procedure using a U.V. spectrophotometer to ascertain the optical density before and after partitioning. From compound Nos. 1-6; compound No. 4 and 5 were found to be very active. Suppressive antimalarial activity was observed at a minimum dose of 5 mg/kg x 4 and above. These two compounds have positive log P values and maximum activity among the entire series of triazines. The bromo benzyloxy analog has log P 2.156 and a positive Π constant of 1.909 which shows that the bromo benzyloxy substituent in the triazine ring contributes a lot to hydrophobic bonding. Similar is the case with compound No.4 though the log P value is slightly lower.

Contrary to expectations that NHCOOCH_3 function may increase lipophilicity, compound No. 6 has a negative log P, thereby comparatively less lipophilicity which probably reflects in its lack of activity. Though the π constant is +0.328 it has contributed little to the whole molecule in increasing the hydrophobic character. Electron withdrawing groups like Cl, and NO_2 do not demonstrate activity. Low or negative log P of compounds 1, 2, 3 and 6 suggest the necessity of $-\text{CH}_2\text{O}-$ linkage.

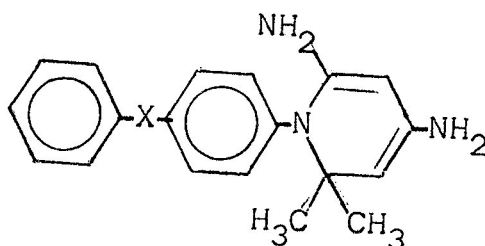
Two other active compounds of the entire triazines series, Nos. 8 and 9 showed positive log P values. Compound No. 8 has a low log P but high antimalarial effect. This may be because the pharmacological efficacy of adamantane derivative is attributed, besides a high lipophilic character, to the high resistance of adamantane substrates towards metabolic degradation, due to which it shows prolonged action in vivo.

Of the entire series only those compounds which showed promising antimalarial action were studied, for this parameter.

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TABLE - XVI : Correlation of antimalarial activity of 4,6-diamino 1-phenyl substituted-1,2-dihydro 2,2-dimethyl s-triazines with log P values :



No.	R	X	Log P	Lipophilic Constant
1.	Cl	-O-	2.199	1.3522
2.	2,4-diel	-O-	-1.137	0.2902
3.	NO ₂	-O-	-1.267	0.4202
4.	Cl	-CH ₂ O-	2.166	1.3187
5.	Br	-CH ₂ O-	2.256	1.409
6.	NHCOOCH ₃	-S-	-1.505	0.0328
7.	-	Cl(Cycloguanil)	0.847	
8.			0.417	-
9.			1.654	-

Note :- For compound Nos. 1-6 the Π constant is defined by the linear free-energy relationship as

$$= \log P_x - \log P_H$$

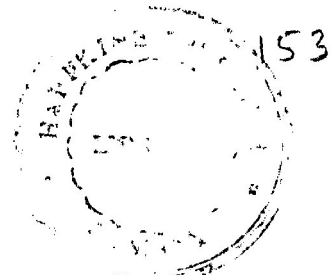
Where, P_x = substituted molecule

P_H = parent molecule (cycloguanil)

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S U M M A R Y

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SUMMARY

The importance and dire necessity of synthesizing new antimalarial agents to combat resistance problems initiated intensive research work in the field of chemotherapy at many reputed organizations all over the world.

Of the newer classes to be explored for activity, the dihydro-s-triazine cycloguanil has been found to show good blood schizontocidal and also causal prophylactic activity. This led us to undertake the research described in the foregoing chapters. A series of compounds of the general nomenclature 4,6-diamino-1,2-dihydro-1-substituted s-triazines were prepared and evaluated for biological action.

In the Introduction is briefly described the world malaria situation according to the recent reports of W.H.O. The Review of Literature gives the details of the chemotherapy of malaria, incorporating the recent advances in the medicinal chemistry of antimalarials and a short account of the known antimalarial compounds and their Mechanisms of Action. The Plan of Work lays down the significance of the proposed synthetic work. The entire set of s-triazines prepared have been, classified according to the variations made in the molecular structure.

This is followed by the Chapter on "Chemistry", wherein, the synthetic chemistry and chemical properties of the dihydro-s-triazines are discussed. Further, the synthetic and experimental aspects of the various arylamines utilized for triazine formation have been discussed under various sections.

Section A deals with the synthesis and chemistry of the first set of 4,6-diamino-1,2-dihydro,2,2-dimethyl 1-(p-substituted phenyl)-s-triazine hydrochlorides. Bisarylamines with a spacer between the two phenyl rings were prepared from the corresponding nitro or acetamido derivatives, by reduction and/or hydrolysis which subsequently yielded the arylamines in 60-70 % yields. These amine hydrochlorides were further utilized for triazine formation.

In Section B are mentioned the chemistry and experimental aspects of o-cresol, 4|4,6-diamino, 1,2-dihydro, 2,2-dialkyl or aryl s-triazine|- -substituted aminomethyl type of compounds.

Section C gives the synthesis of 2,4-diamino-1-|(4'-hydroxy-3'-substituted amino methyl) phenyl| 1,3,5-triazaspiro (5-5) undeca 2,4-diene. The arylamines utilized in both Sections B and C were prepared by the Mannich

Condensation Reaction. The amine hydrochlorides were further treated with dicyandiamide, aromatic aldehydes or aliphatic ketones and cyclic ketones viz; cyclohexanone for triazine cyclization.

Section D deals with the synthesis and experimental procedures of 4,6-diamino, 1,2-dihydro, 2,2-dimethyl s-triazines with heterocyclic substituents at 1-position of the s-triazine ring. Amino functions of alicyclic structure like adamantamine and heterocyclic amine (viz; quinoline and quinazoline) were utilized for the formation of the triazine moiety. 3 Compounds in this section showed antimalarial activity.

Section E consists of preparation and chemistry of compounds of the type 4,6-diamino, 1-|heteroaryl or heteroaryloxy| 1,2-dihydro-2,2-dimethyl s-triazine hydrochlorides. Keeping in mind the structural feature of cycloguanil, the chloro phenyl substituent was replaced by various heteroaryl and heteroaryloxy functions. None of the compounds showed significant biological action, as presumed in this series.

The synthesis of the final compounds involved the two component or three component cyclization of the s-triazines, starting from the various arylamines prepared in the laboratory. In the various modifications attempted

structural changes have been made at the N-1 position and 2-position of the dihydro-s-triazine moiety and effects on biological activity observed.

Thirty five compounds were prepared and screened for antimalarial action in vivo, in the course of the research work. The testing was done in the rodent malaria model against the Plasmodium berghei strain. Two tests were undertaken :-

(1) Rane's Single Dose Test for blood schizontocidal action- Four compounds showed significant antimalarial activity at a minimum dose of 20 mg/kg and above upto 160 mg/kg but no curative results and the animals died between 19-25 days after infection.

(2) Peters' 4-Day Test- The above four compounds which showed blood schizontocidal activity in the single dose treatment were further evaluated for their suppressive blood schizontocidal activity. The compounds were given at dose levels of 2.5, 5, 10 and 20 mg/kg each day for 4 consecutive days, both by the oral and subcutaneous routes. The benzyloxy analogs were found to show better results by the oral route. The benzyloxy and adamantyl triazines showed curative results at 5 mg/kg for 40 days

post infection and 100 % clearance of parasitemia unlike that in the single dose treatment. Though activity was observed at 2.5 mg/kg subcutaneously, the animals did not survive upto 40 days and the parasitemia was found to show an initial suppression, with an increase from the 6th or 7th day until mortality after 13-15 days.

The most promising of the entire set of compounds tested were the 4,6-diamino 1,2-dihydro 1-[(p-bromo phenyl) benzyloxy]-2,2-dimethyl s-triazine of Section A and 4,6-diamino 1,2-dihydro, 1-(1-adamantyl) 2,2-dimethyl s-triazine of Section D which exhibited good activity at 10, 20, 40 and 80 mg/kg. Both these compounds were further studied for their Preliminary Acute Toxicity study. Though the adamantyl derivative has an LD₅₀ of 190 mg/kg, the bromo benzyl analog was comparatively better with an LD₅₀ at 1650.0 mg/kg. No clinical symptoms of malarial infection were observed at low doses and the surviving animals seemed to be cured of parasitemia by 30-40 days. Thus from the biological action point of view it can be concluded that prolongation of survival time and 80-100 % elimination of parasites as observed for all the 4-compounds, proves them to be good suppressive antimalarials.



A few representative compounds were also studied for their spectral data, viz; U.V.; N.M.R.; I.R. and Mass Spectra. The compounds were found in accordance with their assigned structure.

An attempt to discuss the Structure Activity Correlations was made. The introduction of benzyloxy spacer showed active compounds compared to ether and sulfide linkages as seen from Section A.

Presence of gem-dimethyl groups was important for activity. Introduction of higher aryl or alkyl functions resulted in loss of activity as observed from Section B, and Section C.

Alicyclic structures like adamantane and heterocyclic moieties like quinazoline showed curative anti-malarial effects as seen from Section D, indicating further exploration in such type of compounds.

Introduction of heterocyclic moieties at p-position of phenyl ring as in compounds of Section E was not of much value.

The bromo benzyloxy derivative contributed more to decreasing the toxicity as compared to the chloro derivative which was toxic at 160 mg/kg orally.

Few of the compounds were also studied for their physical parameter i.e. Partition co-efficient and lipophilic constant values. The compounds which showed positive log P values also revealed prominent antimalarial activity indicating that positive log P and a positive constant show increased hydrophobicity and slower rate of excretion.

The present research work may give a lead to further exploration of dihydro-s-triazines of possible therapeutic value and probably confirms that 4,6-diamino 1,2-dihydro s-triazine class of compounds are potential antimalarials, which need to be studied are with more structural modification and biological investigations.

The mechanisms of antiplasmodial action of many antimalarial chemicals remain to be fully elucidated at a molecular level. Complete understanding of molecular mechanisms could lead to the design of an ideal chemical, which is the essential need of today.



-O-

R E F E R E N C E S

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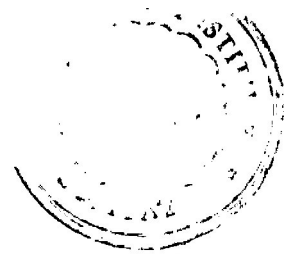
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SYNOPSIS OF
THE THESIS TO BE SUBMITTED FOR THE
DEGREE OF
MASTER OF PHARMACY
TO
THE UNIVERSITY OF BOMBAY

NAME OF THE CANDIDATE : MISS ZARINE KHETY
NAME OF THE GUIDE : DR. B.G. KHADSE.
PLACE OF WORK : HAFFKINE INSTITUTE, PAREL,
BOMBAY 400 012.
TITLE OF THESIS : "SYNTHESIS OF DIHYDRO-s-
TRIAZINES AS POSSIBLE
ANTIMALARIAL AGENTS"

Malaria has been a serious human scourge throughout the recorded history. In recent years the emphasis of the worldwide struggle against malaria has changed from an attempt to eradicate the infection to an attempt to control the disease.

Based on survey data¹ the number of malaria cases in the world in 1981 is estimated to be of the order of 90 million. Resurgences of malaria have occurred in many parts of the world. According to statistical reports of W.H.O. one in every one hundred Indians is found to suffer from malaria.²

According to information available the overall world malaria situation has not improved over the last 15 years. So far chloroquine has been the only successful drug universally used for malaria control. But in 1959 the cases of chloroquine resistance was reported for the first time. The second line of defence sulfadoxine/pyrimethamine combination is also found to be resistant in certain regions more so, when used for its suppressive action.³ Resistance to the good old quinine and of late the new introduction Mefloquine in some cases has spurred chemotherapeutic research to discover new drugs, with more efficacy, safety and those which prevent or delay resistance.

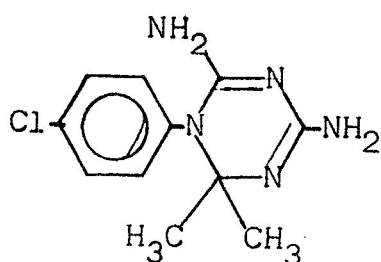
Old drugs like paludrine its active metabolite cycloguanil and other dihydrofolate reductase inhibitors like various dihydro-s-triazines have been extensively worked on. The triazine series of compounds are of interest since some are apparently long acting and are effective against both chloroquine and pyrimethamine resistant parasites.⁴⁻⁷

Thus in the present study synthesis of 4,6-diamino-1,2-dihydro-s-triazines was undertaken and the antimalarial activity of these compounds evaluated.

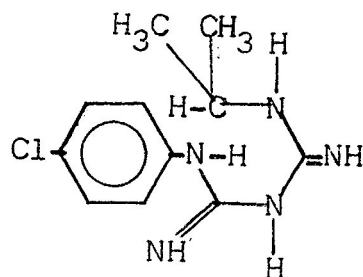
Dihydro-s-triazines :

4,6-Diamino-1,2-dihydro-1-aryl-s-triazines :

After the introduction of proguanil evidence was presented that the drug had essentially no biological action⁸ but an active metabolite was isolated from the urine of proguanil treated rabbits and humans. This metabolite was highly active against P. gallinaceum in vitro and in vivo.⁹ This metabolite was chemically identified as 4,6-diamino-1-(p-chlorophenyl)-1,2-dihydro-2,2-dimethyl-s triazine termed as cycloguanil. The structural relationship of cycloguanil to proguanil can be observed as follows when the latter is drawn in the curved form.



Cycloguanil

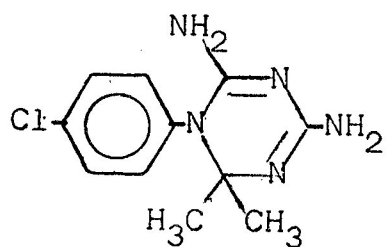


Proguanil

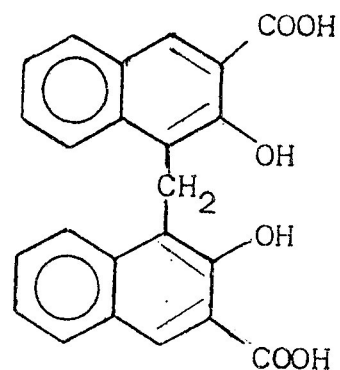
The structure of cycloguanil and its biological activity stimulated the synthesis of many dihydro-s-triazines. 10-11

These compounds showed greater activity than the corresponding biguanides against avian malaria,¹² but not against simian infections or human malaria.¹³ Accumulated evidence shows that the mode of action of cycloguanil analogous dihydro-s-triazines is due to their interference with the folic acid pathways of the parasite.¹⁴ The well-known dihydro-s-triazines that have recently been proved anti-malarials are cycloguanil pamoate (I); clociguanil (II) and WR-99210 (III) which contain the halogen substituted phenyl, benzyloxy and phenoxy alkoxy moieties respectively at 1-position of the s-triazine ring.¹⁵

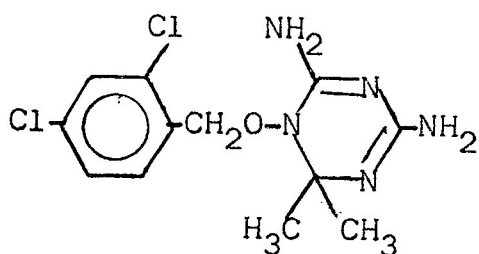




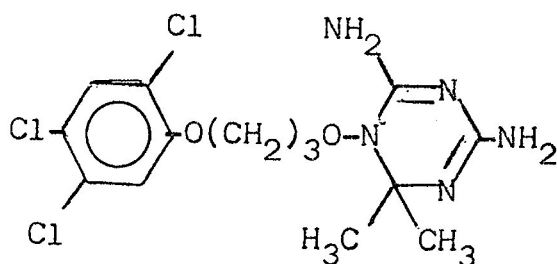
1/2



(I)



(II)



(III)

These compounds have been found effective in humans in combination with sulfadiazine.

Thus we made an attempt to synthesize and evaluate the structural modifications in a series of dihydro-s-triazines. Thirty five compounds have been synthesized and are briefly summarized as follows, under various sections.



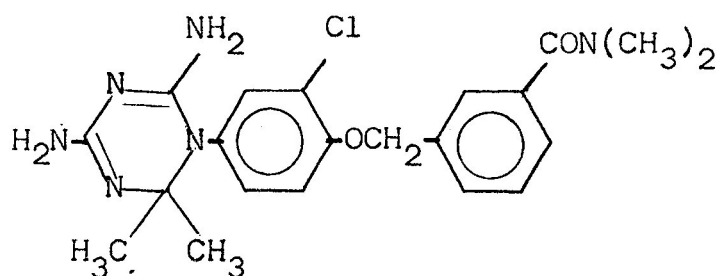


SECTION - A.:

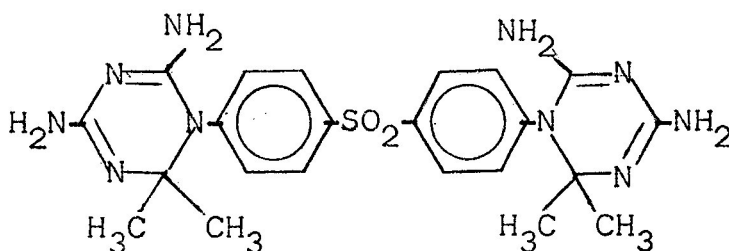
Synthesis of 4,6-diamino-1,2-dihydro 2,2,-
dimethyl-1-(substituted phenyl)-
s-triazine hydrochlorides :

Literature shows that s-triazines have been widely exploited and are still being screened for antihelmintic,¹⁶ antitumor,¹⁷ antitrypanosomal,¹⁸ and the historical anti-malarial activity.

Dihydro-s-triazines which are analogues of cyclo-guanil are DHFR inhibitors and get rapidly excreted from the body. Many triazines attached to bis-(aryl/amino) salts are reported¹⁶⁻¹⁸ viz Baker's antifols (IV) and Capp's sulfonamido triazines (V)

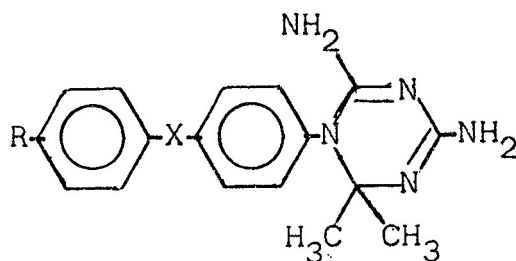


(IV)



(V)

Assuming that introduction and variation of a spacer between two aryl groups may increase lipophilic character of the entire moiety, which may consequently decrease the rate of excretion, compounds of following general structure (VI) have been prepared.



(VI)

Where, R = Cl, Br, NO₂, NHCOCH₃, NHCOOCH₃ etc.

X = -O-, -S-, -CH₂O-

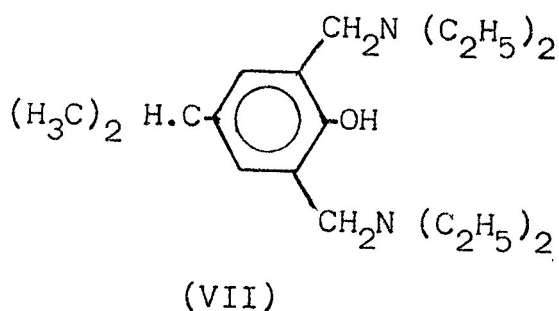
All the compounds have been tested against P. berghei in mice for antimalarial action. Two of them with the benzyloxy linkage between the phenyl groups showed very interesting results.

SECTION - B :

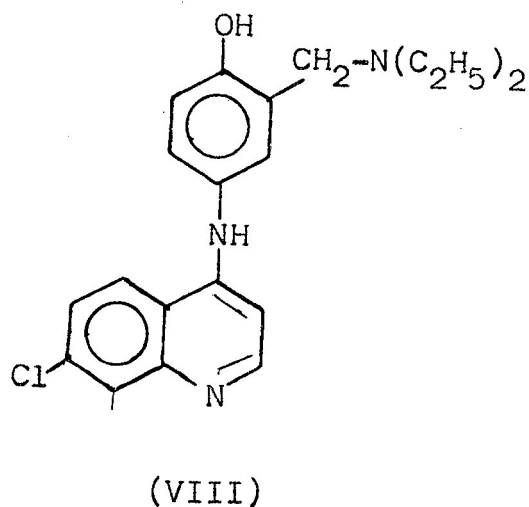
Synthesis of o-cresol, 4- | 4,6-diamino-2,2-dialkyl
or aryl-s-triazine-1-(2H)-yl | - -substituted
amino methyl hydrochloride :

It is known that of the possible positions of the basic side chain in the quinoline nucleus only the 4,6 and 8-positions give rise to active antimalarials.

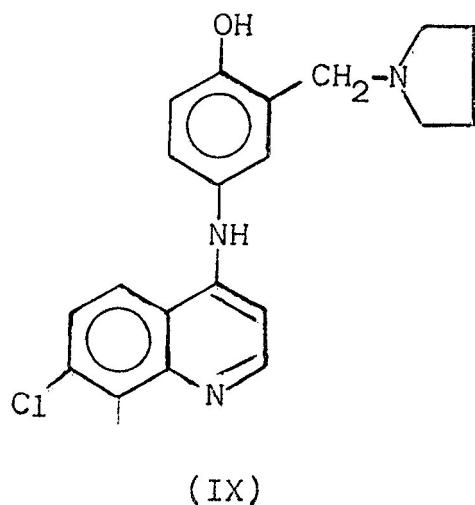
Burckhalter¹⁹ and co-workers prepared a large number of 4-aminoquinolines based on the known activity of certain -dialkyl-amino-o-cresols such as (VII).



This molecule was modified to the 3-diethyl-amino methyl 4-hydroxy anilino side chain and combined with 7-chloroquinoline to give amodiaquine (VIII).



Amodiaquine



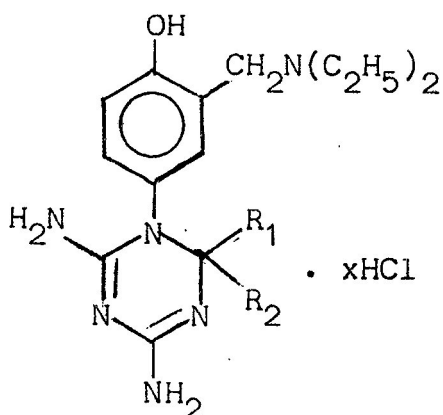
Amopyraquine

Amopyraquine (IX) was formed by the closure of the diethylamino moiety into a pyrrolidine ring. Both these compounds possessed high antimalarial activity. When one

studies the structure activity relations of the 4-amino-quinolines it is observed that those containing a hydroxyl group in the side chain are very active, besides the halogen substitution in the nuclear ring.

Hence, from the history and SAR of the 4-amino-quinolines we thought it fruitful to use the 3-diethylamino methyl-4-hydroxy anilino side chains of amodiaquine and amopyraquine respectively as arylamines to build the s-triazine moiety.

We made an attempt to incorporate substituents in the triazine ring at 2-position with higher alkyl or aryl groups and one compound amongst these showed antimalarial action after a single subcutaneous dose 72 hrs. post infection at 40 mgs/kg. Compounds of general structure XI were therefore synthesized and screened by Rane's Test for antimalarial activity.



Where, $R_1 = \text{H}, \text{CH}_3$

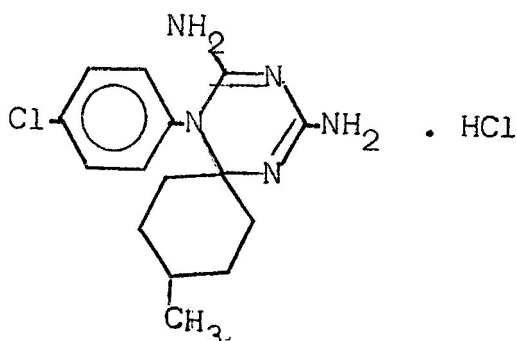
$R_2 = -\text{C}_2\text{H}_5-; -\text{CH}_3(\text{CH}_2\text{CH}_2)_2 \text{CH}_3-; -\text{CH}_3$
 $-\text{C}_6\text{H}_5-\text{N}(\text{CH}_3)_2; (\text{H}_3\text{CO}) (\text{OH}) (\text{Cl})-\text{C}_6\text{H}_5.$

The triazines prepared were checked for correct elemental analysis, after proper purification procedures.

SECTION - C :

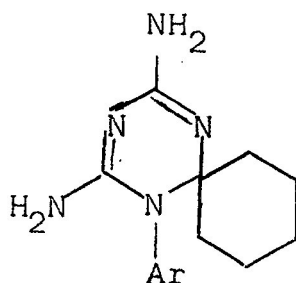
Synthesis of 2,4-diamino-1-| (4'-hydroxy,3'-(substituted amino-methyl) phenyl |-1,3,5-triazaspiro (5-5) undeca 2,4-diene :

Roth et al.²⁰ in their course of studies prepared a compound (XII) analogous to cycloguanil with a slight variation in the triazine ring.



(XII)

This compound was found to be active as an anti-helminthic. The activity was attributed to a hypothesis that a bulky group at 2-position of the triazine ring may enhance activity. Following a similar pattern we attempted to synthesize analogous s-triazines (XIII) on arylamines which possessed some biological action.



(XIII)

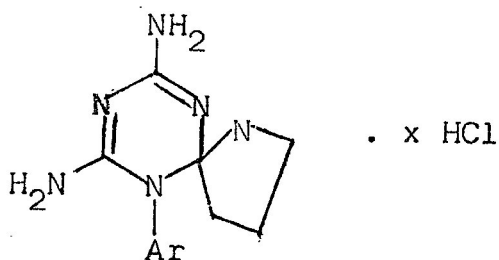
Where, Ar = substituted arylamines, viz;

3-diethyl amino methyl-4-hydroxy aniline,

3-pyrrolidino amino methyl 4-hydroxy aniline,

4-amino-4'-substituted diphenyl sulfide.

In place of cyclohexane we tried to incorporate the cyclopentane moiety to give compounds as XIV.



(XIV)

But the compounds obtained were oily and hygroscopic in nature and could not be characterized in structures by the elemental analysis of carbon, hydrogen, nitrogen. Thus we did not pursue this work further.

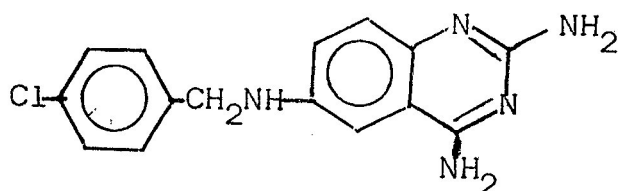


SECTION - D :

Synthesis of 4,6-diamino-1,2-dihydro-2,2-dimethyl-s-triazines with heterocyclic substituents at 1-position of the triazine ring :

Literature studies amply reveal that dihydro-s-triazines attached to various substituted aromatic amines have been extensively worked out as aliphatic amines do not cyclize with dicyandiamide to form a sym-triazine.¹⁰

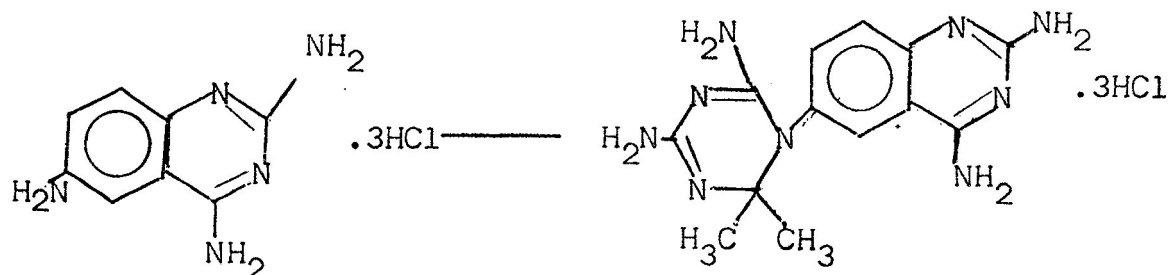
But utilization of amino groups on heterocyclic moieties which are of biological importance is relatively unexplored. Hence we selected a few of these heterocyclic amines and built triazines on them. One of the early non-classical prototype namely 2,4-diamino-6-(3,4-dichloro benzyl amino | quinazoline (XV) displayed strong antimalarial activity against P. berghei in mice and P. gallinaceumin in chicks.



(XV)

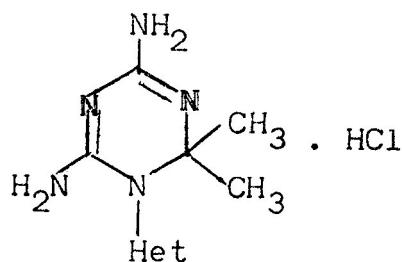
This triamino quinazoline derivative exhibited strong inhibitory effects against S. fecalis at an MIC of 6 ugs/ml which was comparable or superior to pyrimethamine, cycloguanil etc.

Thus the 6-amino group of the 2,4,6-triamino quina-
zoline was utilized by us to build the triazine by the
standard three component synthesis.



The compound was found to have antimalarial action at
20 mgs/kg and suppressive blood schizontocidal effects
at 40 and 80 mgs/kg against P. berghei in mice.

Other compounds synthesized in this series are as
per general structure (XVI)



(XVI)

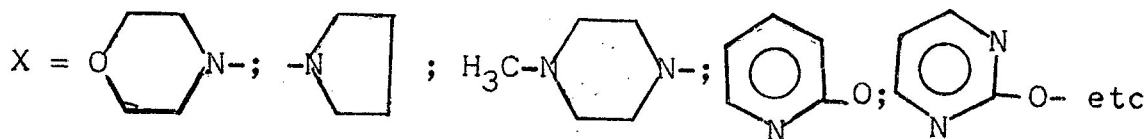
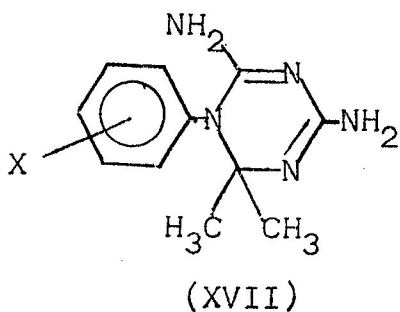
where, Het = quinoline, adamantane.

Efforts in this direction are still in progress
Spectral data of a representative member and elemental
analysis of all compounds are in agreement with the
assigned structure.

SECTION - E :Synthesis of 4,6-diamino-1-(p-substituted phenyl)-1,2-dihydro-2,2-dimethyl-s-triazine hydrochlorides :

Literature shows that pyrrolidine, N-methyl-piperazine, morpholine and piperidine type of moieties confer antimicrobial activity on their parent compounds.²¹

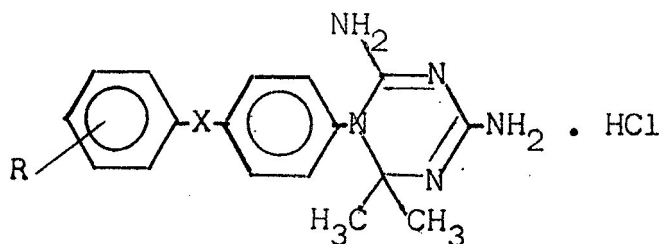
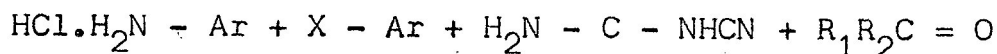
Besides it has been postulated that in cycloguanil the p-chlorophenyl nucleus acts as a carrier moiety for the triazine ring. Assuming that a heterocyclic group can also play a similar role, some heteroaryl and heteroaryloxy amines were prepared and utilized for triazine formation as (XVII).



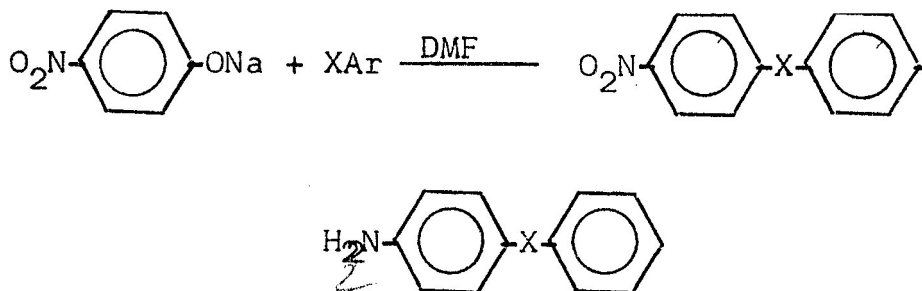
Experimental :

Brief account of the experimental procedures employed is as follows :-

Three major routes exist for the synthesis of 1-aryl 4,6-diamino-1,2-dihydro-s-triazines. But mostly the three component synthesis of Modest¹⁰ was utilized to provide the target compounds as in Scheme - I.

Scheme - I :

Arylamines used in Section-A were prepared as per Scheme-II.

Scheme-II :

The arylamines utilized in Section-B and C were prepared by the Mannich Condensation Reaction in which case a secondary amine hydrochloride is heated at reflux temperature with formaldehyde and paracetamol. The solid obtained is subjected to acidic hydrolysis and the amine hydrochloride is treated with dicyandiamide and ketone or aldehyde as per Scheme-I.

Most of the arylamines required in Section-D were available commercially except the 2,4,6-triamino quinazoline which was synthesized in the laboratory and application of Scheme-I led directly to the required compounds.

Section-E intermediates were prepared by heating p-chloro nitrobenzene in dimethyl formamide with the respective heterocyclic amines and potassium carbonate at 120-140°C; poured into ice water after about 18-20 hours and the solid which precipitated out dried under vacuo at 50-60°C.

The detailed experimental part and chemistry will be discussed in the thesis.

Pharmacological Screening :

(A) Rane's Preliminary Rapid Screening Method²² :

All the compounds synthesized have been evaluated for antimalarial activity by the Rane's method against rodent malarial strain of P. berghei in mice in vivo.

White mice of H.I. strain 22-25 g. average weight were infected with a standard intraperitoneal inoculum of 1×10^6 infected mouse erythrocytes which kills untreated controls in 6.5 ± 1 days average. Test compounds were suspended in Tween-80 and distilled water and injected subcutaneously 72 hrs. post infection. A compound was arbitrarily considered active if treated animals survived at least twice as long as the untreated controls. The test compounds producing mortality within 48 hrs. after administration were classified as toxic. In each test pyrimethamine and cycloguanil hydrochloride were included for comparison. The dose at which double the survival time was attained was considered the Minimum Effective Dose (MED).

(B) Evaluation of Activity by Means of Parasite counts 4-Day Suppressive Test for Blood Schizontocidal action against *P. berghei* in mice²³ :

Male random bred white mice weighing 22-25 g. were inoculated i.p. with one million parasitized red blood cells of laboratory strain of *P. berghei*. Animals were then treated with the test compound once daily for four consecutive days beginning right on the day of infection. Compounds were suspended in Tween-80 and administered

subcutaneously. Dose range selected was 80 mg; 40 mg; 20 mg and 10 mg per kilogram body weight respectively. Blood smears were taken by snipping off a portion of the tail of the mouse and stained with Geimsa. Parasite counts were taken from the day following the last day of treatment i.e. on the fourth day, sixth day, seventh day, ninth day, sixteenth day, etc.

Results and Discussion :

Out of the entire series of structurally modified s-triazines screened some showed activity as shown in Table-I. Those compounds which showed activity by Rane's Test were followed up by Peters' 4-Day Suppressive Test.

On the basis of biological activity attempts have been made to correlate the structure activity relationship.

(A) In Section-A the compounds with the benzyloxy linkage ^b between two aromatic rings produced good results, compared to the ether or sulfide linkage. The dihydro-s-triazines of 4'-halogen substituted-4-amino diphenyl ethers were observed to be toxic when compared to the 4'-halogen substituted-4-amino diphenyl benzyloxy compounds. Preliminary Acute Toxicity study of one of the compounds (2; Table-1) was done and the LD₅₀ was found to be 1650 mg/kg.

- (B) In Section-B the compound where a phenyl nucleus has been introduced in the 2-position of the triazine ring activity was observed (compound 4, Table-I) at 40 and 80 mg/kg. But replacing the phenyl moiety with substituted phenyl groups gave either inactive or toxic compounds.
- (C) Introducing alicyclics like cyclohexane in the triazine nucleus did not show any improvement in activity either as observed in compounds of Section-C.
- (D) Two out of the four compounds of Section-D showed interesting results which indicates that triazine nucleus attached to heterocyclic moieties may show good biological action if explored further. Preliminary Acute Toxicity studies of a compound of this group (Compound No.3, Table-I) showed on LD₅₀ of 190 mg/kg. The optimum doses in these cases were 20 mg/ and 40 mg/kg at lower doses i.e. 10 mg/kg these compounds revealed poor antimalarial action.
- (E) None of the compounds of Section-E showed any significant biological action.

Mice treated with the active test compounds survived almost twice as long as the infected untreated controls by Rane's test and 40 days by Peters' Suppressive Test. The

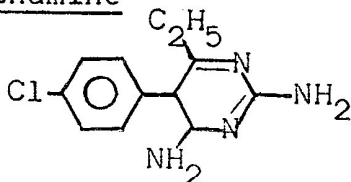
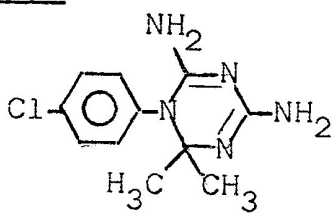
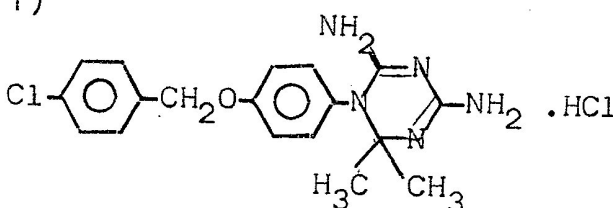
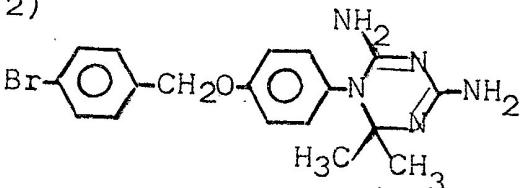
infected controls died on 6.5 ± 1 days (average). The treated mice which survived remained normal and healthy with no clinical symptoms of malarial infection unlike the untreated controls.

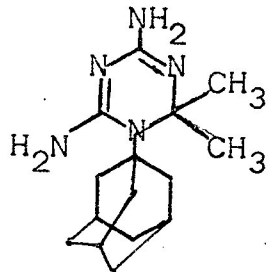
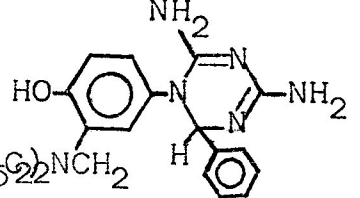
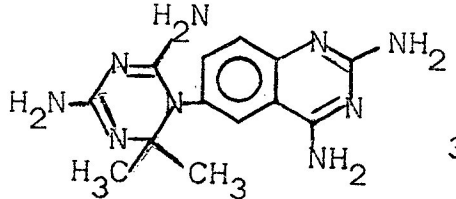
All the blood smears examined during the Peters' Test showed very negligible parasite counts (0.01 %) upto the 16th day post infection. Survival time was monitored for a minimum of 25 days and maximum of 40 days and some compounds showed curative action.

Compound Nos. 1, 2 and 3 in Table-I were also tested by the oral route in the Peters' Suppressive Test and were found to show antimalarial action. In fact a good correlation was observed between subcutaneous and per os results, at certain dose levels.



TABLE - I : Antimalarial Activity against *P. berghei* in mice by Rane's Test.

No.	C O M P O U N D	MST; T; A; C after single S.C. doses in mgs/kg on day* (D + 3)					LD ₅₀
		160	80	40	20	Control	
<u>Pyrimethamine</u>							
		MED= 4.5 mg/kg	C ₁ T ₄	21.6 C ₄ T ₁	13.4 C ₅	12.2 C ₄	6.1
<u>Cycloguanil</u> HCl							
		MED= 20 mg/kg	22.7 C ₄	20.3 C ₄	17.5 C ₃	16.6 C ₃	6.1
1)			18.21 (A)C ₁ T ₁	15.23 (A)C ₂	12.3 (A)	7.2 (A)	5.0
2)			15.01	13.3	9.6	10.3	5.0 1.650 mgs/kg

No.	C O M P O U N D	MST; T; A; C after single S.C. doses in mg/kg on day (D + 3)					
		160	80	40	20	Control	
3)	 HCl	20.1 (A) T ₂	17.9 (A) C ₁	17.0 (A) C ₁	12.9 (A)	6.3	190 mg/kg
4)	 2HCl	7.0 (A)	8.5 (A)	7.7 (A)	4.0	6.1	
5)	 3HCl	12.2 (A) T ₁	10.9 (A)	13.9 (A)	9.3 (A)	6.3	

* MST is the Mean Survival Time (days) of treated mice (MSTT) minus the mean survival time (days) of infected control mice (MSTC).

In the present study the MSTC ranged from 5.0 to 6.5 days.

T = Signifies the number of toxic deaths occurring on days 2-3 after infection which are attributed, to drugs action.

C = Indicates the number of mice surviving at 40 days post infection and termed cured

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(Miss Zarine Khety)
Signature of the Candidate

(Dr. B.G. Khadse)
Signature of the Research Guide.



Statement under University Rule Nos. 0.412 and 0.413

As required by the University Rule No. 0.412 and 0.413, I state that the work presented in this thesis is original in nature and so far to the best of my knowledge has not been submitted for any degree to this or any other University.

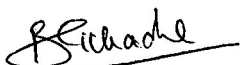
Whenever reference has been made to the work of others, it has been clearly indicated alongwith the source of the information in the "References".



(Miss Zarine Khety)

Signature of the Candidate

Forwarded through the Research Guide.....



(Dr. B.G. Khadse)

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