PART III

Pyrimidines as anti-Malarial agents

CHAPTER – III.1

Literature Survey

Pyrimidines as anti malarial agents

Parasitic infections are still one of the major causes of mortality in the poor or underdeveloped countries. Parasitic protozoan belonging to genus Plasmodium causes malaria, one of the most severe tropical diseases. The four identified species of the parasite responsible for inflicting human malaria are *Plasmodium falciparum, P. vivax, P. ovale, and P. malariae*. Of these, *P. falciparum* and *P. vivax* account for more than 95% of malaria cases in the world. Malaria infections caused by *P. falciparum* are prevalent in the major parts of Africa, sub-Saharan Africa and East Asian countries, whereas *P. vivax* is the causative species primarily of Indian sub-Continent. Interestingly, the disease can be treated in just 48 hr, yet it can cause fatal complications, if the diagnosis and treatment are delayed. The tropical regions provide ideal breeding and living conditions for the anopheles mosquito. Malaria is re-emerging as the biggest infectious killer and is currently the first priority tropical disease of the World Health Organization (WHO).

LIFE CYCLE OF PLASMODIUM FALCIPARUM

Plasmodium species, as members of *Apicomplexa*, share many common morphological features. Each of the developmental stages in the life cycle of malaria parasites exhibits a remarkable conservation and distinct patterns of structural organization[235-236].

the life cycle of malaria parasite (**fig.17**) begins with the invasion of sporozoits by byte of female infected mosquitoes of genus Anopheles. The sporozoits enter in the blood circulation of host and invade in hepatocytes shortly after few time of infection. This process leads to interactions of parasite surface adhesive proteins and host cell surface molecules. Sporozoites infected in the hepatocytes develop into preerythrocytic (exoerythrocytic) schizonts during the next 5–15 days depending on the *Plasmodium* species. *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium cynomolgi* have a dormant stage, named hypnozoite [238-239], that may remain in the liver for weeks to many years before the development of preerythrocytic schizogony. This results in relapses of malaria infection. *Plasmodium falciparum* and *Plasmodium malariae* have no persistent phase.

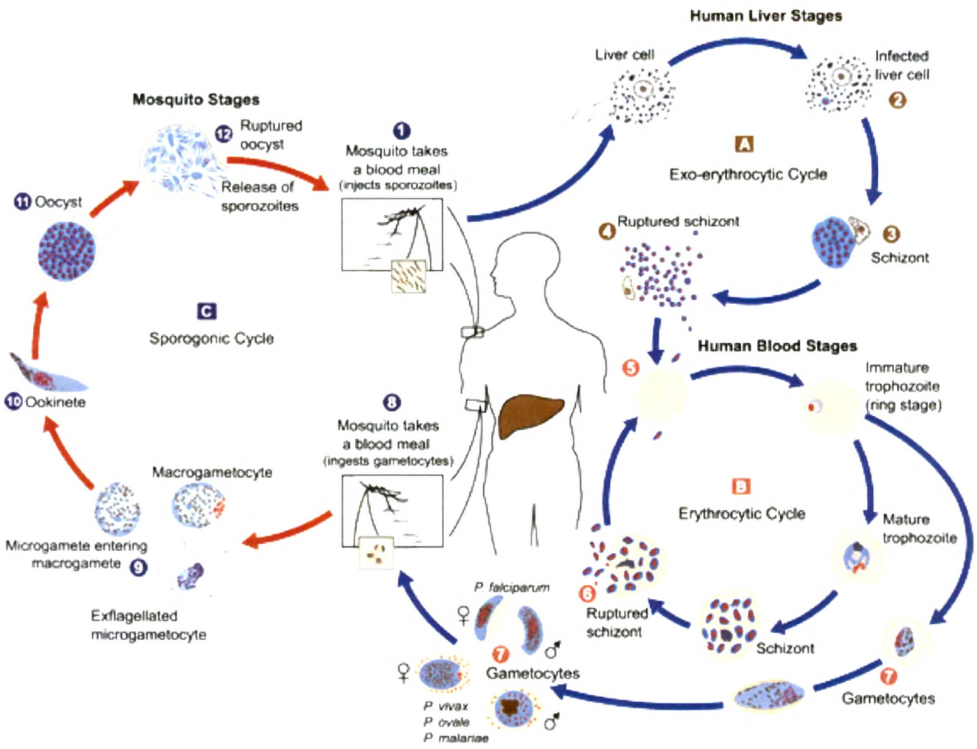


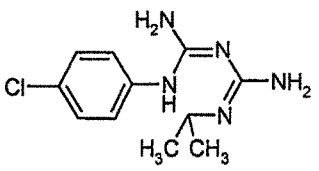
Fig.17.

A preerythrocytic schizont contains 10,000 to 30,000 merozoites, which are released into the blood circulation and invade the red blood cells. The merozoite develops within the erythrocyte through ring, trophozoite and schizont stages (erythrocytic schizogony). The parasite modifies its host cell in several ways to enhance its survival. The erythrocyte containing the segmented schizonts eventually ruptures and releases the newly formed merozoites that invade new erythrocytes. Erythrocyte invasion by merozoites is dependent on the interactions of specific receptors on the erythrocyte membrane with ligands on the surface of the merozoite. The entire invasion process takes about 30 s. Concomitantly, a small portion of the parasites differentiate from newly invaded merozoites into sexual forms, which are macrogametocyte (female) and microgametocyte (male). What triggers this alternative developmental pathway leading to gametocyte formation is unknown. Mature macrogametocytes, taken into the midgut of the *Anopheles* mosquito, escape from the erythrocyte to form macrogametes. Microgametocytes exflagellate, each forming eight haploid motile microgametes after a few minutes in the

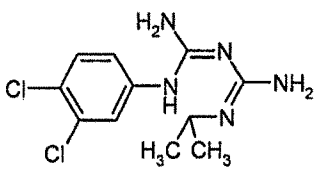
mosquito midgut. The microgamete moves quickly to fertilize a macrogamete and forms a zygote. Within 18–24 h, the non-motile zygotes transform into motile ookinetes. The ookinetes have to cross two barriers: the peritrophic matrix (PM) and midgut epithelium. After traversing the midgut epithelium, the ookinete reaches the extracellular space between the midgut epithelium and the overlaying basal lamina, the salivary gland epithelium. When an infected mosquito bites a susceptible vertebrate host, the *Plasmodium* life cycle begins again.

The global expansion of the disease has been attributed mainly to the failure of vector control programs and spread of resistance to chloroquine and other known antimalarial drugs. As a result, discovering and developing novel antimalarial agents is one of the greatest challenges facing malaria control today. For the development of new molecules effective against resistant strains, a new target for rational drug design is imperative.[240-243]

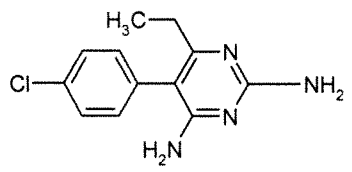
The dihydrofolate reductase (DHFR) domain of P. falciparum is one of the few well-defined targets in malarial chemotherapy.[239] The enzyme catalyzes the nicotinamide adenine dinucleotide phosphate (NADPH) dependent reduction of dihydrofolate to tetrahydrofolate. DHFR has received considerable attention, as it is the target of pyrimethamine **(37)**, trimethoprim **(38)** and other antifolates used for the prophylaxis and treatment of P. falciparum infection. All these antifolates have a higher affinity of binding with P. falciparum than human DHFR.[244] It was accepted that differences of binding affinity account for their good therapeutic index.[245]



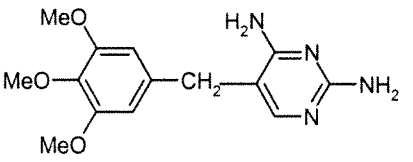
(149)



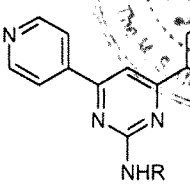
(150)



(37)



(38)



(151)

The first antifolate antimalarial, proguanil **(149)**, was discovered in the 1940s, during the Second World War. Following this discovery, chlorproguanil **(150)** and pyrimethamine **(37)** were identified as potent antimalarial agents. Fifty years later, these three drugs are still the mainstay antifolate drugs used in the treatment and prevention of malaria. For instance, the current most potent prophylactic agent is the combination of proguanil and atovaquone. As the parasite is found to develop resistance to conventional antimalarial drugs, development of drugs attacking crucial targets in the metabolism of the malarial pathogen is the need of the hour.[107]

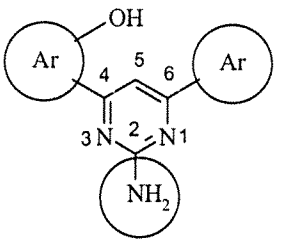


Fig. 18: The common requirement for DHFR inhibitor

Pyrimidine ring is the common feature of the DHFR inhibitors shown above. From the study of various pyrimidine containing DHFR inhibitors the structural requirement of dihydrofolate reductase has been established as given in **Fig. 18.**

As shown in **Fig. 18** for an ideal DHFR inhibitor, the hydrogen donor group at position-2 attaches to buried water molecules of carboxylic group of ASP-54. At 4-position, a hydrogen acceptor group forms a hydrogen bond to the backbone carboxylic oxygen of amino acid residues. The protonated pyrimidine binds ionically to carboxylic group of Asp54 through two hydrogen bonds: one from protonated N-1 and the other from the

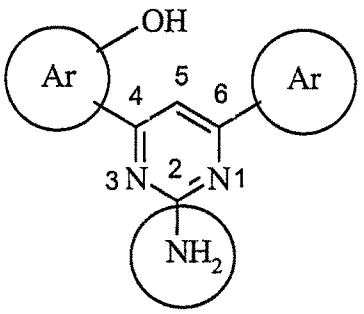
hydrogen donor group at the 2-position. Substitution at the 6-position should be non-polar as it is located in the hydrophobic core of the enzyme active site.[245]

CHAPTER – III.2

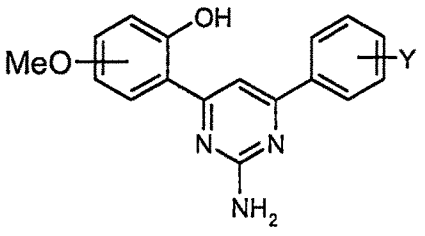
Aims and Objectives

AIMS AND OBJECTIVES

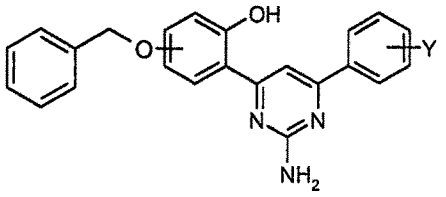
Pyrimidine ring is the common feature of the DHFR inhibitors. From the study of various pyrimidine containing DHFR inhibitors the structural requirement of dihydrofolate reductase has been established as given below:



Keeping the structural requirement for DHFR inhibitor in mind and relating it to our proposed compound we found that they satisfy the requirement for DHFR inhibitors like Pyrimithamine, Trimethoprim etc. as discussed in the previous chapter. We therefore visualized that the designed molecules may have anti malarial activity also.



(27-44)



(45-59)

CHAPTER – III.3

Results and Discussion

RESULTS AND DISCUSSION

The result and discussion work is discussed in two parts:

III.3a. Chemical studies

III.3b. Biological studies

III.3a. Chemical work

The substituted 4,6-diaryl-2-aminopyrimidines were synthesized according to the route presented in **Scheme I.** Condensation of substituted 2-hydroxyacetophenone with various substituted benzoic acids in dry pyridine and $POCl_3$ furnished the esters. The esters were converted into 1,3-diketones by the base catalyzed Baker-Venkatraman transformation reaction. The diketones so obtained, were cyclised to the flavones derivatives in presence of sulphuric acid as the dehydrating agent. Treatment of flavones with a slight excess of guanidine hydrochloride in the alkaline medium afforded 4,6-diaryl-2-aminopyrimidines.

The synthesis carried out has been discussed under following heads:

III.3a.1: Synthesis of 4/5-Benzyloxy-2-hydroxyacetophenone

III.3a.2:Synthesis of 4/5-Benzyloxy-2-acetyl-1-(substituted benzoyloxy)-benzene derivatives

III.3a.3: Synthesis of 1- (4/5-Benzyloxy-2-hydroxyphenyl)- 3-(substituted phenyl)-1,3-propanedione derivatives

III.3a.4: Synthesis of 6/7-Benzyloxy-2-substituted phenyl-4*H*-chromen-4-one derivatives

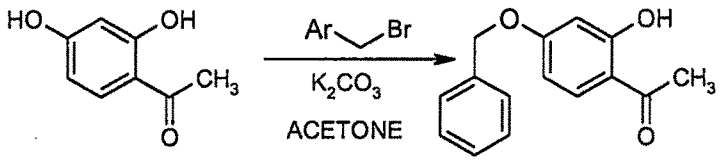
III.3a.5: Synthesis of 4-(4/5-Benzyloxy-2-hydroxyphenyl)-6-(substutited phenyl)-2-aminopyrimidines

III.3a.1: Synthesis of 4/5-Benzyloxy-2-hydroxyacetophenone (A5)

III.3a.1a. Synthesis of 4-Benzyloxy-2-hydroxyacetophenone (A5)

The synthesis of 4-benzyloxy-2-hydroxyactophenone (**A5**) was afforded by selective benzylation of 2,4-dihydroxyacetophenone (**I**) (Scheme II). 2,4-dihydroxyacetophenone was procured commercially. The formation of compound was confirmed by melting point and IR (cm^{-1}).

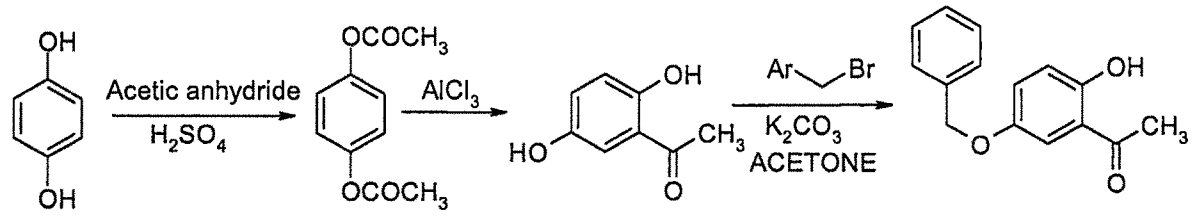
Scheme II



(I) **(A5)**

III.3a.1b. Synthesis of 5-Benzyloxy-2-hydroxyacetophenone (A6)

Scheme III



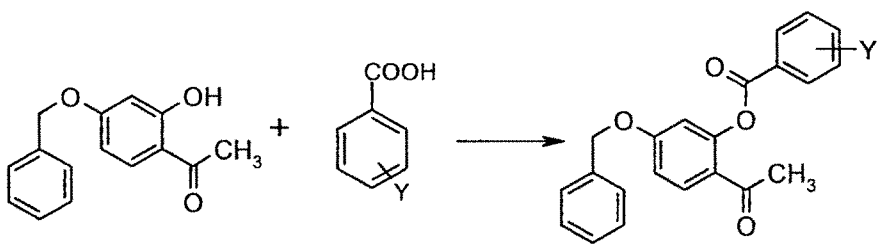
(II) **(III)** **(IV)** **(A6)**

The 5-benzyloxy-2-hydroxyacetophenone (**A6**) was obtained by the selective benzylation of 2,5-dihydroxyacetophenone (**IV**). 2,5-dihydroxyacetophenone was synthesized from fries rearrangement of hydroquinone-1,4-diacetate (**III**) which was synthesized from acetylation of hydroquinone (**II**) in the presence of sulphuric acid. Compound (**A6**) displayed the absorption bands at 3066 (OH), 3000 & 999 cm^{-1} and 1640 cm^{-1} in its IR spectrum.

III.3a.2: Synthesis of 4/5-benzyloxy-2-Acetyl-1-(substituted benzoyloxy)-benzene derivatives

III.3a.2a. Synthesis of 5-benzyloxy-2-Acetyl-1-(substituted benzoyloxy)-benzene derivatives

The benzoate **(C)** esters were obtained by condensation of 4-benzyloxy-2-hydroxyacetophenone **(A5)** and various substituted aryl carboxylic acid derivative in dry pyridine and phosphorus oxychloride . The carbonyl stretching of the esters are given in **Table 28.**



(A5) **(B)** **(C)**

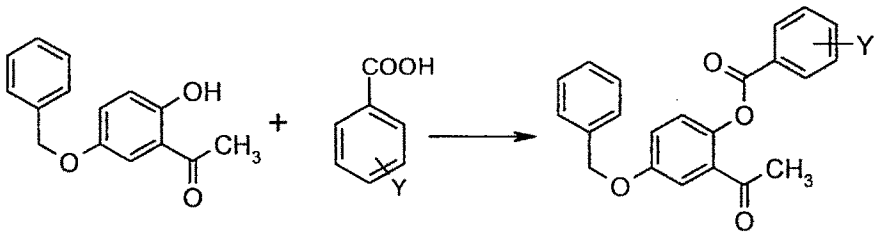
Table 28.

Compound No.	X	Y	M.P. (°C)
C45	5-benzyloxy	H	70-72
C46	5-benzyloxy	4-OMe	68-69
C47	5-benzyloxy	3-OMe	42-44
C48	5-benzyloxy	3,4-di methoxy	67-68
C49	5-benzyloxy	4-F	72-75
C50	5-benzyloxy	4-Br	73-75
C51	5-benzyloxy	2-Cl	87-88
C52	5-benzyloxy	2,4-di chloro	118-117

C53	5-benzyloxy	4-CH_3	63-65
C54	5-benzyloxy	3-CH_3	70-72

III.3a.2b. Synthesis of 4-Benzyloxy-2-acetyl-1-(substituted benzoxy)-benzene derivative

The benzoate **(C)** esters were obtained by condensation of 5-benzyloxy-2-hydroxyacetophenone **(A6)** and various substituted aryl carboxylic acid derivative in dry pyridine and phosphorus oxychloride . The carbonyl stretching of the esters are given in **Table 29.**



(A6) **(B)** **(C55-C59)**

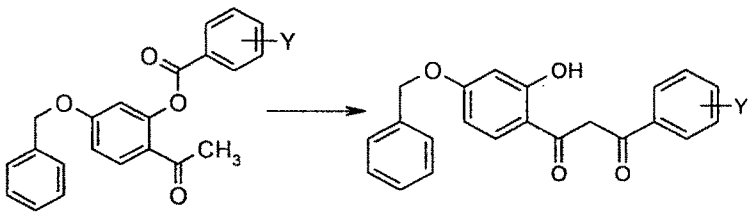
Table 29.

Compound No.	X	Y	M.P. (°C)
C55	4-benzyloxy	4-OMe	90-91
C56	4- benzyloxy	4-CH_3	77-79
C57	4- benzyloxy	4-Cl	60-62
C58	4-benzyloxy	4-F	70-71
C59	4-benzyloxy	4-Br	74-75

III.3a.3: Synthesis of 1- (4/5-Benzyloxy-2-hydroxyphenyl)- 3-(substituted phenyl)-1,3-propanedione derivatives

III.3a.3a. Synthesis of 1-(4-benzyloxy-2-hydroxyphenyl)-3-(substituted phenyl)-1,3-propanedione derivatives (D)

The synthesized esters (**C**) were subjected to Baker-Venkataraman rearrangement to afford yellow colored 1,3-propanediones **(D)**. The IR spectrum of 1,3-propanedione showed two absorption bands of carbonyl at the range of 1615-1625 cm^{-1} and 1685-1695 cm^{-1} as shown in **Table 30**. The yellow crystals of 1,3-propanediones were used for next step.



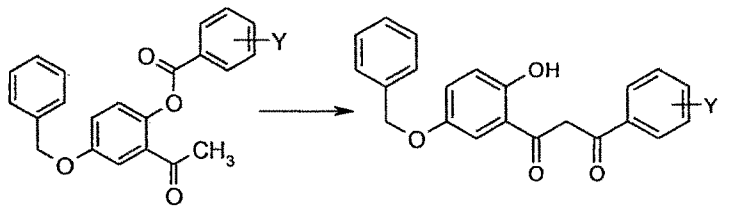
(C) **(D)**

Table 30.

Compound No.	X	Y	M.P. (°C)
D45	4-benzyloxy	H	86-85
D46	4-benzyloxy	4-OMe	145-146
D47	4-benzyloxy	3-OMe	130-131
D48	4-benzyloxy	3,4-di methoxy	88-89
D49	4-benzyloxy	4-F	55-57
D50	4-benzyloxy	4-Br	165-166
D51	4-benzyloxy	2-Cl	72-73
D52	4-benzyloxy	2,4-di chloro	71-73
D53	4-benzyloxy	4-CH3	77-78
D54	4-benzyloxy	3-CH3	83-84

III.3a.3b. Synthesis of 1-(5-benzyloxy-2-hydroxyphenyl)-3-(substituted phenyl)-1,3-propanedione derivative

The synthesized esters **(C)** were subjected to Baker-Venkataraman rearrangement to afford yellow colored 1,3-propanediones. The IR spectrum of 1,3-propanedione showed two absorption bands of carbonyl at the range of 1615-1625 cm^{-1} and 1685-1695 cm^{-1} as shown in **Table 31**. The yellow crystals of 1,3-propanedione **(D)** were used for the next step.



(C) **(D)**

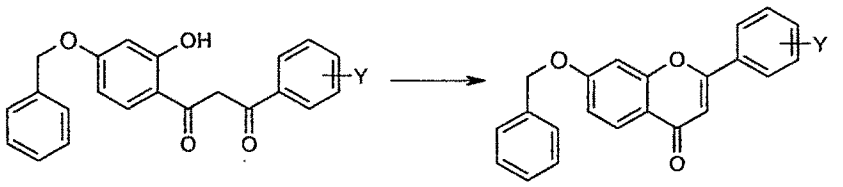
Table 31.

Compound No.	X	Y	M.P. (°C)
D55	5-benzyloxy	4-OMe	151-153
D56	5- benzyloxy	4-CH3	77-78
D57	5- benzyloxy	4-Cl	83-84
D58	5-benzyloxy	4-F	62-63
D59	5-benzyloxy	4-Br	143-145

III.3a.4: Synthesis of 6/7-Benzyloxy-2-substituted phenyl-4*H*-chromen-4-one derivatives

III.3a.4a. Synthesis of 7-Benzyloxy-2-substituted phenyl-4*H*-chromen-4-one derivatives

The chromen derivatives were synthesized from 1,3-propanedione derivative **(D)** by acid catalyzed cyclisation using glacial acetic acid as solvent. It was produce light brown color crystals. The cyclised chromen derivatives **(E)** were confirmed by IR which gives specific stretching of conjugated C=C and C=O at 1675 Cm^{-1} as shown in **Table 32.**



(D) **(E)**

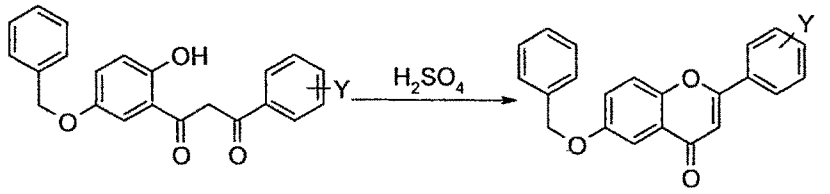
Table 32.

Compound No.	X	Y	M.P. (°C)
E45	7-benzyloxy	H	186-187
E46	7-benzyloxy	4-OMe	154-155
E47	7-benzyloxy	3-OMe	141-142
E48	7-benzyloxy	3,4-di methoxy	191-192
E49	7-benzyloxy	4-F	130-132
E50	7-benzyloxy	4-Br	185-186
E51	7-benzyloxy	2-Cl	102-103
E52	7-benzyloxy	2,4-di chloro	220-221
E53	7-benzyloxy	4-CH3	86-87
E54	7-benzyloxy	3-CH3	107-108

III.3a.4b Synthesis of 6-Benzyloxy-2-substituted phenyl-4*H*-chromen-4-one derivative

The chromen derivatives were synthesized from 1,3-propanedione derivative (**D**) by the acid catalyzed cyclisation using glacial acetic acid as solvent. It produced light brown

color crystals. The cyclised chromen derivatives **(E)** were confirmed by IR which gives specific stretching of conjugated C=C and C=O at 1675 Cm^{-1} as shown in **Table 33.**



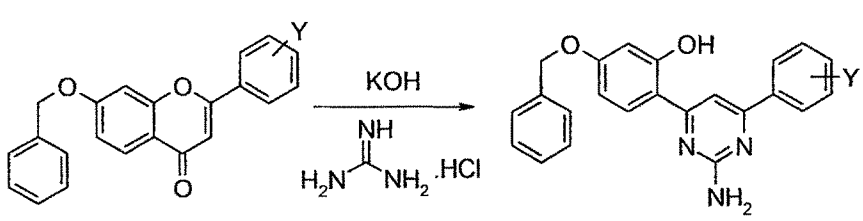
(D) **(E)**

Table 33.

Compound No.	X	Y	M.P. (°C)
E55	6-benzyloxy	4-OMe	NT
E56	6-benzyloxy	4-CH3	180-181
E57	6-benzyloxy	4-Cl	NT
E58	6-benzyloxy	4-F	NT
E59	6-benzyloxy	4-Br	207-209

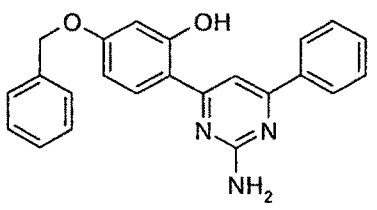
III.3a.5: Synthesis of 4-(4/5-substituted-2-hydroxyphenyl)-6-(substutited phenyl)-2-aminopyrimidines

III.3a.5a. Synthesis of 4-(4-benzyloxy-2-hydroxyphenyl)-6-(substituted phenyl)-2-Aminopyrimidine derivatives

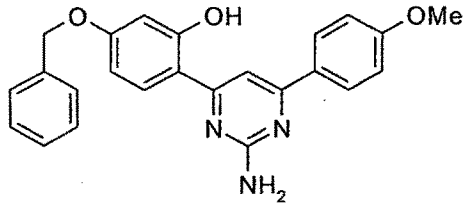


4-(4-Benzyloxy-2-hydroxyphenyl)-6-phenyl-2-aminopyrimidine **(45)** gives stretching of amino group at 3311 and 3191 cm^{-1} and broad peak of hydroxyl stretching at 3036 cm^{-1}.

The C=N ring stretching observed at 1641 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 5.02 (S, 2H, -O***CH***$_2$Ar) for benzylic protons. The amino protons at δ 6.5 (2H, -***NH***$_2$) and aromatic protons displayed at δ 7.25-7.9 (m 13H, Ar***H***), 7.36 (s, 1H, Pyri***H***). The aromatic hydroxyl proton observed at δ 14.12 (1H, -***OH***). The mass spectrum gives M+1 peak at 370.2 (m/z).



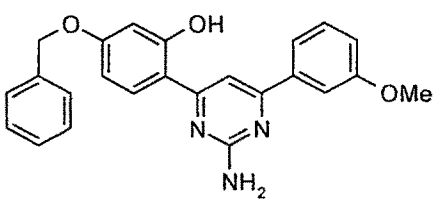
(45)



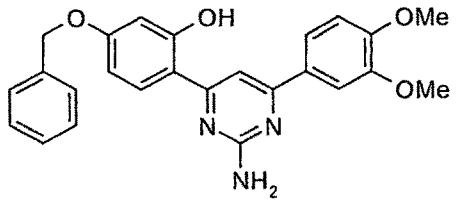
(46)

4-(4-Benzyloxy-2-hydroxyphenyl)-6-(4-methoxyphenyl)-2-aminopyrimidine **(46)** gives stretching of amino group at 3447 and 3363 cm^{-1} and broad peak of hydroxyl stretching at 3100 cm^{-1}. The C=N ring stretching observed at 1631 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 3.4 (s, 3H, -O***CH***$_3$) for methoxy protons and at δ 5.12 (S, 2H, -O***CH***$_2$Ar) for benzylic $-CH_2$ protons. The amino protons at δ 6.5 (2H, -***NH***$_2$) and aromatic protons displayed at δ 6.6-8.1 (m, 12H, Ar***H***), 7.35 (s, 1H, Pyrim***H***). The aromatic hydroxyl proton observed at δ 10.03 (1H, -***OH***). The mass spectrum gives M+1 peak at 400.0 (m/z).

4-(4-Benzyloxy-2-hydroxyphenyl)-6-(3-methoxyphenyl)-2-aminopyrimidine **(47)** gives stretching of amino group at 3400 and 3338 cm^{-1} and broad peak of hydroxyl stretching at 3200 cm^{-1}. The C=N ring stretching observed at 1617 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 3.9 (s, 3H, -O***CH***$_3$) for methoxy protons and at δ 5.12 (S, 2H, -O***CH***$_2$Ar) for benzylic $-CH_2$ protons. The amino protons at δ 5.3 (2H, -***NH***$_2$) and aromatic protons displayed at δ 6.5-7.7 (m 12H, Ar***H***), 7.39 (s, 1H, Ar***H***). The aromatic hydroxyl proton observed at δ 10.53 (1H, -***OH***). The mass spectrum gives M+1 peak at 400.0 (m/z).



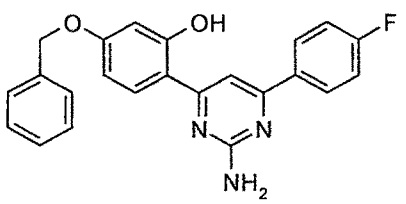
(47)



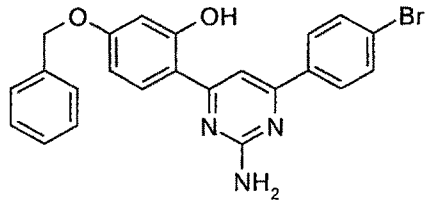
(48)

4-(4-Benzyloxy-2-hydroxyphenyl)-6-(3,4-dimethoxyphenyl)-2-aminopyrimidine **(48)** gives stretching of amino group at 3420 and 3322 cm^{-1} and broad peak of hydroxyl stretching at 3210 cm^{-1}. The C=N ring stretching observed at 1647 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 3.9 (s, 3H, -O***CH₃***), 4.0 (s, 3H, -O***CH₃***) for methoxy protons and at δ 5.12 (S, 2H, -O***CH₂***Ar) for benzylic $-CH_2$ protons. The amino protons at δ 5.3 (2H, -***NH₂***) and aromatic protons displayed at δ 6.9-7.9 (m 11H, Ar***H***), 7.36 (s, 1H, Pyri***H***). The aromatic hydroxyl proton observed at δ 11.03 (1H, -***OH***). The mass spectrum gives M+1 peak at 430.0 (m/z).

4-(4-Benzyloxy-2-hydroxyphenyl)-6-(4-fluorophenyl)-2-aminopyrimidine **(49)** gives stretching of amino group at 3493 and 3328 cm^{-1} and broad peak of hydroxyl stretching at 3200 cm^{-1}. The C=N ring stretching observed at 1647 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 5.12 (S, 2H, -O***CH₂***Ar) for benzylic $-CH_2$ protons. The amino protons at δ 6.5 (2H, -***NH₂***) and aromatic protons displayed at δ 6.6- 8.1 (m 12H, Ar***H***), 7.35 (s,1H, Pyrim***H***). The aromatic hydroxyl proton observed at δ 12.03 (1H, -***OH***). The mass spectrum gives M+1 peak at 388.2 (m/z).



(49)

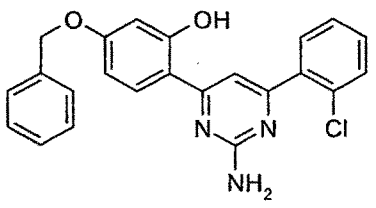


(50)

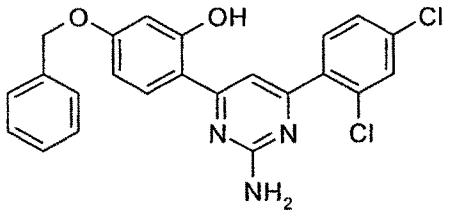
4-(4-Benzyloxy-2-hydroxyphenyl)-6-(4-bromophenyl)-2-aminopyrimidine **(50)** gives stretching of amino group at 3493 and 3347 cm^{-1} and broad peak of hydroxyl stretching at 3223 cm^{-1}. The C=N ring stretching observed at 1637 cm^{-1} in IR spectrum. The PMR

spectrum displayed singlet at δ 5.12 (S, 2H, -O***CH***$_2$Ar) for benzylic –CH_2 protons. The amino protons at δ 6.02 (2H, -***NH***$_2$) and aromatic protons displayed at δ 7.31-7.34 (m,5H, Ar***H***), 6.5-7.9 (m, 7H, Ar***H***), 7.35 (s, 1H, Pyrim***H***.). The aromatic hydroxyl proton observed at δ 14.07 (1H, -***OH***). The mass spectrum gives M+1 peak at 447.9 (m/z).

4-(4-Benzyloxy-2-hydroxyphenyl)-6-(2-chlorophenyl)-2-aminopyrimidine **(51)** gives stretching of amino group at 3315 and 3200 cm^{-1} and broad peak of hydroxyl stretching at 3100 cm^{-1}. The C=N ring stretching observed at 1651 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 5.10 (S, 2H, -O***CH***$_2$Ar) for benzylic –CH_2 protons. The amino protons at δ 5.15 (2H, -***NH***$_2$) and aromatic protons displayed at δ 7.34-7.39 (m, 5H, Ar***H***), 6.55-7.71 (m, 7H, Ar***H***), 7.35(s, 1H, Pyrim***H***). The aromatic hydroxyl proton observed at δ 13.88 (1H, -***OH***). The mass spectrum gives M+1 peak at 404.1 (m/z).



(51)

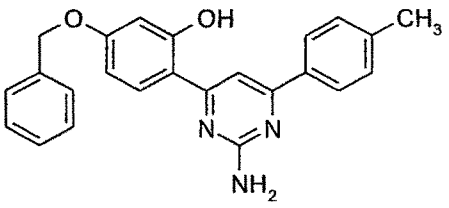


(52)

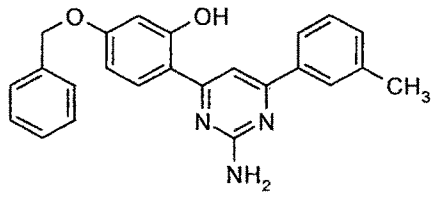
4-(4-Benzyloxy-2-hydroxyphenyl)-6-(2,4-dichlorophenyl)-2-aminopyrimidine **(52)** gives stretching of amino group at 3350 and 3165 cm^{-1} and broad peak of hydroxyl stretching at 3090 cm^{-1}. The C=N ring stretching observed at 1687 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 5.11 (S, 2H, -O***CH***$_2$Ar) for benzylic –CH_2 protons. The amino protons at δ 6.0 (2H, -***NH***$_2$) gives singlet. The aromatic ring protons displayed at δ 7.30-7.35 (m,5H, Ar***H***) for benzylic protons, 6.5-7.9 (m, 6H, Ar***H***) for aromatic protons and at δ 7.35 (s, 1H, Pyrim***H***.) pyrimidine ring proton. The mass spectrum gives M+1 peak at 438 (m/z).

4-(4-Benzyloxy-2-hydroxyphenyl)-6-(4-methylphenyl)-2-aminopyrimidine **(53)** gives stretching of amino group at 3428 and 3323 cm^{-1} and broad peak of hydroxyl stretching at 3204 cm^{-1}. The C=N ring stretching observed at 1637 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 2.4 (s, 3H, -***CH***$_3$) for methyl protons and at δ 5.10 (s, 2H,

-O***CH***$_2$Ar) benzylic –CH_2 protons. The amino protons at δ 6.44 (2H, -***NH***$_2$) gives singlet. The aromatic ring protons displayed at δ 7.2-7.4 (m 5H, Ar***H***) for benzylic protons, 7.4-7.8 (m, 7H, Ar***H***) for aromatic protons and at δ 7.35 (s, 1H, Pyrim***H***) pyrimidine ring proton. The mass spectrum gives M+1 peak at 384 (m/z).



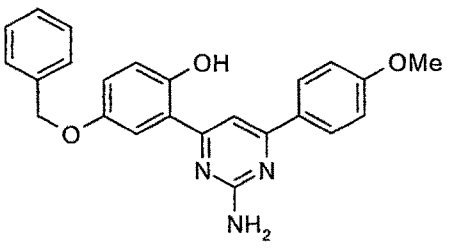
(53)



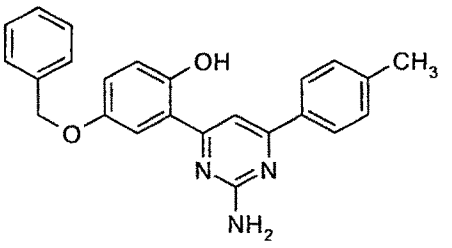
(54)

4-(4-Benzyloxy-2-hydroxyphenyl)-6-(3-methylphenyl)-2-aminopyrimidine **(54)** gives stretching of amino group at 3450 and 3330 cm^{-1} and broad peak of hydroxyl stretching at 3200 cm^{-1}. The C=N ring stretching observed at 1607 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 2.1 (s, 3H, -***CH***$_3$) for methyl protons and at δ 5.11 (s, 2H, -O***CH***$_2$Ar) benzylic –CH_2 protons. The amino protons at δ 6.2 (2H, -***NH***$_2$) gives singlet. The aromatic ring protons displayed at δ 7.2-7.3 (m 5H, Ar***H***) for benzylic protons, 6.9-7.8 (m, 7H, Ar***H***) for aromatic protons and at δ 7.36 (s, 1H, Pyrim***H***.) pyrimidine ring proton. The aromatic hydroxyl proton observed at δ 14.04 (1H, -***OH***). The mass spectrum gives M+1 peak at 384.1 (m/z).

4-(5-Benzyloxy-2-hydroxyphenyl)-6-(4-methoxyphenyl)-2-aminopyrimidine **(55)** gives stretching of amino group at 3447 and 3363 cm^{-1} and broad peak of hydroxyl stretching at 3100 cm^{-1}. The C=N ring stretching observed at 1631 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 3.8 (s, 3H, -O***CH***$_3$) for methoxy protons and at δ 5.0 (s, 2H, -O***CH***$_2$Ar) benzylic –CH_2 protons. The amino protons at δ 5.11(2H, –***NH***$_2$) gives singlet. The aromatic ring protons displayed at δ 6.6-8.1 (m12H, Ar***H***) and the pyrimidne ring proton displayed at δ 7.35 (s, 1H, Pyrim***H***). The aromatic hydroxyl proton observed at δ 13.24 (1H, -***OH***). The mass spectrum gives M+1 peak at 400.1 (m/z).



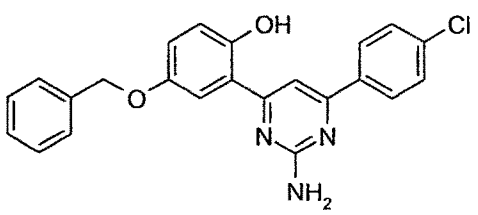
(55)



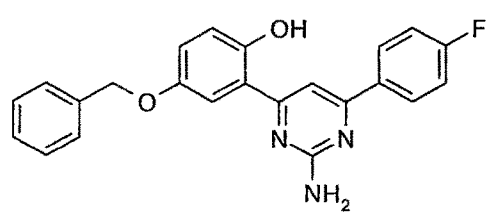
(56)

4-(5-Benzyloxy-2-hydroxyphenyl)-6-(4-methylphenyl)-2-aminopyrimidine **(56)** gives stretching of amino group at 3435 and 3336 cm^{-1} and broad peak of hydroxyl stretching at 3224 cm^{-1}. The C=N ring stretching observed at 1637 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 2.4 (s, 3H, -***CH*$_3$**) for methyl protons and at δ 5.10 (s, 2H, -O***CH*$_2$Ar) benzylic –CH_2 protons. The amino protons at δ 6.44 (2H, –***NH*$_2$**) gives singlet. The aromatic ring protons displayed at δ 6.9-7.4 (m 8H, Ar***H***), 7.4-7.8 (m, 4H, Ar***H***) and the pyrimidne ring proton displayed at δ 7.35 (s, 1H, Pyrim***H***). The aromatic hydroxyl proton observed at δ 14.01 (1H, -***OH***). The mass spectrum gives M+1 peak at 384.1 (m/z).

4-(5-Benzyloxy-2-hydroxyphenyl)-6-(4-chlorophenyl)-2-aminopyrimidine **(57)** gives stretching of amino group at 3315 and 3200 cm^{-1} and broad peak of hydroxyl stretching at 3154 cm^{-1}. The C=N ring stretching observed at 1651 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 5.01 (s, 2H, -***CH*$_2$**) benzylic –CH_2 protons. The amino protons at δ 5.16 (2H, –***NH*$_2$**) gives singlet. The aromatic ring protons displayed at δ 7.34-7.39 (m, 5H, Ar***H***), 6.9-8.0 (m,7H, Ar***H***) and the pyrimidne ring proton displayed at δ 7.35 (s, 1H, Pyrim***H***). The aromatic hydroxyl proton observed at δ 13.87 (1H, -***OH***). The mass spectrum gives M+1 peak at 404.1 (m/z).

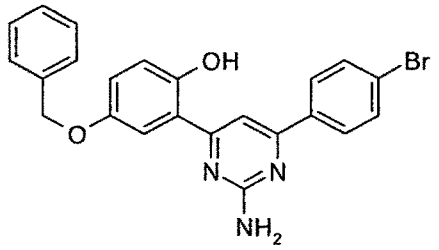


(57)



(58)

4-(5-Benzyloxy-2-hydroxyphenyl)-6-(4-fluorophenyl)-2-aminopyrimidine **(58)** gives stretching of amino group at 3488 and 3336 cm^{-1} and broad peak of hydroxyl stretching at 3218 cm^{-1}. The C=N ring stretching observed at 1643 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 5.1 (s, 2H, -***CH₂***) benzylic $-CH_2$ protons. The amino protons at δ 6.02 (s, 2H, -***NH₂***) gives singlet. The aromatic ring protons displayed at δ 7.31-7.34 (m,5H, Ar***H***), 6.5-7.9 (m, 7H, Ar***H***) and the pyrimidne ring proton displayed at δ 7.35 (s, 1H, Pyrim***H***). The aromatic hydroxyl proton observed at δ 14.17 (1H, -***OH***). The mass spectrum gives M+1 peak at 388.1 (m/z).



(59)

4-(5-Benzyloxy-2-hydroxyphenyl)-6-(4-bromophenyl)-2-aminopyrimidine **(59)** gives stretching of amino group at 3493 and 3318 cm^{-1} and broad peak of hydroxyl stretching at 3200 cm^{-1}. The C=N ring stretching observed at 1647 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 5.0 (s, 2H, -O***CH₂***Ar) benzylic $-CH_2$ protons. The amino protons at δ 5.16 (s, 2H, -***NH₂***) gives singlet. The aromatic ring protons displayed at δ 6.9-8.1 (m12H, Ar***H***) and the pyrimidne ring proton displayed at δ 7.35 (s, 1H, Pyrim***H***). The aromatic hydroxyl proton observed at δ 13.16 (1H, -***OH***). The mass spectrum gives M+1 peak at 448.1 (m/z).

III.3b. Biological studies

The preliminary anti malarial screening of compounds was carried out at Department of Biochemistry, Faculty of Science, The M.S.University of Baroda, Vadodara. The 4-(4/5-methoxy/benzyloxy substituted-2-hydroxyphenyl)-6-phenyl-2-aminopyrimidines were screened for anti malarial activity. Anti malarial activity checked on *Plasmodium falciparum* native strain and drug sensitive strains. Chloroquine sensitive *P. falciparum*, MRC 20 was obtained from the National Institute of Malaria Research (NIMR), New Delhi. All compounds were studied in cultures and the mean was observed for purposes of inferences. The inhibition of parasite growth in the drug-treated groups was calculated as follows: Parasitaemia in the control (non-treated) group minus parasitaemia in the drug treated group, divided by parasitaemia in the control (non-treated) group, expressed as percentages. All values are expressed as percentage growth inhibition. Dose response curves of the fractions were obtained by plotting percentage inhibition against log concentration. The IC_{50} value of Chloroquine and Quinine is around 10 and 29 µg/ ml respectively. The IC $_{50}$ value of test compound was shown in **Table 34**.

Sr. No.	Compound code	IC_{50}value (µg/ml)	Lysis (µg/ml)
1	28	21.35	>25
2	31	64.56(>25)	>25
3	32	**1.78**	>12.5
4	34	15.25	>50
5	35	**8.71**	>25
6	39	>25	>50
7	40	29.35	>50
8	41	**9.53**	>25
9	43	25.52	>50
10	46	**5.01**	>25
11	47	**7.61**	>25
12	48	**1.61**	>12.5
13	49	16.67	>50
14	51	18.87	>25
15	53	14.87	>25
16	54	35.56	>25

Table 34. IC $_{50}$ value of test compound.

* The parasite strain used for the assay is chloroquine sensitive strain of Plasmodium falciparum MRC 20

*The results are represented as a single reading, average was calculated by counting three different fields of culture slide

*The assay is stage specific inhibition assay to check development of schizonts to trophozoites

Compounds **32** and **48** were found most active compared to standard drug with IC_{50} value **1.78** and **1.61** µg/ml respectively. Also compounds **35, 41, 46** and **47** shows IC_{50} value **<10.**

CHAPTER – III.4

Experimental Work

EXPERIMENTAL WORK

The melting points were taken in open capillaries, using the heating block type melting point apparatus and are uncorrected. Thin-Layer Chromatography (TLC) was carried out on precoated silica gel Merk plates. Compounds were visualized by illuminating with UV light (254 nm). Column chromatography was carried out using silica gel (100-200 mesh). The IR spectra were recorded on Shimadzu-8300 FT-IR instrument using KBR pellets. The PMR spectra were recorded in $CDCl_3$ and DMSO on a Brüker spectrometer (300 or 400MHz), using tetramithylsilane as an internal standard. Chemical shift data were reported in parts per million (δ in ppm) where s, d, t, m and bs designate singlet, doublet, triplet multiplet and broad singlet, respectively. Mass spectra were recorded on APISciEX mass spectrometer equipped with an electrospray ionization (ESI) interface. Most of the solvents and chemicals were obtained from S.D.Fine Chemicals, Spectrochem and Loba Chemie and purified using standard purification methods.

III.4a: Chemical work

III.4b: Biological work

III.4a: CHEMICAL WORK

The synthesis carried out has been discussed under following heads:

III.4a.1: Synthesis of 4/5-Benzyloxy-2-hydroxyacetophenone

III.4a.2: Synthesis of 4/5-Benzyloxy-2-Acetyl-1-(substituted benzoyloxy)-benzene derivatives

III.4a.3: Synthesis of 1- (4/5-Benzyloxy-2-hydroxyphenyl)- 3-(substituted phenyl)-1,3-propanedione derivatives

III.4a.4: Synthesis of 6/7-Benzyloxy-2-substituted phenyl-4*H*-chromen-4-one derivatives

III.4a.5: Synthesis of 4-(4/5-Benzyloxy-2-hydroxyphenyl)-6-(substutited phenyl)-2-aminopyrimidines

III.4a.1: Synthesis of 4/5-Benzyloxy-2-hydroxyacetophenone

III.4a.1a. Synthesis of 4-Benzyloxy-2-hydroxyacetophenone (A5)

Benzylbromide (6 ml) and anhydrous potassium carbonate (13.6 g) was added to a solution of 2,4-dihydroxyacetophenone (10 g) **(I)** in acetone (30 ml). The reaction mixture was stirred and refluxed for 4 hr at 60 °C. The reaction mixture was filtered to remove K_2CO_3. Acetone was recovered and the brown solid obtained was recrystalised from methanol to yield (**A5**) (7.8 g, 78 %).

Anal:

M.P. : 88-90 °C

R_f : 0.68 (chloroform)

IR (KBr) : 3100, 1690, 1245 and1055 cm^{-1}

III.4a.1b. Synthesis of 5-Benzyloxy-2-hydroxyacetophenone (A6)

Benzylbromide (6 ml) and anhydrous potassium carbonate (13.6 g) was added to a solution of 2, 5-dihydroxyacetophenone (10 g) in acetone (30 ml). The reaction mixture was stirred and refluxed for 4 hr at 60 °C. The reaction mixture was filtered to remove K_2CO_3. Acetone was recovered and the brown solid obtained was recrystalised from methanol to yield **(A6)** (7 g, 70 %).

Anal:

M.P. : 66-68 °C

R_f : 0.68 (chloroform)

IR (KBr) : 3100, 1690, 1245 and1055 cm^{-1}

III.4a.2. Synthesis of 4/5-Benzyloxy-2-acetyl-(substituted benzoyloxy)-benzene derivatives

5.1b.3. Synthesis of 5-Benzyloxy-2-acetyl-1-(substitutedbenzoyloxy)-benzene derivatives

Table 35. Details of 5-Benzyloxy-2-acetyl-1-(substitutedbenzoyloxy)-benzene derivatives

No.	A5	B (substituted benzoic acid)	$POCl_3$	Method	R_f ($CHCl_3$)	Yield (%)	IR (cm^{-1})	M.P. (°C)
C45	3.0 g, 12.3 mmol	1.80 g, 14.85 mmol	2.28 g, 14.85 mmol	I	0.72	2.7 g 90 %	1735, 1690, 1499, 856 and 780	71-72
C46	3.0 g, 12.38 mmol	2.25 g, 14.85 mmol	2.28 g, 14.85 mmol	I	0.78	2.9 g, 93 %	1750, 1685, 1485, 879 and 749	68-69
C47	3.0 g, 12.38 mmol	2.25 g, 14.85 mmol	2.28 g, 14.85 mmol	I	0.59	2.3 g, 78 %	1748, 1685, 1490, 879 and 760	42-44
C48	3.0 g, 12.38 mmol	2.7 g, 14.85 mmol	2.28 g, 14.85 mmol	I	0.55	2.3 g, 76 %	1740, 1675, 1500, 860 and 740	67-68
C49	3.0 g, 12.38 mmol	2.32 g, 14.85 mmol	2.28 g, 14.85 mmol	I	0.69	2.8 g, 93 %	1735, 1685, 1490, 870 and 750	72-75
C50	3.0 g, 12.38 mmol	2.98 g, 14.85 mmol	2.28 g, 14.85 mmol	I	0.82	2.6 g, 86 %	1738, 1690, 1480, 880 and 745	73-75
C51	3.0 g, 12.38 mmol	2.32 g, 14.85 mmol	2.28 g, 14.85 mmol	I	0.48	2.6 g, 80 %	1735, 1698, 1490, 856 and 780	87-88

C52	3.0 g, 12.38 mmol	2.66 g, 14.85 mmol	2.28 g, 14.85 mmol	I	0.73	2.7 g, 90 %	1730, 1695, 1485, 880 and 775	118-117
C53	3.0 g, 12.38 mmol	2.25 g, 14.85 mmol	2.28 g, 14.85 mmol	I	0.75	2.7 g, 93 %	1742, 1678, 1498, 868 and 743	63-65
C54	3.0 g, 12.38 mmol	2.25 g, 14.85 mmol	2.28 g, 14.85 mmol	I	0.58	2.3 g, 77 %	1740, 1680, 1491, 860 and 743	70-72

III.3a.2b. Synthesis of 4-Benzyloxy-2-acetyl-1-(substituted benzoyloxy)-benzene derivatives

Table 36. Details of 4-Benzyloxy-2-acetyl-1-(substituted benzoyloxy)-benzene derivatives

No.	A6	B (substituted benzoic acid)	$POCl_3$	Method	R_f ($CHCl_3$)	Yield (%)	IR (cm^{-1})	M.P. (°C)
C55	3.0 g, 12.38 mmol	2.25 g, 14.85 mmol	2.28 g, 14.85 mmol	I	0.78	3.0 g, 96 %	1741, 1692, 1485, 890 and 760	90-91
C56	3.0 g, 12.38 mmol	2.21 g, 14.85 mmol	2.28 g, 14.85 mmol	I	0.73	2.4 g, 80 %	1736, 1692, 1486, 890 and 769	80-81
C57	3.0 g, 12.38 mmol	2.32 g, 14.85 mmol	2.28 g, 14.85 mmol	I	0.69	2.3 g, 77 %	1735, 1685, 1490, 860 and 745	78-79
C58	3.0 g, 12.38 mmol	2.32 g, 14.85 mmol	2.28 g, 14.85 mmol	i	0.73	2.8 g, 80 %	1740, 1690, 1485, 850 and 750	90-92

C59	3.0 g, 12.38 mmol	2.98 g, 14.85 mmol	2.28 g, 14.85 mmol	I	0.74	2.6 g, 86 %	1738, 1698, 1488, 858 and 750	74-75

III.4a.3: Synthesis of 1- (4/5-Benzyloxy-2-hydroxyphenyl)- 3-(substituted phenyl)-1,3-propanedione derivatives

III.4a.3a. Synthesis of 1-(4-Benzyloxy-2-hydroxyphenyl)-3-(substiutedphenyl)-1,3-propanedione derivatives (D)

Table 37. Details of propanedione derivatives.

No.	C	KOH	Method	R_f ($CHCl_3$)	Yield (%)	IR (cm^{-1})	M.P. (°C)
D45	2.5 g, 7.2 mmol	1 g, 1.7 mmol	I	0.80	2.1 g, 84%	1618, 1592, 1480, 1249, 1079 and 870	86-85
D46	2.8 g, 7.4 mmol	1 g, 1.7 mmol	I	0.62	2.6 g, 92%	1615, 1590, 1480, 1245, 1060 and 878	145-146
D47	2.2 g, 8.6 mmol	1 g, 1.7 mmol	I	0.73	1.8 g, 81.8%	1608, 1585, 1480, 1255, 1060 and 800	130-131
D48	2.2 g, 5.412 mmol	1 g, 1.7 mmol	I	0.72	1.8 g, 80 %	1620, 1599, 1485, 1240, 1080 and 810	88-89
D49	2.5 g, 6.82 mmol	1 g, 1.7 mmol	I	0.61	2.3 g, 92%	1620, 1610, 1490, 1250, 1080 and 840	55-57
D50	2.5 g, 5.64	1 g, 1.7 mmol	I	0.64	1.9 g, 79%	1620, 1590, 1480, 1260,	165-166

	mmol					1059 and 850	
D51	2.4 g, 6.30 mmol	1 g, 1.7 mmol	I	0.59	2.1 g, 87.5%	1620, 1599, 1485, 1240, 1080 and 810	72-73
D52	2.5 g, 6.02 mmol	1 g, 1.7 mmol	I	0.54	2.1 g, 84 %	1620, 1599, 1485, 1240, 1080 and 810	70-71
D53	2.5 g, 6.9 mmol	1 g, 1.7 mmol	I	0.72	2.2 g, 88%	1618, 1590, 1490, 1241, 1088 and 800	77-78
D54	2.1 g, 5.8 mmol	1 g, 1.7 mmol	I	0.69	1.8 g, 85.7 %	1620, 1610, 1490, 1250, 1080 and 840	83-84

III.4a.3b. Synthesis of 1-(5-benzyloxy-2-hydroxyphenyl)-3-(substiutedphenyl)-1,3-propanedione derivatives (D)

Table 38. Details of propanedione derivatives.

No.	C	KOH	Method	R_f ($CHCl_3$)	Yield (%)	IR (cm^{-1})	M.P. (°C)
D55	2.8 g, 7.4 mmol	1 g, 1.7 mmol	I	0.52	2.6 g, 92%	1625, 1598, 1488, 1249, 1080 and 850	151-153
D56	2.5 g, 6.9 mmol	1 g, 1.7 mmol	I	0.68	2.2 g, 88%	1619, 1590, 1488, 1247, 1080 and 845	77-78
D57	2.1 g, 5.8 mmol	1 g, 1.7 mmol	I	0.72	1.8 g, 85.7 %	1622, 1592, 1485, 1241, 1088 and 800	83-84
D58	2.5 g,	1 g, 1.7	I	0.71	2.3 g,	1615, 1580,	62-63

	6.82 mmol	mmol			92%	1488, 1249, 1080 and 850	
D59	2.8 g, 7.4 mmol	1 g, 1.7 mmol	I	0.60	2.6 g, 92%	1624, 1590, 1489, 1255, 1080 and 850	143-145

III.4a.4: Synthesis of 6/7-Benzyloxy-2-substituted phenyl-4*H*-chromen-4-one derivatives

III.4a.4a. Synthesis of 7-Benzyloxy-2-substituted phenyl-4*H*-chromen-4-one derivatives

Table 39. Details of 7-Benzyloxy-2-substituted phenyl-4*H*-chromen-4-one derivatives

No.	D	Method	R_f ($CHCl_3$)	Yield (%)	IR (cm^{-1})	M.P. (°C)
E45	2.0 g, 5.77 mmol	I	0.50	1.2 g, 60%	1690, 1635, 1492, 1230, 1065 and 850	186-187
E46	2.5 g, 6.64 mmol	I	0.45	1.2 g, 48%	1680, 1627, 1498, 1230, 1065 and 850	154-155
E47	1.6 g, 4.25 mmol	I	0.47	1.2 g, 75%	1699, 1620, 1485, 1235, 1080 and 850	141-142
E48	1.5 g, 3.6 mmol	I	0.40	0.95 g, 63%	1698, 1640, 1470, 1235, 1079 and 850	191-192
E49	2.2 g, 6.0 mmol	I	0.55	1.3g, 59 %	1688, 1630, 1489, 1225, 1080 and 850	130-132

E50	1.7 g, 3.9 mmol	I	0.48	1.2 g, 70%	1695, 1628, 1480, 1230, 1089 and 850	185-186
E51	1.8 g, 4.72 mmol	I	0.58	0.95 g, 52.7%	1690, 1615, 1475, 1225, 1090 and 850	102-103
E52	1.8 g, 4.33 mmol	I	0.56	1.2 g, 66.6%	1695, 1625, 1470, 1220, 1075 and 850	220-221
E53	2.04 g, 5.54 mmol	I	0.57	1.34 g, 65%	1685, 1620, 1480, 1230, 1089 and 850	86-87
E54	1.7 g, 4.71 mmol	I	0.46	1.1 g, 64%	1690, 1615, 1475, 1225, 1090 and 850	107-108

III.4a.4b Synthesis of 6-Benzyloxy-2-substituted phenyl-4*H*-chromen-4-one derivatives

Table 40. Details of 6-Benzyloxy-2-substituted phenyl-4*H*-chromen-4-one derivatives

No.	D	Method	R_f ($CHCl_3$)	Yield (%)	IR (cm^{-1})	M.P. (°C)
E55	2.2 g, 3.173 mmol	II	0.50	1.5 g, 68%	-------	NT
E56	2.2 g, 6.104 mmol	I	0.45	1.2 g, 54%	1680, 1627, 1498, 1230, 1065 and 850	180-181
E57	1.7 g, 4.71 mmol	II	0.47	1.1 g, 64%	-------	NT

E58	1.7 g, 4.71 mmol	II	0.40	1.1 g, 64%	-------	NT
E59	2.2 g, 3.173 mmol	I	0.55	1.5 g, 68%	1688, 1630, 1489, 1225, 1080 and 850	207-209

III.4a.5: Synthesis of 4-(4/5-substituted-2-hydroxyphenyl)-6-(substutited phenyl)-2-Aminopyrimidines

4-(4-Benzyloxy-2-hydroxyphenyl)-6-phenyl-2-aminopyrimidine (45)

A mixture of 7-benzyloxy-2-phenyl-4*H*-chromen-4-one **(E45)** (0.5 g, 1.522 mmol), guanidine hydrochloride (0.436 g, 4.5 mmol) and potassium hydroxide (0.1 g, 2.2 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up is as described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(45)**, (0.2 g, 40 %) m.p. 183-84 °C

Anal:

TLC : 0.51 (benzene)

IR (KBr): 3490, 3311, 3220, 1652, 1230, 890 and 760 cm^{-1}

PMR : δ 5.02 (S, 2H, -O***CH***$_2$Ar), 6.5 (2H, -***NH***$_2$), 7.25-7.9 (m 13H, Ar***H***), 7.36 (s, 1H, Pyri***H***), 14.12 (1H, -OH)

Mass : 370.2 (m/z) (M+1)

4-(4-Benzyloxy-2-hydroxyphenyl)-6-(4-methoxyphenyl)-2-aminopyrimidine (46)

A mixture of 7-benzyloxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one **(E46)** (0.5 g, 1.3 mmol), guanidine hydrochloride (0.39 g, 4.13 mmol) and potassium hydroxide (0.11 g, 2.02 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up is as described for

(1). The solid so obtained was filtered, washed with water and recrystallised from to yield the title product **(46)**, (0.28 g, 56 %) m.p. 226-27 °C

Anal:

TLC : 0.51 (benzene)

IR (KBr): 3495, 3347, 3223, 1652, 1230, 890 and 760 cm^{-1}

PMR : δ 3.4 (s, 3H, -O***CH₃***), 5.12 (S, 2H, -O***CH₂***Ar), 6.5 (2H, -***NH₂***), 6.6-8.1 (m, 12H, Ar***H***), 7.35 (s, 1H, Pyrim***H***), 10.07 (1H, -OH)

Mass : 400.2 (m/z) (M+1)

4-(4-Benzyloxy-2-hydroxyphenyl)-6-(3-methoxyphenyl)-2-aminopyrimidine (47)

A mixture of 7-benzyloxy-2-(3-methoxyphenyl)-4*H*-chromen-4-one **(E47)** (0.5 g, 1.3 mmol), guanidine hydrochloride (0.39 g, 4.13 mmol) and potassium hydroxide (0.11 g, 2.02 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up is as described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(47)**, (0.23 g, 47 %) m.p. 154-55 °C

Anal:

TLC : 0.51 (benzene)

IR (KBr): 3400, 3338, 3223, 1652, 1230, 890 and 760 cm^{-1}

PMR : δ 3.9 (s, 3H, -O***CH₃***), 5.12 (S, 2H, -O***CH₂***Ar), 5.3 (2H, -***NH₂***), 6.6-8.1 (m, 12H, Ar***H***), 7.39 (s, 1H, Pyrim***H***), 10.53 (1H, -OH)

Mass : 400.2 (m/z) (M+1)

4-(4-Benzyloxy-2-hydroxyphenyl)-6-(3,4-dimethoxyphenyl)-2-aminopyrimidine (48)

A mixture of 7-benzyloxy-2-(3,4-dimethoxyphenyl)-4*H*-chromen-4-one **(E48)** (0.5 g, 1.2 mmol), guanidine hydrochloride (0.39 g, 4.13 mmol) and potassium hydroxide (0.11 g, 2.02 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up is as described for

(1). The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(48)**, (0.16 g, 32 %) m.p. 198-200 °C

Anal:

TLC : 0.51 (benzene)

IR (KBr): 3420, 3322, 3210, 1647, 1230, 890 and 760 cm^{-1}

PMR : δ 3.9 (s, 3H, -O***CH*$_3$**), 4.0 (s, 3H, -O***CH*$_3$**), 5.12 (S, 2H, -O***CH*$_2$**Ar), 5.3 (2H, -***NH*$_2$**), 6.9-7.9 (m 11H, Ar***H***), 7.36 (s, 1H, Pyri***H***), 11.03 (1H, -OH)

Mass : 430.2 (m/z) (M+1)

4-(4-Benzyloxy-2-hydroxyphenyl)-6-(4-fluorophenyl)-2-aminopyrimidine (49)

A mixture of 7-benzyloxy-2-(4-fluorophenyl)-4*H*-chromen-4-one **(E49)** (0.5 g, 1.4 mmol), guanidine hydrochloride (0.39 g, 4.13 mmol) and potassium hydroxide (0.11 g, 2.02 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up is as described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(49)**, (0.22 g, 45 %) m.p. 195-96 °C

Anal:

TLC : 0.51 (benzene)

IR (KBr): 3493, 3318, 3220, 1652, 1230, 890 and 760 cm^{-1}

PMR : δ 5.12 (S, 2H, -O***CH*$_2$**Ar), 6.5 (2H, -***NH*$_2$**), 6.6- 8.1 (m 12H, Ar***H***), 7.35 (s,1H, Pyrim***H***), 12.01 (1H, -OH)

Mass : 388.2 (m/z) (M+1)

4-(4-Benzyloxy-2-hydroxyphenyl)-6-(4-bromophenyl)-2-aminopyrimidine (50)

A mixture of 7-benzyloxy-2-(4-bromophenyl)-4*H*-chromen-4-one **(E50)** (0.5 g, 1.2 mmol), guanidine hydrochloride (0.39 g, 4.13 mmol) and potassium hydroxide (0.11 g, 2.02 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up is as described for

(1). The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(50)**, (0.23 g, 46 %) m.p. 198-99 °C

Anal:

TLC : 0.51 (benzene)

IR (KBr): 3495, 3347, 3223, 1652, 1230, 890 and 760 cm^{-1}

PMR : δ 5.12 (S, 2H, -O***CH***$_2$Ar), 6.02 (2H, -***NH***$_2$), 7.31-7.34 (m,5H, Ar***H***), 6.5-7.9 (m, 7H, Ar***H***), 7.35 (s, 1H, Pyrim***H***.) 14.07 (1H, -OH)

Mass : 447.2 (m/z) (M+1)

4-(4-Benzyloxy-2-hydroxyphenyl)-6-(2-chlorophenyl)-2-aminopyrimidine (51)

A mixture of 7-benzyloxy-2-(2-chlorophenyl)-4*H*-chromen-4-one **(E51)** (0.5 g, 1.3 mmol), guanidine hydrochloride (0.39 g, 4.13 mmol) and potassium hydroxide (0.11 g, 2.02 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up is as described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(51)**, (0.17 g, 35 %) m.p. 161-62 °C

Anal:

TLC : 0.51 (benzene)

IR (KBr): 3450, 3315, 3220, 1652, 1230, 890 and 760 cm^{-1}

PMR : δ 5.10 (S, 2H, -O***CH***$_2$Ar), 5.15 (2H, -***NH***$_2$), 7.34-7.39 (m, 5H, Ar***H***), 6.55-7.71 (m, 7H, Ar***H***), 7.35(s, 1H, Pyrim***H***), 13.88 (1H, -OH)

Mass : 404.1 (m/z) (M+1)

4-(4-Benzyloxy-2-hydroxyphenyl)-6-(2,4-dichlorophenyl)-2-aminopyrimidine (52)

A mixture of 7-benzyloxy-2-(2,4-dichlorophenyl)-4*H*-chromen-4-one **(E52)** (0.5 g, 1.2 mmol), guanidine hydrochloride (0.39 g, 4.13 mmol) and potassium hydroxide (0.11 g, 2.02 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up is as described for

(1). The solid so obtained was filtered, washed with water and recrystallised from to yield the title product **(52)**, (0.2 g, 40 %) m.p. 240-42 °C

Anal:

TLC : 0.51 (benzene)

IR (KBr): 3400, 3317, 3220, 1652, 1230, 890 and 760 cm^{-1}

PMR : δ 5.11 (S, 2H, -O***CH***$_2$Ar), 6.0 (2H, -***NH***$_2$), 7.30-7.35 (m, 5H, Ar***H***), 6.55-7.71 (m, 7H, Ar***H***), 7.35(s, 1H, Pyrim***H***), 13.88 (1H, -OH)

Mass : 438.1 (m/z) (M+1)

4-(4-Benzyloxy-2-hydroxyphenyl)-6-(4-methylphenyl)-2-aminopyrimidine (53)

A mixture of 7-benzyloxy-2-(4-methylphenyl)-4*H*-chromen-4-one **(E53)** (0.5 g, 1.4 mmol), guanidine hydrochloride (0.39 g, 4.13 mmol) and potassium hydroxide (0.11 g, 2.02 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up is as described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(53)**, (0.28 g, 56 %) m.p. 215-216 °C.

Anal:

TLC : 0.55 (benzene)

IR (KBr): 3428, 3323, 3204, 1647, 1230, 890 and 760 cm^{-1}

PMR : δ 2.4 (s, 3H, -***CH***$_3$), 5.10 (S, 2H, -O***CH***$_2$Ar), 6.44 (2H, -***NH***$_2$), 7.2-7.4 (m 5H, Ar***H***), 7.4-7.8 (m, 7H, Ar***H***), 7.35 (s, 1H, Pyrim***H***)

Mass : 384.2 (m/z) (M+1)

4-(4-Benzyloxy-2-hydroxyphenyl)-6-(3-methylphenyl)-2-aminopyrimidine (54)

A mixture of 7-benzyloxy-2-(4-methylphenyl)-4*H*-chromen-4-one **(E54)** (0.5 g, 1.4 mmol), guanidine hydrochloride (0.39 g, 4.13 mmol) and potassium hydroxide (0.11 g, 2.02 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up is as described for

(1). The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(54)**, (0.14 g, 29 %) m.p. 165-67 °C

Anal:

TLC : 0.58 (benzene)

IR (KBr): 3450, 3330, 3200, 1607, 1230, 890 and 760 cm^{-1}

PMR : δ 2.1 (s, 3H, -***CH*$_3$**), 5.11 (S, 2H, -O***CH*$_2$Ar), 6.2 (2H, -***NH*$_2$**), 7.2-7.4 (m 5H, Ar***H***), 6.9-7.8 (m, 7H, Ar***H***), 7.36 (s, 1H, Pyrim***H***)

Mass : 384.2 (m/z) (M+1)

4-(5-Benzyloxy-2-hydroxyphenyl)-6-(4-methoxyphenyl)-2-aminopyrimidine (55)

A mixture of 6-benzyloxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one **(E55)** (0.5 g, 1.4 mmol), guanidine hydrochloride (0.39 g, 4.13 mmol) and potassium hydroxide (0.11 g, 2.02 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up is as described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from to yield the title product **(55)**, (0.21 g, 46 %) m.p. °C

Anal:

TLC : 0.58 (benzene)

IR (KBr): 3493, 3336, 3218, 1647, 1230, 890 and 760 cm^{-1}

PMR : δ 3.8 (s, 3H, -O***CH*$_3$**), 5.0 (S, 2H, -O***CH*$_2$Ar), 5.16 (2H, -***NH*$_2$**), 7.6-8.1 (m12H, Ar***H***) 7.35 (s, 1H, Pyrim***H***), 13.24 (1H, -***OH***)

Mass : 400.2 (m/z) (M+1)

4-(5-Benzyloxy-2-hydroxyphenyl)-6-(4-methylphenyl)-2-aminopyrimidine (56)

A mixture of 6-benzyloxy-2-(4-methylphenyl)-4*H*-chromen-4-one **(E56)** (0.5 g, 1.4 mmol), guanidine hydrochloride (0.39 g, 4.13 mmol) and potassium hydroxide (0.11 g,

2.02 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up is as described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from to yield the title product **(56)**, (0.18 g, 36 %) m.p. 177-78 °C

Anal:

TLC : 0.58 (benzene)

IR (KBr): 3493, 3336, 3218, 1647, 1230, 890 and 760 cm^{-1}

PMR : δ 2.4 (s, 3H, -CH_3), 5.10 (S, 2H, -OCH_2Ar), 6.44 (2H, -NH_2), 6.9-7.4 (m, 8H, Ar*H*) 7.4-7.8 (m, 4H, Ar*H*), 7.35 (s, 1H, Pyrim*H*), 14.01 (1H, -*OH*)

Mass : 384.2 (m/z) (M+1)

4-(5-Benzyloxy-2-hydroxyphenyl)-6-(4-chlorophenyl)-2-aminopyrimidine (57)

A mixture of 6-benzyloxy-2-(4-chlorophenyl)-4*H*-chromen-4-one **(E57)** (0.5 g, 1.4 mmol), guanidine hydrochloride (0.39 g, 4.13 mmol) and potassium hydroxide (0.11 g, 2.02 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up is as described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(57)**, (0.3 g, 49 %) m.p. 190-92 °C

Anal:

TLC : 0.58 (benzene)

IR (KBr): 3315, 3200, 3254, 1651, 1230, 890 and 760 cm^{-1}

PMR : δ 5.01 (S, 2H, -OCH_2Ar), 5.16 (2H, -NH_2), 7.34-7.39 (m, 5H, Ar*H*), 6.9-8.0 (m,7H, Ar*H*)7.35 (s, 1H, Pyrim*H*), 13.87 (1H, -*OH*)

Mass : 404.2 (m/z) (M+1)

4-(5-Benzyloxy-2-hydroxyphenyl)-6-(4-fluorophenyl)-2-aminopyrimidine (58)

A mixture of 6-benzyloxy-2-(4-fluorophenyl)-4*H*-chromen-4-one **(E58)** (0.5 g, 1.4 mmol), guanidine hydrochloride (0.39 g, 4.13 mmol) and potassium hydroxide (0.11 g, 2.02 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up is as described for

(1). The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(58)**, (0.28 g, 42 %) m.p. °C

Anal:

TLC : 0.58 (benzene)

IR (KBr): 3488, 3336, 3218, 1634, 1230, 890 and 760 cm^{-1}

PMR : δ 5.1 (S, 2H, -O***CH***$_2$Ar), 6.02 (2H, -***NH***$_2$), 7.31-7.34 (m, 5H, Ar***H***), 6.5-7.9 (m,7H, Ar***H***) 7.35 (s, 1H, Pyrim***H***), 14.17 (1H, -***OH***)

Mass : 388.2 (m/z) (M+1)

4-(5-Benzyloxy-2-hydroxyphenyl)-6-(4-bromophenyl)-2-aminopyrimidine (59)

A mixture of 6-benzyloxy-2-(4-bromophenyl)-4*H*-chromen-4-one **(E59)** (0.5 g, 1.4 mmol), guanidine hydrochloride (0.39 g, 4.13 mmol) and potassium hydroxide (0.11 g, 2.02 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up is as described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(59)**, (0.28 g, 56 %) m.p. 214-16 °C

Anal:

TLC : 0.58 (benzene)

IR (KBr): 3493, 3336, 3218, 1647, 1230, 890 and 760 cm^{-1}

PMR : δ 5.0 (S, 2H, -O***CH***$_2$Ar), 5.16 (2H, -***NH***$_2$), 6.9-8.1 (m12H, Ar***H***) 7.35 (s, 1H, Pyrim***H***), 13.16 (1H, -***OH***)

Mass : 448.2 (m/z) (M+1)

5.2c. Anti malarial activity

The preliminary anti malarial screening of compounds were carried out at Department of Biochemistry, The M.S.University of Baroda, Vadodara. The 4-(4/5-methoxy/benzyloxy substituted-2-hydroxyphenyl)-6-phenyl-2-aminopyrimidines were screened for anti malarial activity. Anti malarial activity as checked on *Plasmodium falciparum* native strain and drug sensitive strains.

Experimental process for anti malarial activity:

Strain of *P. falciparum* was cultured continuously according to the candle jar method of Trager and Jensen, *in vitro* in human red blood cells (blood type B+) with 5% hematocrit in LIQUID RPMI 1640 medium (HIMEDIA) supplemented with 25 mM HEPES (Sigma), 0.2% sodium bicarbonate (Sigma) and 10% human B+ serum. Slides of culture were observed after 3, 6 and 24 h for regular development of parasite stages.

Chloroquine sensitive *P. falciparum*, MRC 20 was obtained from the National Institute of Malaria Research (NIMR), New Delhi. The culture was synchronized using sorbitol and parasitaemia was adjusted to 1-1.5% by diluting with fresh human erythrocytes. The cells were diluted with complete media to make 8% haematocrit. Again the slides of culture were prepared and observed for the calculation of parasitemia, particularly for young trophozoites or ring stages. One mg of each compound/extract was dissolved in 100 µL DMSO and 900 µL RPMI-1640 to obtain a stock of 1 mg ml^{-1} (stock solution). A series of eight concentrations were prepared from the stock solutions by 2 fold dilutions. After 24 h, thin films of the contents of each well were prepared and examined under the microscope. Parasite count for each blood film was made using a compound microscope under oil immersion with X 100 objective after staining the film with eosin yellow and methylene blue. Each film was observed at three different visual fields. The number of schizonts per 200 parasites were noted and compared between control and test wells for the determination of the % inhibition. All doses were studied in cultures and the mean was observed for purposes of inferences. The inhibition of parasite growth in the drug-treated groups was calculated as follows: Parasitaemia in the control (non-treated) group

minus parasitaemia in the drug treated group, divided by parasitaemia in the control (non-treated) group, expressed as percentages. All values are expressed as percentage growth inhibition. Dose response curves of the fractions were obtained by plotting percentage inhibition against log concentration. The values of the compounds provided a mid-point value where parasite growth would be 50%. Linear regression analysis was applied to the linear portion of the sigmoidal curve was plotted and IC50 values were derived for each fraction.

The IC50 value of Chloroquine and Quinine is around 10 and 29 μg ml^{-1} respectively.

Compound code	IC_{50}value (μg/ml)	Lysis (μg/ml)
28	21.35	>25
31	64.56(>25)	>25
32	**1.78**	>12.5
34	15.25	>50
35	**8.71**	>25
39	>25	>50
40	29.35	>50
41	**9.53**	>25
43	25.52	>50
46	**5.01**	>25
47	**7.61**	>25
48	**1.61**	>12.5
49	16.67	>50
51	18.87	>25
53	14.87	>25
54	35.56	>25

Table 41. IC_{50} value of test compounds.