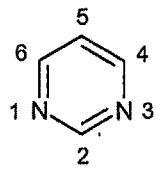
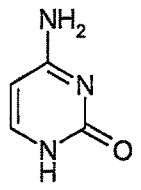
CHAPTER – 1

'PYRIMIDINE-A Perspective'

Pyrimidine (**1**) derivatives are an important class of nitrogen containing heterocycles and have shown a broad spectrum of biological activities. The pyrimidine ring contains two nitrogen atoms at positions 1 and 3 of the six membered ring. Several pyrimidine compounds were isolated between 1837 and 1864, but their structures were not recognized until 1868.[1] Some well-known pyrimidine compounds include cytosine (**2**), thymine (**3**) and uracil (**4**) present in nucleic acids, thiamine (vitamin B_1),[2] sulfadiazine, sulfamerazine and sulfamethazine drugs used in therapy of bacterial and viral diseases.[3]



(**1**)



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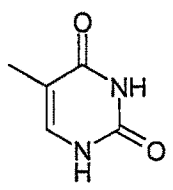

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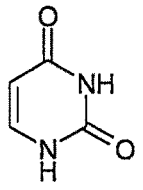
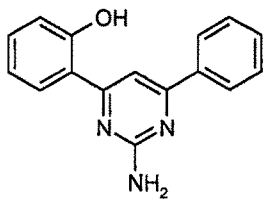


(**4**)

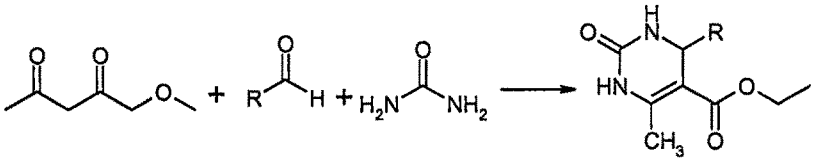
As a part of building blocks of DNA and RNA, pyrimidine derivatives are important for anti cancer and anti parasitic drug design.[4] Various types of pyrimidines derivatives were reported in literature like fused pyrimidines, dihydropyrimidines, pyrimidinones, pyrimidinediones etc. Pyrimidine derivatives possess several biological activities like antitumor, antimalarial, antiviral, antifungal, antimicrobial and anti platelet.[5] The present thesis embodies the work done on 4,6-diaryl-2-aminopyrimidines (**5**). The work was an improvisation of the studies previously carried out in our laboratory for HIV-1 activity with a view to improve the selectivity index.[6] During the course of the anti HIV work it was also visualized that the designed molecules had promising anti platelet activity and anti malarial activity as well.



(**5**)

Chemistry of pyrimidines

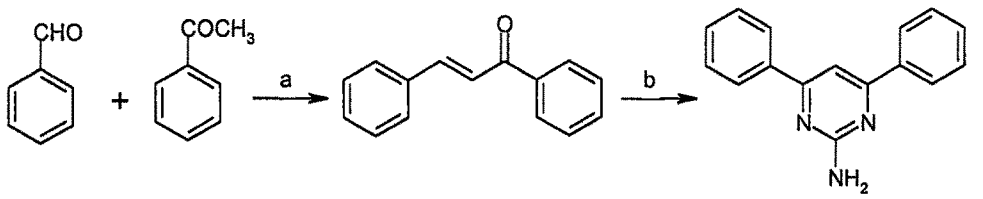
In view of the importance of pyrimidine derivatives due to its varied biological activities, numerous methods of synthesis were reported in literature. After establishing the structure in the 18th century the first efficient synthesis was reported by Biginelli in 1891.[7] He was the pioneer of the single step synthesis of dihydropyrimidine. Later that reaction was known as Biginelli pyrimidine synthesis. This reaction involves condensation of β-keto esters with aldehyde and urea to yield 3, 4-Dihydro-1*H*-pyrimidine-2-one esters.[8] (Scheme 1)



(6) (7)

Scheme 1. Reaction condition: HCl, EtOH, Reflux

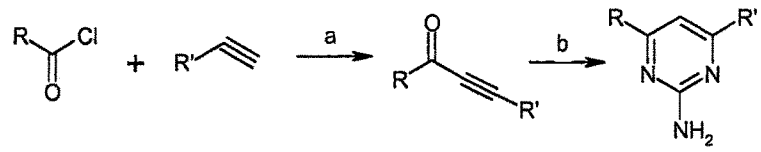
The most efficient method for the synthesis of diarylpyrimidine **(11)** was reported from chalcones **(10)**, which was synthesized from condensation of different aldehyde **(8)** and acetophenone **(9)** in methanolic KOH. The reaction of chalcones with alcoholic solution of guanidine carbonate in aqueous sodium hydroxide solution, produced the corresponding 2-amino-4,6-diarylpyrimidines.[9] (Scheme 2)



(8) **(9)** **(10)** **(11)**

Scheme 2. Reaction condition: (a) MeOH, KOH, (b) MeOH, aqueous NaOH, guanidine carbonate.

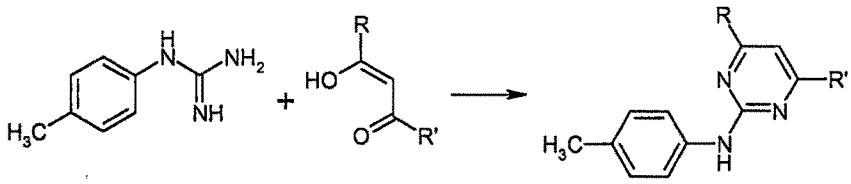
A new approach for the synthesis of 2-aminopyrimidines **(15)** was reported in 2003 using Sonogashira reaction condition. The coupling of acid chlorides with terminal alkynes using one equivalent of triethylamine under Sonogashira conditions followed by subsequent addition of amines or amidinium salts to the intermediate alkynones allows a straightforward access to enaminones and pyrimidines under mild conditions and in excellent yields.[10] (Scheme 3)



(12) **(13)** **(14)** **(15)**

Scheme 3. Reaction condition: (a) $Pd(Ph_3)_2Cl_2$, CuI, triethylamine, (b) guanidine HCl, Na_2CO_3, THF.

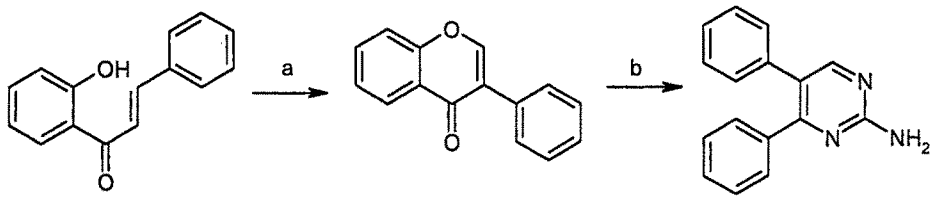
In 1985, Alfred Kreutzberger reported the synthesis of 2-aminopyrimidine **(18)** by condensation of 4-tolylgunidine **(16)** and β-diketonen **(17)** in alcoholic KOH medium. [11] (Scheme 4)



(16) **(17)** **(18)**

Scheme 4.

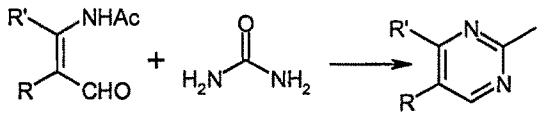
Synthesis of vicinal aryl 2-aminopyrimidine **(21)** was reported in 1983, from isoflavone **(20)**. The isoflavone was synthesized from hydroxyl chalcones using thalium trinitrate (TTN) in methanol. The isoflavone was refluxed in xylene with guanidine carbonate to give 2-aminopyrimidine.[12] (Scheme 5)



(19) **(20)** **(21)**

Scheme 5. Reaction condition: (a) Thalium trinitrate, methanol, (b) guanidine carbonate, xylene.

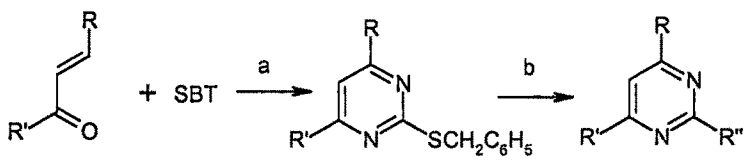
Synthesis of 2-aminopyrimidines **(24)** was also reported using microwave synthesis. A novel and efficient synthesis of pyrimidine from β-formyl enamide **(22)** involves samarium chloride catalysed cyclisation of β-formyl enamides using urea as source of ammonia under microwave irradiation.[13] (Scheme 6)



(22) **(23)** **(24)**

Scheme 6. Reaction condition: $SmCl_3$, MW, (<300 W, 140 °C) 8-10 min.

The synthesis of 4,6-substituted 2-aminopyrimidines **(27)** using microwave method was reported in 2003. In this method chalcones was condensed with S-benzylthiuronium chloride in morpholine/piperidine/pyrrolidine in neutral alumina in microwave synthesizer at 100-200 W.[14] (Scheme 7).



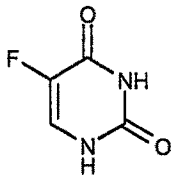
(25) **(26)** **(27)**

Scheme 7. Reaction condition: (a) SBT, neutral alumina, morpholine, MW, (b) R_3H, Basic alumina, MW

Various bioactivities of pyrimidines

Pyrimidines as anti neoplastic agents:

Due to structural resemblance with the biological nucleosides, pyrimidine derivatives are found to act as antimetabolites in anti cancer therapy. Many pyrimidines were reported to act as false precursors and act as antagonists in antineoplastic therapy. One of the early metabolites prepared was 5-fluorouracil (5-FU) [15,16] **(28)**, a pyrimidine derivative. 5-Thiouracil **(29)**[17] also exhibits some useful antineoplastic activity. The antineoplastic compounds possessing the guanine nucleus[17] like mercaptopurine **(30)**,[19] tegafur **(31)**,[20] etc. were discovered after formulation of the antimetabolite theory by Woods and Fildes in 1940. These drugs prevent the utilization of normal cellular metabolites.[17] There are many more in recent times, like nimustine **(32)**, uramustine **(33)**[21] and trimetrixate **(34)**.[22] Gemcitabine **(35)**, a pyrimidine antimetabolite, shows excellent antitumour activity against murine solid tumours.[23]



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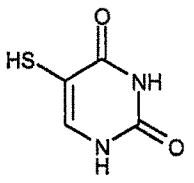

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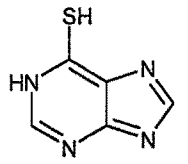

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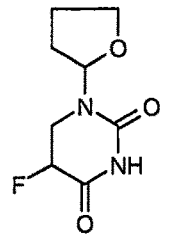

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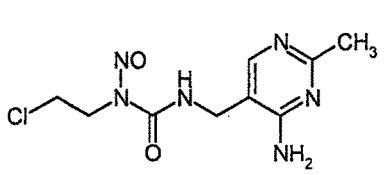

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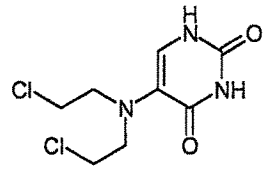

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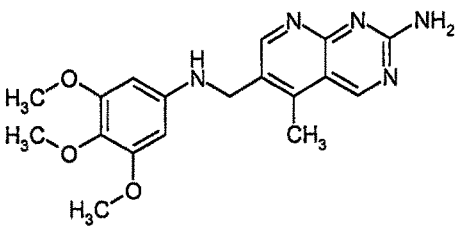

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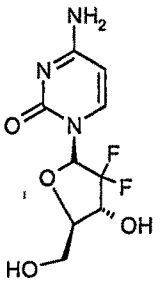

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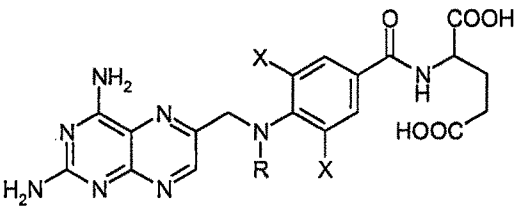

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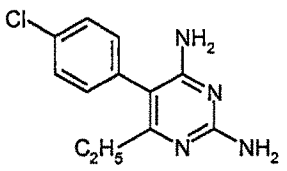
Pyrimidines as antifolates and antimalarial agents:

In 1948, Hitchings made an important observation that a large number of 2,4-diaminopyrimidines and some 2-amino-4-hydroxypyrimidines are antagonists of folic acid.[24] On the basis of that observstion methotrexate **(36,** R=CH_3) and aminopterin **(36,** R=H) were reported as selective inhibitors of DHFR which is useful in cancer chemotherapy.[25] These derivatives were fused ring heterocycles. Another pyrimidine derivative was also reported which acts as DHFR inhibitor for anti protozoal and anti bacterial chemotherapy like pyrimethamine **(37),** a selective inhibitor of the DHFR of malarial plasmodia; trimethoprim **(38)**[25] an antibacterial drug which selectively inhibits bacterial DHFR.



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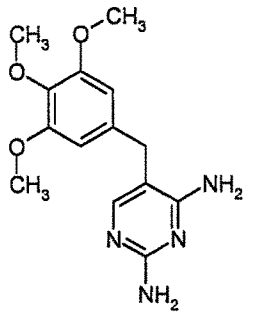

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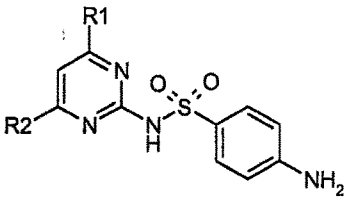
Pyrimidines as part of sulfa drugs:

Pyrimidine derivatives of sulfa drugs, namely sulfadiazine **(39a)**, sulfamerazine **(39b)** and sulfadimidine **(39c)** are superior to many other sulfonamides and are used in some acute UT infections, cerebrospinal meningitis and for patients allergic to pencillins.[26] Sulfonamide–trimethoprim combinations are used extensively for opportunistic infections in patients with AIDS.[27] Sulfadiazine, sulfamerzine and sulfadimidine possess good water solubility and therefore carry minimum risk of kidney damage, which makes them safe even for patients with impaired renal function.



sulphadiazine (39a)	H	H
sulfamerazine (39b)	CH_3	H
sulphadimidine (39c)	CH_3	CH_3

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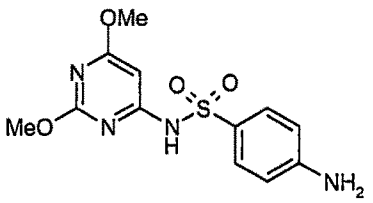

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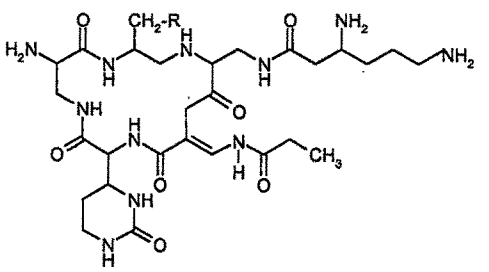
A new broad-spectrum sulfonamide, sulfamethomidine **(40)** is relatively nontoxic and patients do not need extra fluid intake or alkalization.[28]

Pyrimidines as anti tubercular drugs:

Capreomycin **(41)** produced by *Streptomyces capreolus* is a second-line bacteriostatic antituberculin drug containing pyrimidine.[29] Viomycin **(42)** is more tuberculostatic than *p*-aminosalicyclic acid. It is effective in the treatment of experimental tuberculosis.[29,30]



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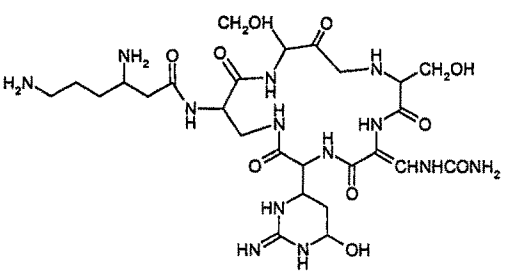

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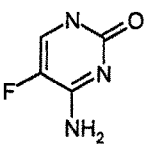
Pyrimidine derivatives as antifungal agents:

Pyrimidines also exhibit antifungal properties. Flucytosine **(43)** is a fluorinated pyrimidine used as nucleosidal anti fungal agent for the treatment of serious systemic infections caused by susceptible strains of candida and cryptococcus.[31,32] Hexitidine **(44)** is mainly used for the treatment of aphthous ulceration.[33]



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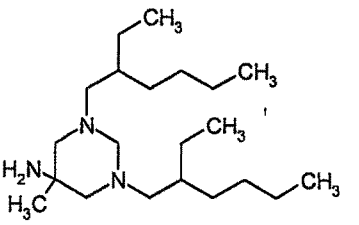

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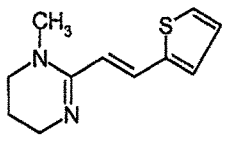
Pyrimidine derivatives as anthelmentics:

These drugs have the ability of ridding the body of parasitic worms. Pyrantel pamoate **(45)** a pyrimidine derivative, is a depolarizing neuromuscular blocking agent that causes spastic paralysis in helminths and is employed in the treatment of infestations caused by pinworms and roundworms.[34]



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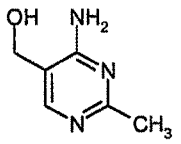
Pyrimidine derivatives as antibiotics:

There are few examples of pyrimidine antibiotics, the simplest of all is bacimethrin (5-hydroxymethyl-2-methoxypyrimidin-4-amine) **(46)**, which is active against several staphylococcal infections.[35] Amicetin **(47)** and plicacetin **(48)** are cytosine derivatives which exhibit activity against acid fast and Gram-positive bacteria as well as other organisms.[35] Puromycin has a wide spectrum of antitrypanosomal activity. Aminoglycoside antibiotics phleomycin, bleomycin and related families are wide-spectrum antibiotics containing the pyrimidine ring. Bleomycin is already in clinical use against certain tumours like Hodgkin's lymphoma and disseminated testicular cancer.[36]



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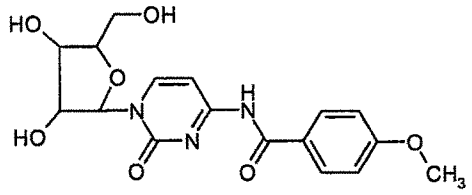

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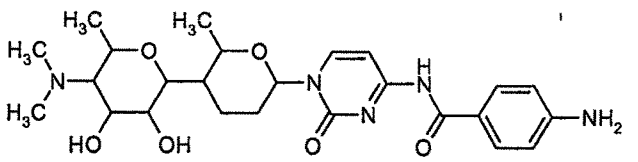
A few pyrimidine derivatives are also used as anxiolytics. Most important of these is buspirone **(54)**, indicated in the management of anxiety disorders accompanied with or



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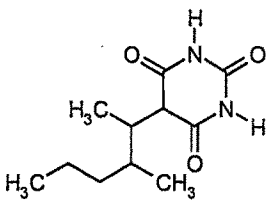
Pyrimidines as sedative, hypnotic and antiepileptic agents:

Agents of the anxiolytic, sedative and hypnotic group include a wide variety of barbiturates and are classified as drugs having short, intermediate and long duration of action.[37,38] Pentobarbital **(49)**, Phenobarbital **(50)** and secobarbital **(51)** are frequently used clinical hypnotic barbiturates.[39] Hexobarbital **(52)** and propallylonal **(53)** are some of the current drugs in the market used as sedative hypnotics.[40]



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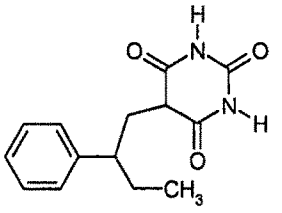

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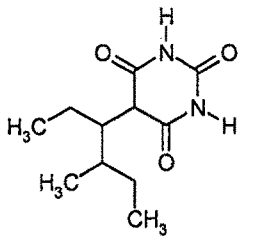

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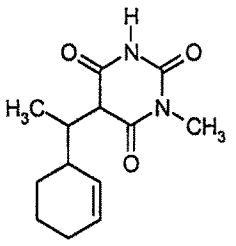

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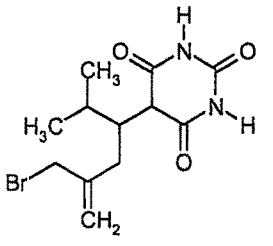

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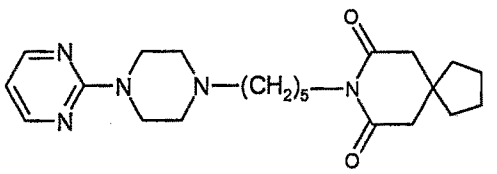
Pyrimidine derivatives as anxiolytic agents:

A few pyrimidine derivatives are also used as anxiolytics. Most important of these is buspirone **(54)**, indicated in the management of anxiety disorders accompanied with or

without depression.[41] Ritanserin **(55)**, a $5HT_2$ antagonist with anxiolytic activity is a pyrimidine derivative.[42] Risoperidone **(56)** is an antipsychotic drug, which is a structural hybrid of butyrophenone and can be used as anxiolytic, antidepressant and antiparkinsonian drug.[43]



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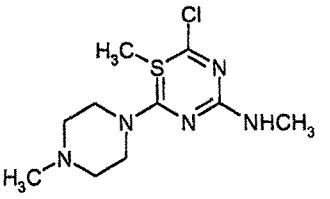

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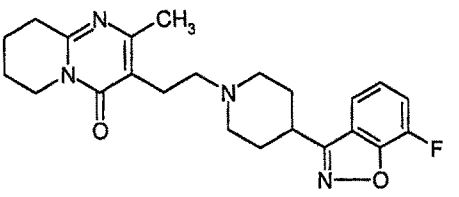
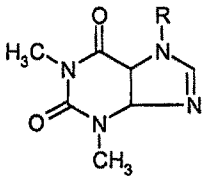


(56)

Pyrimidines as diuretics:

Several xanthine derivatives **(57)** containing fused pyrimidine ring systems like caffeine,[44] etamiphylline,[45] lomiphylline,[46] etophylline,[47] theophylline[44] and theodrendaline[48] are known to promote weak diuresis by stimulation of cardiac function and by a direct action on the nephron, acting as adenosine receptor antagonists.[44]



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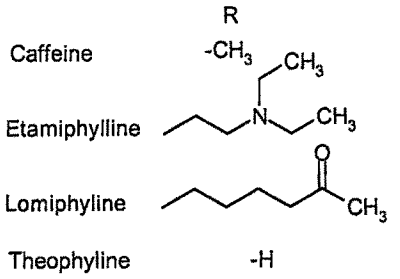
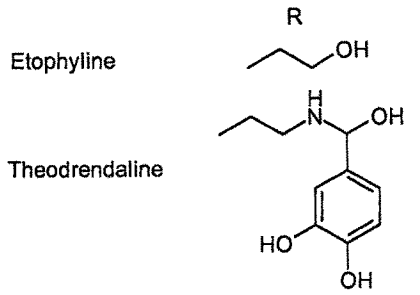
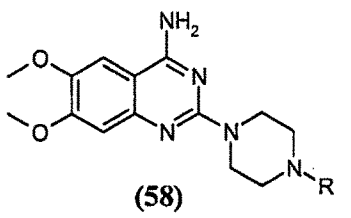




Pyrimidines as antihypertensive agents:

Several pyrimidine ring-containing drugs have exhibited antihypertensive activity. Prazosin, a quinozoline derivative **(58)**, is a selective a_1-adrenergic antagonist.[49, 50] Its related analogues bunazosin,[51] terazosin[52] and trimazosin[53] are potent antihypertensive agents. Another quinazoline derivative, ketanserin **(59)**[54] having a similar effect is an antagonist of both a_1-adrenergic and serotonin-S_2 receptors. Another pyrimidine derivative alfuzocin **(60)**[55] a prazocin analogue and an $a1$-adrenoceptor antagonist, as well as urapidil **(61)**[56] are used especially in urinary obstruction caused by benign prostate hyperplasia.



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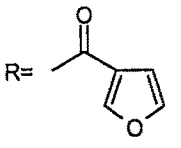
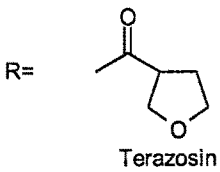


Prazosin

R= $-COCH_2CH_2CH_3$

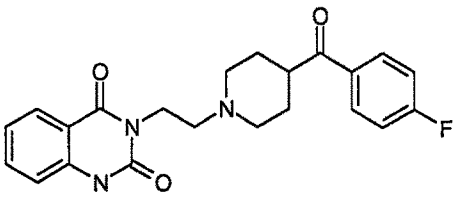
Bunazosin



Terazosin

R= $-COOCH_2COH(CH_3)_2$

Trimazosin



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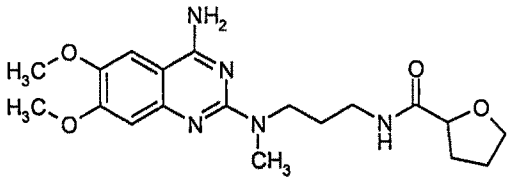

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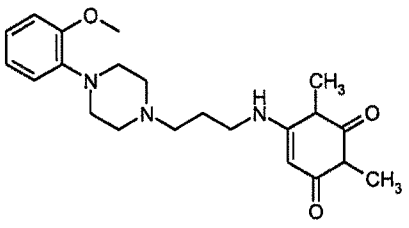
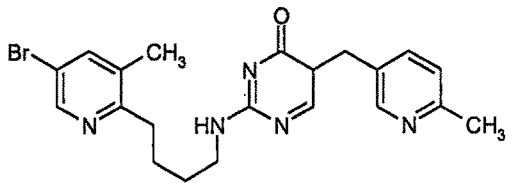


(61)

Pyrimidines as antihistaminic agents:

Pyrimidine ring containing compounds also possess potent anti histaminic activity. Temelastine **(62)** is a good example of them. Radiolabelled studies have indicated that it does not penetrate the CNS appreciably.[57] Icotidine **(63)**, a structural analogue of temelastine lacks CNS activity and is a dual antagonist of both H_1 and H_2 receptors.[58] Pemirolast **(64)**, a new oral nonbronchodilator antihistaminic agent is also a pyrimidine derivative. It has demonstrated sufficient antihistaminic activity to warrant its use in severe asthma.[59]



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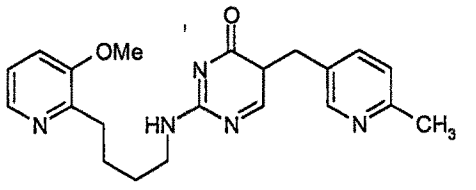

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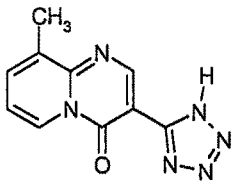
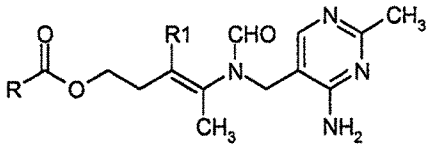


(64)

Pyrimidines as anti inflammatory agents:

Acetiamine,[60] bentiamine[60] and fursultiamine[61] are new pyrimidines **(64)** having therapeutic use in beriberi, polyneuritis, encephalopathy, pain, malnutrition, alcoholism and especially in the treatment of long-standing insulin-dependent diabetes mellitus. Fursultamine has been reported to inhibit the arachadonic acid cascade-line activation and reverse the increase in CBF (Coronary Blood Flow). Afloqualone **(65)**[62] has been evaluated as a successful anti-inflammatory agent with lower back pain patients.

Epirazole **(66)**[63] another NSAID, is suggested to be a COX-2 inhibitor. Ademetionine **(67)**[64] is primarily used in conjunction to glucosamine and chondroitin therapy.



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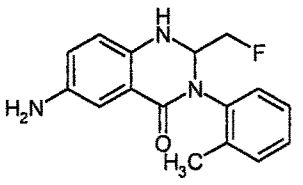

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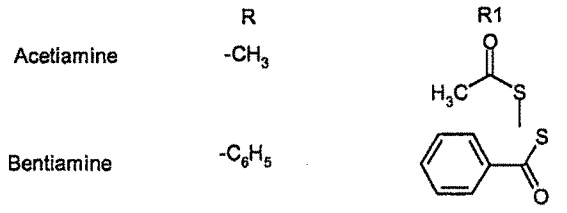

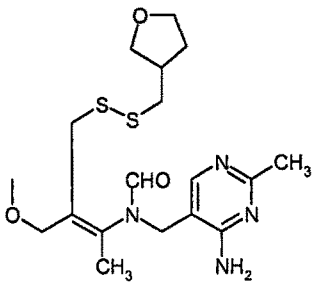

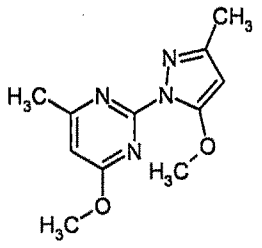

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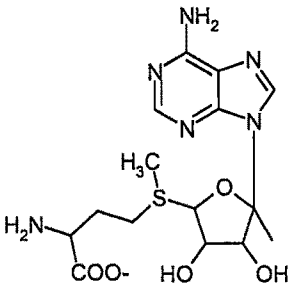
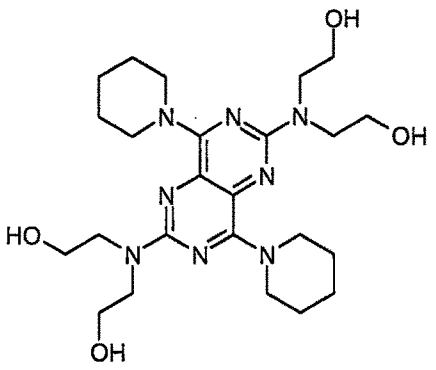


(67)

Pyrimidines as anti platelet agents:

Dipyridamol **(68)**[65] is a pyrimidine derivative which was marketed drug for antiplatelet therapy. Monocyclic as well as fused pyrimidines possess potential antiplatelet activity. Dipyridamol and benzopyran-2-one **(69)**[66] are fused pyrimidines. In the last decades, because of the adverse effects of available drugs, the search for selective or dually acting inhibitor preferred. The molecular modeling studies of these inhibitors shows the specific

role of the guanidine fragment. Many types of derivatives were designed with free guanidine and condensed guanidine fragment. Among them vicinal diarylpyrimidines **(70)** [67] were found to possess potent antiplatlet activity.



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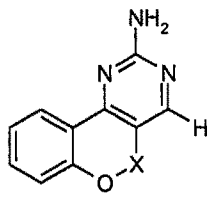

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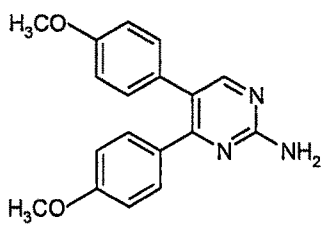
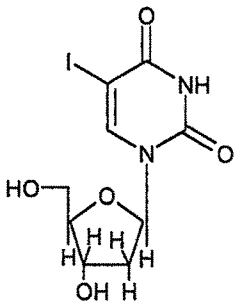


(70)

Pyrimidines as anti viral agents:

Pyrimidine derivatives have generated widespread interest due to their antiviral properties. 5-Iododeoxyuridine **(71)**[68] is an antiviral agent of high selectivity. 5-Trifluromethyl-2'-deoxyuridine (F3 TDR, **72**) has been found useful against infections resistant to IDU therapy.[68] It is especially effective against IDU-resistant herpes virus. Retrovir (AZT-16, **73**) is a potent inhibitor of the *in vivo* replication and cytopathic effects of HIV and has been recently approved for use against AIDS.[69]



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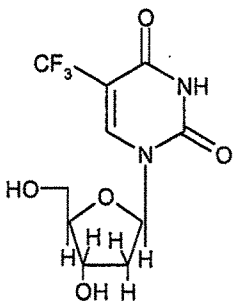

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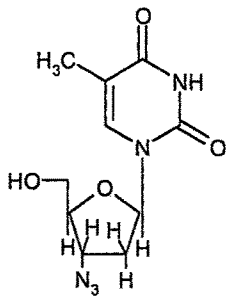
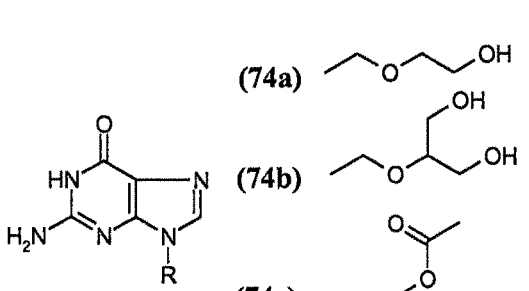
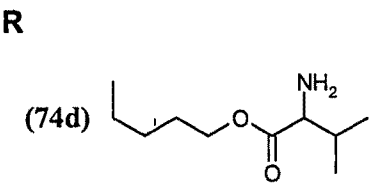


(73)

At present Acyclovir (**74a**) is the only remedy for genital herpes.[70] Ganciclovir (DHPG-2, **74b**) has shown good *in vivo* activity against HCV1 and HCV2.[71] Several members of a series of acyclic nucleosides, which contain a fused pyrimidine ring (mainly purine), are found to be effective antivirals. Famiciclovir (**74c**) and valacyclovir (**74d**)[72] are drugs used for several DNA viruses, including Hsv types 1 and 2, Varicella-zoster virus and Epstein-Barr virus. Penciclovir (**74e**) is useful for topical treatment of recurrent herpes, *Libialis*. Cidofovir (**75a**),[73] an antimetabolite for deoxycytosine triphosphate is used for treatment of cytomegalovirus (CMV) in AIDS patients.



(74a) **(74b)** **(74c)**



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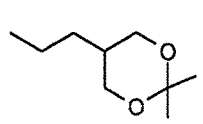

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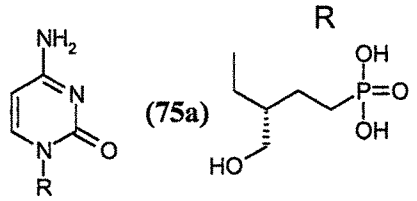
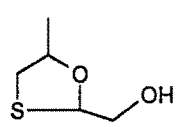


(75a)



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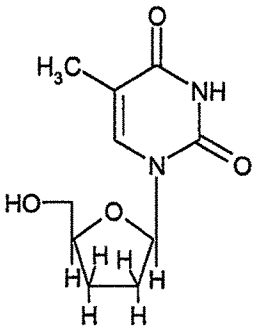

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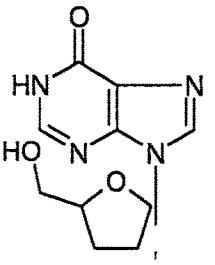


(77)

Lamivudine **(75b)**[73] is an effective anti-AIDS drug when used in combination with zidovudine (**73**). Zidovudine is an analogue of thymidine in which the azido group is substituted at the 3-position of the dideoxyribose moiety. It is active against RNA tumour viruses (retroviruses) that are the causative agents of AIDS and T-cell leukaemia. It is used in AIDS and AIDS-related complex (ARC) to control opportunistic infections by raising absolute CD4+ lymphocyte counts. Also, zalcitabine **(76)** is another useful alternative drug to zidovudine. It is given in combination with zidovudine, when CD4+ cell counts fall below 300 cells/mm^3. Didanosine **(77)** is a purine dideoxynucleoside, which is an analogue of inosine.[73]

From the previous work of the diaryl pyrimidine in our lab it was seen that they possess anti-HIV activity. The work presented here is based on diaryl pyrimidines with anti-HIV activity, anti platelet and anti malarial activity is described in PART I, PART II, PART III respectively.

1	**PART I**	**Pyrimidines as Anti-HIV agents**
2	**PART II**	**Pyrimidines as Anti platelet agents**
3	**PART III**	**Pyrimidines as Anti malarial agents**

PART I

Pyrimidines as anti-HIV agents

CHAPTER – I.1

Literature Survey

Pyrimidines as anti HIV agents

Human immunodeficiency virus type 1 (HIV-1) which causes AIDS has become the leading pandemic disease cause of death worldwide. Data from the World Health Organization AIDS Epidemic Update at the end of 2008 list 3.1 million deaths, 33 million people currently living with AIDS.[74]

HIV-1 is the etiological agent of acquired immunodeficiency syndrome (AIDS). Following infection, this retrovirus uses three key enzymes to complete its life cycle (**Fig.1**); reverse transcriptase (RT), integrase (IN) and protease (PR). The replication cycle of HIV involves three main stages: **entry into the cell, replication and transcription of the viral RNA to DNA**, and finally **assembly and release.**[75-78]

Entry into the cell

The virus binds itself to the target monocytes/macrophages and CD4 T-cells by adsorbing its glycoprotein to two host-cell receptors (proteins), the CD4 molecule receptor and CCR5 or CXCR4 co-receptors (also known as CC chemokine receptor 5 and CXC chemokine receptor 4). The gp41 protein facilitates the fusion of the viral envelope and the host-cell membranes. This fusion allows the release of the capsid of HIV into the target cell. However, the fusion mechanism influenced by the gp41 protein is still unclear. As HIV has attached to the host cell, the HIV-RNA and enzymes such as reverse transcriptase (RT), integrase (IN) and protease (PR) are able to enter into the host cell cytoplasmic compartment.[75]

Replication and transcription

The RT converts the single-stranded RNA genome of the virus into double-stranded DNA, a process known as reverse transcription. The DNA thus formed is transported to the cell nucleus to be integrated into the host chromosome. At this stage, the virus is known as a provirus. The integration process requires the IN enzyme. Thereafter, the virus can enter a latent stage of HIV infection, because the 'proviral DNA remains permanently within the target cell in a productive or latent state. The factors that may

affect this stage are the HIV variant, the cell type, and the expression capacity of the host cell. Eventually, the transcription of the HIV genomic materials and viral proteins (mRNA) forms the HIV messenger (mRNA) and proteins required for the assembly of the virus. This production is exported from the cell nucleus into the cell cytoplasm.[75-76]

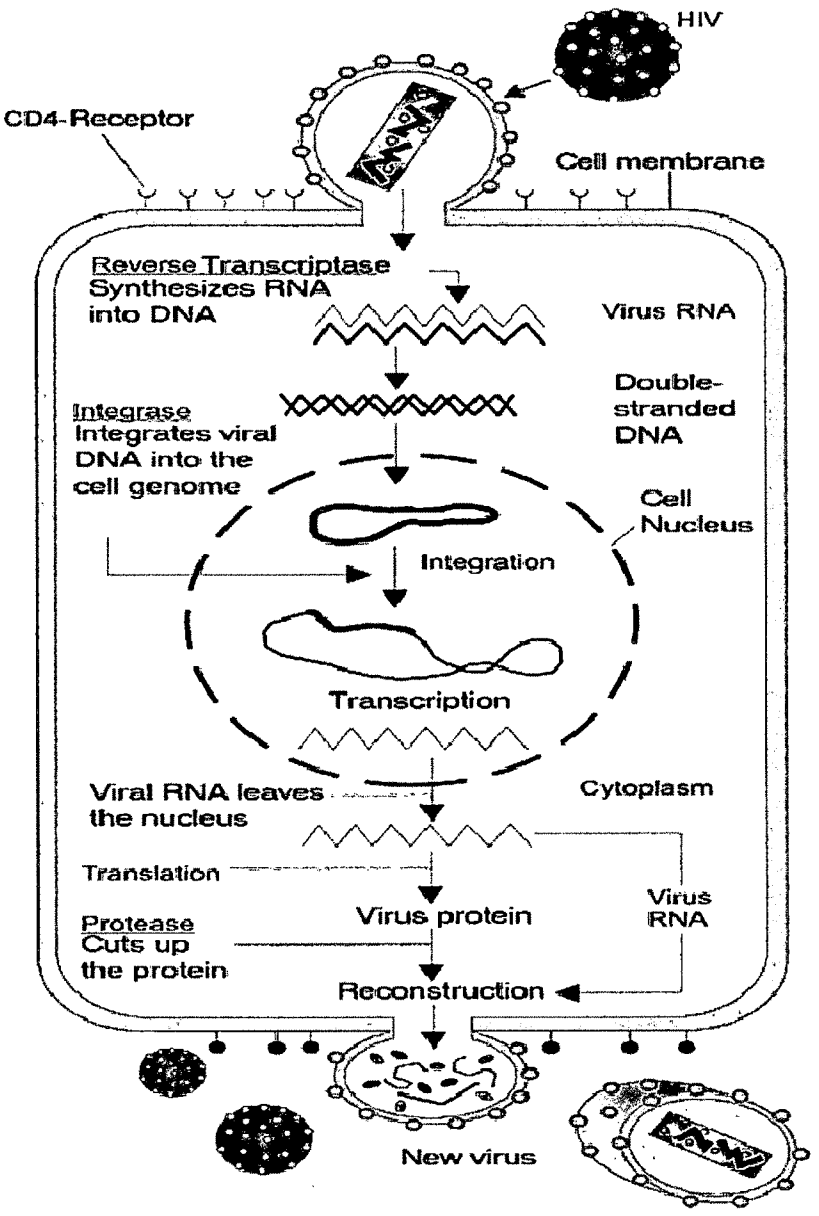
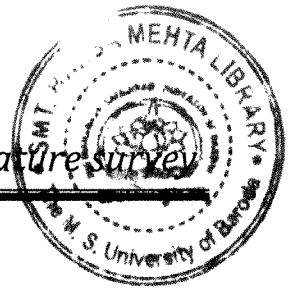


Fig.1: Life cycle of HIV.



Assembly and release

The new mRNA codes for the new viral proteins that contributes to the reconstruction of the HIV-RNA. The viral proteins help the mRNA and the reconstruction proteins to transport into the cell membrane. The structural components of the virus accumulate at the membrane of the infected cell to construct the HIV virion. Left over proteins (cleaved by the protease) associated with the inner surface of the host-cell membrane, along with the HIV RNA, are released to form a bud from the host cell, and can proceed to infect other healthy cells.[76]

HIV undergoes rapid genetic variation caused primarily by the enormous number of viruses produced daily in an infected individual. Because of this variation, HIV presents a moving target for drug and vaccine development. The variation within individuals has led to the generation of diverse HIV-1 subtypes, which further complicates the development of effective drugs and vaccines. In general, it is more difficult to hit a moving target than a stationary target. Presently a combination of different inhibitors is used to inhibit this moving target.[78] This combination is called Highly Active Anti Retroviral Therapy (HAART).

RT of the human immunodeficiency virus type 1 (HIV-1) is a crucial target for inhibition of HIV-1 replication, which can be inhibited by two classes of drugs belonging either to the nucleoside reverse transcriptase inhibitors (NRTIs) or to the non-nucleoside reverse transcriptase inhibitors (NNRTIs). The first RT inhibitors approved were NRTIs which compete as triphosphates with normal nucleoside substrates for incorporation into the viral genome, thus behaving as chain terminators. Unlike NRTIs, NNRTIs bind in a noncompetitive manner to a specific pocket of the HIV-1 RT, which is closely associated with but distinct from the substrate binding site, altering its ability to function. NNRTIs gained the greatest importance for anti-HIV activity because of their specificity and low cytotoxicity. All NNRTIs bind to a hydrophobic pocket near the polymerase active site. NNRTIs were found to be a more potential class than the NRTIs and nucleotide reverse transcriptase inhibitors (NtRTIs) because they differ structurally from the nucleoside

analogs. They do not interfere with the human cell cycle and are specific inhibitors of reverse transcriptase enzyme of HIV-1.[79]

Reverse transcriptase

RT is the replicative enzyme of HIV and other retroviruses. RT copies the single-stranded viral genomic RNA into double-stranded DNA **(Fig.1)**, which is subsequently integrated into host cell DNA. RT has two enzymatic activities: a polymerase that can copy either RNA or DNA and an RNase H that degrades the RNA strand of RNA–DNA intermediates formed during viral DNA synthesis. HIV-1 RT is composed of two subunits, p66 and p51 both having the same N terminus. p66 has 560 amino acid residues and p51 has 440 residues.[80] p66 contains two domains: polymerase and RNase H. p51 lacks the RNase H domain. The polymerase domain of p66 and p51 contains four common subdomains, termed 'fingers', 'palm', 'thumb' and 'connection'. The folding of the individual subdomains is similar in p66 and p51, but the spatial arrangement of the subdomains differs markedly. p66 contains the active sites for both polymerase and RNase H; p51 primarily plays a structural role. Highly conserved regions in the fingers and palm subdomains of p66, together with two helices of the thumb subdomain, act as a clamp that helps position the template primer **(Fig.2)**. One of these regions (part of the palm subdomain) is the DNA 'primer grip'. The primer grip is responsible for the appropriate placement of the primer terminus at the polymerase active site and is involved in translocation of the template–primer following nucleotide incorporation.[81-84] Appropriate binding/positioning of the template–primer is also important for appropriate cleavage of the RNA–DNA substrate by the RNase H activity of RT. HIV-1 RT inhibitors currently available as anti-AIDS drugs, target the polymerase activity of the enzyme.[84-86]

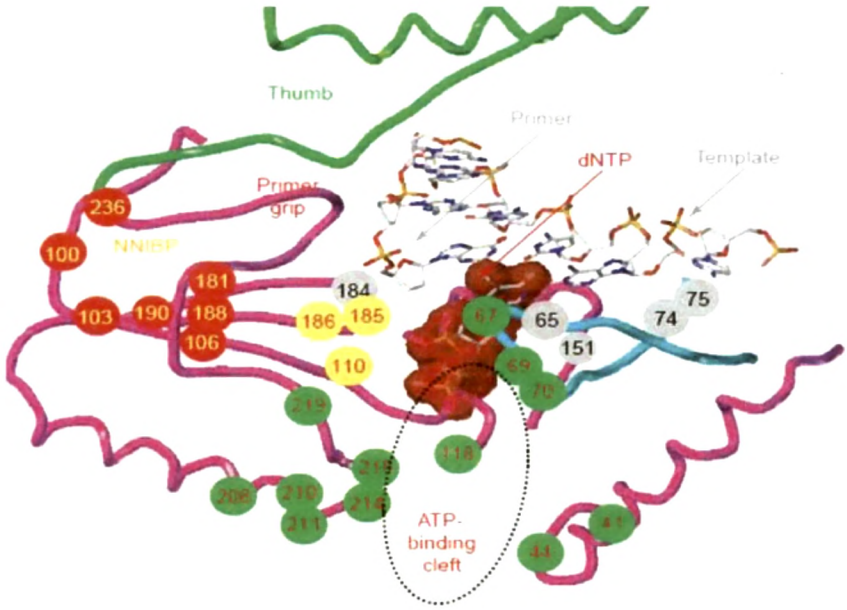
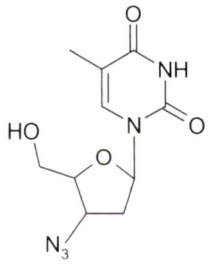


Fig.2. The structure of HIV-1 RT in the region near the polymerase active site.

NRTIs

Taking into account that the virus encodes its own polymerase, the development of RT inhibitors began and active inhibitors were developed within a few years of HIV discovery. The first inhibitor that received much attention was zidovudine (**73**),[87-88] approved by the FDA in 1987, which was followed by the discovery of didanosine (ddI) **(80)**,[89] dideoxycytidine (ddC) (**81**),[89] didehydrodideoxythymidine (d4T) (**82**),[90-91] 3'-thiadideoxycytidine (3TC) (**83**),[92] abacavir (**84**),[93] emitricitabine ((-)FTC) (**85**)[94] and tenofovir disoproxil fumarate (TDF) **(86)**.[95-96]



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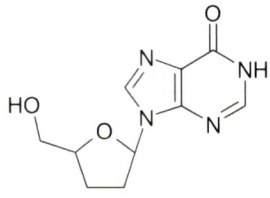

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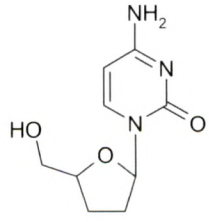

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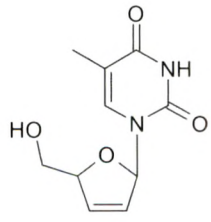

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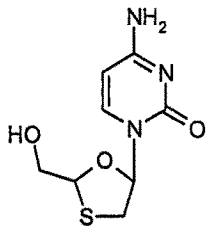

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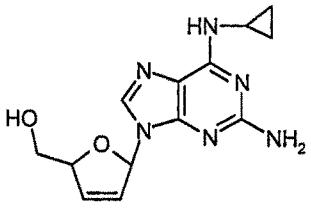

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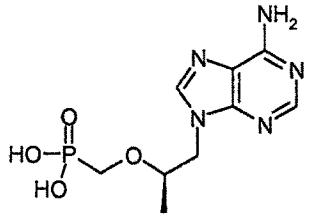

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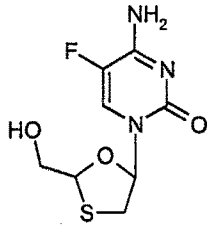
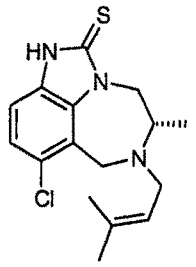


(86)

The above eight compounds were approved by FDA for clinical use alone and/or in combination. NRTIs inhibit viral replication because they lack a hydroxyl group at the 3' position of the ribose ring and, when incorporated into viral DNA, act as chain terminators. For NRTIs to be effective against HIV, they must be taken up by the host cell, phosphorylated by a series of cellular enzymes to the triphosphate form, bind at the polymerase active site and act as substrate mimics that can be incorporated into viral DNA by HIV-1 RT. Because NRTIs are analogs of normal nucleotides, they can also be incorporated into the DNA of the host, thereby resulting in toxicity.

NNRTIs

The era of NNRTIs began before two decades, with the discovery of HEPT[97] and TIBO[98-99] as specific inhibitors of reverse transcriptase. Following this, thirty different structural classes of compounds were reported as NNRTIs viz., TIBO derivatives [i.e. 8-chloro-TIBO (R86183, tivirapine)] **(87),**[100] HEPT derivatives [MKC-442] **(88),**[101] dipyridodiazepinones [nevirapine (BIRG-587)] **(89),**[102-103] pyridinones (L-697,661) **(90),**[104] BHAP derivatives [delavirdine (U-90152)] **(91),**[105] TSAO derivatives (TSAO-m^3T) **(92),**[106-107]



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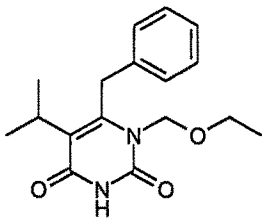

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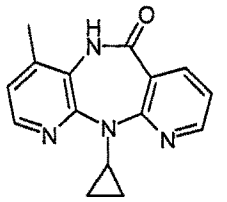

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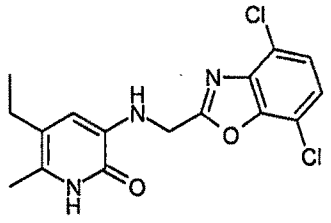

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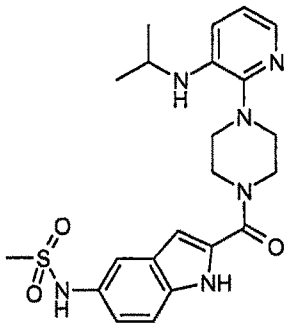

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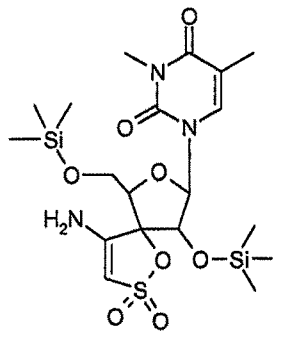
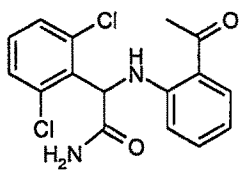


(92)

α-APA derivatives [loviride] **(93)**,[108] PETT derivatives [trovirdine] **(94)**,[109] thiocarboxanilide derivatives (UC-781) **(95)**,[110-111] quinoxaline derivatives (HBY 097) **(96)**,[112-113] thiazolobenzimidazole (NSC 625487) **(97)**,[114-115] thiazoloisoindolinones **(98)**,[116-117]



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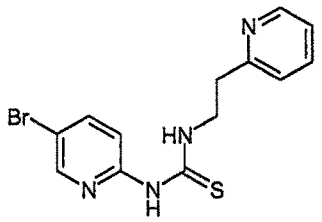

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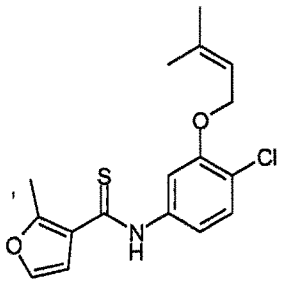

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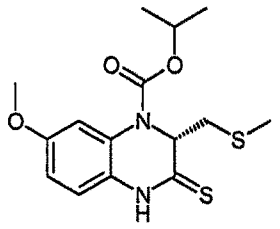

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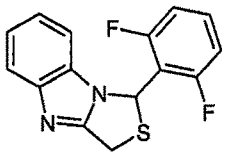

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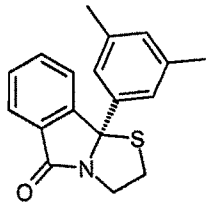
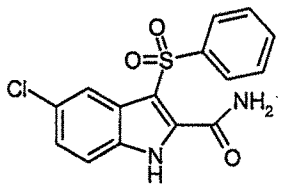


(98)

indole carboxamide L-737,126 **(99)**,[118] benzothiadiazine NSC 287474 **(100)**,[119] quinazolinones **(101)**,[120] benzoxazinones (efavirenz) **(102)**,[121] calanolide A **(103)**,[122-123] pyrrolobenzodiazepinones **(104)**,[124]



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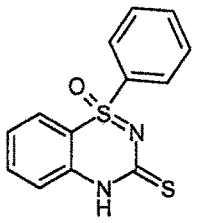

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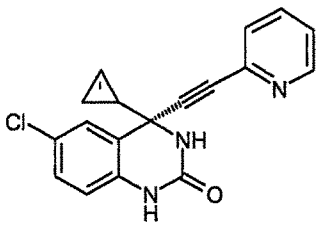

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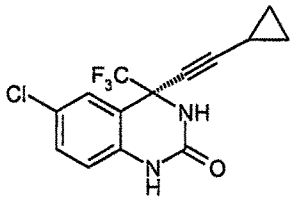

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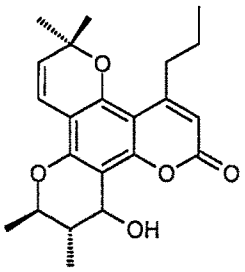

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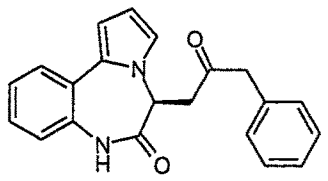

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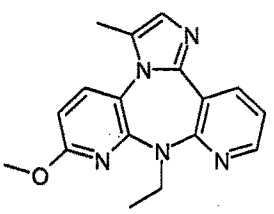
imidazodipyridodiazepine UK-129,485 **(105)**,[125] imidazopyridazines **(106)**,[126] thiadiazolyl dialkylcarbamates (TDA RD-4-2024) **(107)**,[127-128] arylpyridodiazepine and -thiodiazepine derivatives (MEN 10979) **(108)**,[129] DABO derivatives **(109)**,[130-131] HEPT-pyridinone hybrids **(110)**,[132]



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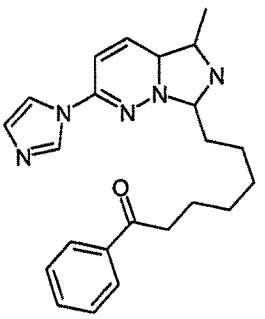

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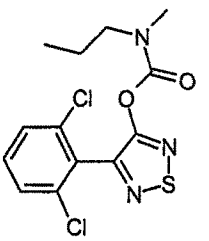

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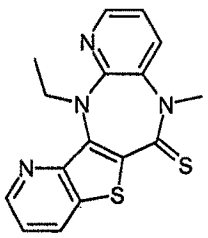

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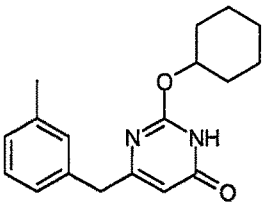

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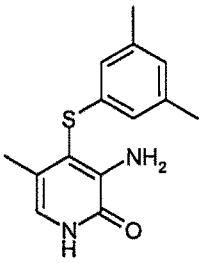
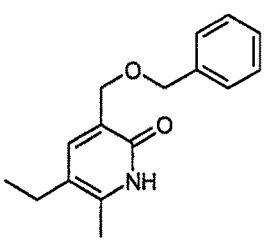


(110)

benzyloxymethylpyridinones **(111)**,[133] alkoxy(arylthio)uracils **(112)**,[134] indolyl dipyridodiazepinones **(113)**,[135] pyrrolobenzoxazepinones **(114)**,[136] highly substituted pyrroles **(115)**,[137] the benzylthiopyrimidines U-31355**(116)**[138] and pyridazinobenzoxazepinones,[139] pyrrolobenzothiadiazepines,[140] indolobenzo-thiazepines,[141] and trioxothienothiadiazine (TTD) derivatives.[142]



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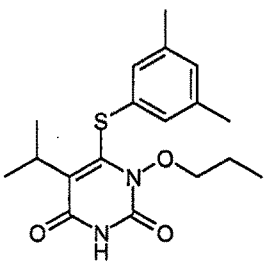

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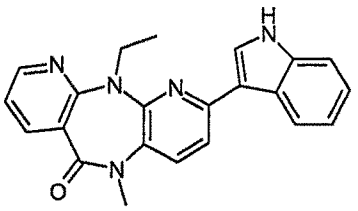

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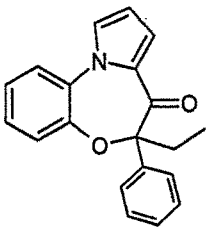
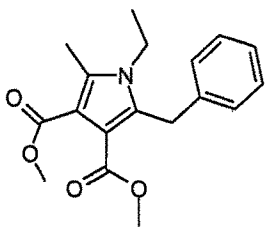
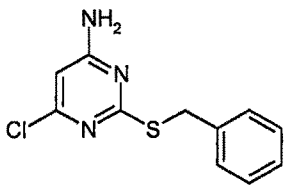






(114) **(115)** **(116)**

NNRTIs are highly specific and potent inhibitors of HIV-1 RT, and do not interfere with cellular or mitochondrial DNA synthesis.[143] However, the rapid emergence of resistant virus variants and the problem of resistance have limited their clinical use.[144]

Drawbacks of currently available inhibitors

Currently, HAART[145] is used for the treatment of AIDS. It is a combination of drugs to circumvent the problem of resistance development. It successfully decreases the blood viral load, and increases the CD-4 cell count in the body. The major drawback of this therapy is serious CNS complications, including mainly psychiatric and neurological complications.[146] AIDS dementia complex (ADC) is the most serious complication in AIDS patients requiring withdrawal of therapy, which further causes the emergence of blood viral load.[147] Currently approved drugs are not able to act on the virus which remains in the brain reservoirs.[148] therefore requiring the drugs to cross the blood brain barrier (BBB). Although some drugs from the NRTIs class are able to cross the BBB they have their own side effects.[149]

Role of small inhibitor

The binding of NNRTI forces RT residue W229 to change its position slightly, leading Y181 and Y188 residues to adopt another rotamer conformation. Consequently, the binding pocket would be substantially larger than it was before NNRTI binding, forcing the primer-template into an inactive binding conformation and rendering the protein inactive. This volume change is a direct consequence of the different positions of the Y181, Y188 and W229 side chains before and after NNRTI binding. When Y181 and

Y188 are mutated to cysteine residues, the volume change due to NNRTI binding is smaller and the impact of NNRTI inhibiting the RT mutants would be attenuated.[150-153]

The existence of CNS viral reservoirs is the main cause of recurrence of HIV infection when medication is withdrawn. This problem remains unsolved because of poor CNS penetration of the existing anti retroviral drugs. To meet the demand of crossing the blood-brain barrier (BBB) the compound should have a polar surface area (PSA) value in the limit 60-70 Å as reported by Kelder et al.[154] For meeting this requirement the compound should be a small hydrophobic compound. TMC-125 and TMC-278 with a size of 110 Å and 98 Å respectively inhibit viral growth belonging to wild type as well as mutant species. But, because of requirement of high dose and inability to cross the BBB, the problem remains. To solve this problem the inhibitor must be small to achieve sufficient drug concentrations in the brain.

NNRTIs as small inhibitors

NNRTIs are a potential class of HIV-1- RT inhibitors due to their structural diversity and specificity towards the HIV-1-RT. As discussed before, they bind to the hydrophobic region of the enzyme which is far from NRTIs binding site. This site is called the non nucleoside inhibitor binding pocket (NNIBP), which is near the polymerase active site. It is 10 Å from the polymerase active site and 60 Å from RNase H region.[155-156] The NNIBP lies between two β sheets, one containing the catalytic aspartates (β9–β10) and the other containing the primer grip (β12–β13–β14). Structures of HIV-1 RT without a bound NNRTI do not have an NNIBP, the binding cavity is created by torsional rotations of the side chains of Y181 and Y188, and repositioning of the second β sheet, containing F227 and W229.[212] Binding of an NNRTI traps a conformation of the NNIBP that may be accessible during the polymerization reaction, perhaps accompanying displacement of the primer grip during translocation following nucleotide incorporation. The NNIBP is formed primarily by residues from the p66 subunit, including aromatic residues Y181, Y188, F227, W229 and Y318, and hydrophobic residues P95, L100, V106, V179, L234 and P236. A small portion of the NNIBP is formed by p51; a single amino acid from the p51 subunit (E138) interacts with some NNRTIs.[157-158]

From recently reported literature of binding modes of inhibitor and enzyme [159] and the role of specific regions on the enzyme in reverse transcription process, two strategies for the design of novel inhibitors are suggested:

The first strategy can be addressed by understanding the mechanism(s) of drug resistance and developing drugs that effectively inhibit mutant viruses. There are three primary mechanisms, by which a mutation can confer resistance to NNRTIs, **1)** Loss or changes of interactions: Mutation of either Y181 or Y188 to non-aromatic hydrophobic residues (usually Y181C and Y188L) causes resistance to many NNRTIs by reducing the contribution of aromatic interactions that are important for the binding of these NNRTIs,[213,159-160] **2)** steric hindrance: The G190A/S mutations appear in response to treatment with HBY 097 and cause resistance to NNRTIs. Modeling an alanine or serine at glysine residue 190 shows that the sidechains of these amino acids would be in steric conflict with HBY 097. The G190A/S mutations may also cause similar steric conflicts with other NNRTIs, including nevirapine and efavirenz.[161-162] and **3)** indirect effects: Its mainly includes mutation of K103, L100, V108 and V106 amino acids. The K103N mutation is both unusual and particularly clinically important because it causes broad-spectrum resistance to most NNRTIs. The structure of the unliganded HIV-1 RT K103N mutant showed that there is a hydrogen bond between the phenoxyl group of Y188 and the side chain of N103. This interaction is not present in the wild-type enzyme; the interaction helps stabilize the structure of the unliganded K103N mutant in a conformation in which the entrance to the NNIBP is closed. This makes it difficult for an NNRTI to enter the NNIBP of the K103N mutant, resulting in resistance to multiple NNRTIs. The other mutation of L100I shows that an isoleucine at position 100 of these complexes would cause steric hindrance. This steric hindrance would cause changes either in the conformation of the inhibitor and/or in the geometry of the NNIBP. Mutations at residues relatively distal to NNRTIs in the NNIBP (V108I and to a lesser extent V106A) appear to have an indirect effect on NNRTI binding by changing the positioning of amino acids that interact directly with a bound NNRTI. The V108I mutation appears to cause NNRTI resistance by affecting the way Y181 and Y188 interact with the inhibitor. [213,163-164] From the thirty structurally diverse classes of

compounds the diaryl pyrimidines, diaryl triazines, TIBO, HEPT, DABO, imidazoles and thiozolidones were found to be effective. Interaction studies of all classes revealed that the second generation NNRTIs effectively encountered three primary mechanisms of resistance by assuming conformational as well as rotational changes. They are flexible, highly hydrophobic, and tightly bind in the NNIBP. All have inhibitory activity in subnanomolar concentrations but the disadvantage observed with these classes is their larger size and higher hydrophobicity. This creates pharmacokinetic problems like higher dose (TMC-125) requirement, and not reaching the viral reservoirs of the brain.[162]

The second strategy is to target the amino acid or the highly conserved area like the primer grip region where least amount of mutation was observed. It includes four amino acids in the highly conserved region W229, F227, L234, Y318. The first three are part of the primer grip. The role of primer grip is to maintain the primer terminus in appropriate orientation for nucleophilic attack on an incoming dNTP.[167] The crucial role of the primer grip was recently investigated and found that several mutations in this region significantly compromise RNA and DNA-dependent DNA polymerase activities.[168-169] In particular, mutations of W229, including the conservative mutations W229F and W229Y, reduced RT activity to less than 2% .[170] Moreover, mutation of the key W229 has never been observed in combination with other mutations, indicating that compensatory mutations to restore RT activity of the W229 mutated enzyme do not occur easily.[171] Hence, W229 has been proposed to be crucial for correct protein folding and/or for stabilizing the complex between RT and the template-primer. This proposal is supported by the experimentally determined structures of HIV-1 RT in complex with a double stranded DNA template- primer12 (binary complex)[172] and of the covalently trapped catalytic complex of HIV-1 RT (mimicking the ternary complex).[173] HIV-1 RT X-ray structures prove that the movement of the primer grip region (W229) with respect to Y181 and Y188 (located, together with the catalytic residues D185 and D186, in the β9-β10 hairpin) is crucial for enzyme function. On the other hand, the locking of the W229 position represents the molecular basis of NNRTI activity.[174]

In conclusion, results indicate that Trp-229 (W229) is a prime amino acid candidate within the HIV-1 RT for targeted design of NNRTIs due to the following reasoning: (1) It is not possible to mutate Trp-229 without severe loss of RT activity and virus infectivity, (2) mutating Trp-229 does not result in a high resistance profile to NNRTIs and (3) it is feasible to target Trp-229 with NNRTIs because of its physical participation in creating the NNRTI-characteristic binding pocket. Because targeting one crucial amino acid in the RT is insufficient to afford efficient resistance suppression, it is believed that designing new drugs should be concomitantly targeted at different immutable amino acids, like Trp-229.

Accordingly, design of compounds based on the hypothesis that targeting the conservative residue W229 for more extensive interaction with an inhibitor may lead to the discovery of potent NNRTIs that are less sensitive to resistant mutations. The design of compounds based on this hypothesis was by modifications made on the pyrimidines, pyridines, pyrimidininones, pyrrolobenzoxazepines, thiazolidines and imidazoles. From interaction studies it was found that unsaturated group on the meta position of the aryl ring was necessary.

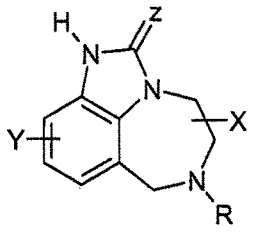
2-Aminopyrimidines as NNRTIs

The discovery of pyrimidines as NNRTIs is the outcome of eighteen years of research of its lead compound 4,5,6,7-tetrahydro-5-methylimidazo[4,5,l-[j 1k,]4]benzodiazepin-2(1H)-ones (TIBO) (**87**) derivative.[175] The structure based lead discovery started with this finding.

4,5,6,7-Tetrahydro-5-methylimidazo[4,5,l-[j 1k,]4]benzodiazepin-2(1H)-ones (TIBO)

The first of the lead compounds in the NNRTI class is TIBO (**117**). The anti HIV activity of this class was reported in 1990.[175] Compound (**118**) is the prototype of this class. The potency of compound (**118**) was rather weak, with an EC_{50} of 62 μM but with specific

activity.[176] Compound (**119**) (trivirapine) was the most active compound in this series having activity with EC_{50} of 0.0043μM.[177]



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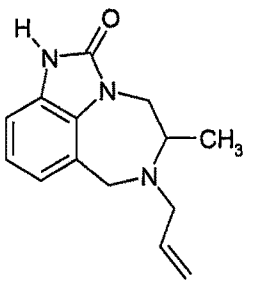

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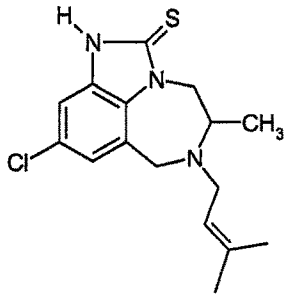

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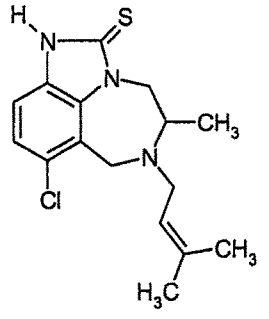


(87)

Each compound binds to a hydrophobic pocket close to, but distinct from, the substrate binding site, and each compound adopts a butterfly-like shape (**Fig.3**).[178] In the case of the TIBO compounds, the dimethylallyl substituent interacts with the side chains of Tyr181, Tyr188, and Trp229, and the chlorophenyl group interacts with Leu100, Lys101, and Tyr318. The thiourea NH group of TIBO forms a critical hydrogen bond with the main chain carbonyl oxygen atom of Lys101.[179-181] The distance between amino acid of NNIBP and 8-Cl of TIBO is shown in **Fig.4.**

The potency of (**118**) was rather weak, with an EC_{50} of 62 uM and the advantage of specificity. The activity was improved by synthesizing compounds with modification of X, Y, Z and R substitution (**117**), whereby compound (**119**) with 8-Cl substitution on aromatic ring and unsaturated dimethylallyl group on the diazepine ring system was

found to be the most active with high activity against wild type and some mutant strains of HIV-1 virus.[182] It was seen that except at Y, other modifications do not show any remarkable change in activity.

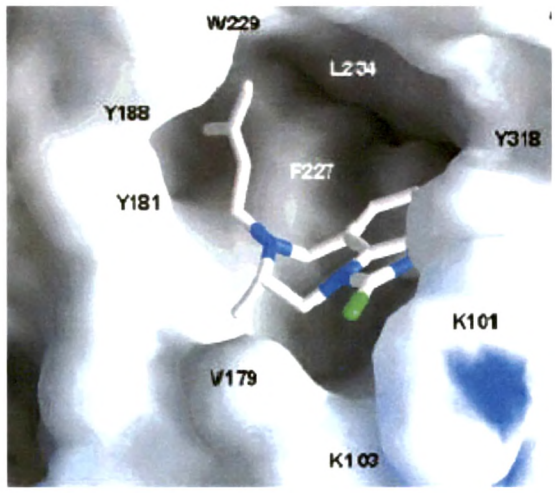


Fig.3. Molecular surface diagram showing the structure of the non-nucleoside inhibitor binding pocket and the interactions with TIBO derivative.

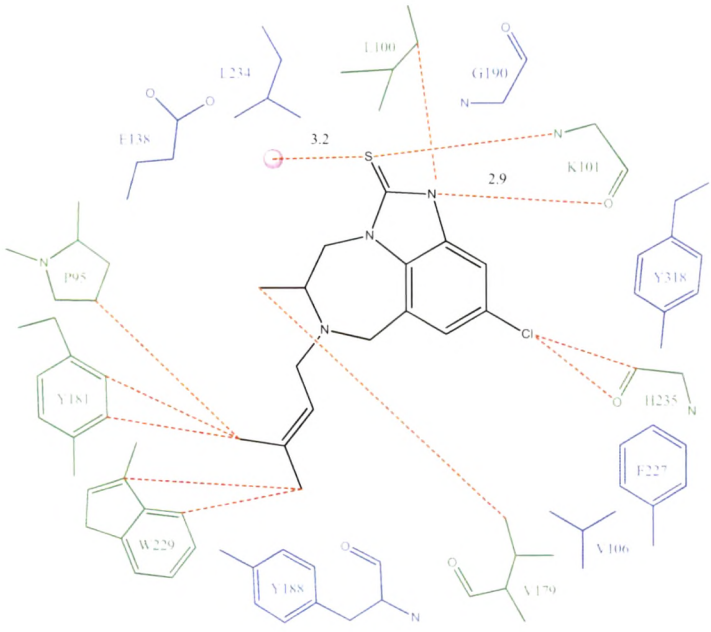
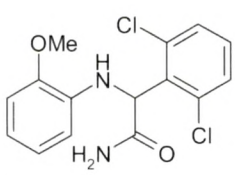


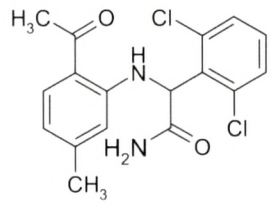
Fig.4. TIBO (119) positioned in the NNRTI-binding pocket of HIV-1 RT

α-Anilinophenylacetamides (α -APAs)

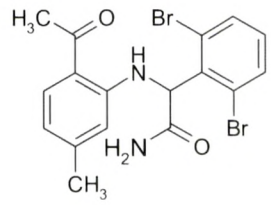
With the discovery of α-anilinophenylacetamides (α –APAs) **(120)**, the era of flexible derivatives started. These exhibited EC_{50} values of 610 nM.[183] The most active compound in this series found is loviride (**121**) with an EC_{50} of 13 nM, and an inhibitory concentration 10,000 fold less than the cytotoxic concentration. The compounds in this series were found to bind to the allosteric pocket of RT. X-ray analysis of a cocrystal of loviride analog R95845 (**122**) with HIV-RT revealed that the 4-methylacetophenone group occupies the western section of the binding pocket, whereas the 2,6-dihalophenyl substituent is positioned in the eastern section **(Fig.5)**.[184]



HIV-1=610 nM **(120)**



HIV-2=13nM **(121)**



(122)

Loviride **(121)** was chosen and pursued through Phase II clinical trials. However its development was discontinued when it became apparent that there was no significant advantage over the NNRTI therapies already approved at that time.

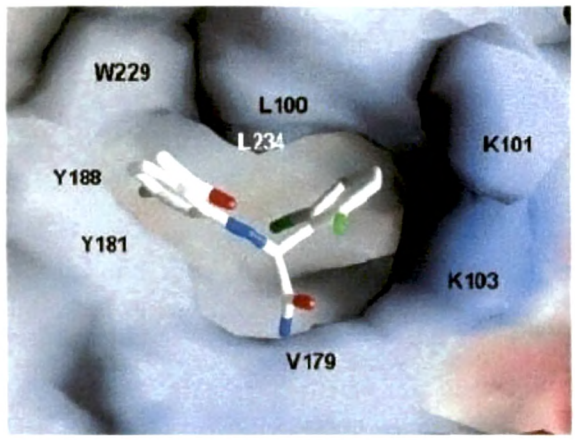
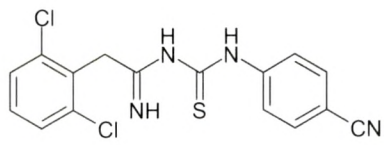


Fig.5. Molecular surface diagram showing the structure of the non-nucleoside inhibitor binding pocket and the interactions with loviride analogue (121).

Imidoyl Thiourea (ITU)

Imidoyl thiourea was derived from the SAR studies of loviride **(121)** and resulted from the modification of the basic structure. The changes made were keeping p-cyanophenyl substitution on one side producing the most active compound **(123)**. It was found that this compound had superior activity against commonly mutant species of HIV-1.[185]



(123)

However, the hydrolytic instability of the imidoyl thiourea functionality posed a problem during formulation studies. An obvious solution to this problem was to synthesize the well known cyanoguanidine bioisostere. But, this compound was not stable as it immediately cyclized to the triazine. Evaluation of this unexpected product indicated that it was nearly as active as **(123)**.

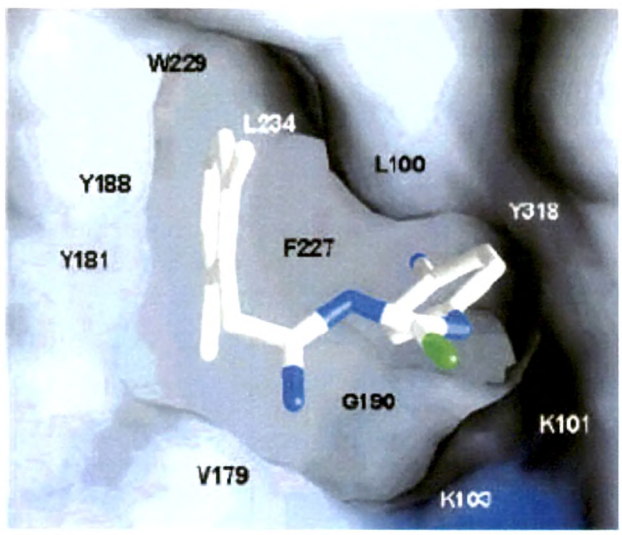
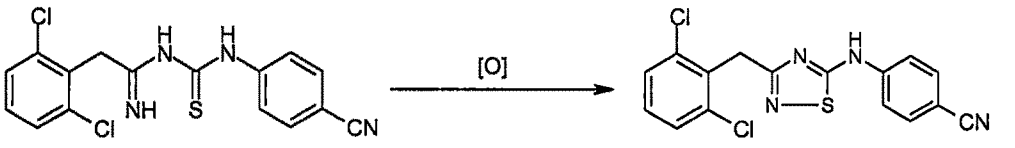


Fig.6. Molecular surface diagram showing the structure of the non-nucleoside inhibitor binding pocket and the interactions with (123).

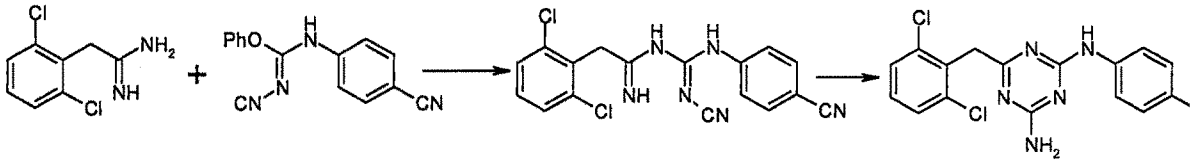
The interaction of **(123)** shown in **Fig.6**, indicated the di-chloro substituted phenyl ring had strong hydrophobic interactions in Y181, Y188 and W229 pocket.[137]

Diaryl triazine (DATA)

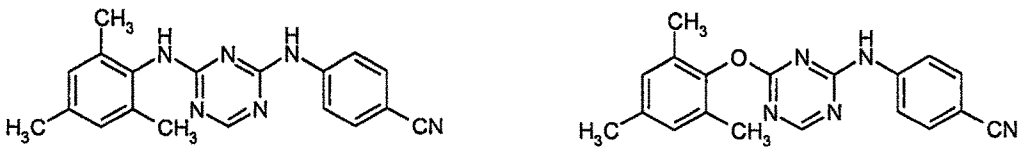
DATA is the fourth modification in the TIBO family. It is an outcome of the bioisosteric modifications of ITUs. As mentioned above imidoyl thiourea **(123)** was quickly prone to both hydrolytic lability and oxidative instability. The latter was shown by the facile conversion of **(123)** the to thiadiazole **(124)**, a compound that was completely devoid of HIV inhibitory activity. This suggested that compounds in the ITU series were able to achieve favorable conformations for binding to HIV-1 RT, a property not available to the more rigid thiadiazole system. Hence, a strategy of attempting to stabilize the ITUs while maintaining their conformational flexibility was proposed. In this context, an attempt was made to prepare compound **(127)**, which incorporated a cyanoguanidine moiety, a well-known thiourea bioisostere.[186] It gets cyclized to a triazine derivative **(128)** .[187]



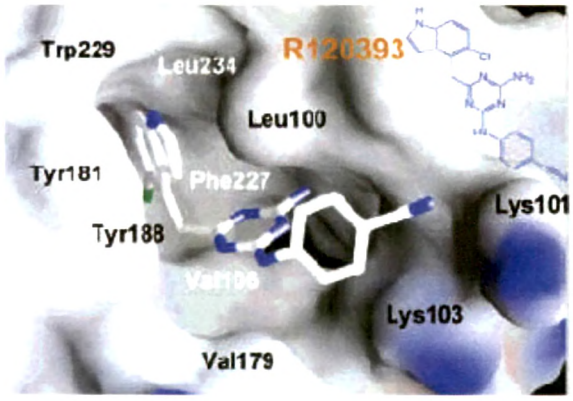
(123) **(124)**

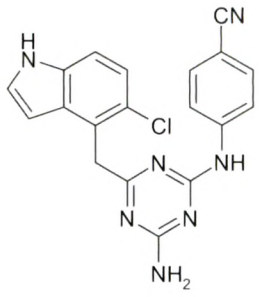


(125) **(126)** **(127)** **(128)**



(129) **(130)**





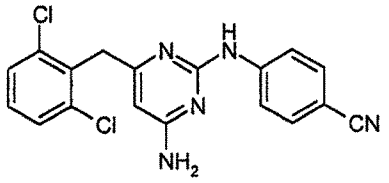
R 120393

Fig.7. **Binding modes of DATA analogue.** **(131)**

Molecular modeling studies of compound **(129)** and **(130)** showed the 2,4,6-trimethyl ring was positioned in the important Y181, Y188, W229 region of the enzyme, while the C-4 position was located at the opening of the binding pocket. More extensive interaction was found with the more potent derivative of this series R120393 **(131)** where the 5-chloro substituted indole ring placed in the hydrophobic tunnel of the RT enzyme as shown in **Fig. 7.**[137]

Diaryl Pyrimidines (DAPY)

With the discovery of a group of diaryltriazines (DATAs) as a novel class of NNRTIs, the goal of discovering hydrolytically stable inhibitors was achieved. As previously discussed, HIV is a moving target having a very high mutation rate. The DATA compounds show decreased activity against the double mutant. This consideration led to the development of the next series of TIBO family, the diarylpyrimidines (DAPY).[188] The structure–activity relationship of a variety of dichlorobenzyltriazines **(128)** was explored and synthesis of the three isomeric pyrimidine analogs [**(132)**, **(133)** and **(134)**] of triazine was pursued in an attempt to gain insight into the importance of the central heterocycle.



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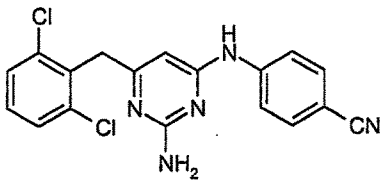

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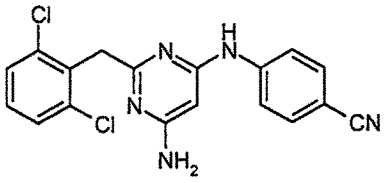
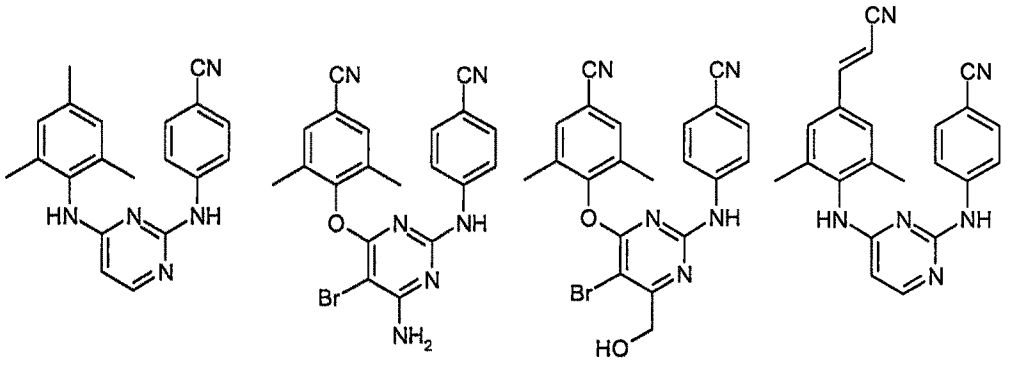


(134)

Compounds **(132)**, **(133)** and **(134)** were designed by the replacement of nitrogen by carbon from the triazine ring of DATA compounds. The conformational adaptability and flexibility of the DAPY compounds makes it active against most of the mutant species of the virus.



TMC-120 **(135)** TMC-125**(136)** R-1545508**(137)** TMC-278**(138)**

In this series TMC-120 **(135)**, TMC-125 **(136)**, R-185545 **(137)** and TMC-278 **(138)** were the most active compounds. TMC-125 was marketed. The receptor binding study of this class reveals that they have ability to bind in different conformations in the binding pocket. Surprisingly, four types of binding conformations were observed with this compound which makes it active against mutant species possibly due to the ability to

undergo rotational and conformational changes due to its flexibility. The compound assumed binding conformation according to changes in the binding pocket, a feature observed in the mutant species of the reverse transcriptase enzyme **(Fig. 8)**.[137]

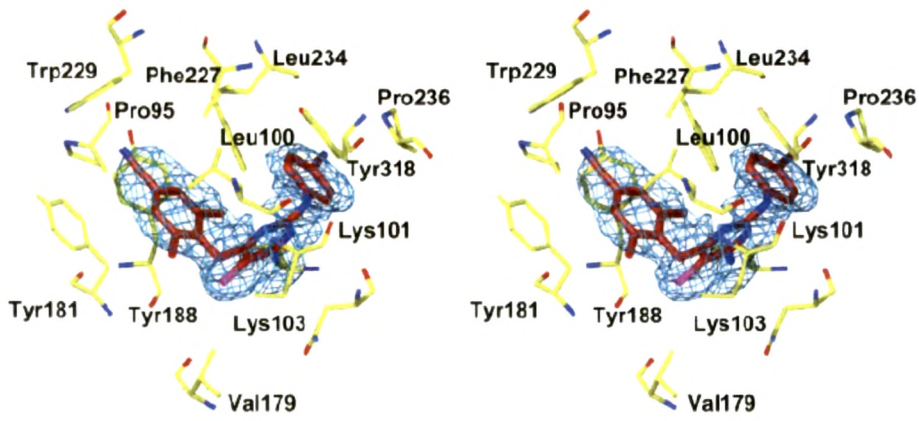


Fig.8. Different binding conformations of TMC-125 observed in mutant species of the reverse transcriptase enzyme.

In a study it was found that the cyanophenyl ring occupies the eastern pocket made up of hydrophobic residues like Tyr181, Tyr188 and W229.[181] Molecular modeling studies suggested that extension of the wing I pharmacophore in the direction of the conserved Trp229 residue could greatly enhance the activity of the ligand against the wild-type virus and its resilience to mutation. Also, since substitutions of Tyr181 and Tyr188 led to resistance to many NNRTIs, the plan favored reduction of binding dependence on interactions with Tyr181 and Tyr188. The improved activity of TMC-278 **(138)** on wild-type and mutant HIV-1 strains may involve a specific interaction of the cyano group in wing I of the molecule with the indole ring of Trp229 **(Fig.9)**.[189] The presence of an additional torsional degree of freedom in TMC-278 (the flexible dihedral angle between the anilino ring and cyanovinyl moiety) relative to earlier DAPY analogs is also likely to contribute to the excellent resistance profile of TMC-278.[190]

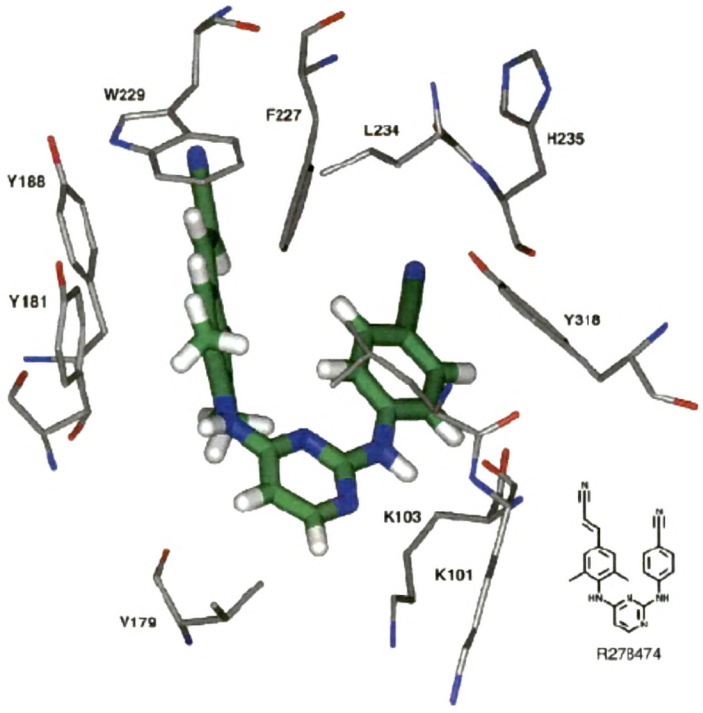


Fig.9. The binding modes of TMC-278 (138).

2-Aminopyrimidines as small inhibitors

The era of 2-aminopyrimidines as NNRTIs started with the discovery of diarylpyrimidines (DAPY and TMC-120) in 2001. TMC-278 was the most active compound in the series, and TMC-125 (etravirine) was marketed. The discovery of DAPY was the result of the structure based drug discovery of the lead compound TIBO, the first of the NNRTIs. A step by step modification of this compound is TMC-125.[181] The discovery tree of 2-aminopyrimidines from TIBO shown in **Fig.10**.

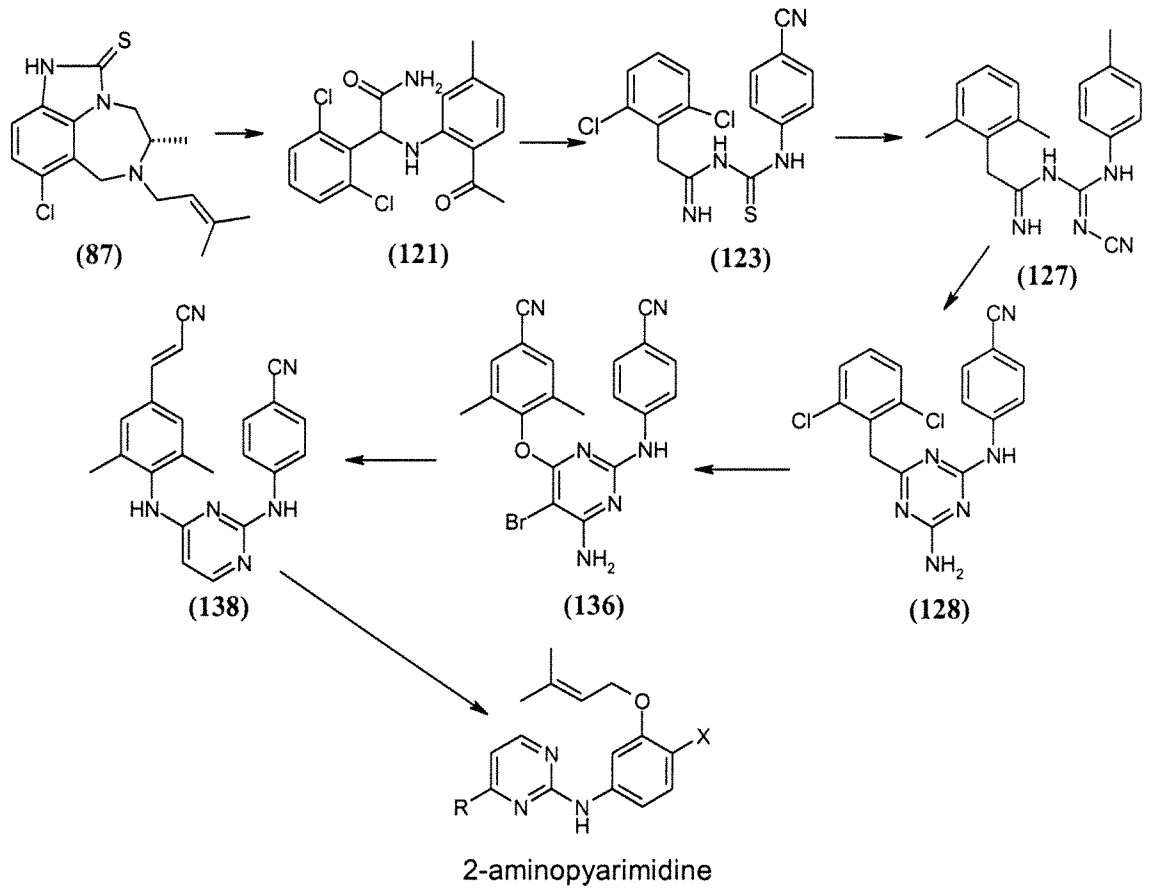
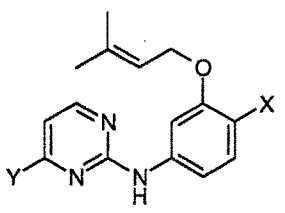


Fig.10. The evolution of 2-aminopyrimidnes.

TIBO (*t*etrahydro*i*midazo*b*enzodiazepin*o*ne) **(87)** analogues, the first NNRTIs, were discovered in 1987 by screening a subset of the Janssen compound library of pharmacologically "inactive" compounds in a cell-based anti-HIV test at the Rega Institute. Subsequent screening of Janssen compounds led to the discovery of the α-APA (α-*a*nilino*p*henyl*a*cetamide) **(121)** class of NNRTIs. Further chemical modification led to the class of potent ITU (*i*mino*t*hio*u*rea) **(123)** NNRTIs. In an attempt to synthesize the corresponding imino-*N*-cyanoguanidine derivatives **(127)** of ITU analogues, an unexpected ring closure occurred, producing R106168 **(128)**, the first compound of the DATA (*dia*ryl*tria*zine) class of NNRTIs. In 1996, molecular modeling studies suggested replacing the central aminotriazine ring of DATA with a pyrimidine ring. This led to the

class of DAPY (*diarylpyrimidine*) NNRTIs, of which TMC125 **(136)** is the prototype. DAPY compounds were found to be most active against the wild type as well as mutant species of HIV virus. The conformational adaptability and rotational flexibility of these compounds makes them active against the commonly observed mutations of K101N, Y188L and Y181C. The disadvantage with the DAPY compounds was their very high therapeutic dose requirement (900 mg bid).[190-191] Also the higher molecular size of these compounds makes them impenetrable into the brain, due to which they are not able to act on the virus present in the brain reservoir. Hence, therapy is an almost a life time requirement. Therefore, efforts were made to design novel inhibitors for RT using computer aided drug design. This lead generation was performed using BOMB programme. The output from BOMB includes a spreadsheet with a row for each ligand containing its predicted activities, protein–ligand energetic and structural results, and QikProp results for predicted properties including solubility and cell permeability. Taking the above design considerations, focused virtual libraries were generated in two motifs, U–Het–NH–Ph and Het–NH–Ph–U, where U is an unsaturated group as the hydrophobic group and HET represents the heterocycle.[192]



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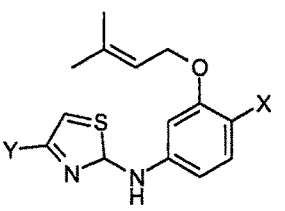
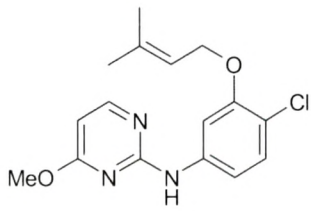


(140)

In 2006, many compounds were synthesised with changes in the various heterocycles in the reported motif for NNRTIs. These compounds were termed diamines. Pyrimidines **(139)** and thiazole **(140)** as the heterocycle were first optimized.[193-194]



(141)

The interactions of ligand **(141)** a pyrimidine heterocycle and reverse transcriptase enzyme is shown in **Fig.11**,

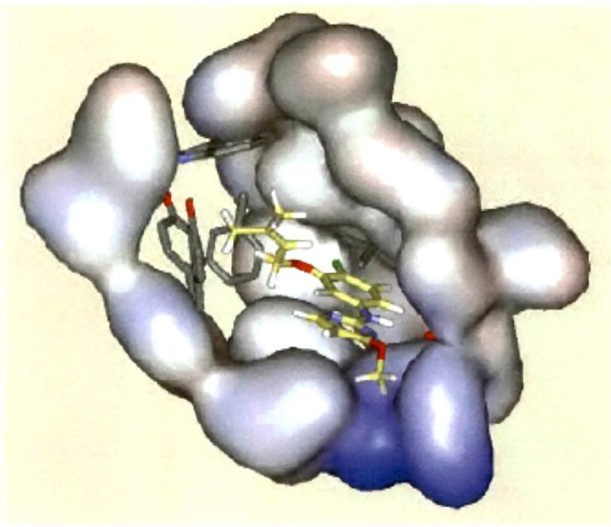


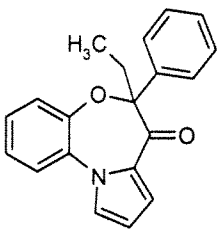
Fig.11. Computed structure for (141) bound to RT.

Computed structure for **(141)** bound to RT; Y181, Y188, F227, W229 on the left; L100 and the K101 C=O on the right. The methoxy group of **(141)** can be accommodated either to the right, as shown, or left.

Pyrrolobenzoxazepinones (PBOs)

The anti HIV activity of pyrrolobenzoxazepinones (PBOs) **(114)** was reported in 1996. This compound was designed to overcome the drawbacks of poor brain barrier

penetration of the available drugs and emergence of resistance. This class of compounds resulted from a random screening of tricyclic compounds. PBOs were designed to be used in combination with AZT (**73**) (Zidovudine) to achieve high brain concentration[195-194] with compound **(142)** having an activity 0.05 μM in wild type enzyme and 0.022 μM in K103N mutant enzyme. This compound is however less active against other mutant species. The main structural difference that arises at the NNBS level after K103N mutation is that W229 bends down to Y181 and Y188 and the side chain of the latter establishes a H-bond interaction with N103 which consequently closes the NNRTI binding site.[167] In 2002, the role of primer grip in reverse transcription process and the role of hydrophobic tunnel (Y181, Y188, and W229) were reported. Especially the role of some highly conserved amino acid residues in the hydrophobic region. W229 is one of the important amino acid residues where less mutation was observed and with a role in the tight binding of inhibitor with the enzyme. The novel PBOs were designed to specifically bind to the highly conserved residue W229.[168-169]



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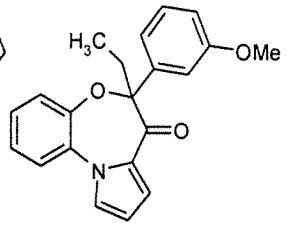

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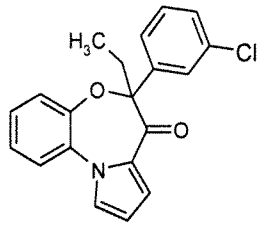
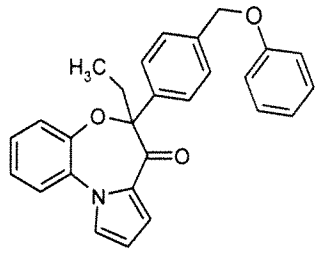


(143)



(144)

From docking studies of the compounds it was hypothesized that the capability of NNRTIs to override K103N resistance could be related to their ability to penetrate the binding site by establishing a strong interaction with W229. The 3-methoxysubstituted phenyl ring of **(142)** penetrates into the aromatic cleft formed by W229, Y181 and Y188 more deeply in comparison to the benzo-fused moiety of 3-chloro substituted phenyl **(143)** thereby establishing larger contacts with W229. A new series of compounds were synthesized by an extended aromatic system at C-6, to enhance PBO interaction with the primer grip region (F227-H235) and in particular with the conserved amino acid W229.

From the synthesized series, compound **(144)** exhibited activity against wild type and comparable activity with mutant enzyme. Its docking studies showed extended phenyl ring interactions with W229. The pendant 4-(phenyloxymethyl)-phenyl moiety elongates in the aromatic pocket bound by Y188, Y181, and W229.[167]

The docking of the designed compounds is shown in **Fig.12** below:

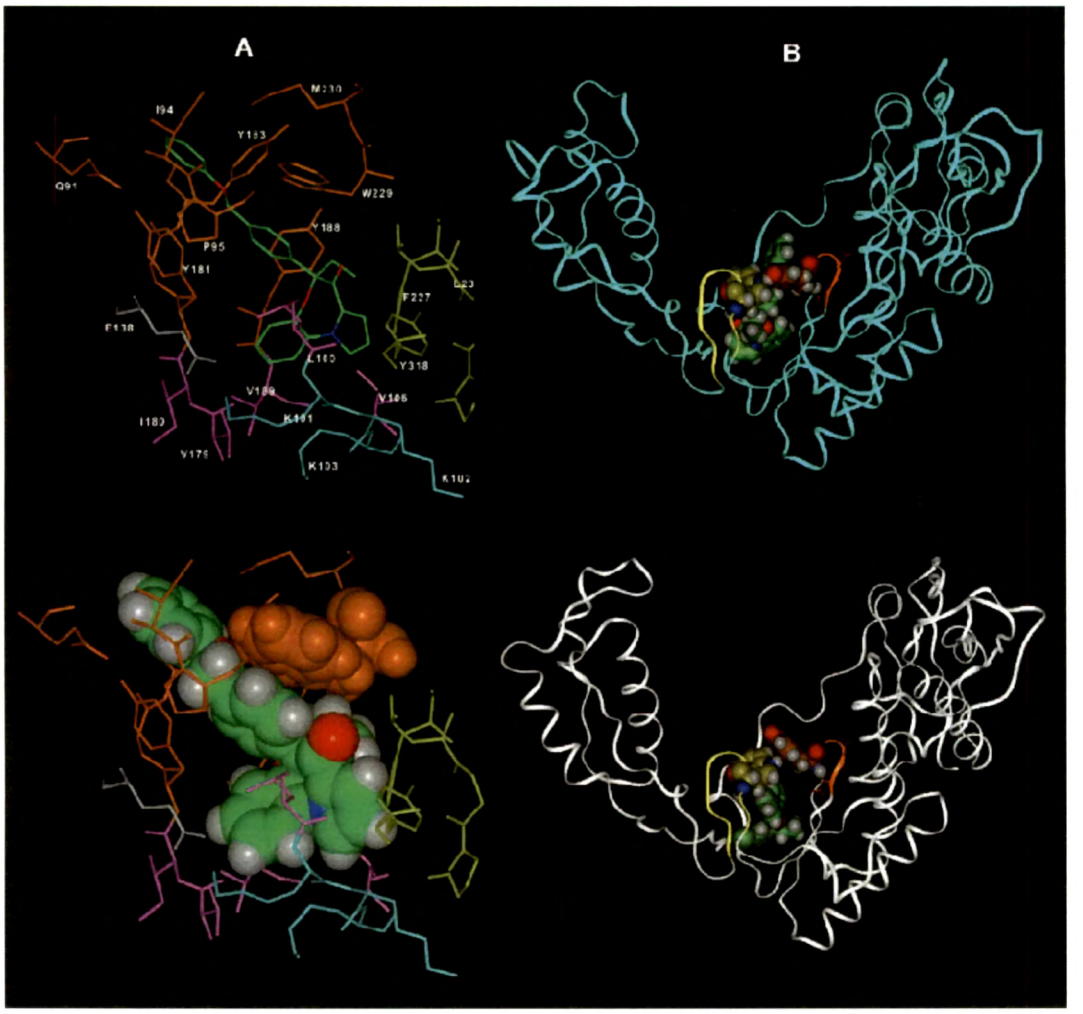


Fig.12. Compound (S)-(144) (colored by atom type) docked into the HIV-1 RT NNBS.

Interaction studies clearly indicate that a phenyloxymethyl substituent in the para position of **(144)** pendant phenyl ring at C-6 is able to establish favorable interactions within wild-type HIV-1-RT NNBS This is demonstrated by the fact that **(144)** is almost seven times more active than a PBOs analogue bearing a methyl group at the same position. The second phenyl ring of **(144)** protrudes toward the catalytic site of the enzyme through an extensive interaction with crucial residues in the primer grip region (F227, W229, M230) and in the β9-β10 hairpin (Y181, Y188, Y183).

Pyridinone Derivatives

The pyridinone compounds (developed by Merck Research Laboratories) are chemically related to the HEPT (1-[(2-hydroxyethoxy) methyl]-6-(phenylthio) thymine) series of NNRTIs and reported in 1991.[197] From QSAR approach a number of derivatives were synthesized among which three compounds were found to be the most active. Of these three, R157208 (5-ethyl -3-[(2-methoxyethyl)methylamino]-6-methyl-4-(3-methylbenzyl)pyridin-2 (1H)-one) **(145)** is a member of the benzylpyridinone subclass of NNRTIs.[198] The other two, R165481 (E-3-[3-(5-ethyl-3-iodo-6-methyl-2-oxo-1,2-dihydropyridin-4-yloxy) phenyl] acrylo -nitrile) **(146)** and R221239 (4-(3,5-dimethylphenoxy)- 5-(furan-2-yl methyl sulfanyl methyl)-3-iodo-6-methylpyridin-2(1H)-one) **(147)**, belong to the 3-iodo-4-aryloxypyridinone (IOPY) subclass.[199]

The pyridinone compounds R157208, R165481, and R221239 are chemically related to the HEPT (1-[(2-hydroxyethoxy) methyl]-6-(phenylthio) thymine) series of NNRTIs, with the central pyrimidinone ring of HEPT being replaced with a pyridin-2-one ring in the pyridinone compounds, and different substituents on the ring. When bound to RT, the two aromatic rings of R157208, R165481, and R221239 are in a butterfly-like conformation, which is seen in other RT/NNRTI complexes, although the ring orientations are different **(Fig.13)**. The substituted benzyl or phenoxyl ring corresponds to "wing I" of the butterfly and the pyridinone ring to "wing II".[200-201]

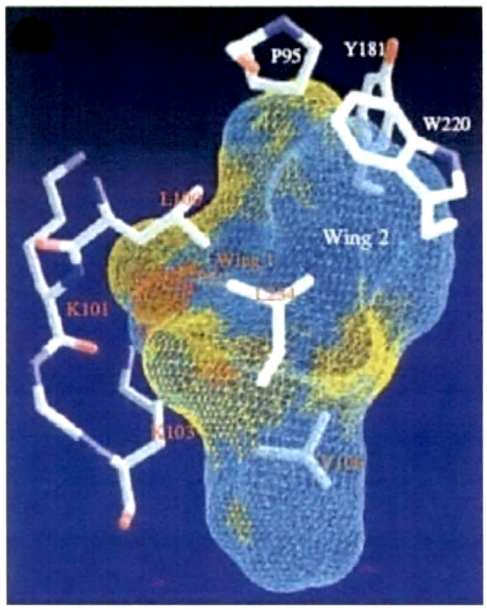
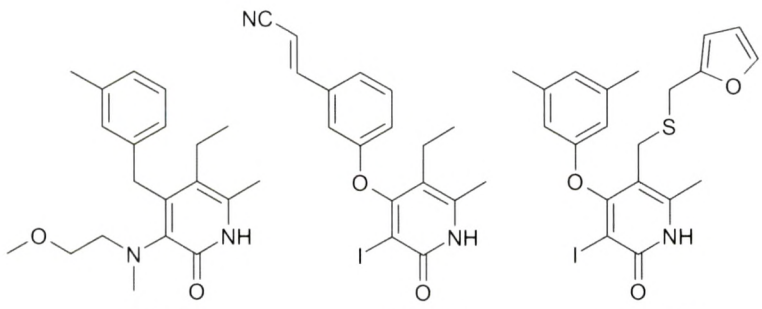


Fig.13. The composite binding pocket of the NNRTI active site of HIV-1 RT, which is illustrated as grid lines representing the collective van der Waals and is overlaid with the residues that constitute Wing 1 and Wing 2 of the butterfly-shaped binding pocket.



R157208 R165481 R221239

(145) **(146)** **(147)**

The interaction of R157208 (**145**) with the 3'-methylsubstituted benzyl ring at the 4-position of the pyridinone ring of R157208 makes extensive hydrophobic contacts with Trp229. This highly conserved residue is part of the primer grip and appears to be essential for normal polymerase activity.[202-203] The 4'-and 5-carbon atoms and the 3'-methyl group of R157208 interact with Trp229.[204]

Compound R165481 **(146)**, having the methylene linker between the two rings of R157208 replaced by an oxygen atom in R165481 and an acrylonitrile group substituted for the methyl substituent at the 3'-position of that ring resulted in substantially improved interactions of this inhibitor with the protein. The 3'-acrylonitrile substituent on the phenoxyl ring of R165481 **(146)** extends into a "tunnel". The amino acid residues Val108, Tyr188, Phe227, Leu228, and Trp229 form the walls of this tunnel. The tunnel leads out of the NNIBP toward the catalytic site, suggesting that structure-based modifications at this location might produce an inhibitor that can interact with the conserved amino acids of the polymerase active site.[202-203] The extensive interactions between the protein and the acrylonitrile substituent contribute to the inhibitor's subnanomolar activity against wild-type RT. These interactions may help to stabilize the position of the inhibitor in the pocket when NNRTI resistance mutations elsewhere in the NNIBP alter the interactions of the protein with the inhibitor. The C-β carbon of W229 have extensive interactions with the nitrile group of the acrylonitrile **(Fig.14)**.[204]

In the third compound R221239 **(147)**, the acrylonitrile substituent of the phenoxyl ring is replaced by methyl groups at the 3'- and 5'-positions of **(145)**, and the 5-ethyl substituent on the pyridinone ring replaced by a furfuryl methyl thioether **(Fig.14)**.[204-210]

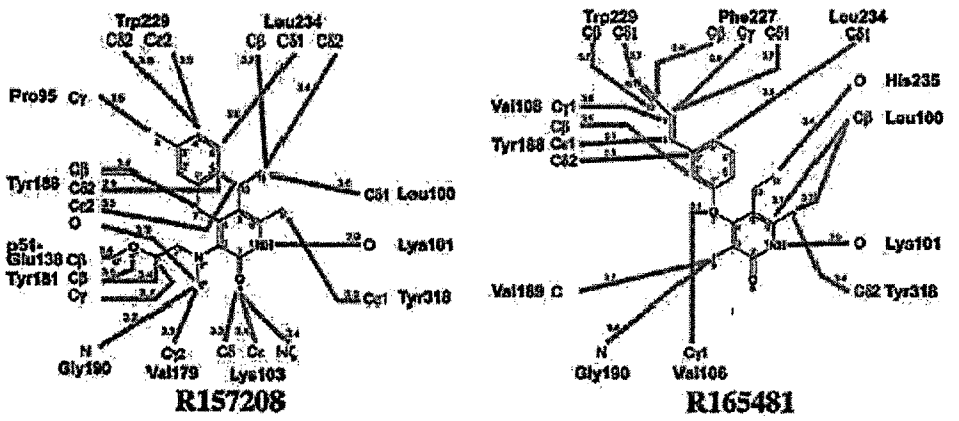


Fig.14. Schematic diagrams showing the contacts between HIV-1 RT and inhibitor R157208 and R165481.

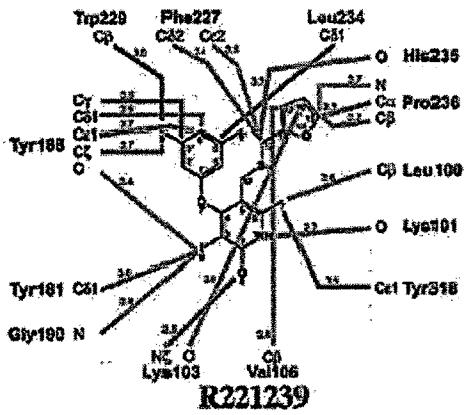
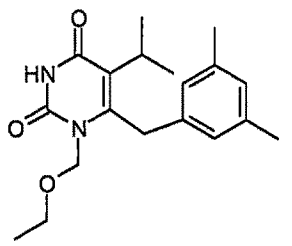
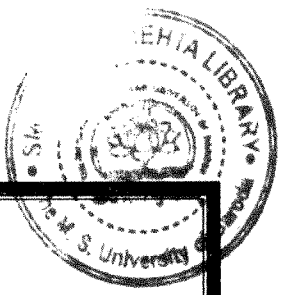


Fig.15. Schematic diagrams showing the contacts between HIV-1 RT and inhibitor R221239 respectively.

The *m*-methyl groups of the dimethylphenoxyl ring of R221239 **(147)** present a large surface area for contact with Trp229[206] similar to what has been seen in a structure of RT complexed with GCA-186 (6-(3',5'-dimethybenzyl)-1-ethoxymethyl-5-isopropyluracil) **(148)**, a HEPT derivative an analog of emivirine **(Fig.15)**.[204]



(148)

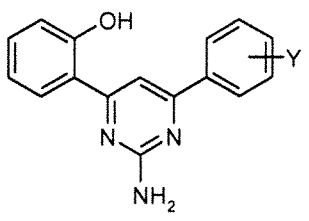


CHAPTER – I.2

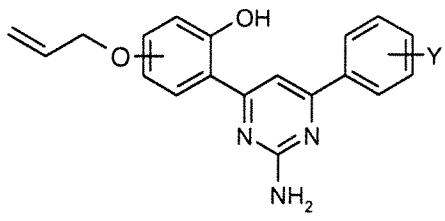
Aims and Objectives

AIMS AND OBJECTIVES

From literature review it is evident that in the treatment of HIV infection the role of NNRTI's are inevitable. The crystal structure of the enzyme and its binding sites are explored. Ideally, a molecule which has the butterfly or horse shoe shape was found to have better binding on the catalytic site of the enzyme. Both the side (wings) would have л interactions with the enzyme. These л donors should have a guanidine moiety at the center (body). All the currently available NNRTI's confirm this hypothesis. Diarylpyrimidines exactly match the basic requirements for an NNRTI. However, all the currently available drugs develop resistance due to change in the amino acid sequence of the enzyme. The change in amino acid sequence observed in the aromatic amino acids like tyrosine and tryptophan ultimately leads to the poor л interaction between the drug and the enzyme. Most often the wing A was found to have poor binding in the resistant mutants.[35] We have developed some diarylpyrimidines (DRR series) as NNRTI in the recent past.[16] We got promising activity from that series, however with poor selectivity indices. In order to improvise the selectivity indices and activity of these series of compounds, docking studies on those compounds were done.

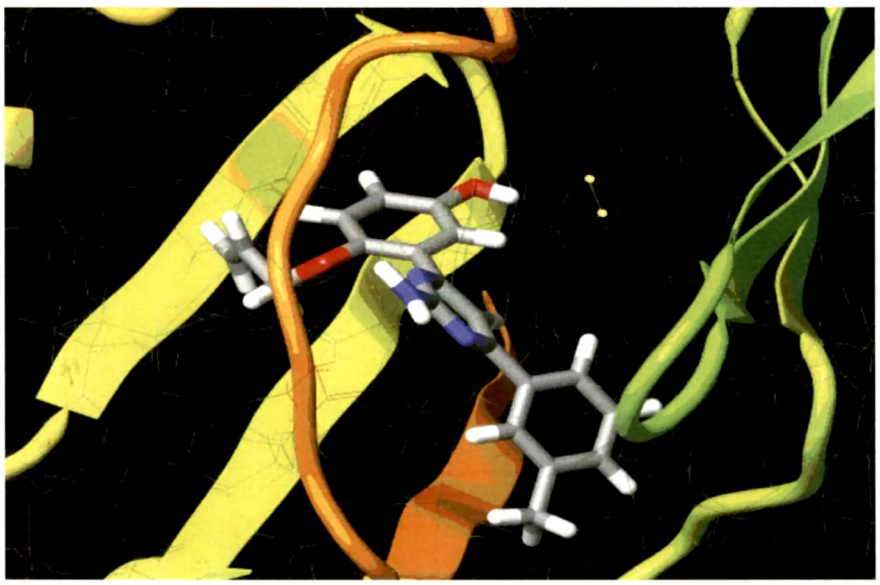


(DRR series)



(1-26)

Incorporation of an allyloxy group in one wing and performing docking studies gave us the following interaction:



Compound (**1**) showed interaction with amino acid residues like Tyr 181, Tyr 188, Trp 229, Phe 227, Gly 223.

Hence, it was proposed to introduce an allyloxy group in one ring for achieving anti-HIV activity.

CHAPTER – I.3

Results and Discussion

RESULTS AND DISCUSSION

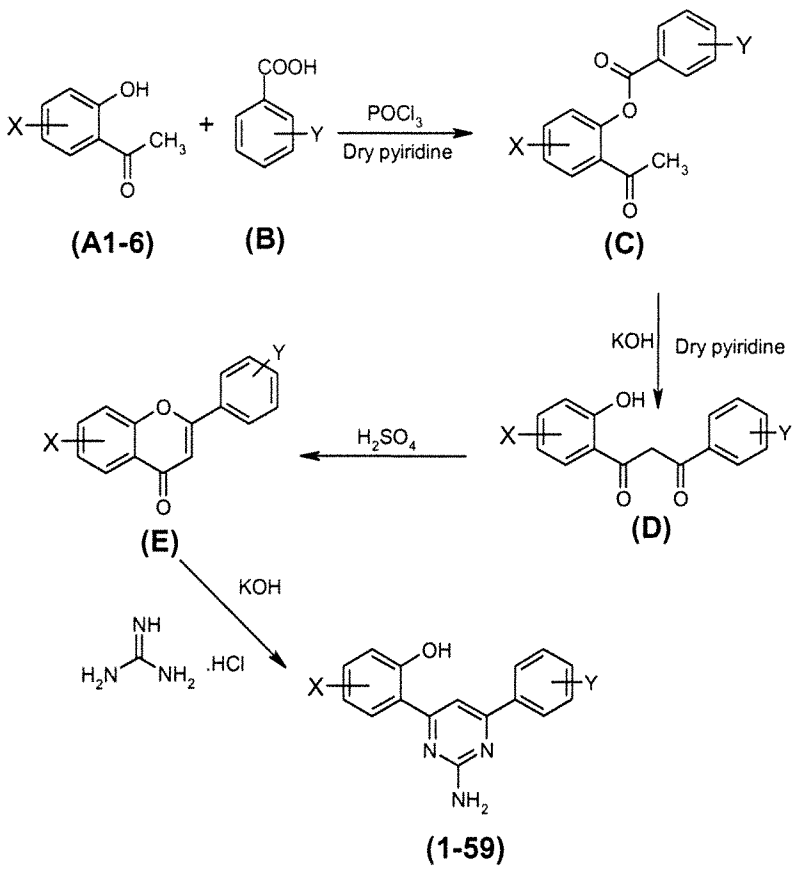
The result and discussion work is discussed in two parts:

I.3a. Chemical work

I.3b. Biological studies

I.3a. Chemical work

The synthesis of the compounds is presented below in **Scheme I**



X= **A1**=4-Allyloxy, **A2**=5-Allyloxy,

Y= H, 4-Methoxy, 3-Methoxy, 3,4-dimethoxy, 2-Methyl, 3-Methyl, 4-Methyl, 2-Cl, 3-Cl, 4-Cl, 2,4-Dichloro, 4-F, 4-Br, 4-NO_2,4-Allyloxy, furan

Scheme I

The substituted 4,6-diaryl-2-aminopyrimidines were synthesized according to the route presented in **Scheme I.** Condensation of 4/5-allyloxy-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 flavone derivatives in presence of sulphuric acid as the dehydrating agent. Treatment of flavones with a slight excess of guanidine hydrochloride in alkaline medium afforded 4,6-diaryl-2-aminopyrimidines.

The synthesis carried out has been discussed under following heads:

I.3a.1: Synthesis of 4/5-Allyloxy-2-hydroxyacetophenone

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

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

I.3a.4: Synthesis of 6/7-Allyloxy-2-(substituted phenyl)-4*H*-chromen-4-one derivatives

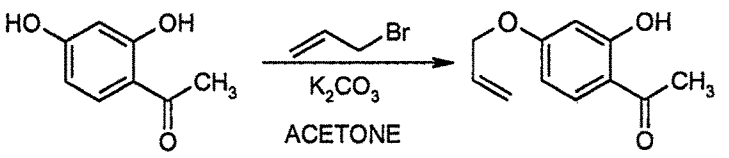
I.3a.5: Synthesis of 4-(4/5-Allyloxy-2-hydroxyphenyl)-6-(substutited phenyl)-2-aminopyrimidines

I.3a.1: Synthesis of 4/5-Allyloxy-2-hydroxyacetophenone (A1,2)

I.3a.1a. Synthesis of 4-allyloxy-2-hydroxyacetophenone (A1)

The synthesis of 4-allyloxy-2-hydroxyactophenone (**A1**) was afforded by selective allylation of 2,4-dihydroxyacetophenone (**I**) (Scheme II). 2,4-dihydroxyacetophenone was procured commercially. The formation of compound was confirmed by melting point and IR.

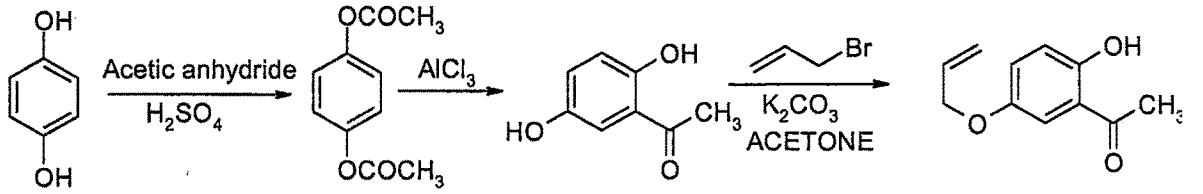
Scheme II



(I) **(A1)**

I.3a.1b. Synthesis of 5-Allyloxy-2-hydroxyacetophenone (A2)

Scheme III



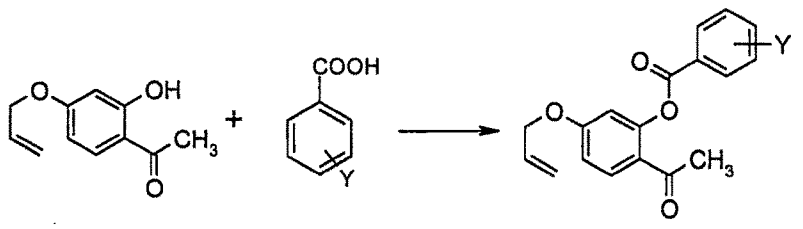
(II) **(III)** **(IV)** **(A2)**

The 5-allyloxy-2-hydroxyacetophenone (**A2**) was obtained by the selective allylation of 2,5-dihydroxyacetophenone (**IV**). 2,5-dihydroxyacetophenone was synthesized by Fries rearrangement of hydroquinone-1,4-diacetate (**III**) which was obtained by acetylation of hydroquinone (**II**) in presence of sulphuric acid. Compound (**A2**) displayed the absorption bands at 3066 (OH), 3000 and 999 cm^{-1} and 1640 cm^{-1} in its IR spectrum.

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

I.3a.2a. Synthesis of 5-allyloxy-2-acetyl-1-(substituted benzoyloxy) benzene derivatives (C1-C14)

The phenoxybenzoate **(C1-C14)** esters were obtained by condensation of 4-allyloxy-2-hydroxy -acetophenones and various substituted aryl/heteroaryl carboxylic acid derivatives in dry pyridine and phosphorus oxychloride. The carbonyl stretching of the esters are given in **Table 1**.



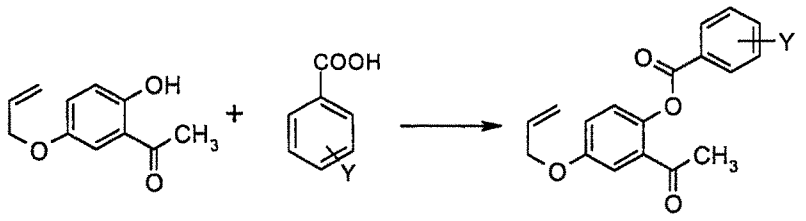
(A1) **(B)** **(C1-14)**

Table 1.

Compound No.	X	Y	M.P. (°C)
C1	5-allyloxy	H	68-70
C2	5-allyloxy	4-Cl	68-69
C3	5-allyloxy	3-Cl	Liquid
C4	5-allyloxy	2-Cl	57-58
C5	5-allyloxy	2,4-dichloro	Liquid
C6	5-allyloxy	4-F	84-85
C7	5-allyloxy	4-Br	Liquid
C8	5-allyloxy	4-NO_2	52-54
C9	5-allyloxy	4-OCH_3	80-81
C10	5-allyloxy	3-OCH_3	79-80
C11	5-allyloxy	3,4-OMe	91-93
C12	5-allyloxy	4-CH_3	43-45
C13	5-allyloxy	3-CH_3	Liquid
C14	5-allyloxy	Furan	57-58

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

The phenoxybenzoate (**C15-C26**) esters were obtained by condensation of 5-allyloxy-2-hydroxyacetophenones and various substituted aryl carboxylic acid derivatives in dry pyridine and phosphorus oxychloride . The carbonyl stretching of the esters are given in **Table 2.**



(A2) **(B)** **(C15-26)**

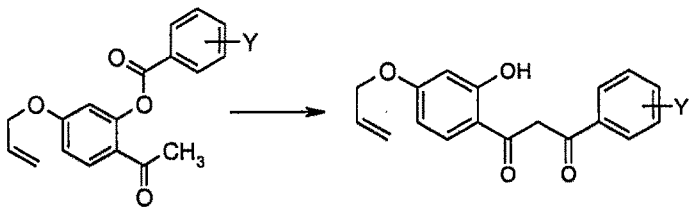
Table 2.

Compound No.	X	Y	M.P. (°C)
C15	4-allyloxy	H	Liquid
C16	4-allyloxy	4-Cl	61-63
C17	4-allyloxy	3-Cl	52-53
C18	4-allyloxy	2-Cl	71-72
C19	4-allyloxy	2,4-dichloro	74-75
C20	4-allyloxy	4-F	98-99
C21	4-allyloxy	4-Br	86-87
C22	4-allyloxy	4-NO_2	110-111
C23	4-allyloxy	4-OCH_3	70-72
C24	4-allyloxy	3-OCH_3	65-67
C25	4-allyloxy	4-CH3	83-85
C26	4-allyloxy	3-CH_3	Liquid

I.3a.3: Synthesis of 1- (4/5-allyloxy-2-hydroxyphenyl)-3-(substituted-phenyl)-1,3-propanedione derivative

I.3a.3a. Synthesis of 1-(4-allyloxy-2-hydroxyphenyl)-3-(substituted phenyl)-1,3-propanedione derivative (D1-D14)

The synthesized esters **(C1-C14)** 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 3.** The yellow crystals of 1,3-propanediones **(D1-D14)** were used for the next step.



(C1-C14) **(D1-D14)**

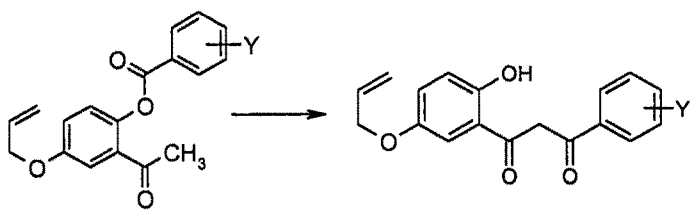
Table 3.

Compound No.	X	Y	M.P. (°C)
D1	4-allyloxy	H	80-82
D2	4-allyloxy	4-Cl	159-160
D3	4-allyloxy	3-Cl	89-90
D4	4-allyloxy	2-Cl	Liquid
D5	4-allyloxy	2,4-dichloro	Liquid
D6	4-allyloxy	4-F	Liquid
D7	4-allyloxy	4-Br	159-161
D8	4-allyloxy	4-NO_2	148-149
D9	4-allyloxy	4-OCH_3	Liquid

D10	4-allyloxy	3-OCH_3	128-129
D11	4-allyloxy	3,4-OMe	136-137
D12	4-allyloxy	4-CH_3	103-105
D13	4-allyloxy	3-CH_3	, 89-91
D14	4-allyloxy	Furan	102-103

I.3a.3b. Synthesis of 1-(5-allyloxy-2-hydroxyphenyl)-3-(substituted phenyl)-1,3-propanedione derivative (D15-D26)

The synthesized esters (**C15-C26**) 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 4.** The yellow crystals of 1,3-propanediones **(D15-D26)** obtained were used for the next step.



(C15-C26) **(D15-D26)**

Table 4.

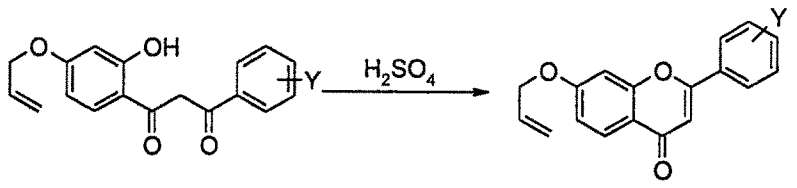
Compound No.	X	Y	M.P. (°C)
D15	5-allyloxy	H	Liquid
D16	5-allyloxy	4-Cl	84-86
D17	5-allyloxy	3-Cl	96-97
D18	5-allyloxy	2-Cl	90-91
D19	5-allyloxy	2,4-dichloro	93-94

D20	5-allyloxy	4-F	113-114
D21	5-allyloxy	4-Br	104-105
D22	5-allyloxy	4-NO_2	130-131
D23	5-allyloxy	4-OCH_3	149-150
D24	5-allyloxy	3-OCH_3	90-93
D25	5-allyloxy	4-CH_3	Liquid
D26	5-allyloxy	3-CH_3	86-87

I.3a.4: Synthesis of 6/7-allyloxy-2-(substituted phenyl)-4*H*-chromen-4-one derivatives

I.3a.4a. Synthesis of 7-allyloxy-2-substituted phenyl-4*H*-chromen-4-one derivatives (E1-E14)

The chromen derivatives were synthesized from 1,3-propanedione derivative **(D1-D14)** by the acid catalyzed cyclisation using glacial acetic acid as solvent. It produced light brown color crystals. The cyclised chromen derivatives **(E1-E14)** were confirmed by IR which gives specific stretching of conjugated C=C and C=O at 1675 cm^{-1} as shown in **Table 5.**



(D1-D14) **(E1-E14)**

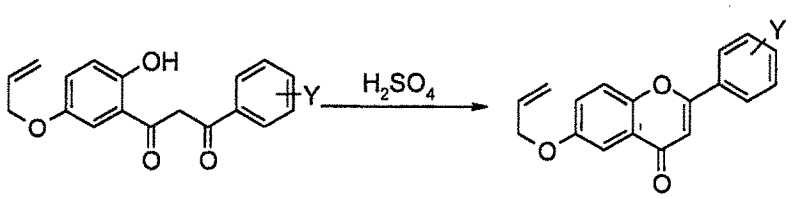
Table 5.

Compound No.	X	Y	M.P. (°C)
E1	7-allyloxy	H	99-100

E2	7-allyloxy	4-Cl	228-229
E3	7-allyloxy	3-Cl	118-119
E4	7-allyloxy	2-Cl	80-83
E5	7-allyloxy	2,4-dichloro	98-99
E6	7-allyloxy	4-F	123-124
E7	7-allyloxy	4-Br	181-182
E8	7-allyloxy	4-NO_2	187-189
E9	7-allyloxy	4-OCH_3	163-165
E10	7-allyloxy	3-OCH_3	148-149
E11	7-allyloxy	3,4-OMe	154-155
E12	7-allyloxy	4-CH_3	89-90
E13	7-allyloxy	3-CH_3	80-81
E14	7-allyloxy	Furan	169-171

I.3a.4b. Synthesis of 6-allyloxy-2-substituted phenyl-4*H*-chromen-4-one derivatives (E15-26)

The chromen derivatives were synthesized from 1,3-propanedione derivative (**D15-26**) by the acid catalyzed cyclisation using glacial acetic acid as solvent. It produces light brown colored crystals. The cyclised chromen derivatives **(E15-26)** were confirmed by IR which gives specific stretching of conjugated C=C and C=O at 1675 cm^{-1} as shown in **Table 6.**

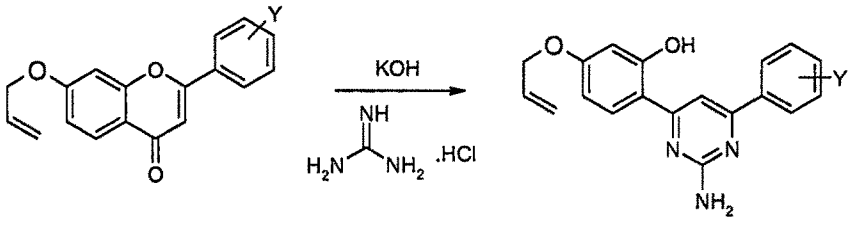


(D15-D26) **(E15-E26)**

Table 6.

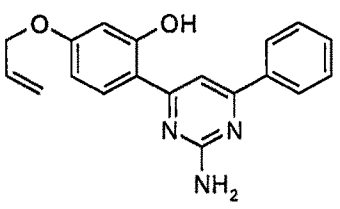
Compound No.	X	Y	M.P. (°C)
E15	6-allyloxy	H	NT
E16	6-allyloxy	4-Cl	151-152
E17	6-allyloxy	3-Cl	140-141
E18	6-allyloxy	2-Cl	160-161
E19	6-allyloxy	2,4-dichloro	104-106
E20	6-allyloxy	4-F	189-190
E21	6-allyloxy	4-Br	147-148
E22	6-allyloxy	4-NO_2	198-200
E23	6-allyloxy	4-OCH_3	180-182
E24	6-allyloxy	3-OCH_3	120-121
E25	6-allyloxy	4-CH_3	liquid
E26	6-allyloxy	3-CH_3	118-119

I.3a.5a: Synthesis of 4-(4-allyloxy-2-hydroxyphenyl)-6-(substutited phenyl)- 2-Aminopyrimidines

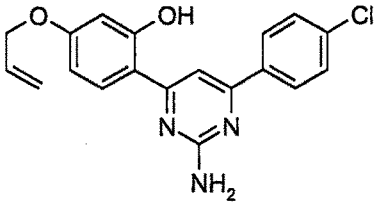


4-(4-allyloxy-2-hydroxyphenyl)-6-phenyl-2-aminopyrimidines (**1**) showed a characteristic peak of –OH stretching at 3207 cm^{-1} in the IR spectrum. The asymmetric and symmetric stretching of NH were observed at 3506 and 3369 cm^{-1} respectively. The characteristic C=N stretching of the pyrimidine ring system found at 1639 cm^{-1}. The

PMR spectrum displayed at δ 4.58 (2H, -O***CH***$_2$CH=CH$_2$) of allyloxy, 5.3-5.4 for terminal protons of allyloxy group (2H, OCH$_2$CH=***CH***$_2$), 6.0 (2H, -*NH*$_2$), 6.1 for alkene proton (1H, OCH$_2$***CH***=CH$_2$), aromatic proton at 6.4-6.5 (2H, Ar***H***), 7.4-8.0 (7H, Ar***H***) and hydroxyl proton at 14.15 (1H, -***OH***). The mass spectrum shows the M+1 peak at 319.9 (m/z).



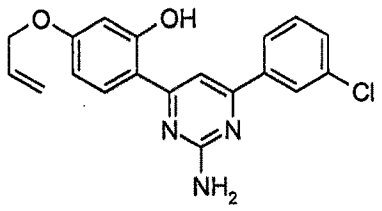
(1)



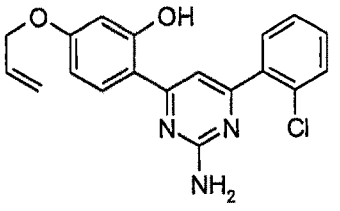
(2)

4-(4-allyloxy-2-hydroxyphenyl)-6-(4-chlorophenyl)-2-aminopyrinidine **(2)** was confirmed by its analytical data which shows stretching of amino group at 3501 and 3370 cm^{-1} in the IR spectrum. The –OH stretching observed at 3216 cm $^{-1}$ and C=N stretching observed at 1615 cm^{-1}. The PMR spectrum displayed at δ 4.57 (2H, O***CH***$_2$CH=CH$_2$) of allyloxy, 5.2-5.4 for terminal protons of allyloxy group (2H, OCH$_2$CH=***CH***$_2$), for amino proton 5.46 (2H, -*NH*$_2$), 6.0 (1H OCH$_2$***CH***=CH$_2$), 6.4-6.5 (2H, Ar***H***), 7.4-8.0 (6H, Ar***H***), 14.22 hydroxyl proton of (1H, -***OH***). The mass spectrum shows the M+1 peak at 354.1 (m/z).

4-(4-allyloxy-2-hydroxyphenyl)-6-(3-chlorophenyl)-2-aminopyrimidine **(3)** gives stretching of –NH at 3501 and 3361 cm^{-1} and -OH stretching broad peak at 3211 cm^{-1}. The C=N ring stretching observed at 1639 cm^{-1}. The PMR spectrum displayed at δ 4.59 (2H, O***CH***$_2$CH=CH$_2$) for allyloxy group, 5.3-5.4 (2H, OCH$_2$CH=***CH***$_2$) for terminal unsaturated protons, 6.0 (1H, -OCH$_2$***CH***=CH$_2$) for CH proton, 6.3 (2H, -*NH*$_2$), 6.4-6.5 (2H, Ar***H***), 7.4-8.1 (6H, Ar***H***) aromatic protons, 14.15 (1H, -***OH***) for aromatic hydroxyl group. The mass spectrum gives M+1 peak at 354.1 (m/z).



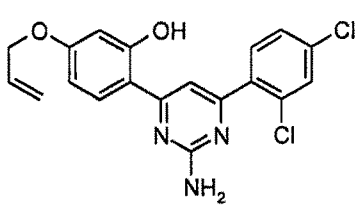
(3)



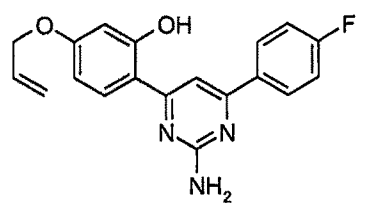
(4)

4-(4-allyloxy-2-hydroxyphenyl)-6-(2-chlorophenyl)-2-aminopyrimidine **(4)** gives stretching of amino group at3481 and 3390 cm^{-1} and hydroxyl stretching at 3303 cm^{-1}. The C=N ring stretching observed at 1645 cm^{-1}. The PMR displayed doublet at δ 4.56 (2H, -O***CH₂***CH=CH_2) of allyloxy group, δ 5.2-5.43 (2H, OCH_2CH=***CH_2***) of terminal protons of allyloxy group, sharp peak at δ 5.8 (2H, NH_2) of amino group, 6.0 (1H, OCH_2***CH***=CH_2) multiplet of CH proton of allyloxy group, the aromatic protons observed at δ 6.4-6.5 (2H, Ar***H***), 7.4-8.3 (6H, Ar***H***), 14.12 (1H, -***OH***) for aromatic hydroxyl group. The mass spectrum gives M+1 peak at 354.1 (m/z).

4-(4-allyloxy-2-hydroxyphenyl)-6-(2,4-dichlorophenyl)-2-aminopyrimidine **(5)** gives stretching of 3343 and 3220 cm^{-1} and hydroxyl stretching at 3075 cm^{-1}. The C=N ring stretching observed at 1645 cm^{-1}. The PMR spectrum shows peak of allyloxy saturated protons at δ 4.57 (2H, O***CH₂***CH=CH_2), and unsaturated terminal protons at δ 5.2-5.4 (2H, OCH_2CH=***CH_2***) and the CH proton of allyloxy group observed at δ 6.0 (1H, OCH_2***CH***=CH_2) which gives pentate, at δ 6.3 (2H, -***NH_2***) amino protons observed. The 7 aromatic protons observed at δ 6.4-6.5 (2H, Ar***H***), 7.4-7.7 (5H, Ar***H***) and δ 14.00 (1H, -***OH***) for aromatic hydroxyl group. The mass spectrum gives M+1 peak at 389.1 (m/z).



(5)

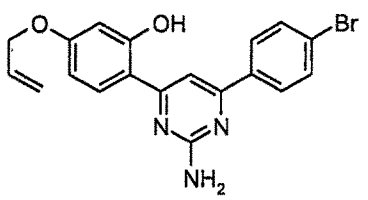


(6)

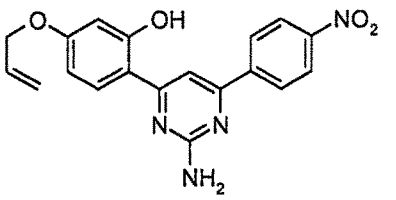
4-(4-allyloxy-2-hydroxyphenyl)-6-(4-fluorophenyl)-2-aminopyrimidine **(6)** gives stretching of 3505 and 3367 cm^{-1} and hydroxyl stretching at 3221 cm^{-1}. The C=N ring

stretching observed at 1642 cm^{-1}. The PMR spectrum shows peak of allyloxy saturated protons at δ 4.58 (2H, $OCH_2CH=CH_2$), and unsaturated terminal protons at δ 5.2-5.4 (2H, $OCH_2CH=CH_2$), the CH proton at δ 6.0 (1H, $OCH_2CH=CH_2$), amino proton at δ 6.3 (2H, $-NH_2$) and the aromatic protons observed at δ 6.4-6.5 (2H, Ar*H*), 7.4-8.1 (6H, Ar*H*). The aromatic hydroxyl proton observed at 14.18 (1H, -*OH*). The mass spectrum gives M+1 peak at 338.1 (m/z).

4-(4-allyloxy-2-hydroxyphenyl)-6-(4-bromophenyl)-2-aminopyrimidine **(7)** gives stretching of 3498 and 3373 cm^{-1} and hydroxyl stretching at 3201 cm^{-1}. The C=N ring stretching observed at 1639 cm^{-1}. The PMR spectrum shows peak of allyloxy saturated protons at δ 4.58 (2H, $-OCH_2CH=CH_2$), 5.2-5.4 (2H, $-OCH_2CH=CH_2$) displayed terminal protons of allyloxy group and CH protons at 6.0 (1H, - $OCH_2CH=CH_2$). The amino proton observed at δ 6.3 (2H, $-NH_2$) and aromatic protons at δ 6.4-6.5 (2H, Ar*H*), 7.3 (1H, Pyri*H*), 7.4-8.0 (5H, Ar*H*). The aromatic hydroxyl proton observed at δ 14.11(1H, -*OH*). The mass spectrum gives M+1 peak at 398.1 (m/z).



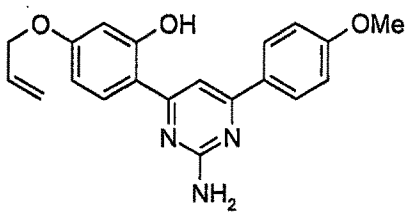
(7)



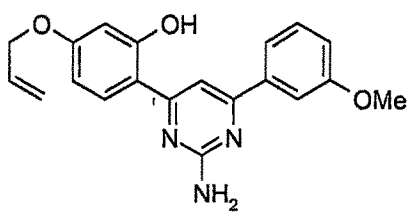
(8)

4-(4-allyloxy-2-hydroxyphenyl)-6-(4-nitrophenyl)-2-aminopyrimidine **(8)** gives stretching of 3495 and 3369 cm^{-1} and broad peak of hydroxyl stretching at 3221 cm^{-1}. The C=N ring stretching observed at 1645 cm^{-1}. The PMR spectrum displayed doublet of allyloxy saturated protons at δ 4.58 (2H, - $OCH_2CH=CH_2$), the terminal unsaturated protons at δ 5.2-5.4 (2H, $OCH_2CH=CH_2$) and CH proton at δ 6.0 (1H, $OCH_2CH=CH_2$). The amino protons observed at 6.9 (2H, $-NH_2$) and aromatic protons observed at δ 6.4-6.5 (2H, Ar*H*), 7.4-8.0 (6H, Ar*H*). The aromatic hydroxyl proton observed at δ 14.15(1H, -*OH*). The mass spectrum gives M+1 peak at 365.0 (m/z).

4-(4-allyloxy-2-hydroxyphenyl)-6-(4-methoxyphenyl)-2-aminopyrimidine **(9)** gives stretching of 3499 cm^{-1} and broad peak of hydroxyl stretching at 3220 cm^{-1}. The C=N ring stretching observed at 1630 cm^{-1}. The PMR spectrum displayed singlet at δ 3.8 (3H, -O***CH₃***), the allyloxy protons at δ 4.58 (2H, O***CH₂***CH=CH_2), 5.2-5.4 (2H, OCH_2CH=***CH₂***), 6.0 (1H, OCH_2***CH***=CH_2). The amino protons displayed at δ 6.4 (2H, -***NH₂***), and aromatic protons at δ 6.4-6.5 (2H, Ar***H***), 7.4 (1H, Pyri***H***) 7.0-8.0 (5H, Ar***H***). The aromatic hydroxyl proton observed at δ 14.07 (1H, -***OH***). The mass spectrum gives M+1 peak at 350.2 (m/z).



(9)

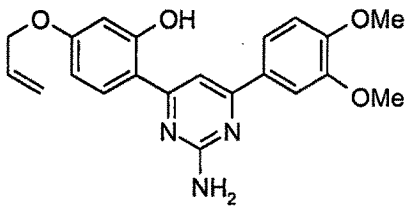


(10)

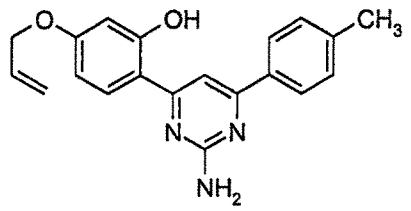
4-(4-allyloxy-2-hydroxyphenyl)-6-(3-methoxyphenyl)-2-aminopyrimidine **(10)** gives stretching of 3490 and 3362 cm^{-1} and broad peak of hydroxyl stretching at 3200 cm^{-1}. The C=N ring stretching observed at 1599 cm^{-1}. The PMR spectrum displayed singlet at δ 3.9 (3H, -O***CH₃***) for methoxy group. The allyloxy protons observed at δ 4.58 (2H, -O***CH₂***CH=CH_2), 5.2-5.4 (2H, -OCH_2CH=***CH₂***), 6.0 (1H, -OCH_2***CH***=CH_2) with doublet, doublet and multiplate respectively. The amino protons observed at δ 6.4 (2H, -***NH₂***) and aromatic protons observed at δ 6.4-6.5 (2H, Ar***H***), 7.0-7.8 (5H, Ar***H***), the pyrimidine proton observed at δ 7.4 (1H, Pyri***H***) as singlet. The aromatic hydroxyl proton observed at δ 14.18 (1H, -***OH***). The mass spectrum gives M+1 peak at 350.0 (m/z).

4-(4-allyloxy-2-hydroxyphenyl)-6-(3,4-dimethoxyphenyl)-2-aminopyrimidine **(11)** gives stretching of 3493 and 3370 cm^{-1} and broad peak of hydroxyl stretching at 3194 cm^{-1}. The C=N ring stretching observed at 1604 cm^{-1}. The PMR spectrum displayed singlet at δ 3.9 (3H, -O***CH₃***) and 4.0 (3H, -O***CH₃***) for both the methoxy group. The saturated allyloxy protons observed at 4.58 (2H, -O***CH₂***CH=CH_2) and unsaturated protons observed at δ 5.2-5.4 (2H, -OCH_2CH=***CH₂***), 6.0 (1H, -OCH_2***CH***=CH_2). The amino

protons observed at δ 6.9 (2H, -***NH₂***) and the aromatic protons at δ 6.4-6.5 (2H, Ar***H***), 7.5-7.8 (5H, Ar***H***). The aromatic hydroxyl proton observed at δ 14.27 (1H, -***OH***). The mass spectrum gives M+1 peak at 380.0 (m/z).



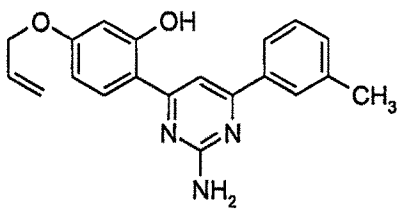
(11)



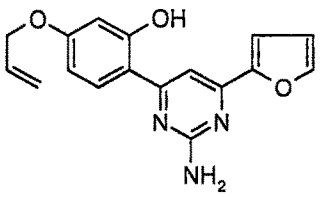
(12)

4-(4-allyloxy-2-hydroxyphenyl)-6-(4-methylphenyl)-2-aminopyrimidine **(12)** gives stretching of 3508 and 3200 cm^{-1} and broad peak of hydroxyl stretching at 3072 cm^{-1}. The C=N ring stretching observed at 1639 cm^{-1}. The PMR spectrum displayed singlet at δ 2.4 (3H, -***CH₃***) for methyl group. The allyloxy protons observed at δ 4.57 (2H, -O***CH₂***CH=CH_2), the unsaturated protons at δ 5.2-5.4 (2H, -OCH_2CH=***CH₂***), 6.0 (1H, -OCH_2***CH***=CH_2) and the amino protons observed at δ 6.2 (2H, -***NH₂***). The aromatic protons observed at δ 6.4-6.5 (2H, Ar***H***), 7.4 (1H, Pyri***H***), 7.2-7.9 (5H, Ar***H***). The aromatic hydroxyl proton observed at δ 14.15 (1H, -***OH***). The mass spectrum gives M+1 peak at 334.0 (m/z).

4-(4-allyloxy-2-hydroxyphenyl)-6-(3-methylphenyl)-2-aminopyrimidine **(13)** gives stretching of 3508 and 3375 cm^{-1} and broad peak of hydroxyl stretching at 3209 cm^{-1}. The C=N ring stretching observed at 1639 cm^{-1}. The PMR spectrum displayed singlet at δ 2.4 (3H, -***CH₃***) for methyl group. The allyloxy protons observed at δ 4.57 (2H, -O***CH₂***CH=CH_2), the unsaturated protons at δ 5.2-5.4 (2H, -OCH_2CH=***CH₂***), 6.0 (1H, -OCH_2***CH***=CH_2) and the amino protons observed at δ 6.2 (2H, ***NH₂***). The aromatic protons observed at δ 6.4-6.5 (2H, Ar***H***), 7.4 (1H, Pyri***H***), 7.2-7.9 (5H, Ar***H***). The aromatic hydroxyl proton observed at δ 14.15 (1H, -***OH***). The mass spectrum gives M+1 peak at 334.0 (m/z).



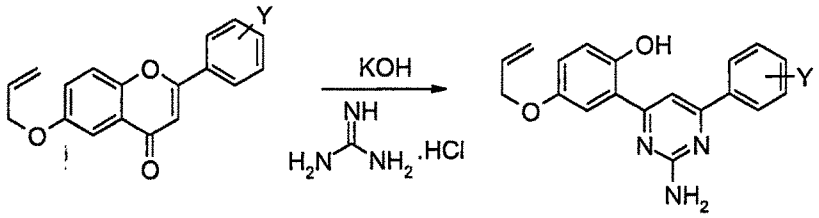
(13)



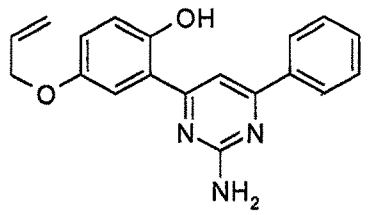
(14)

4-(4-allyloxy-2-hydroxyphenyl)-6-furan-2-aminopyrimidine **(14)** gives stretching of 3360 and 3315 cm^{-1} and broad peak of hydroxyl stretching at 3195 cm^{-1}. The C=N ring stretching observed at 1627cm^{-1}. The PMR spectrum displayed singlet at δ 4.57 (2H, -O***CH₂***CH=CH_2), 5.2-5.4 (2H, -OCH_2CH=***CH_2***), 6.0 (1H, - OCH_2***CH***=CH_2) for allyloxy group. The amino protons at δ 6.2 (2H, -***NH_2***) and the aromatic protons observed at δ 6.4-6.5 (2H, Ar***H***), 7.4 (1H, Pyri***H***) 7.4 (3H, ***Furan***), 7.34 (1H, Ar***H***). The aromatic hydroxyl proton observed at δ 13.18 (1H, -***OH***). The mass spectrum gives M+1 peak at 310.0 (m/z).

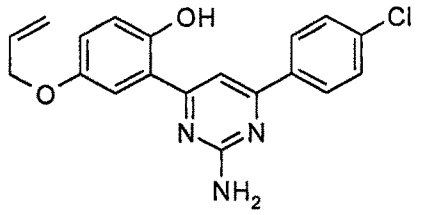
I.3a.5b. Synthesis of 4-(5-allyloxy-2-hydroxyphenyl)-6-(substitutedphenyl)-2-amino pyrimidine derivatives



4-(5-allyloxy-2-hydroxyphenyl)-6-phenyl-2-aminopyrimidine **(15)** gives stretching of 3428 and 3384 cm^{-1} and broad peak of hydroxyl stretching at 3210 cm^{-1}. The C=N ring stretching observed at 1635 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 4.48 (2H, -O***CH_2***CH=CH_2) of allyloxy protons of oxygen attached carbon, at δ 5.0 (2H, -***NH_2***) the amino protons displayed as singlet. The unsaturated group attached protons observed at δ 5.2-5.3 (2H, -OCH_2CH=***CH_2***) and 5.9 (1H, - OCH_2***CH***=CH_2). The nine aromatic protons observed at δ 6.8-6.9 (2H, Ar***H***), 7.5-7.8 (7H, Ar***H***). The aromatic hydroxyl proton observed at δ 12.05 (1H, -***OH***). The mass spectrum gives M+1 peak at 319.0 (m/z).



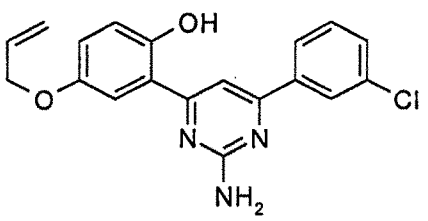
(15)



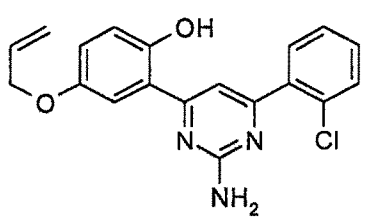
(16)

4-(5-allyloxy-2-hydroxyphenyl)-6-(4-chlorophenyl)-2-aminopyrimidine **(16)** gives stretching of 3499 and 3348 cm^{-1} and broad peak of hydroxyl stretching at 3200 cm^{-1}. The C=N ring stretching observed at 1618 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 4.44 (2H, - O***CH₂***CH=CH_2) of allyloxy protons of oxygen attached carbon, at δ 5.1 (2H, -***NH₂***) the amino protons displayed as singlet. The unsaturated group attached protons observed at δ 5.2-5.3 (2H, OCH_2CH=***CH₂***) as doublet and the multiplate of CH at δ 5.9 (1H, OCH_2***CH***=CH_2). The nine aromatic protons observed at δ 6.8-6.9 (2H, Ar***H***), 7.5-7.8 (7H, Ar***H***). The aromatic hydroxyl proton observed at δ 12.12 (1H, -***OH***). The mass spectrum gives M+1 peak at 354.0 (m/z).

4-(5-allyloxy-2-hydroxyphenyl)-6-(3-chlorophenyl)-2-aminopyrimidine **(17)** gives stretching of 3451 and 3351 cm^{-1} and broad peak of hydroxyl stretching at 3229 cm^{-1}. The C=N ring stretching observed at 1640 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 4.57 (2H, -O***CH₂***CH=CH_2) of CH_2 protons and at δ 5.2-5.4 (2H, - OCH_2CH=***CH₂***) and 6.0 (1H, - OCH_2***CH***=CH_2) the other protons of allyloxy group was displayed. The two protons of amine group of pyrimidine observed at δ 6.5 (2H, -***NH₂***) and the aromatic protons observed at δ 6.8-6.9 (2H, Ar***H***), 7.0-8.1 (6H, Ar***H***). The aromatic hydroxyl proton observed at δ 13.44 (1H, -***OH***). The mass spectrum gives M+1 peak at 354.0 (m/z).



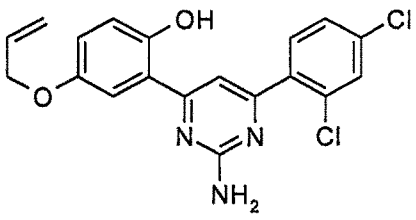
(17)



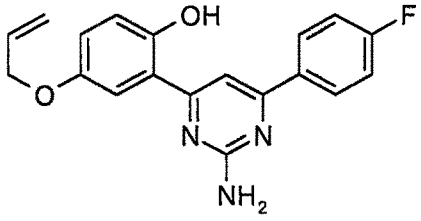
(18)

4-(5-allyloxy-2-hydroxyphenyl)-6-(2-chlorophenyl)-2-aminopyrimidine **(18)** gives stretching of 3499 and 3348 cm^{-1} and broad peak of hydroxyl stretching at 3233 cm^{-1}. The C=N ring stretching observed at 1618 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 4.6 (2H, -O***CH₂***CH=CH_2) of CH_2 protons and at δ 5.2-5.4 (2H, OCH_2CH=***CH₂***) and 6.0 (1H, - OCH_2***CH***=CH_2) the other protons of allyloxy group was displayed. The two protons of amine group of pyrimidine observed at δ 6.6 (2H, -***NH₂***) and the aromatic protons observed at δ 7.3-7.9 (8H, Ar***H***). The aromatic hydroxyl proton observed at δ 13.44 (1H, -***OH***). The mass spectrum gives M+1 peak at 354.0 (m/z).

4-(5-allyloxy-2-hydroxyphenyl)-6-(2,4-dichlorophenyl)-2-aminopyrimidine **(19)** gives stretching of 3506 and 3371 cm^{-1} and broad peak of hydroxyl stretching at 3075 cm^{-1}. The C=N ring stretching observed at 1645 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 4.4 (2H, -O***CH₂***CH=CH_2) of - CH_2 protons and at δ 5.1 (2H, OCH_2CH=**CH₂**) and 5.97 (1H, - OCH_2**CH**=CH_2) the other protons of allyloxy group was displayed. The two protons of amine group of pyrimidine observed at δ 5.0 (2H, -***NH₂***) and the aromatic protons observed at δ 7.2-7.6 (7H, Ar***H***). The aromatic hydroxyl proton observed at δ 12.81 (1H, -***OH***). The mass spectrum gives M+1 peak at 389.0 (m/z).



(19)

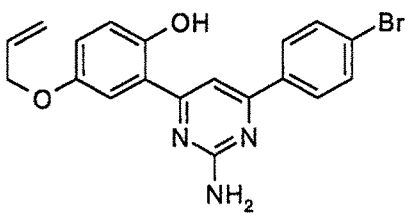


(20)

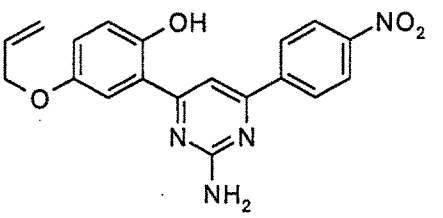
4-(5-allyloxy-2-hydroxyphenyl)-6-(4-flourophenyl)-2-aminopyrimidine **(20)** gives stretching of 3501 and 3329 cm^{-1} and broad peak of hydroxyl stretching at 3216 cm^{-1}. The C=N ring stretching observed at 1624 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet of saturated protons of allyloxy group at δ 4.5 (2H, -O***CH₂***CH=CH_2) and the protons of alkene group observed at δ 5.2-5.3 (2H, OCH_2CH=***CH₂***) and 5.97 (1H, OCH_2***CH***=CH_2). The two protons of amine group of pyrimidine observed at δ 6.1 (2H, -***NH₂***) and the aromatic protons observed at δ 6.8-8.1 (8H, Ar***H***). The aromatic hydroxyl

proton observed at δ 13.44 (1H, ***-OH***). The mass spectrum gives M+1 peak at 338.0 (m/z).

4-(5-allyloxy-2-hydroxyphenyl)-6-(4-bromophenyl)-2-aminopyrimidine **(21)** gives stretching of 3491 and 3353 cm^{-1} and broad peak of hydroxyl stretching at 3216 cm^{-1}. The C=N ring stretching observed at 1624 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet of saturated protons of allyloxy group at δ 4.46 (2H, $-OCH_2CH=CH_2$) and the protons of alkene group observed at δ 5.0-5.2 (2H, $OCH_2CH=CH_2$) and 6.6 (1H, $OCH_2CH=CH_2$). The two protons of amine group of pyrimidine observed at δ 5.2 (2H, $-NH_2$) and the aromatic protons observed at δ 6.8-8.1 (8H, Ar***H***). The aromatic hydroxyl proton observed at δ 13.44 (1H, ***-OH***). The mass spectrum gives M+1 peak at 398.0 (m/z).



(21)

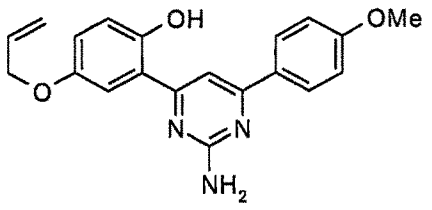


(22)

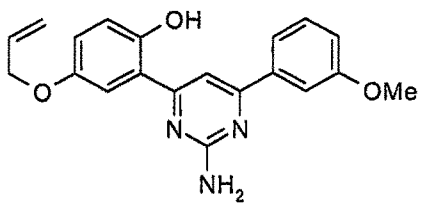
4-(5-allyloxy-2-hydroxyphenyl)-6-(4-nitrophenyl)-2-aminopyrimidine **(22)** gives stretching of 3495 and 3369 cm^{-1} and broad peak of hydroxyl stretching at 3221 cm^{-1}. The C=N ring stretching observed at 1645 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet of saturated protons of allyloxy group at δ 4.65 (2H, $-OCH_2CH=CH_2$), the terminal CH_2 protons observed at δ 5.3-5.4 (2H, $OCH_2CH=CH_2$) and CH proton displayed as multiplate at δ 6.0 (1H, $-OCH_2CH=CH_2$). The amine protons displayed at δ 6.9 (2H, $-NH_2$) and aromatic protons observed at δ 7.3-8.3 (8H, Ar***H***). The aromatic hydroxyl proton observed at δ 14.15 (1H, ***-OH***). The mass spectrum gives M+1 peak at 365.0 (m/z).

4-(5-allyloxy-2-hydroxyphenyl)-6-(4-methoxyphenyl)-2-aminopyrimidine **(23)** gives stretching of 3497 and 3438 cm^{-1} and broad peak of hydroxyl stretching at 3182 cm^{-1}. The C=N ring stretching observed at 1643 cm^{-1} in IR spectrum. The PMR spectrum

displayed singlet at δ 3.8 (3H, -O***CH₃***), the allyloxy protons at δ 4.58 (2H, -O***CH₂***CH=CH₂), 5.3-5.4 (2H, OCH₂CH=***CH₂***), 6.0 (1H, - OCH₂***CH***=CH₂). The amino protons at δ 6.9 (2H, -***NH₂***), and remaining aromatic protons displayed at δ 7.38 (1H, Pyri***H***) and 7.0-8.0 (7H, Ar***H***). The aromatic hydroxyl proton observed at δ 14.07 (1H,-***OH***). The mass spectrum gives M+1 peak at 349.2 (m/z).



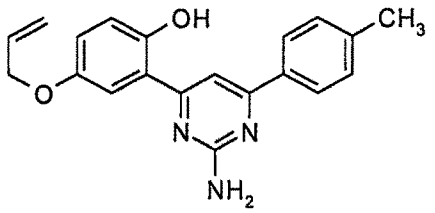
(23)



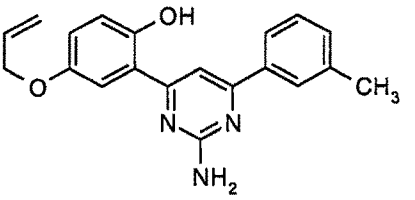
(24)

4-(5-allyloxy-2-hydroxyphenyl)-6-(3-methoxyphenyl)-2-aminopyrimidine **(24)** gives stretching of 3457 and 3353 cm^{-1} and broad peak of hydroxyl stretching at 3236 cm^{-1}. The C=N ring stretching observed at 1645 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 3.9 (3H, -O***CH₃***), the allyloxy protons at δ 4.5 (2H, -O***CH₂***CH=CH₂), 5.3-5.4 (2H, -OCH₂CH=***CH₂***), 6.0 (1H, - OCH₂***CH***=CH₂). The amino protons at δ 6.9 (2H, -***NH₂***), and remaining aromatic protons displayed at δ 7.38 (1H, Pyri***H***) and 7.2-8.0 (7H, Ar***H***). The aromatic hydroxyl proton observed at δ 14.11 (1H, -***OH***). The mass spectrum gives M+1 peak at 349.2 (m/z).

4-(5-allyloxy-2-hydroxyphenyl)-6-(4-methylphenyl)-2-aminopyrimidine **(25)** gives stretching of 3492 and 3354 cm^{-1} and broad peak of hydroxyl stretching at 3240 cm^{-1}. The C=N ring stretching observed at 1630 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 2.4 (3H, -***CH₃***), the allyloxy protons at δ 4.7 (2H, -O***CH₂***CH=CH₂), 5.0-51 (2H, -OCH₂CH=***CH₂***), 5.99 (1H, -OCH₂***CH***=CH₂). The amino protons at δ 5.2 (2H, -***NH₂***) and remaining aromatic protons displayed at δ 7.2-7.56 (9H, Ar***H***). The aromatic hydroxyl proton observed at δ 12.87 (1H, -***OH***). The mass spectrum gives M+1 peak at 334.2 (m/z).



(25)



(26)

4-(5-allyloxy-2-hydroxyphenyl)-6-(3-methylphenyl)-2-aminopyrimidine **(26)** gives stretching of 3508 and 3375 cm^{-1} and broad peak of hydroxyl stretching at 3209 cm^{-1}. The C=N ring stretching observed at 1636 cm^{-1} in IR spectrum. The PMR spectrum displayed singlet at δ 2.4 (3H, ***-CH₃***), the allyloxy protons at δ 4.7 (2H, -O***CH₂***CH=CH_2), 5.0-51 (2H, -OCH_2CH=***CH_2***), 5.99 (1H, - OCH_2***CH***=CH_2). The amino protons at δ 5.2 (2H, ***-NH₂***) and remaining aromatic protons displayed at δ 7.2-7.56 (9H, Ar***H***). The aromatic hydroxyl proton observed at δ 12.87 (1H, ***-OH***). The mass spectrum gives M+1 peak at 334.2 (m/z).

I.3b. Biological studies

The anti-HIV studies of allyloxy substituted 4,6-diaryl-2-aminopyrimidine were carried out at Rega Institute for Medical Research, Katholieke Universiteit Leuven, Belgium. Inhibition of the HIV-induced cytopathic effect was used as the end point. The viability of both HIV and mock-infected cells was assessed spectrophotometrically via the *in situ* reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into formazan.

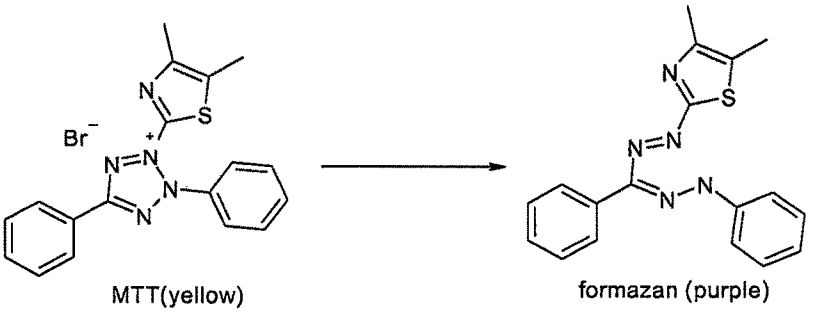


Table 7. The Anti-HIV activity and cytotoxicity of allylated 4,6-diaryl-2-aminopyrimidines.

Sr.no	Compound	IC $_{50}$ (μM) HIV-1-III$_B$	CC$_{50}$(μM) HIV-1-III$_B$	IC $_{50}$ (μM) HIV-1-ROD	CC$_{50}$(μM) HIV-1-ROD
1	1	>125	>125	>125	>125
2	2	>125	>125	>125	>125
3	3	>125	>125	>125	>125
4	4	>125	>125	>125	>125
5	5	>125	>125	>125	>125
6	6	>125	>125	>125	>125
7	7	>125	>125	>125	>125
8	8	>125	>125	>125	>125
9	9	>125	>125	>125	>125
10	10	>125	>125	>125	>125
11	11	>125	>125	>125	>125
12	12	>125	>125	>125	>125
13	13	>125	>125	>125	>125
14	14	>125	>125	>125	>125
15	15	>125	>125	>125	>125
16	16	>125	>125	>125	>125
17	17	>125	>125	>125	>125
18	**18**	**1.34**	**1.34**	**2.33**	**2.33**
19	**19**	**0.34**	**0.34**	**0.40**	**0.40**
20	20	>125	>125	>125	>125
21	21	>125	>125	>125	>125
22	22	>125	>125	>125	>125
23	23	>125	>125	>125	>125
24	24	>125	>125	>125	>125
25	25	>125	>125	>125	>125
26	**26**	**1.88**	**1.88**	**2.11**	**2.11**
27	**27**	**16.73**	**16.73**	**16.73**	**16.73**
28	**29**	**6.44**	**6.44**	**6.44**	**6.44**
29	**31**	**0.52**	**0.52**	**1.53**	**1.53**
30	**33**	**13.56**	**13.56**	**13.56**	**13.56**
31	**36**	**13.25**	**13.25**	**13.25**	**13.25**
32	**40**	**9.49**	**9.49**	**9.49**	**9.49**

Concentration required to reduce HIV-1 induced cytopathic effect by 50% in MT-4 cells.

Concentration required to reduce MT-4 cell viability by 50%.

The primary anti-HIV screening of newly synthesized 2-aminopyrimdines shows that compound **18, 19** and **26** possess IC_{50} value 1.34, 0.34 and 1.88 in μM respectively. Unfortunately, this compound also possesses cytotoxicity at same concentration. As per discussed in literature survey Methoxy substituted compound also shows anti HIV activity. So we also screened the 4-(4/5-methoxy-2-hydroxy phenyl)-6-(substituted phenyl)-2-aminopyrimidnes which is discussed in Part II. In that series compounds **29** and **31** possess IC_{50} value 6.44 and 0.52 in μM respectively.

CHAPTER – I.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 by standard purification methods.

I.4a: Chemical work

I.4b: Biological work

I.4a: CHEMICAL WORK

The synthesis carried out has been discussed under following heads:

I.4a.1: Synthesis of 4/5-Allyloxy-2-hydroxyacetophenone

I.4a.2: Synthesis of 4/5-Allyloxy 2-acetyl-1-(substituted benzoyloxy) benzene derivatives

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

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

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

I.4a.1: Synthesis of 4/5-allyloxy-2-hydroxyacetophenone (A)

I.4a.1a. Synthesis of 4-allyloxy-2-hydroxyacetophenone (A1)

Allylbromide (5.6 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 6-8 hr at 60 °C. It was filtered to remove K_2CO_3. Acetone was recovered and the brown liquid was dissolved in chloroform and washed with water successively to remove traces of base. After removal of solvent in vacuum, the resulting oily compound was subjected to silica gel column chromatography using chloroform as eluent. The yield is 7.2 g, 60 %.

Anal:

R_f : 0.69 (chloroform)

IR (KBr) : 3066, 1695, 1625, 1218, 850 and 769 cm^{-1}

I.4a.1b. Synthesis of 5-allyloxy-2-hydroxyacetophenone (A2)

Synthesis of Hydroquinone diacetate (III)

To a mixture of 10 g (0.0908 mole) of hydroquinone **(II)** and 12.966 g (0.12712 mole) of acetic anhydride in a 250 ml conical flask, few drops of con. H_2SO_4 was added. The mixture was stirred gently and warmed to dissolve hydroquinone. After 5 minutes the clear solution was poured on to 400 ml of crushed ice. A white solid which was obtained was filtered, washed with water and recrystalized from methanol to afford white crystals.

Yield : 8.5 g (85%)

M.P. : 154-156 °C

R_f : 0.82 ($CHCl_3$: MeOH) (10 : 1)

2,5-Di hydroxyacetophenone (IV)

Dry Hydroquinone diacetate 8.0 g (0.0411 mole) **(III)** and 6.5 g (0.0494 mole) of anhydrous $AlCl_3$ was taken in a 250 ml RBF. The contents were heated in an oil bath by raising the temperature slowly to 160-165 °C and maintained for 3 hrs. HCl gas evolved

was taken up by the trap. When the evolution of gas ceases the flask was removed from oil bath. The contents were poured in ice HCl mixture wherein a green colored solid was obtained. It was filtered washed with water and recrystallized from methanol to afford green crystals of 2,5-dihydroxyacetophenone.

Yield : 4.2 g (52.5%)

M.P. : 200-201 °C

R_f : 0.82 ($CHCl_3$: MeOH) (10 : 1)

5-allyloxy-2-hydroxyacetophenone (A2)

Allylbromide (5.6 ml) and anhydrous potassium carbonate (13.6 g) was added to a solution of 2,5-dihydroxyacetophenone (10 g) **(IV)** in acetone (30 ml). The reaction mixture was stirred and refluxed for six to eight hour at 60 °C. The reaction mixture was purified as per compound **(A1).** Yield-7.2 g, 60 %.

Anal:

R_f : 0.66 (chloroform)

IR (KBr) : 3066, 1695, 1625, 1218, 850 and 769 cm^{-1}

I.4a.2. Synthesis of 4/5-Allyloxy-2-acetyl-1-(substituted benzoyloxy) benzene derivatives (C)

The synthesis of the derivatives was carried out using either of the two methods.

Method I

To a cold solution of 4/5-allyloxy-2-hydroxyacetophenone **(A1, A2)** and substituted benzoic acid **(B)** (1.1-1.3 eq.) in dry pyridine (30 ml) $POCl_3$ (1.3 eq.) was added dropwise with stirring. After completion of addition the contents were further stirred for 3 hrs at room temperature. The reaction mixture was poured into ice and acidified with Con. HCl. The solid so obtained was filtered, washed free from acid and dried. Recrystallisation from methanol gave white crystals of **(C).**

Method II

To a cold solution of 4/5-(methoxy/benzyloxy/allyloxy)-2-hydroxyacetophenone **(A1,2)** and substituted benzoic acid (1.1-1.3 eq.) in dry pyridine (30 ml) $POCl_3$ (1.3 eq.) was added dropwise with stirring. After completion of addition the contents were further stirred for 3 hrs at room temperature. The reaction mixture was poured into ice and acidified with Con. HCl. The oily material obtained was extracted with chloroform successively. It was washed with dil. HCl, sodium bicarbonate solution and water. After successive extractions the organic layer was dried over anhydrous sodium sulphate. It was purifed by column chromatography using chloroform as eluent.

I.4a.2a. Synthesis of 5-Allyloxy-2-acetyl-1-(substituted benzoyloxy) benzene derivatives (C1-C14)

Table 8. Details of 5-Allyloxy-2-acetyl-1-(substituted benzoyloxy) benzene derivatives

No.	A1	(B) (substituted benzoic acid)	$POCl_3$	Method	R_f ($CHCl_3$)	Yield (%)	IR (cm^{-1})	M.P. (°C)
C1	4.7 g, 24.0 mmol	3.2 g, 26.0 mmol	5.1 gm, 36.0 mmol	I	0.77	4.2 g, 58 %	1735, 1692, 1660, 1270 and 769	68-70
C2	4.93 g, 22.0 mmol	4.8 g, 30.0 mmol	5.7 g, 37.0 mmol	I	0.69	8 g 94 %	1740, 1680, 1650, 1220 and 769	68-69
C3	3.5 g, 18.0 mmol	3.4 g, 20.0 mmol	5.7 g, 37.0 mmol	II	0.77	3.8 g, 63 %	-------	NT
C4	4.31 g, 22.0 mmol	3.8 g, 23.0 mmol	5.2 g, 53.0 mmol	I	0.79	2.6 g, 36 %	1739, 1679, 1655, 1240 and 769	57-58
C5	6.0 g, 31.0 mmol	7.12 g, 37.0 mmol	7.17 g, 46.0	II	0.76	10.5 g, 84	1735, 1670, 1630, 1270	NT

			mmol			%	and 750	
C6	4.12 g, 21.0 mmol	3.6 g, 25.0 mmol	4.18 g, 31.0 mmol	I	0.63	6.3 g, 94 %	1750, 1670, 1630, 1170 and 765	84-85
C7	4.6 g, 23.0 mmol	5.7 g, 28.0 mmol	5.2 g, 34.0 mmol	II	0.76	6.8 g, 77 %	-----	NT
C8	4.26 g, 22.0 mmol	4.07 g, 24.0 mmol	5.05 g, 33.0 mmol	I	0.78	4.8 g, 64 %	1749, 1680, 1620, 1110 and 800	52-54
C9	3.0 g, 15.0 mmol	2.84 g, 18.0 mmol	3.4 g, 22.0 mmol	I	0.80	4.2 g, 83 %	1750, 1685, 1630, 1130 and 800	80-81
C10	2.5 g, 13.0 mmol	2.34 g, 15.0 mmol	2.4 g, 16.0 mmol	I	0.74	2.6 g, 61 %	1745, 1685, 1635, 1115 and 800	79-80
C11	3.2 g, 16.0 mmol	3.3 g, 18.0 mmol	3.4 g, 22.0 mmol	I	0.74	2.8 g, 63 %	1736, 1680, 1620, 1125 and 800	91-93
C12	4.0 g, 20.0 mmol	3.2 g, 23.0 mmol	4.5 g, 30.0 mmol	I	0.79	3.7 g, 58 %	1739, 1685, 1625, 1160 and 888	43-45
C13	3.3 g, 16.0 mmol	2.6 g, 19.0 mmol	4.5 g, 30.0 mmol	II	0.77	2.1 g, 58 %	1735, 1680, 1610, 1100 and 800	NT
C14	4 g, 20.0 mmol	2.7 g, 23.0 mmol	4.5 g, 30.0 mmol	I	0.69	3.6 g, 88 %	1745, 1685, 1640, 1200 and 890	57-58

I.4a.2b. Synthesis of 4-Allyloxy-2-acetyl-1-(substituted benzoyloxy) benzene derivatives (C14-C26)

Table 9. Details of 4-Allyloxy-2-acetyl-1-(substituted benzoyloxy) benzene derivatives

No.	A2	(B) (substituted benzoic acid)	$POCl_3$	Method	R_f ($CHCl_3$)	Yield (%)	IR (cm^{-1})	M.P. (°C)
C15	3 g, 15.0 mmol	2.2 g, 16.5 mmol	2.5 g, 16.3 mmol	II	0.88	3.1 g, 67 %	----	NT
C16	3 g, 15.0 mmol	2.9 g, 18 mmol	3.4g, 22 mmol	I	0.78	3.7 g, 72 %	1745, 1689, 1635, 1215 and 845	61-63
C17	3 g, 15.0 mmol	2.9 g, 18 mmol	3.4g, 22 mmol	I	0.75	3.2 g, 62 %	1741, 1685, 1632, 1220and 845	52-53
C18	4.31 g, 22.0 mmol	3.8 g, 23.0 mmol	5.2 g, 33.0 mmol	I	0.79	2.6 g 36 %	1740, 1685, 1632, 1220 and 845	71-72
C19	4 g, 20.0 mmol	4.3 g, 22.0 mmol	4.5 g, 30.0 mmol	I	0.77	4.2 g (59 %)	1744, 1689, 1635, 1225 and 855	74-75
C20	4 g, 20.0 mmol	3.4 g, 24.0 mmol	4.5 g, 30.0 mmol	I	0.73	6.3 g 94 %	1745, 1687, 1638, 1250 and 846	98-99
C21	2.5 g, 13.0 mmol	3.1 g, 15.0 mmol	2.9 g, 19.0 mmol	I	0.76	2.8 g 58 %	1749, 1685, 1635, 1242 and 840	86-87

C22	4.26 g, 22.0 mmol	4.07 g, 24.0 mmol	5.05 g, 33.0 mmol	I	0.70	3.8g 78 %	1749, 1685, 1635, 1242 and 840	110-111
C23	2 g, 10.0 mmol	2.8 g, 15.0 mmol	2.2 g, 15.0 mmol	I	0.78	2.3 g 58 %	1745, 1687, 1640, 1238 and 848	70-72
C24	3 g, 15.0 mmol	3.2 g, 18.0 mmol	2.8 g, 18.0 mmol	I	0.79	2.0 g, 50 %	1735, 1685, 1642, 1234 and 848	65-67
C25	3 g, 15.0 mmol	2.8 g, 18.0 mmol	3.4 g, 22.0 mmol	I	0.78	4.1 gm 63 %	1735, 1685, 1642, 1230 and 855	83-85
C26	2.5 g, 13.0 mmol	2.6 g, 19.0 mmol	2.9 g, 19.0 mmol	II	0.78	3.8 gm 95 %	------	Liquid

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

The synthesis of propanedione derivative was carried out using either of the two methods.

Method I

2-acetyl-4/5-substitutedphenyl-benzoate **(C)** was dissolved in dry pyridine (30 ml). Freshly fused and powdered potassium hydroxide was added and stirred for 2 hrs. The yellow colored mass obtained was poured into ice containing Con. HCl. The yellow solid obtained was washed free from acid with water, filtered and dried. Recrystalisation from methanol afforded yellow crystals of **(D).**

Method II

2-acetyl-4/5-substitutedphenyl-benzoate **(C)** was dissolved in dry pyridine (30 ml). Freshly fused and powdered potassium hydroxide was added and stirred for 2 hrs. The yellow colored mass obtained was poured into ice containing Con. HCl. The oily material

obtained was extracted by chloroform successively. It was washed with dil. HCl, sodium bicarbonate solution and water. After successive extractions organic layer was dried over anhydrous sodium sulphate. The compound was purified by column chromatography using chloroform as eluent **(D).**

I.4a.3a. Synthesis of 1-(4-allyloxy-2-hydroxyphenyl)-3-(substituted phenyl)-1,3-propanedione derivatives (D1-D14)

Table 10. Details of propanedione derivatives.

No.	C	KOH	Method	R_f ($CHCl_3$)	Yield (%)	IR (cm^{-1})	M.P. (°C)
D1	1.4 g, 4.0 mmol	1 g, 1.7 mmol	I	0.79	1.2 g, 85 %	1660, 1624, 1590, 1489, 1255, 1080 and 850	80-82
D2	7.7 g, 23.0 mmol	1 g, 1.7 mmol	I	0.74	6.9 g, 89 %	1665, 1610, 1580, 1490, 1255, 1080 and 850	159-160
D3	1.2 g, 3.6 mmol	1 g, 1.7 mmol	I	0.78	0.9 g, 81%	1654, 1615, 1588, 1492, 1250, 1080 and 850	89-90
D4	2.4 g, 7.2 mmol	1 g, 1.7 mmol	II	0.80	2.3 g, 95 %	------	NT
D5	2.5 g, 6.8 mmol	1 g, 1.7 mmol	II	0.77	1.8 g, 78 %	-------	NT
D6	6 g, 19.0 mmol	1 g, 1.7 mmol	II	0.76	5.1 g, 85 %	-------	NT
D7	3 g, 7.9	1 g, 1.7	I	0.80	2.8 g,	1660, 1618, 1579, 1491, 1254, 1080	159-161

	mmol	mmol			93 %	and 850	
D8	3 g, 8.7 mmol	1 g, 1.7 mmol	I	0.70	2.9 g, 90 %	1665, 1620, 1580, 1495, 1260, 1080 and 850	148-149
D9	3 g, 9.0 mmol	1 g, 1.7 mmol	II	0.68	2.4 g, 80 %	1649, 1616, 1585, 1489, 1260, 1080 and 850	NT
D10	2 g, 6.1 mmol	1 g, 1.7 mmol	I	0.71	1.7 g, 85 %	1655, 1620, 1590, 1490, 1265, 1050 and 850	128-129
D11	3 g, 9.0 mmol	1 g, 1.7 mmol	I	0.72	3.1 g, 96 %	1670, 1621, 1595, 1495, 1265, 1079 and 850	136-137
D12	2.5 g, 8.0 mmol	1 g, 1.7 mmol	I	0.68	2.2 g, 88 %	1650, 1610, 1585, 1493, 1265, 1050 and 850	103-105
D13	2.5 g, 8.0 mmol	1 g, 1.7 mmol	I	0.71	2.2 g, 88 %	1662, 1625, 1590, 1490, 1265, 1079 and 850	89-91
D14	2.5 g, 8.0 mmol	1 g, 1.7 mmol	I	0.67	2.2 g, 88 %	1665, 1615, 1585, 1480, 1265, 1079 and 850	102-103

I.4a.2b. Synthesis of 1-(5-allyloxy-2-hydroxyphenyl)-3-(substituted phenyl)-1,3-propanedione derivatives (D15-D26)

Table 11. Details of propane dione derivatives

No.	C	KOH	Method	R_f ($CHCl_3$)	Yield (%)	IR (cm^{-1})	M.P. (°C)
D15	2 g, 6.7 mmol	1 g, 1.7 mmol	II	0.59	1.8 g, 90 %	------	liquid

D16	3 g, 9.0 mmol	1 g, 1.7 mmol	I	0.62	2.7 g, 90 %	1660, 1620, 1580, 1485, 1265, 1079 and 850	84-86
D17	3 g, 9.0 mmol	1 g, 1.7 mmol	II	0.70	2.7 g, 90 %	1663, 1615, 1590, 1480, 1265, 1079 and 850	96-97
D18	2.4 g 7.2 mmol	1 g, 1.7 mmol	I	0.75	2.3 g, 95 %	1665, 1620, 1590, 1480, 1265, 1079 and 850	90-91
D19	3.1 g, 12.0 mmol	1 g, 1.7 mmol	I	0.70	2.9 g, 93 %	1660, 1618, 1586, 1490, 1265, 1079 and 850	93-94
D20	3 g, 9.5 mmol	1 g, 1.7 mmol	I	0.73	2.6 g, 86 %	1665, 1615, 1586, 1485, 1265, 1079 and 850	113-114
D21	2 g, 5.3 mmol	1 g, 1.7 mmol	I	0.76	1.8 g, 90 %	1666, 1618, 1585, 1490, 1270, 1079 and 850	104-105
D22	2 g, 5.3 mmol	1 g, 1.7 mmol	I	0.80	1.8 g, 90 %	1655, 1615, 1580, 1477, 1270, 1079 and 849	130-131
D23	2 g, 6.1 mmol	1 g, 1.7 mmol	I	0.79	1.7 g, 85 %	1667, 1618, 1595, 1495, 1265, 1079 and 850	149-150
D24	2 g, 6.1 mmol	1 g, 1.7 mmol	I	0.72	1.6 g, 82 %	1665, 1620, 1590, 1480, 1265, 1079 and 850	90-93
D25	2.5 g, 8.0 mmol	1 g, 1.7 mmol	II	0.70	2.2 g, 88 %	--------	NT
D26	3.5 g, 11.0	1 g, 1.7 mmol	I	0.76	3.2 g, 91 %	1659, 1615, 1590, 1480, 1265, 1079	86-87

	mmol					and 850	

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

The synthesis of ester derivative was carried out using either of the two methods.

Method I

1-(4/5-substituted-2-hydroxyphenyl)-3-(substitutedphenyl)-1,3-propanedione **(D)** was dissolved in 20 ml of glacial acetic acid. To this a few drops of Con. H_2SO_4 were added and the contents were refluxed for 2 hrs on water bath. The reaction mixture was poured onto crushed ice. The brownish white solid so obtained was washed with water and filtered. The dried product was recrystallised from methanol to afford **(E)**

Method II

1-(4/5-substituted-2-hydroxyphenyl)-3-(substitutedphenyl)-1,3-propanedione **(D)** was dissolved in 20 ml of glacial acetic acid. To this a few drops of Con. H_2SO_4 were added and the contents were refluxed for 2 hrs on water bath. The reaction mixture was poured onto crushed ice. The oily material obtained was extracted successively with chloroform. It was washed with sat. sodium bicarbonate solution and water. After successive extractions the organic layer was dried over anhydrous sodium sulphate and purified by column chromatography using chloroform as eluent to afford **(E)**.

I.4a.4a. Synthesis of 7-allyloxy-2-substituted phenyl-4*H*-chromen-4-one derivatives (E)

Table 12. Details of 7-allyloxy-2-substituted phenyl-4*H*-chromen-4-one derivatives

No.	D	Method	R_f ($CHCl_3$)	Yield (%)	IR (cm^{-1})	M.P. (°C)
E1	0.6 g, 2 mmol	I	0.49	0.45 g, 80 %	1660, 1590, 1489, 1255,	99-100

					1080 and 850	
E2	4 g, 12.0 mmol	I	0.51	2.1 g, 55 %	1665, 1580, 1490, 1255, 1080 and 850	228-229
E3	1.2 g, 3.6 mmol	I	0.45	0.9 g, 81 %	1654, 1615, 1492, 1250, 1080 and 850	118-119
E4	1 g, 3.0 mmol	I	0.58	0.6 g, 64 %	1665, 1580, 1490, 1255, 1080 and 850	80-83
E5	2.5 g, 6.8 mmol	I	0.55	1.8 g, 78 %	1667, 1589, 1480, 1255, 1080 and 850	98-99
E6	1.9 g, 6.0 mmol	I	0.43	1.6 g, 94 %	1665, 1580, 1490, 1255, 1080 and 850	123-124
E7	1 g, 2.6 mmol	I	0.48	0.78 g, 82 %	1660, 1618, 1491, 1254, 1080 and 850	181-182
E8	1.5 g, 4.3 mmol	I	0.41	1.2 g, 92 %	1665, 1620, 1495, 1260, 1080 and 850	187-189
E9	1 g, 3 mmol	I	0.42	0,7 g, 78 %	1649, 1616, 1489, 1260, 1080 and 850	163-165
E10	0.6 g, 1.8 mmol	I	0.43	0.48 g, 85 %	1654, 1615, 1492, 1250, 1080 and 850	148-149
E11	1.2 g, 3 mmol	I	0.54	0.9 g, 74 %	1665, 1580, 1490, 1255, 1080 and 850	154-155

E12	1.9 g, 6.1 mmol	I	0.50	1.1 g, 64 %	1667, 1589, 1480, 1255, 1080 and 850	89-90
E13	1.9 g, 6.1 mmol	I	0.56	1.2 g, 68 %	1665, 1580, 1490, 1255, 1080 and 850	80-81
E14	1.9 g, 6.1 mmol	I	0.45	1.6 g, 80 %	1660, 1618, 1491, 1254, 1080 and 850	169-171

I.4a.4b. Synthesis of 6-allyloxy-2-substituted phenyl-4*H*-chromen-4-one derivatives (E15-26)

Table 13. Details of 6-allyloxy-2-substituted phenyl-4*H*-chromen-4-one derivatives

No.	D	Method	R_f ($CHCl_3$)	Yield (%)	IR (cm^{-1})	M.P. (°C)
E15	1.5 g, 5.0 mmol	II	0.50	1.1 g, 78 %	--------	NT
E16	2.5 g, 7.5 mmol	I	0.56	2.1 g, 91 %	1665, 1580, 1490, 1255, 1080 and 850	151-152
E17	3 g, 10 mmol	I	0.51	2.4 g, 88 %	1654, 1615, 1492, 1250, 1080 and 850	140-141
E18	3 g, 10 mmol	I	0.45	1 g, 3.0%	1665, 1580, 1490, 1255, 1080 and 850	160-161
E19	2.3 g, 6.2 mmol	I	0.54	1.7 g, 77 %	1667, 1589, 1480, 1255, 1080 and 850	104-106
E20	1.5 g, 4.7	I	0.59	1.1 g,	1665, 1580, 1490, 1255,	189-190

	mmol			78 %	1080 and 850	
E21	1.2 g, 3.1 mmol	I	0.49	0.9 g, 81 %	1660, 1618, 1491, 1254, 1080 and 850	147-148
E22	1.4 g, 3.1 mmol	I	0.55	1.2 g, 79 %	1665, 1620, 1495, 1260, 1080 and 850	198-200
E23	1 g, 3 mmol	I	0.50	0.7 g, 74 %	1649, 1616, 1489, 1260, 1080 and 850	180-182
E24	1.1 g, 3.5 mmol	I	0.49	0.9 g, 84 %	1654, 1615, 1492, 1250, 1080 and 850	120-121
E25	2 g, 6.4 mmol	II	0.51	1.5 g, 86 %	------	NT
E26	2.5 g, 8 mmol	I	0.45	1.5 g, 86 %	1667, 1589, 1480, 1255, 1080 and 850	118-119

I.4a.5: Synthesis of 4-(4/5-substituted-2-hydroxyphenyl)-6-(substutited phenyl)-2-aminopyrimidines (1-14)

4-(4-Allyloxy-2-hydroxyphenyl)-6-phenyl-2-aminopyrimidine (1)

A mixture of 7-allyloxy-2-phenyl-4*H*-chromen-4-one **(E1)** (0.2 g, 0.71 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. After removal of excess methanol the reaction mixture was poured on crushed ice containing acetic acid. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(1)**, (0.110 g, 50 %) m.p. 160-62 °C

Anal:

TLC : 0.51 (benzene)

IR (KBr): 3506, 3369, 3207, 1639, 1230, 890 and 760 cm^{-1}

PMR : δ 4.58 (2H, -O***CH***$_2$CH=CH_2), 5.3-5.4 (2H, OCH_2CH=***CH***$_2$), 6.0 (2H, -***NH***$_2$), 6.1, (1H, OCH_2***CH***=CH_2), 6.4-6.5 (2H, Ar***H***), 7.4-8.0 (7H, Ar***H***), 14.15 (1H, -***OH***)

Mass : 319.2 (m/z) (M+1)

4-(4-Allyloxy-2-hydroxyphenyl)-6-(4-chlorophenyl)-2-aminopyrimidine (2)

A mixture of 7-allyloxy-2-(4-chlorophenyl)-4*H*-chromen-4-one **(E2)** (1 g, 3.0 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 as per described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(2)**, (0.31 g, 28 %) m.p. 180-82 °C

Anal:

TLC : 0.59 (benzene)

IR (KBr): 3501, 3370, 3216, 1615, 1230, 890 and 760 cm^{-1}

PMR : δ 4.57 (2H, -O***CH***$_2$CH=CH_2), 5.2-5.4 (2H, OCH_2CH=***CH***$_2$), 5.46 (2H, -***NH***$_2$), 6.0, (1H, OCH_2***CH***=CH_2), 6.4-6.5 (2H, Ar***H***), 7.4-8.0 (7H, Ar***H***), 14.22 (1H, -***OH***)

Mass : 354.2 (m/z) (M+1)

4-(4-Allyloxy-2-hydroxyphenyl)-6-(3-chlorophenyl)-2-aminopyrimidine (3)

A mixture of 7-allyloxy-2-(3-chlorophenyl)-4*H*-chromen-4-one **(E3)** (0.4 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 as per described for **(1)**.

The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(3)**, (0.19 g, 42 %) m.p. 156-57 °C

Anal:

TLC : 0.59 (benzene)

IR (KBr): 3501, 3361, 3216, 1615, 1230, 890 and 760 cm^{-1}

PMR : δ 4.59 (2H, -O***CH₂***CH=CH_2), 5.3-5.4 (2H, OCH_2CH=***CH_2***), 6.3 (2H, -***NH_2***), 6.0 (1H, OCH_2***CH***=CH_2), 6.4-6.5 (2H, Ar***H***), 7.4-8.0 (7H, Ar***H***), 14.18 (1H, ***-OH***)

Mass : 354.2 (m/z) (M+1)

4-(4-Allyloxy-2-hydroxyphenyl)-6-(2-chlorophenyl)-2-aminopyrimidine (4)

A mixture of 7-allyloxy-2-(2-chlorophenyl)-4*H*-chromen-4-one **(E4)** (0.35 g, 1.1 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 as per described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(4)**, (0.12 g, 30 %) m.p. 191-92 °C

Anal:

TLC : 0.59 (benzene)

IR (KBr): 3481, 3390, 3216, 1615, 1230, 890 and 760 cm^{-1}

PMR : δ 4.56 (2H, -O***CH₂***CH=CH_2), 5.2-5.43 (2H, OCH_2CH=***CH_2***), 5.8 (2H, -***NH_2***), 6.0 (1H, OCH_2***CH***=CH_2), 6.4-6.5 (2H, Ar***H***), 7.4-8.3 (7H, Ar***H***), 14.12 (1H, ***-OH***)

Mass : 354.2 (m/z) (M+1)

4-(4-Allyloxy-2-hydroxyphenyl)-6-(2,4-dichlorophenyl)-2-aminopyrimidine (5)

A mixture of 7-allyloxy-2-(2,4-dichlorophenyl)-4*H*-chromen-4-one **(E5)** (0.8 g, 2.4 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 as per described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(5)**, (0.45 g, 50 %) m.p. 173-75 °C

Anal:

TLC : 0.51 (benzene)

IR (KBr): 3343, 3220, 3075, 1645, 1230, 890 and 760 cm^{-1}

PMR : δ 4.57 (2H, -O***CH₂***CH=CH_2), 5.2-5.43 (2H, OCH_2CH=***CH_2***), 6.3 (2H, -***NH_2***), 6.0 (1H, OCH_2***CH***=CH_2), 6.4-6.5 (2H, Ar***H***), 7.4-8.3 (7H, Ar***H***), 14.00 (1H, -***OH***)

Mass : 389.2 (m/z) (M+1)

4-(4-Allyloxy-2-hydroxyphenyl)-6-(4-fluorophenyl)-2-aminopyrimidine (6)

A mixture of 7-allyloxy-2-(4-fluorophenyl)-4*H*-chromen-4-one **(E6)** (0.8 g, 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 as per described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(6)**, (0.27 g, 30 %) m.p. 197-98 °C

Anal:

TLC : 0.46 (benzene)

IR (KBr): 3505, 3367, 3221, 1642, 1230, 890 and 760 cm^{-1}

PMR : δ 4.58 (2H, -O***CH₂***CH=CH_2), 5.2-5.43 (2H, OCH_2CH=***CH_2***), 6.3 (2H, -***NH_2***), 6.0 (1H, OCH_2***CH***=CH_2), 6.4-6.5 (2H, Ar***H***), 7.4-8.3 (7H, Ar***H***), 14.18 (1H, -***OH***)

Mass : 338.2 (m/z) (M+1)

4-(4-Allyloxy-2-hydroxyphenyl)-6-(4-bromophenyl)-2-aminopyrimidine (7)

A mixture of 7-allyloxy-2-(4-bromophenyl)-4*H*-chromen-4-one (**E7**) (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 as per described for (**1**). The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product (**7**), (0.3 g, 54 %) m.p. 196-97 °C

Anal:

TLC : 0.51 (benzene)

IR (KBr): 3498, 3373, 3201, 1639, 1230, 890 and 760 cm^{-1}

PMR : δ 4.58 (2H, -O***CH₂***CH=CH_2), 5.2-5.43 (2H, OCH_2CH=***CH₂***), 6.3 (2H, -***NH₂***), 6.0 (1H, OCH_2***CH***=CH_2), 6.4-6.5 (2H, Ar***H***), 7.4-8.3 (7H, Ar***H***), 14.11 (1H, -***OH***)

Mass : 398.2 (m/z) (M+1)

4-(4-Allyloxy-2-hydroxyphenyl)-6-(4-nitrophenyl)-2-aminopyrimidine (8)

A mixture of 7-allyloxy-2-(4-nitrophenyl)-4*H*-chromen-4-one (**E8**) (0.4 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 as per described for (**1**). The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product (**8**), (0.29 g, 64 %) m.p. 200-202 °C

Anal:

TLC : 0.59 (benzene)

IR (KBr): 3495, 3369, 3201, 1639, 1230, 890 and 760 cm^{-1}

PMR : δ 4.58 (2H, -O***CH₂***CH=CH_2), 5.2-5.43 (2H, OCH_2CH=***CH₂***), 6.3 (2H, -***NH₂***), 6.0 (1H, OCH_2***CH***=CH_2), 6.4-6.5 (2H, Ar***H***), 7.4-8.3 (7H, Ar***H***),

14.15 (1H, ***-OH***)

Mass : 365.2 (m/z) (M+1)

4-(4-Allyloxy-2-hydroxyphenyl)-6-(4-methoxyphenyl)-2-aminopyrimidine (9)

A mixture of 7-allyloxy-2-(4-methoxyhenyl)-4*H*-chromen-4-one **(E9)** (0.2 g, 0.64 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 as per described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(9)**, (0.11 g, 50 %) m.p. 199-200 °C

Anal:

TLC : 0.59 (benzene)

IR (KBr): 3499, 3220, 1639, 1230, 890 and 760 cm^{-1}

PMR : δ 3.8 (3H, -O***CH₃***),4.58 (2H, -O***CH₂***CH=CH_2), 5.2-5.43 (2H, OCH_2CH=***CH_2***), 6.3 (2H, -***NH_2***), 6.0, (1H, OCH_2***CH***=CH_2), 6.4-6.5 (2H, Ar***H***), 7.4-8.3 (7H, Ar***H***),

14.07 (1H, ***-OH***)

Mass : 350.2 (m/z) (M+1)

4-(4-Allyloxy-2-hydroxyphenyl)-6-(3-methoxyphenyl)-2-aminopyrimidine (10)

A mixture of 7-allyloxy-2-(3-methoxyhenyl)-4*H*-chromen-4-one **(E10)** (0.2 g, 0.64 mmol), guanidine hydrochloride (0.39 g, 4.0 mmol) and potassium hydroxide (0.11 g, 1.7 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up as per described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(10)**, (0.14 g, 63 %) 173-175 m.p. °C

Anal:

TLC : 0.48 (benzene)

IR (KBr): 3490, 3362, 3200, 1600, 1230, 890 and 760 cm^{-1}

PMR : δ 3.9 (3H, -O***CH₃***),4.58 (2H, -O***CH₂***CH=CH_2), 5.2-5.43 (2H, OCH_2CH=***CH_2***), 6.3 (2H, -***NH_2***), 6.0, (1H, OCH_2***CH***=CH_2), 6.4-6.5 (2H, Ar***H***), 7.4-8.3 (7H, Ar***H***), 14.18 (1H, -***OH***)

Mass : 350.2 (m/z) (M+1)

4-(4-Allyloxy-2-hydroxyphenyl)-6-(3,4-dimethoxyphenyl)-2-aminopyrimidine (11)

A mixture of 7-allyloxy-2-(3,4-dimethoxyhenyl)-4*H*-chromen-4-one **(E11)** (0.2 g, 0.64 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 as per described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(11)**, (0.099 g, 41 %) 208-209 m.p. °C

Anal:

TLC : 0.59 (benzene)

IR (KBr): 3493, 3370, 3194, 1604, 1230, 890 and 760 cm^{-1}

PMR : δ 3.9 (3H, -O***CH₃***),4.0 (3H, -***OCH₃***) ,4.58 (2H, -O***CH₂***CH=CH_2), 5.2-5.43 (2H, OCH_2CH=***CH_2***), 6.3(2H, -***NH_2***), 6.0, (1H, OCH_2***CH***=CH_2), 6.4-6.5 (2H, Ar***H***), 7.4-8.3 (7H, Ar***H***), 14.21 (1H, -***OH***)

Mass : 380.2 (m/z) (M+1)

4-(4-Allyloxy-2-hydroxyphenyl)-6-(4-methylphenyl)-2-aminopyrimidine (12)

A mixture of 7-allyloxy-2-(4-methylphenyl)-4*H*-chromen-4-one **(E12)** (0.2 g, 0.64 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 as per described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(12)**, (0.19 g, 42 %) m.p. 165-67 °C

Anal:

TLC : 0.49 (benzene)

IR (KBr): 3490, 3362, 3200, 1600, 1230, 890 and 760 cm^{-1}

PMR : δ 2.4 (3H, -***CH₃***), 4.57 (2H, -O***CH₂***CH=CH_2), 5.2-5.43 (2H, OCH_2CH=***CH₂***), 6.2 (2H, -***NH₂***), 6.0, (1H, OCH_2***CH***=CH_2), 6.4-6.5 (2H, Ar***H***), 7.4-8.3 (7H, Ar***H***), 14.18 (1H, -***OH***)

Mass : 334.2 (m/z) (M+1)

4-(4-Allyloxy-2-hydroxyphenyl)-6-(3-methylphenyl)-2-aminopyrimidine (13)

A mixture of 7-allyloxy-2-(3-methylphenyl)-4*H*-chromen-4-one **(E13)** (0.2 g, 0.64 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 as per described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(13)**, (0.19 g, 42 %) m.p. 165-67 °C

Anal:

TLC : 0.49 (benzene)

IR (KBr): 3490, 3362, 3200, 1600, 1230, 890 and 760 cm^{-1}

PMR : δ 2.4 (3H, -***CH₃***), 4.57 (2H, -O***CH₂***CH=CH_2), 5.2-5.43 (2H, OCH_2CH=***CH₂***), 6.2 (2H, -***NH₂***), 6.0, (1H, OCH_2***CH***=CH_2), 6.4-6.5 (2H, Ar***H***), 7.4-8.3 (7H, Ar***H***), 14.18 (1H, -***OH***)

Mass : 334.2 (m/z) (M+1)

4-(4-Allyloxy-2-hydroxyphenyl)-6-furan-2-aminopyrimidine (14)

A mixture of 7-allyloxy-2-furan-4*H*-chromen-4-one **(E14)** (0.2 g, 0.64 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 as per described for **(1)**. The solid

so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(14)**, (0.19 g, 42 %) m.p. 165-67 °C

Anal:

TLC : 0.49 (benzene)

IR (KBr): 3490, 3362, 3200, 1600, 1230, 890 and 760 cm^{-1}

PMR : δ 4.57 (2H, -O***CH₂***CH=CH_2), 5.2-5.43 (2H, OCH_2CH=***CH_2***), 6.2 (2H, -***NH_2***), 6.0, (1H, OCH_2***CH***=CH_2), 6.4-6.5 (2H, Ar***H***), 7.4 (1H, Pyri***H***) 7.4 (3H, ***Furan***), 7.34 (1H, Ar***H***), 13.18 (1H, -***OH***)

Mass : 310.2 (m/z) (M+1)

4-(5-Allyloxy-2-hydroxyphenyl)-6-phenyl-2-aminopyrimidine (15)

A mixture of 6-allyloxy-2-phenyl-4*H*-chromen-4-one **(E15)** (0.5 g, 1.7 mmol), guanidine hydrochloride (0.42 g, 4.4 mmol) and potassium hydroxide (0.2 g, 3.5 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up as per described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(15)**, (0.14 g, 24 %) m.p. 183-84 °C

Anal:

TLC : 0.58 (benzene)

IR (KBr): 3428, 3384, 3210, 1635, 1230, 890 and 760 cm^{-1}

PMR : δ 4.48 (2H, -O***CH₂***CH=CH_2), 5.2-5.43 (2H, OCH_2CH=***CH_2***), 5.0 (2H, -***NH_2***), 5.9, (1H, OCH_2***CH***=CH_2), 6.8-6.9 (2H, Ar***H***), 7.5-7.8 (7H, Ar***H***), 12.05 (1H, -***OH***)

Mass : 319.2 (m/z) (M+1)

4-(5-Allyloxy-2-hydroxyphenyl)-6-(4-chlorophenyl)-2-aminopyrimidine (16)

A mixture of 6-allyloxy-2-(4-chlorophenyl)-4*H*-chromen-4-one (**E16**) (0.5 g, 1.5 mmol), guanidine hydrochloride (0.35 g, 3.7 mmol) and potassium hydroxide (0.3 g, 5.3 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up as pe described for (**1**). The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product (**16**), (0.21 g, 41 %) m.p. 188-89 °C

Anal:

TLC : 0.57 (benzene)

IR (KBr): 3499, 3348, 3200, 1618, 1230, 890 and 760 cm^{-1}

PMR : δ 4.44 (2H, -O***CH₂***CH=CH_2), 5.2-5.43 (2H, OCH_2CH=***CH_2***), 5.1 (2H, -***NH_2***), 5.9 (1H, OCH_2***CH***=CH_2), 6.8-6.9 (2H, Ar***H***), 7.5-7.8 (6H, Ar***H***), 12.12 (1H, -***OH***)

Mass : 354.2 (m/z) (M+1)

4-(5-Allyloxy-2-hydroxyphenyl)-6-(3-chlorophenyl)-2-aminopyrimidine (17)

A mixture of 6-allyloxy-2-(3-chlorophenyl)-4*H*-chromen-4-one (**E17**) (0.5 g, 1.5 mmol), guanidine hydrochloride (0.35 g, 3.7 mmol) and potassium hydroxide (0.3 g, 5.3 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up as per described for (**1**). The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product (**17**), (0.13 g, 30 %) m.p. 159-60 °C

Anal:

TLC : 0.57 (benzene)

IR (KBr): 3451, 3351, 3229, 1646, 1230, 890 and 760 cm^{-1}

PMR : δ 4.57 (2H, -O***CH₂***CH=CH_2), 5.2-5.4 (2H, OCH_2CH=***CH_2***), 6.5 (2H, -***NH_2***), 6.0 (1H, OCH_2***CH***=CH_2), 6.8-6.9 (2H, Ar***H***), 7.0-8.1 (6H, Ar***H***), 12.12 (1H, -***OH***)

Mass : 354.2 (m/z) (M+1)

4-(5-Allyloxy-2-hydroxyphenyl)-6-(2-chlorophenyl)-2-aminopyrimidine (18)

A mixture of 6-allyloxy-2-(2-chlorophenyl)-4*H*-chromen-4-one **(E18)** (0.8 g, 2.5 mmol), guanidine hydrochloride (0.6 g, 6.3 mmol) and potassium hydroxide (0.21 g, 3.7 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up as per described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(18)**, (0.54 g, 64 %) m.p. 149-50 °C

Anal:

TLC : 0.57 (benzene)

IR (KBr): 3499, 3348, 3333, 1618, 1230, 890 and 760 cm^{-1}

PMR : δ 4.6 (2H, -O***CH₂***CH=CH_2), 5.2-5.4 (2H, OCH_2CH=***CH_2***), 6.6 (2H, -***NH_2***), 6.0 (1H, OCH_2***CH***=CH_2), 7.3-7.9 (8H, Ar***H***), 13.44 (1H, -***OH***)

Mass : 354.2 (m/z) (M+1)

4-(5-Allyloxy-2-hydroxyphenyl)-6-(2,4-dichlorophenyl)-2-aminopyrimidine (19)

A mixture of 6-allyloxy-2-(2,4-dichlorophenyl)-4*H*-chromen-4-one **(E19)** (1 g, 2.8 mmol), guanidine hydrochloride (0.68 g, 7.2 mmol) and potassium hydroxide (0.21 g, 3.7 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up as per described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(19)**, (0.68 g, 61 %) m.p. 192-93 °C

Anal:

TLC : 0.51 (benzene)

IR (KBr): 3506, 3371, 3075, 1645, 1230, 890 and 760 cm^{-1}

PMR : δ 4.4 (2H, -O***CH₂***CH=CH_2), 5.1 (2H, OCH_2CH=***CH_2***), 5.0 (2H, -***NH_2***), 5.97 (1H, OCH_2***CH***=CH_2), 7.2-7.9 (7H, Ar***H***),

12.81 (1H, -***OH***)

Mass : 389.2 (m/z) (M+1)

4-(5-Allyloxy-2-hydroxyphenyl)-6-(4-fluorophenyl)-2-aminopyrimidine (20)

A mixture of 6-allyloxy-2-(4-fluorophenyl)-4*H*-chromen-4-one **(E20)** (0.8 g, 2.6 mmol), guanidine hydrochloride (0.5 g, 5.2 mmol) and potassium hydroxide (0.3 g, 5.3 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up as per described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from to yield the title product **(20)**, (0.48 g, 53 %) m.p. 159-60 °C

Anal:

TLC : 0.51 (benzene)

IR (KBr): 3501, 3329, 3216, 1624, 1230, 890 and 760 cm^{-1}

PMR : δ 4.5 (2H, -O***CH₂***CH=CH_2), 5.2-5.3 (2H, OCH_2CH=***CH_2***), 6.1 (2H, -***NH_2***), 5.97 (1H, OCH_2***CH***=CH_2), 6.8-8.1 (8H, Ar***H***), 13.44 (1H, -***OH***)

Mass : 338.2 (m/z) (M+1)

4-(5-Allyloxy-2-hydroxyphenyl)-6-(4-bromophenyl)-2-aminopyrimidine (21)

A mixture of 6-allyloxy-2-(4-bromophenyl)-4*H*-chromen-4-one **(E21)** (0.5 g, 1.3 mmol), guanidine hydrochloride (0.33 g, 3.2 mmol) and potassium hydroxide (0.2 g, 3.5 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up as per described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(21)**, (0.28 g, 52 %) m.p. 184-85 °C

Anal:

TLC : 0.51 (benzene)

IR (KBr): 3491, 3353, 3216, 1624, 1230, 890 and 760 cm^{-1}

PMR : δ 4.46 (2H, -O***CH₂***CH=CH_2), 5.0-5.2 (2H, OCH_2CH=***CH_2***), 5.2

(2H, -NH_2), 6.6 (1H, $OCH_2CH{=}CH_2$), 6.8-8.1 (8H, Ar*H*),

13.40 (1H, -*OH*)

Mass : 398.2 (m/z) (M+1)

4-(5-Allyloxy-2-hydroxyphenyl)-6-(4-nitrophenyl)-2-aminopyrimidine (22)

A mixture of 6-allyloxy-2-(4-nitrophenyl)-4*H*-chromen-4-one **(E22)** (0.5 g, 1.5 mmol), guanidine hydrochloride (0.33 g, 3.2 mmol) and potassium hydroxide (0.2 g, 3.5 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up as per described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(22)**, (0.3 g, 53 %) m.p. 190-92 °C

Anal:

TLC : 0.51 (benzene)

IR (KBr): 3495, 3369, 3221, 1645, 1230, 890 and 760 cm^{-1}

PMR : δ 4.65 (2H, -$OCH_2CH{=}CH_2$), 5.3-5.4 (2H, $OCH_2CH{=}CH_2$), 6.9

(2H, -NH_2), 6.0 (1H, $OCH_2CH{=}CH_2$), 7.3-8.3 (8H, Ar*H*),

14.15 (1H, -*OH*)

Mass : 365.2 (m/z) (M+1)

4-(5-Allyloxy-2-hydroxyphenyl)-6-(4-methoxyphenyl)-2-aminopyrimidine (23)

A mixture of 6-allyloxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one **(E23)** (0.5 g, 1.6 mmol), guanidine hydrochloride (0.38 g, 4.0 mmol) and potassium hydroxide (0.2 g, 3.5 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up as per described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(23)**, (0.19 g, 34 %) m.p. 113-15 °C

Anal:

TLC : 0.49 (benzene)

IR (KBr): 3497, 3438, 3182, 1643, 1230, 890 and 760 cm^{-1}

PMR : δ 3.8 (3H, -O***CH₃***), 4.58 (2H, -O***CH₂***CH=CH_2), 5.3-5.4 (2H, OCH_2CH=***CH₂***), 6.9 (2H, -***NH₂***), 6.0 (1H, OCH_2***CH***=CH_2), 7.38 (1H, Pyri***H***) and 7.0-8.0 (7H, Ar***H***), 14.07 (1H, -***OH***)

Mass : 349.2 (m/z) (M+1)

4-(5-Allyloxy-2-hydroxyphenyl)-6-(3-methoxyphenyl)-2-aminopyrimidine (24)

A mixture of 6-allyloxy-2-(3-methoxyphenyl)-4*H*-chromen-4-one **(E24)** (0.5 g, 1.6 mmol), guanidine hydrochloride (0.38 g, 4.0 mmol) and potassium hydroxide (0.2 g, 3.5 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up as per described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(24)**, (0.2 g, 40 %) m.p. 110-12 °C

Anal:

TLC : 0.49 (benzene)

IR (KBr): 3457, 3353, 3236, 1645, 1230, 890 and 760 cm^{-1}

PMR : δ 3.9 (3H, -O***CH₃***), 4.5 (2H, -O***CH₂***CH=CH_2), 5.3-5.4 (2H, OCH_2CH=***CH₂***), 6.9 (2H, -***NH₂***), 6.0 (1H, OCH_2***CH***=CH_2), 7.38 (1H, Pyri***H***) and 7.0-8.0 (7H, Ar***H***), 14.11 (1H, -***OH***)

Mass : 349.2 (m/z) (M+1)

4-(5-Allyloxy-2-hydroxyphenyl)-6-(4-methylphenyl)-2-aminopyrimidine (25)

A mixture of 6-allyloxy-2-(4-methylphenyl)-4*H*-chromen-4-one **(E25)** (0.6 g, 2 mmol), guanidine hydrochloride (0.48 g, 5.1 mmol) and potassium hydroxide (0.17 g, 3 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up as per described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(25)**, (0.29 g, 42 %) m.p. 160-161°C

Anal:

TLC : 0.59 (benzene)

IR (KBr): 3492, 3354, 1630, 1230, 890 and 760 cm^{-1}

PMR : δ 2.4 (3H, -***CH*$_3$**), 4.7 (2H, -O***CH*$_2$**CH=CH_2), 5.0-5.1 (2H, OCH_2CH=***CH*$_2$**), 5.2 (2H, -***NH*$_2$**), 5.99 (1H, OCH_2***CH***=CH_2), . 7.2-7.56 (9H, Ar***H***), 12.87 (1H, -***OH***)

Mass : 334.2 (m/z) (M+1)

4-(5-Allyloxy-2-hydroxyphenyl)-6-(3-methylphenyl)-2-aminopyrimidine (26)

A mixture of 6-allyloxy-2-(3-methylphenyl)-4*H*-chromen-4-one **(E26)** (1 g, 3.4 mmol), guanidine hydrochloride (0.8 g, 8.1 mmol) and potassium hydroxide (0.3 g, 5.3 mmol) was refluxed in methanol (30 ml) for 12 hrs. The work up as per described for **(1)**. The solid so obtained was filtered, washed with water and recrystallised from methanol to yield the title product **(26)**, (0.65 g, 57 %) m.p. 125-26°C

Anal:

TLC : 0.59 (benzene)

IR (KBr): 3508, 3375, 3209, 1636, 1230, 890 and 760 cm^{-1}

PMR : δ 2.4 (3H, -***CH*$_3$**), 4.7 (2H, -O***CH*$_2$**CH=CH_2), 5.0-5.1 (2H, OCH_2CH=***CH*$_2$**), 5.2 (2H, -***NH*$_2$**), 5.99 (1H, OCH_2***CH***=CH_2), . 7.2-7.56 (9H, Ar***H***), 12.87 (1H, -***OH***)

Mass : 334.2 (m/z) (M+1)

5.2 Biological Work

The biological studies carried out have been discussed under following heads:

5.2b. Anti HIV activity

Preliminary anti-HIV evaluation was carried out at Rega Institute for Medical Research, Katholieke Universiteit Leuven, Belgium, adopting the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. 4,6-diaryl pyrimidines were evaluated for anti-HIV activity by determining their ability to inhibit the replication of HIV-1 (IIIB) in MT-4 cells. Cytotoxicity (CC_{50}) of the compounds was evaluated in parallel with antiviral activity (IC_{50}) for determining their selectivity index (SI). The results are given in **Table.7.**

Anti-HIV Assay

The antiviral activity of the test compounds was tested against HIV-IIIB, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay as previously described. Briefly, various concentrations of the test compounds were added to wells of a flat-bottom microtiter plate. Subsequently, virus and MT-4 cells were added to a final concentration of 200 CCID50/well and 30 000 cells/well, respectively. To determine the toxicity of the test compound, mock-infected cell cultures, containing an identical compound concentration range were incubated in parallel with the virus infected cell cultures. After 5 days of incubation (37 °C, 5% CO_2), the viability of the cells was determined using MTT. The results of drug susceptibility assays were expressed as an EC_{50} defined as the concentration of drug at which there was 50% infection compared with the drug-free control. Toxicity results are expressed as CC_{50} which is defined as the concentration of the drug at which cell viability was reduced by 50% as compared to the drug-free control.