Part B: Synthesis, Anti-HIV Activity and Docking Studies of 4,6-diaryl-2-aminopyrimidines

5

Introduction

Acquired Immunodeficiency Syndrome (AIDS)

The human immunodeficiency virus (HIV) is the cause of the acquired immunodeficiency syndrome (AIDS), which was first identified in the Western world in 1981. Since then, AIDS has developed into a worldwide pandemic of disastrous proportions[214], with more than 42 million people infected, the vast majority residing in resource-limited countries. The cumulative death toll from the epidemic is at least 23 million persons[215].

Present AntiHIV therapy and its complications

The current scenario in the treatment of HIV is highly active antiretroviral therapy (HAART), in which potent combinations of inhibitors are used to suppress viral replication maximally, thereby reducing the number of viral variants generated and the opportunity to select for resistant mutations. The most common combinations used in HAART generally include two or three reverse transcriptase (RT) inhibitors and one or two protease inhibitors. As of now, the FDA has approved[216] 14 anti-HIV agents as drugs for clinical use, including 6 nucleoside reverse transcriptase inhibitors (NRTIs), 3 non nucleoside reverse transcriptase inhibitors (NNRTIs), and 5 protease inhibitors (PIs). All these drugs are currently used alone or as part of a combination regimen to treat HIV infection/AIDS disease. Still, the problem is not completely answered because of the serious CNS complications produced by the presently available anti-HIV drugs. Investigators have begun to realize that HIV cannot be completely eradicated with the currently available treatments and that long-term HAART may have side-effects that are severe or health complicating. The reappearance of the HIV in the blood suggests that this treatment may not eradicate the virus in certain reservoirs, mainly CNS, where

it is replicated and delivered to the periphery. This is because the present drugs have limited CNS penetration. Due to this inability to affect CNS viral load, effective treatment of neurologic and psychiatric components of HIV infection is thought to be limited[217]. Moreover, the patient has to continue HAART treatment, life time. In such circumstances the patient's health condition worsens because of the CNS complications like toxoplasmosis, cryptococcal meningitis, primary CNS lymphoma, and progressive multifocal leukoencephalopathy (PML), peripheral or sensory neuropathy and acquired immune deficiency syndrome (AIDS) dementia complex[218, 219] (ADC). The neurological complications are due interactions of virus and macrophages in the brain and is incompletely understood[220].

Reverse Transcriptase (RT)

RT of human immunodeficiency virus type 1 (HIV-1) (Fig) is a key enzyme[221] for the cause of AIDS. Structurally, RT is a heterodimer composed of a 66-kDa subunit (p66) and a 51-kDa subunit (p51) derived from p66 by proteolytic removal of the C-terminal domain. RT possesses both DNA polymerase activity, which ultimately produces double-stranded DNA from the viral genomic RNA, and a ribonuclease H (RNase H) activity, which cleaves the viral genome after it is copied.

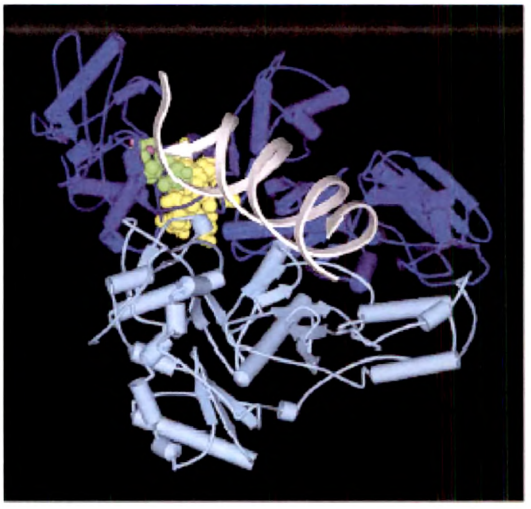
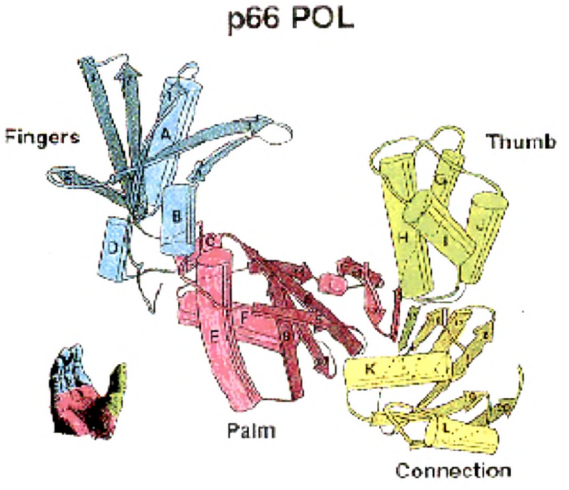


Figure HIV RT complexed with DNA template:primer (white flat arrows). The RT heterodimer consists of a p66 subunit (dark blue) and a p51 subunit (light blue). The two magnesium ions in the active site are shown as purple balls. The side-chains of active-site amino acids Tyr-183, Met-184, Asp-185, Asp-186 and Asp-110 are represented as green-colored Van der Waals spheres. Residues of the NNRTI binding site (Leu-100, Lys-101, Lys-103, Val-106, Val-108, Val-179, Tyr-181, Tyr-188, Pro-225, Phe-227, Trp-229, Leu-234, Pro-236 and Tyr-318) are represented as yellow-colored Van der Waals spheres. Based on X-ray structure (PDB code: 1RTD) with a resolution of 3.2 Å. Schematic presentation of the protein (helices = cylinders, sheets = flat arrows, and coils and turns = strand).

The 66kDa monomer has identifiable 'finger', 'palm', and 'thumb' components which are functionally analogous to those in the Klenow fragment structure[222]. The Klenow fragment is a large protein fragment which is produced when DNA polymerase I from *E. coli* is cleaved by the protease enzyme subtilisin. It exhibits the 5′ → 3′ polymerase activity and the 3′ → 5′ exonuclease activity for removal of precoding nucleotides, but does not retain the 5' → 3' exonuclease activity.



Mechanism of action of NRTI and NNRTIs

Most of the antiHIV agents bind to the p66 palm near the polymerase activity, a region that is a well-packed hydrophobic core in the unliganded enzyme. Room

for the drug is provided by the movement of β-sheet within the palm domain. As a result, rearrangement within the palm, thumb and as well as domain shifts relative to the enzyme core, leads to prevent the correct placement of the nucleotide substrate when the drug is bound[223].

In 1987, zidovudine, a nucleoside RT inhihibitor (NRTI), was approved in the USA as the first chemotherapeutic agent against HIV[224].

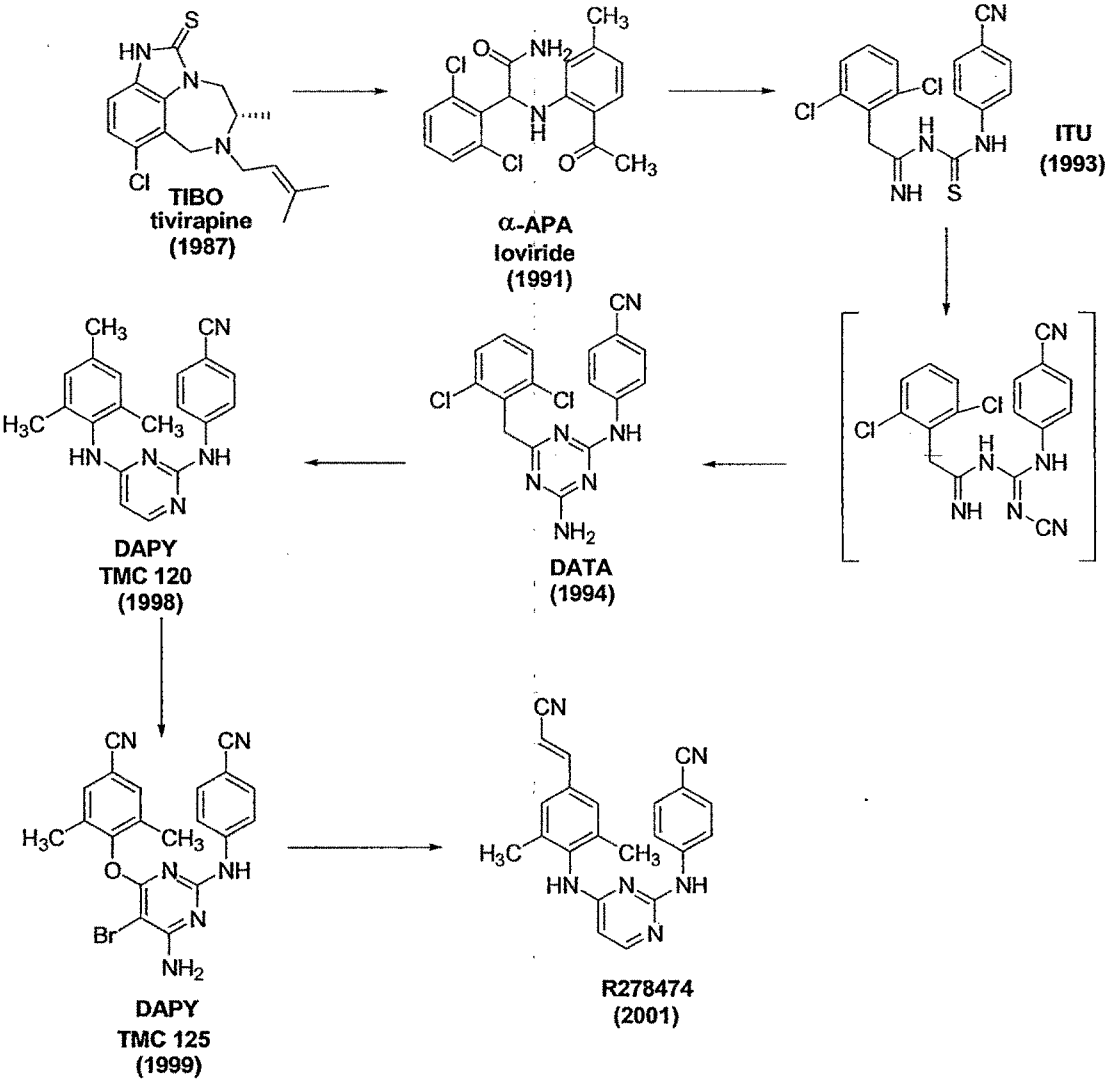
Development of NNRTI

Later, it was found that the function of RT could also be blocked by noncompetitive inhibitors that show no chemical resemblance to nucleosides, the non-nucleoside reverse transcriptase inhibitors (NNRTIs) like Nevirapine[225] and TIBO[226]. This mode of inhibition is attractive for drug design because, unlike for the nucleoside inhibitors there is little interference with human systems and therefore low toxicity and side effects.

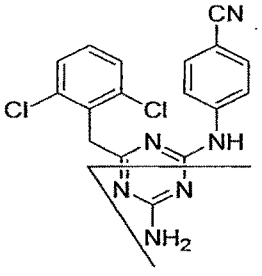
Evolution of Diaryl pyrimidines (DAPY) as NNRTIs

As mentioned in the Part-1 introduction, TIBO analogues, the first NNRTIs, were discovered at the Rega Institute, Belgium. Subsequent screening of the Janssen compounds led to the discovery of the *R*-APA (*R*-anilino*ph*enyl*a*cetamide) class of NNRTIs[227]. Further chemical modification[228] led to the class of potent ITU (iminothiourea) NNRTIs. In an attempt to synthesize the corresponding imino-*N*-cyanoguanidine derivatives of ITU analogues, an unexpected ring closure occurred, producing R106168, the first compound of the DATA (diaryltriazine) class of NNRTIs[229]. 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 TMC120 (R147681) is the prototype. In phase II studies on treatment of HIV-infected patients, TMC120 and TMC125 proved to be highly active in reducing viral loads[230, 231]. TMC125 was also found to significantly

reduce viral load after 7 days of treatment in anti-retroviral-experienced patients whose HIV viruses carried RT mutations selected by previous exposure to NNRTIs[232]. Further collaboration among medicinal chemists, crystallographers, and molecular modelers led in 2001 to the discovery of the cyanovinyl DAPY compounds, of which R278474 is the prototype[233]. The latter is the *E*-isomer of the *p*-cyanovinyl analogue of TMC120. The development of the NNRTIs is depicted in the chart below:



Incorporation of a structural fragment or pharmacophore into a molecule is the one of the approaches adopted in searching for lead compounds. Common fragments present in various compounds contribute to similarity in biological activity. However, they usually exhibit different potencies. The common fragments, guanidine and the diaryl wings, present in the structures of the diaryl pyrimidines, an NNRTI class of compounds, were recognized by us.



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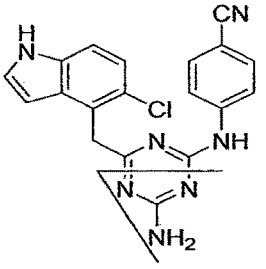

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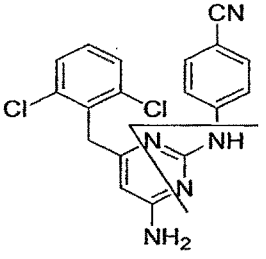

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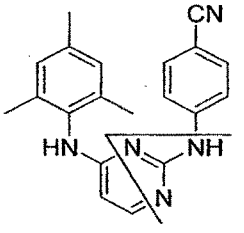

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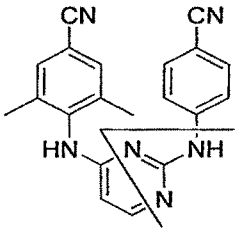

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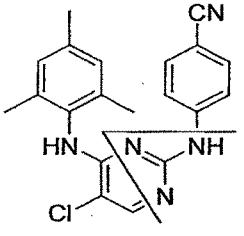

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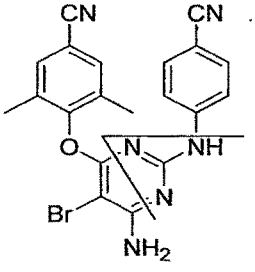

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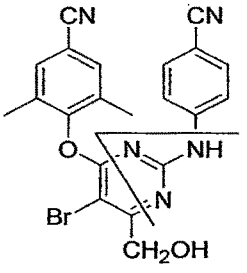

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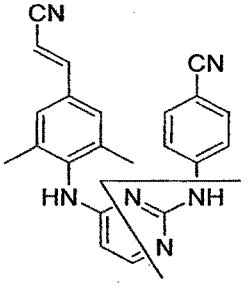

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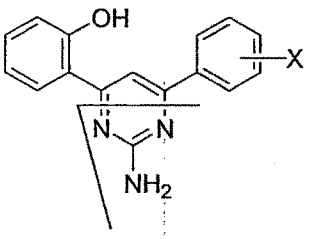

Proposed compounds (50a-q)

Compounds containing such common fragments can be synthesized by reacting the starting materials containing such fragments or by incorporating the fragments during the synthesis. It is also known that diaryl pyrimidines could be synthesized from a common intermediate chromen-4-one derivatives **(47a-q)**, from which we reported the synthesis of different 5,7-diaryl diazepines presented in Part I of this thesis. The reaction of flavones with guanidine hydrochloride leads to the formation of the diaryl pyrimidines which contain both the common fragments.

Hence, it was proposed to synthesize diaryl pyrimidines and evaluate their anti-HIV activity.