Review Article

Diarylpyrimidine derivatives– Potent NNRTIs against resistant HIV-1 and wild type strains

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ABSTRACT

Drug resistance is a particularly difficult problem in the treatment of human immunodeficiency virus (HIV) the virus that causes AIDS. Emergence of drug-resistant HIV variants in patients is the primary cause of treatment failure. Etravirine (TMC125-R165335) and other diarylpyrimidine derivatives are non-nucleoside reverse transcriptase inhibitor a flexible molecule that can fit in the active pocket of HIV's reverse transcriptase in different ways, even when the shape of that pocket changes because of viral mutations that would defeat other drugs. TMC125 is a highly flexible compound with low in vitro toxicity. TMC125 has garnered attention because of its activity against NNRTI-resistant HIV strains..

Key words: *Diarylpyrimidine, HIV, Reverse transcriptase, NNRTIs, AIDS*

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INTRODUCTION

HIV type 1, a causative agent of AIDS, is a source of worldwide morbidity and mortality. There are an estimated 1 million people in North America currently living with HIV infection, and more than 40,000 new cases occur annually. Before the advent of highly active antiretroviral therapy (HAART), the mortality rate of HIV infection was nearly 100%, and life expectancy was short. However, successful HAART delays the onset of AIDS, allowing patients to live with chronic HIV infection for 20 years or more. HAART usually consists of a combination of protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs), and/or non nucleoside reverse transcriptase inhibitors (NNRTIs). Although these agents are highly efficacious in delaying the onset of AIDS, their clinical utility is limited by viral resistance, non adherence to therapy, and drug toxicity.[1] The successful development of about 20 new anti-HIV drugs during the last two decades is ample proof that antiviral discovery has truly come of age and that selective antiviral drugs can achieve important clinical benefits. The introduction of these anti-HIV drugs as part of a so-called highly active antiretroviral therapy (HAART) has led to a dramatic reduction of patient mortality and morbidity.[3]

The approved anti- HIV drugs inhibit either the viral envelope gp41-mediated

fusion of the viral and host cell membrane (1 drug, for review see [4], the HIV reverse transcriptase (RT)-catalyzed transcription of the viral RNA genome to a DNA copy (11 drugs, 2 mechanistic classes differing in binding sites and mechanism of drug inhibition) or (iii) the HIV protease (PR) mediated cleavage of immature viral proteins into new enzymatic and structural HIV proteins (9 drugs). These well-characterized viral steps in the HIV replication cycle are thus extensively clinically validated since daily and continued administration of certain combinations of these agents (HAART) reduces the HIV load to levels below the limits of detection, prevents ongoing host cell destruction and even allows restoration of CD4 cell numbers and function [2].

HIV and drug resistance

Drug resistance is a serious clinical concern in the chemotherapeutic treatment of an infection, whether bacterial or viral, or with anticancer mutations. Drug resistance is a particularly difficult problem in the treatment of human immunodeficiency virus (HIV) [7], the virus that causes AIDS. Emergence of drug-resistant HIV variants in patients is the primary cause of treatment failure. HIV is a retrovirus and the genome in the virion is encoded in RNA. This RNA genome is generated by the host cell‟s DNA-dependent RNA polymerase. In an infected cell, the viral RNA is reverse transcribed to produce a double-stranded DNA (dsDNA). Reverse transcription is carried out by the retroviral enzyme reverse transcriptase (RT), using the following catalytic activities:

(i) RNA-dependent DNA polymerization to form an RNA:DNA hybrid; (ii) RNase H degradation of the RNA strand from RNA:DNA hybrids; and (iii) DNA dependent DNA polymerization to form dsDNA. Neither the host RNA- dependent DNA polymerase nor the RT have proofreading or other error correction capabilities. The error rate per nucleotide introduced in a single cycle of retroviral replication is between 10_4 and 10_5: HIV undergoes a cycle of replication approximately every 2 days. The relatively fast turnover of HIV combined with a high mutation rate results in the production of a large pool of viral variants (referred to as a "quasispecies").[6]

Many of the variants are capable of carrying out normal HIV functions though mutations affect the efficiency of replication to varying degrees. In the presence of drugs that suppress the replication of wild-type HIV, drug- resistant variants are selected and eventually these mutants dominate the viral population [9]. Drug- resistant variants arise if the virus can replicate in the presence of the drugs. To try to completely block replication, AIDS patients are commonly treated with combinations or "cocktails" of drugs targeting the viral enzymes RT and protease, a treatment strategy known as HAART (highly active antiretroviral therapy.[10]

Unfortunately, multi-drug-resistant HIV variants can emerge when patients are treated with drug cocktails [11]. New drugs and treatment strategies are designed to block the replication of viruses that are resistant to the available drugs. One such new drug, the fusion inhibitor enfuvirtide (Fuzeon) [12] was recently approved for treatment of patients with advanced HIV infection. Resistance to enfuvirtide has been recently reported [13]. New inhibitors against the existing and novel viral targets

are being developed for clinical use [8]. Among the most promising new anti-AIDS drug candidates, etravirine (TMC125-R165335) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that is effective against a wide range of existing drug- resistant HIV-1 viral variants.

HIV-1 genome & reverse transcriptase (RT)

The human immunodeficiency virus type 1 (HIV-1) genome encodes a variety of different proteins, including the essential viral enzymes protease (PR), reverse transcriptase (RT), and integrase (IN). These viral enzymes are translated not as discrete units but rather as segments of a much larger polyprotein termed Pr160gag-pol, and nascent virions assemble using these polyprotein precursors. The individual active enzymes are subsequently formed by proteolytic cleavage at specific sites on Pr160gag-pol during virion assembly and budding, a "maturation" process catalyzed by PR.[25,34] The monomeric subunits of the HIV-1 enzymes are inactive; each enzyme must oligomerize to at least a dimer for enzymatic activity. [3,16] PR and IN are homodimers (or perhaps higher-order homo-oligomers in the case of IN) with subunits of the size predicted from their genes. The mature active form of HIV-1 PR is a symmetrical homodimer released from Pr160gag-pol upon autoprocessing carried out by the polyprotein form of the enzyme [44].

RT and IN are formed after activation of PR, but it is still unclear whether the PR mediated proteolytic processing of these Pol proteins occurs in *cis*, in *trans*, or in some combination of these [55] [56].

Like HIV-1 PR, active HIV-1 IN is at least a homodimer, although higher- order homo-oligomers may play a role in the multiple activities of this enzyme [30] [31]. In contrast, RT in mature infectious virions is a heterodimer with subunits of 66 kDa (p66) and 51 kDa (p51), even though the gene for HIV-1 RT encodes only a protein of 66 kDa. The smaller p51 subunit is derived from the larger p66 subunit by proteolytic cleavage between RT amino acid residues F440/Y441 during virion maturation.[9,18,27].

Although both p66 and p51 subunits have identical amino acid sequences, their folding in the context of the active RT heterodimer differs, resulting in an asymmetric dimer structure [37]. The catalytic activities of HIV-1 RT, namely, DNA polymerase and RNase H (RNH), are carried out solely by the p66 subunit of the RT heterodimer [40]. The function of the p51 subunit is not entirely clear, but it may play a primarily structural role [1]. Thus, formation of mature active RT from the Pr160gag-pol polyprotein precursor in HIV-1 virions requires an additional internal PR-catalyzed cleavage event compared to the formation of active IN or PR. This suggests that the heterodimeric form of HIV-1 RT is essential for virus replication. However, recombinant RT p66/p66 homodimers have enzymatic activities (DNA polymerase and RNH) comparable to RT p66/p51 heterodimers [5,20]. While functional PR and IN are released as separate entities in all retroviruses, the proteolytic events that generate RT appear to differ. Moloney murine leukemia virus consists of a single subunit [29], suggesting that the catalytically active enzyme is a monomer or perhaps homodimer. In addition, chimeras constructed from the first

425 amino acid residues of HIV-1 RT and the last 200 amino acid residues of Moloney murine leukemia virus RT have a single subunit composition and possess substantial enzymatic activity [47]. Further, RT isolated from avian sarcoma leucosis virus (ASLV) virions includes both heterodimers and homodimers [2] [26] neither of which are processed internally between the polymerase and RNH domain [3].

HIV-1 RT is a heterodimer consisting of p66 and p51 subunits. The p66 subunit contains an N-terminal polymerase domain and a C-terminal RNase H domain. The p51 subunit is derived either from p66 or from a large gag-pol precursor by proteolytic cleavage with HIV-1 protease and has the same amino acid sequence as the polymerase domain of p66. Crystal structures of HIV-1 RT [30,39] revealed that the p66 subunit resembles a right hand containing fingers, palm, thumb, and connection sub domains (Fig. 1a). The nucleic acid binding cleft extends from the polymerase active site to the RNase H active site in the p66 subunit, covering a distance of approximately 17–18 base pairs. The p51 subunit is not directly involved in catalysis but instead appears to provide structural support which allows the p66 subunit to carry out polymerase and RNase H activities.RT undergoes conformational changes while carrying out its enzymatic functions. Steps including binding of RNA, DNA, and dNTP, nucleotide incorporation and translocation of the nucleic acid substrate can affect the conformation of RT. In particular, the p66 thumb [40,41] and fingers [42,43] move when nucleic acids and dNTP bind. The non-nucleoside inhibitor-binding pocket (NNIBP) is located in the palm sub domain of p66 (Fig.-1). This pocket lies near the polymerase active site, the polymerase primer grip, and the base of the p66 thumb sub domain, which serves as the hinge for the movement of the thumb. Binding of an NNRTI apparently blocks the chemical steps of polymerization [44,45] possibly by affecting the conformational changes required for polymerase catalysis [46,47]. The NNRTI-binding region of RT is dynamic; the NNIBP does not exist unless an NNRTI is bound to RT [40,41].

RT changes its conformation to create an NNIBP which can accommodate an NNRTI (Fig. 1b). [4] RT converts the single-stranded viral genomic RNA into a linear double- stranded DNA that can be integrated into the host chromosomes (reviewed in ref. 1). The enzyme has two activities, (i) a DNA polymerase that can use either RNA or DNA as a template and (ii) an RNase H (RNH) that selectively degrades theRNA strand of an RNA–DNA heteroduplex. The RNH activity of RT is required for virus replication; cellular RNH cannot substitute for the retroviral enzyme (*2*). The RNH activity degrades the genomic RNA during first strand (“minus-strand”) DNA synthesis, which allows the newly synthesized DNA to be used as a template for second-strand (“plus-strand”) DNA synthesis. [5]

Non-nucleoside RT inhibitors (NNRTIs)

Reverse transcriptase inhibitors serve as a mainstay of most frontline HIV combination therapies. The reverse transcriptase enzyme 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).[3]. NRTIs act at the active site of RT. They are incorporated in the growing DNA strand and prevent further elongation of the DNA-

chain, which terminates DNA synthesis. This class of inhibitors is active in HIV-1 as well as HIV-2 strains. NNRTIs, however, bind to an allosteric site, in the proximity of the active site of the enzyme and are specifically active against HIV-1 [4,5].

The application of the approved NNRTIs is limited to some extent. This is mainly due to relatively low genetic barrier of this class of drugs, and cross-resistance between these structurally unrelated drugs. The intensive use of approved NNRTIs (efavirenz, nevirapine and delavirdine, Fig. 1) [6e8] has led to the emergence of HIV-resistant strains. Actually, for the first generation of NNRTIs, only a single mutation is sufficient to confer resistance, leaving some patients with no further NNRTI therapeutic solutions [9]. Issues related to the use of NNRTI drugs have stirred effort and allowed the discovery of new series of NNRTIs that belong to the diarylpyrimidine (DAPY) family [10]. DAPY compounds, with TMC125 (Fig. 1) as prototype of the early generation of DAPY series, display a broad anti- HIV activity spectrum against wild-type and mutant strains. TMC125, which is currently in phase III clinical trials [11], is the first NNRTI to demonstrate beneficial effects on heavily experienced HIV-infected patients failing the traditional NNRTI therapies.[12,13]

Structure activity relationship studies combined with molecular modeling and X-ray analyses have provided keys on the capability of DAPY compounds to accommodate in the binding pocket, to amino acid residues that confer resistance to NNRTIs. This has resulted into the synthesis of a second-generation of DAPY compounds displaying high potency against a whole panel of HIV-1 relevant mutant strains. One of the second-generation of DAPY compounds, TMC278 (Fig.1) [14,15] is now in phase II clinical trials.[6,16] Professor Eddy Arnold recently reported to why it is relatively difficult for the AIDS virus to develop resistance to the drug, tenofovir, or the DAPY (diarylpyrimidine) family of compounds, and offers explanations of the mechanisms involved. Tenofovir and the DAPY compounds are different types of reverse transcriptase (RT) inhibitors. RT is the enzyme or molecular machine the AIDS virus uses to replicate its genetic material. The two life-saving drugs approach the problem of drug resistance in different ways. HIV RT uses ingredients available within the cell as building blocks to make the genetic copies that allow the virus to proliferate. These building blocks or substrates are fitted together to create new copies of the viral genetic information.

TMC125 was designed by Belgian scientists to reduce drug resistance, partly by making a flexible molecule that can fit in the active pocket of HIV's reverse transcriptase in different ways, even when the shape of that pocket changes because of viral mutations that would defeat other drugs. [4] TMC125 is a highly flexible compound with low in vitro toxicity. [5] TMC125 has garnered attention because of its activity against NNRTI- resistant HIV strains.[6,7]

Chemistry OF DAPY derivatives

A series of DAPY derivatives (compounds 2-11) are synthesized following two main pathways based on classical reactions namely the Heck reaction and the Wittige Wadsw or the Emmons reaction, which has been developed for the synthesis of 1 (TMC278) (Scheme 1) [14]. These two

synthetic routes start from the same building block, chloropyrimidine derivative 12. [10]

On one hand, the Wittig precursor, the aldehyde derivative C, is synthesized in three steps from 12 with moderate yields. The ester intermediate A is first obtained by heating the chloropyrimidine 12 with the desired 3,5-disubstituted ethyl 4-aminobenzoate at 150°C or 110°C in the presence of 3 N hydrochloric acid. Thereafter, the ester function is classically reduced with lithium aluminum hydride and the resulting alcohol B underwent manganese- mediated oxidation to give the expected aldehyde C. The latter is thus involved in a key Wittig reaction and condensed with diethyl cyanomethyl phosphonate in the presence of t-BuOK to afford the following acrylonitrile derivatives 2, 3, 10 and 11.

These products are isolated and further crystallized with a complete (E) – stereo selectivity, except for compound 3, which exhibited a (E)/(Z) ratio of 80/20. On the other hand, the Heck precursor is readily prepared from 12. The bromo-derivative D is synthesized either by heating (140°C) the chloropyrimidine 12 and the corresponding bromo-aniline in N- methyl-pyrrolidinone as solvent (compounds 4 and 9) or according to the protocols described above for carboxylic esters of type A (7 and 8). The coupling reaction is then conducted on different bromo- derivatives of D according to classical Heck conditions using palladium (II) diacetate as a catalyst in the presence of triethylamine in acetonitrile. The following acrylonitrile derivatives 4, 7, 8 and 9 were thus prepared according to this procedure and obtained with moderate yields. The stereoselectivity ranged from 80/20 to 96/4 ratio in favour of the (E) diastereoisomer. The synthesis of the corresponding phenoxy and phenylsulfanyl derivatives 5 and 6 is also based on a Wittig reaction in order to introduce the acrylonitrile function (Scheme 2).

Compound 5 is synthesized in two steps from derivative 12. Introduction of the phenoxy moiety on the pyrimidine scaffold is realized by heating (150 _C) the chloropyrimidine 12 and commercially available 3, 5-dimethyl-4-hydroxy-benzaldehyde with sodium hydride in a 1:1 mixture of NMP: dioxane. A Wittig reaction on 5a allowed us to isolate 5 with a complete (E)-stereoselectivity and an excellent yield. The same coupling conditions are used, started from 12, to synthesize bromo-derivative 6a, which is then submitted to a palladium (II) catalyzed CO-insertion leading to the corresponding ethyl ester 6b with a moderate yield.

A standard sequence $LiAlH_4$-reduction/MnO_2- oxidation / Wittig reaction is finally performed to achieve the synthesis of 6 with a (E)/ (Z) ratio of 85/15. All these A-ring analogues of 1 are synthesized to allow further exploration of the influence of a heteroatom linker X and the effect of a 2,6-disubstitution on the activity.

The anti-HIV activity of compounds 2e11 was measured using an HIV-1 replication assay and compared to TMC278 (1). The cells were infected with HIV-1 wild-type virus (LAI) or with single (L100I, K103N, Y181C, Y188L, F227C) or double mutant (L100I/K103N, K103N/Y181C, F227C/V106A) strains derived from wild-type LAI. The results are reported as the concentration required achieving 50% inhibition of cellular activity(EC50). In addition, the cytotoxicity (CC50) of the

compounds was determined. The selective index (SI ¼ CC50/ EC50), which indicates the specificity of the antiviral effect, is listed for the wild-type virus. Tables 1 and 2 list the results for compounds 2e11 in comparison with those for compound 1 and three reference compounds efavirenz (13), nevirapine (14) and delavirdine (15). All DAPY derivatives were considerably more potent than 13, 14 or 15 on the whole panel of viruses. First the influence on activity of the X spacer connecting the left phenyl ring and the pyrimidine was evaluated (Table 1). Compounds 5 and 6, in which the NH-linker of TMC278 (1) was respectively replaced by an O- and S-linker, were thus compared to TMC278 (1) and to the reference compounds.[13, 14, 15]

In line with the results obtained for 1, compounds 5 and 6 demonstrated high potency on the whole panel with an enhanced resistance profile on the double mutant strains as compared to marketed drugs (13, 14 and 15). The K103N single mutant strain showed hypersusceptibility towards the DAPY compounds. However, the sulfanyl derivative 6 displayed a slight decrease of activity to some extent compared to TMC278 (1). These results prompted us to consider the NH-group as the most appropriate spacer to achieve high-level potency on single and double mutant strains. Therefore, we pursued the optimization process with this linker.

The effect of the left phenyl A-ring‟s 2,6-disubstitution on activity was then investigated. Modeling data with a 2,6-dimethyl DAPY derivative had shown that л-л main interactions were present between the phenyl ring A of the substrate and residues Tyr181 and Tyr188 of the binding pocket [12]. The conformation favouring these specific interactions was partially due to the presence of the two methyl groups at positions 2 and 6, which prevented great conformational shifts of this left wing A, especially by limiting the rotational freedom. Removal of the substituents might therefore increase the conformational degrees of freedom and weaken the л- л interaction. In addition, the two methyl groups were involved in hydrophobic interactions with a number of side chains exposed in the NNRTI pocket. One methyl group interacted with Pro95, Leu100 and Tyr181 and to a lesser extent with Glu138 and Trp229, while the other methyl group was located close to Val106 and Val179 and might also interact weakly with the side chains of K103 and Y188. The mono-substituted derivative 2 exhibited an excellent potency against wild-type virus but a rather moderate activity on mutant strains.

The decrease in activity on the single mutant strains L100I, Y181C and Y188L was due to the loss of favourable hydrophobic interactions and the weakening of the pep interactions. In the double mutant L100I/K103N, compound 2 was more than 100 times less active than in the wild-type enzyme. The non-2,6- substituted compound 3 had submicromolar activity against wild-type (EC50 ¼ 0.034 mM) and was considerably less potent compared to compound 2. This decrease in activity of 3 might be explained by additional degrees of freedom for the molecule due to the absence of substituents on the phenyl ring, causing further weakening of the pep interactions. Interesting results were also observed when considering the methylation of the NH-linker (compound 4).

This compound showed a nanomolar activity on wild-type and single mutant

strains that were comparable to the other substituted DAPY derivatives. However, a drop in activity was observed when considering L100I/K103N and K103N/Y181C mutations. Superposition of 1 and 4 after docking in the NNRTI pocket showed that the methyl group of the NeCH3 linker in compound 4 is located further away from residues Pro95, Leu100 and Tyr181. It is also interesting to notice that introduction of an ethyl (7) or isopropyl (8) group at position 6 had only minor effects on the activity. In the series methylethylisopropyl, the size of the substituent seems to be inversely proportional to the activity.

This could be explained by the presence of some steric hindrance generated by the larger substituents. However, the double mutants L100I/K103N and K103N/Y181C, which were fully resistant to efavirenz (13) and nevirapine (14) /delavirdine (15), respectively, remained strongly inhibited by any of the substituents. Introduction of small R1 and R2 groups, with electronic properties different from the previous alkyl substitutions, at positions 2 and 6 of the phenyl ring was also investigated. Electron-donating and electron- withdrawing groups were considered (9e11). The results showed antiviral profiles for these three compounds similar to the 2,6-dimethyl substituted compound TMC278 (1); the electronic nature of the substituents did not seem to highly impact the activity. The potency of mpounds 9 and 11 on the double mutant strain L100I/ K103N was slightly decreased compared to 1. Derivatives 10 and 11 were selected for further in vitro and in vivo studies and compared to TMC278 (1).

The metabolic stability profile of these compounds was assessed using rat, dog and human liver microsomes (Table 3). As outlined in Table 3, the metabolic stability in rat, dog and human liver microsomes of compounds 1, 10 and 11 was similar. In rat and dog liver microsomes, high rates of recovery were observed (60e70%) whereas in human microsomes, 10 and 11 seemed to be less metabolically stable (up tom 74% metabolized). However, since TMC278 demonstrated the same metabolic stability, further evaluation of the pharmacokinetic profile of the two new compounds 10 and 11 in rat and dog species was sensible.

Binding of NNRTI to HIV-1 RT

Crystal structures of HIV-1 RT have been determined in various forms including apo-enzyme [40,41] in complexes with nucleic acid substrates: ds-DNA.[39,43,47] ds-DNA/dNTP [42] and RNA/DNA [43] and with different NNRTIs. These structures have provided information on effects of substrate and inhibitor binding on the enzyme, snapshots of different functional conformations, roles of resistance mutations, etc. A number of crystal structures of HIV-1 RT/NNRTI complexes have also been determined. These include nevirapine [30] tivirapine [47] delavirdine [52] efavirenz, the quinoxaline derivative HBY 097 [47] imidoylthiourea (ITU), diaryltriazine (DATA), and DAPY compounds [46] complexed with HIV-1 RT. Among the salient principles regarding NNRTI binding to RT learned from various chemical, biochemical, structural, and thermodynamic studies are:

1. NNRTIs are chemically diverse. One class of NNRTIs may be considerably different from another class in terms of its chemical composition and size.

2. Binding an NNRTI to HIV-1 RT does not prevent the binding of nucleic acid or

nucleotide triphosphate substrates to the enzyme, but blocks the chemical step of nucleotide incorporation [44] [45]

3. NNRTIs bind to HIV-1 RT in a hydrophobic pocket (NNIBP) that contains side chains of aromatic amino acid residues Y181, Y188, F227, W229, and Y318 and of hydrophobic amino acid residues P95, L100, V106, V108, V179, L234, and P236 from the p66 subunit. E138 is the only amino acid residue of the p51 subunit that interacts with NNRTIs; E138 does not directly interact with all NNRTIs.

4. The extent of interaction of amino acid residues in the NNIBP is different for different NNRTIs.

5. NNRTIs develop extensive hydrophobic interactions with NNIBP residues including van der Waals and p2p interactions with aromatic rings. Many NNRTIs have a hydrogen bond with the K101 main-chain carbonyl group.

6. The first generation NNRTIs including nevirapine, TIBO, and a-APA bind HIV-1 RT in a common "butterfly- like" binding mode despite their chemical diversity. K. Das et al. / Progress in Biophysics and Molecular Biology 88 (2005) 209–231 215 However, many of the more recent NNRTIs have been found to bind to RT in different modes. [46,47,52]

7. The hydrophobic binding site or NNIBP is not present in structures of HIV-1 RT that do not have a bound NNRTI („closed pocket form"). Expansion or opening of the NNIBP region, „to produce the open pocket form" (Fig. 1b), involves flipping of the aromatic side chains of Y181 and Y188 and an angular displacement of the b122b132b14 sheet that results in a movement of the „„primer grip"" away from the polymerization site present in the b62b92b10 sheet [41]; the primer grip is thought to assist in appropriately directing the template- primer so that the 30-end of the DNA primer aligns with the catalytic site for incorporation of the next nucleotide.[39,53]; It is possible that the NNRTI-bound "open pocket form"might correspond to an intermediate state(s) of RT that occurs during DNA polymerization.

8. The NNIBP is elastic and its conformation depends on the size, shape, specific chemical composition, and binding mode of an NNRTI. The limit of the flexibility of the pocket is not fully understood. Therefore, accurate prediction of the structures of HIV-1 RT/NNRTI complexes by molecular modelling is a very challenging problem. Reliable molecular modelling of HIV-1 RT/NNRTI complexes requires experimentally determined structures of related NNRTIs in complexes with HIV-1 RT. The binding of an NNRTI also has long-range effects on the relative arrangement of the RT segments. These long-range effects also vary according to the nature of the bound NNRTI.

Effect of mutation on the binding of NNRTIs with RT

Resistance to NNRTIs occurs when there are mutations in the binding pocket, and affinity for the drug is reduced. At first, these drugs did not look promising because it took very few mutations to develop high resistance. After more research, new drugs that inhibit RT were developed but do not have the same cross resistant problems. Now

the drugs target amino acids, such as Trp 229, that are present in the binding pocket in many genetically diverse strands. This amino acid is essential for RT to function and if a mutation in RT were to occur at this site, the enzyme would not be able to function. The K103N mutation is the most common mutation found with the NNRTIs. This is selected for in the presence of efavirenz, but leads to cross resistance of all NNRTIs (Table 1).

The next most common mutation is Y181C, which is selected for by nevirapine. Efavirnez, though, is still effective in the presence of this mutation.The amino acid residues Y181 and Y188 form part of a hydrophobic core that interacts with Wing I of the butterfly-like NNRTIs. Mutations such as L100I, Y181C, or Y188L affect inhibitor–protein interactions and the size, shape, and chemical environment of the binding pocket. The Y181Cand Y188L mutations appear to cause decreased NNRTI binding because the mutations result in the loss of aromatic ring interactions with inhibitors ("loss of contact" mechanism). The potency of relatively rigid inhibitors such as nevirapine and a-APA, which interact extensively with the aromatic side-chains of Y181 and Y188, is severely impaired by these two mutations. The amino acid residue L100 interacts with the top portion of a bound NNRTI (Fig. 3) by interacting with Wing I and the central part of the inhibitor. The L100I mutation can cause steric interference between the b-branched isoleucine and a bound NNRTI.

The G190A mutation can cause resistance through steric conflict of the methyl side chain and a bound NNRTI. The K103N mutation apparently has minimal influence on the bound conformation of an NNRTI. The K103N mutant HIV- RT bound conformations of efavirenz, and HBY 097 was almost identical to their respective wild-type HIV-1 RT and K103N mutant structures. The K103N mutation appears to affect the kinetics of the inhibitor-binding process by stabilizing the unbound state of RT; a hydrogen bond between the Y188 phenoxyl group and the N103 side chain is formed in K103N mutant apo enzyme structure which is not present in the wild-type apo HIV-1 RT structure. However, this mutation enhances the binding of NNRTIs like etravirine [46] (Table 1). In summary, NNRTI- resistance mutations appear to affect NNRTI binding directly, by altering the size, shape, and polarity of different parts of the NNIBP or, indirectly, by affecting access to the pocket.

Conformational flexibility of diaryl pyrimidine derivatives

Diarylpyrimidines dapivirine (TMC120, R147681) etravirine (TMC125, R165335) and rilpivirine (R278474). 38m adopt a "horseshoe" conformation in the NNRTI binding site of the RT, with the central pyrimidine ring sandwiched between the side- chains of Leu-100 and Asn-103 or Lys-103.34 However, these inhibitors have remarkable flexibility and can adopt different conformations within the binding site, allowing significant repositioning and reorientation within the NNRTI binding pocket. This ability appears to be critical to retain potency against a wide range of drug-resistant HIV-1 RTs.[34]

It has been shown that, *in vitro*, etravirine retained meaningful activity (EC50<100nM) against 97% of 1,081 clinically derived recombinant viruses resistant to at least one of the currently

marketed NNRTIs.[39] Moreover, preliminary clinical investigations have shown that etravirine treatment (900mg bid for seven days) of patients harbouring NNRTI-resistant variants resulted in an approximate 10-fold decrease of the viral load.40 However, HIV strains showing high-level resistance to etravirine (>100-fold increase of the EC50 relative to the wild-type) have been selected *in vitro*,[41] and recombinant viruses having mutations Y181I, F227C, or the combination L100I/K103N also showed reduced susceptibility to the inhibitor[39]. Rilpivirine was found to be more potent than nevirapine, efavirenz, dapivirine and etravirine against the wild-type virus and many single and double mutants conferring high-level resistance to NNRTIs.[42] In addition, no sign of virus breakthrough was observed in cultures treated with the inhibitor at 1μM for 30 days. These observations, together with data revealing its high oral bioavailability and minimal adverse effects in preclinical studies, suggest a promising future for rilpivirine.

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Hughes SH, Janssen PA, ArnoldE. Roles of conformational and positional adaptability in structure-based design of TMC125- R165335 (etravirine) and related non- nucleoside reverse transcriptase inhibitors that are highly potent and effective against wild-type and drug-resistant HIV-1 variants. Journal of Medicinal Chemistry 2004, 47, 2550-60

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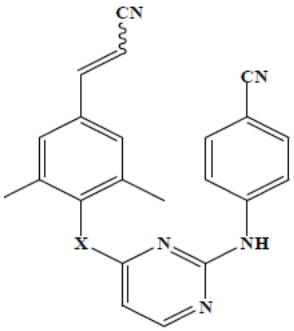
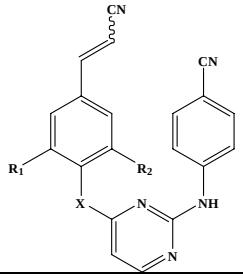
Table 1: Influence of the X Spacer on the inhibition of wt LAI and mutant strains of HIV-1



Comp	X	E/Z	LA I	CC_{50}	SI[a]	EC_{50} (nM)							
						L100I	K103N	Y181C	Y188L	F227C	L100I+K103N	K103N+Y181C	F227C+V106A
1	NH	100/0	1.0	2000	2020	0.5	0.1	1.3	1.2	0.5	7.5	3.2	0.5
5	O	100/0	0.9	>25000	28302	06	0.2	3.2	1.6	0.7	3.7	4.3	0.6
6	S	85/15	3.7	>25000	>6821	2.6	0.7	5.8	2.8	1.4	79.2	18.6	1.1
13	Efavirenz	-	1.6	32314	19850	31.0	46.4	3.9	55.0	46.9	1460	55.0	4.1
14	Nevirapine	-	33.3	31631	>949	209	1980	7078	5910	827	3532	14505	2697
15	Delavirdine	-	122.1	67188	>550	9530	10220	9035	1022	4560	20583	38352	721

[a]SI= CC50 /EC50

Table 2: Influence of the 2,6-substitution on the inhibition of wt LAI and mutant strains of HIV-1



Comp	X	R 1	R 2	E/ Z	L AI	CC_{50}	SIa	EC_{50} (nM)							
								L1 00 I	K10 3N	Y181 C	Y1 88L	F227C	L100I/ K103N	K103N/ Y181C	F227C/ V106A
1	NH	Me	Me	100/0	1.0	2000	2020	0.5	0.1	1.3	1.2	0.5	7.5	3.2	0.5
2	NH	H	Me	100/0	0.9	>25000	>29372	9.2	0.6	13.4	43.6	1.2	478.7	89.6	1.2
3	NH	H	H	80/20	33.9	>25000	>737	585.1	57.7	1523	303.3	98.4	6440	3952	76.1
4	NMe	H	Me	80/20	1.0	11625	11351	2.5	0.7	12.0	16.8	1.0	103.8	160.3	1.5
7	NH	Me	Et	85/15	3.0	2553	841	0.9	0.5	4.0	2.6	1.1	8.2	5.3	0.9
8	NH	Me	iPr	96/04	4.0	11791	2947	3.1	1.3	11.1	3.8	3.9	38.3	10.6	3.2
9	NH	Me	OMe	91/09	0.7	633	846	0.6	<0.1	1.9	0.9	0.3	21.1	4.3	0.3
10	NH	Me	Cl	100/0	0.8	2606	3316	0.4	0.1	2.3	2.3	0.5	6.6	4.1	0.5
11	NH	OMe	Cl	100/0	0.1	398	3981	0.2	0.3	1.0	2.5	0.8	15.8	2.0	0.3

[a]SI= CC_{50} /EC_{50}

Table 3: Metabolic stability of compounds 1,10,11

Compound	microsome stability (% recovered after 15 min)		
	Rat	Dog	Human
1 (TMC278)	73	65	26
10	72	60	39
11	53	58	26

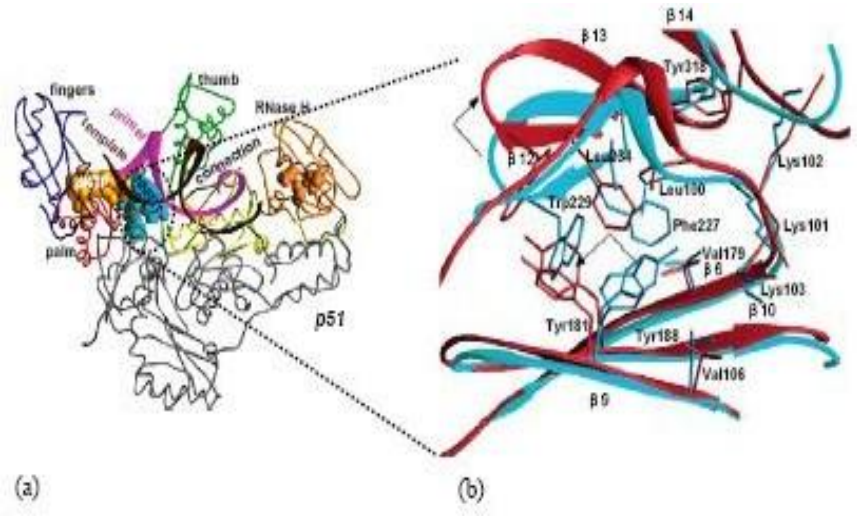


Fig.1 (a) A cartoon representation of the structure of HIV-1 RT in which the fingers, palm, thumb, connection, and RNase H domains in p66 are colored blue, red, green, yellow, and orange, respectively. The p51 subunit is colored gray .Important sites including the dNTP-binding site (in gold), RNase H active site (in orange), and NNRTI-binding site (incyan), are highlighted. The nucleic acid (brown and purple ribbons) binding cleft extends from the polymerase active site to RNase H active site. (b) A closer view of the NNIBP region showing comparison of its NNRTI bound (in red) and unbound (in cyan) conformations.

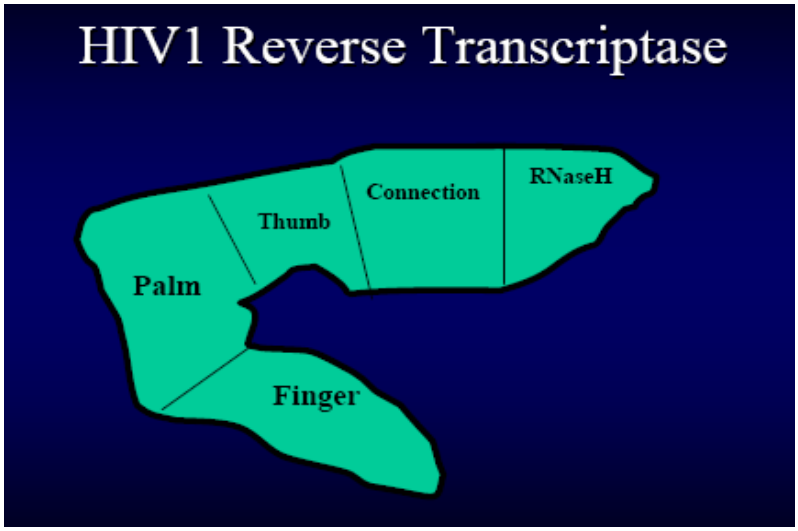


Fig.2 structure of enzyme reverse transcriptase.

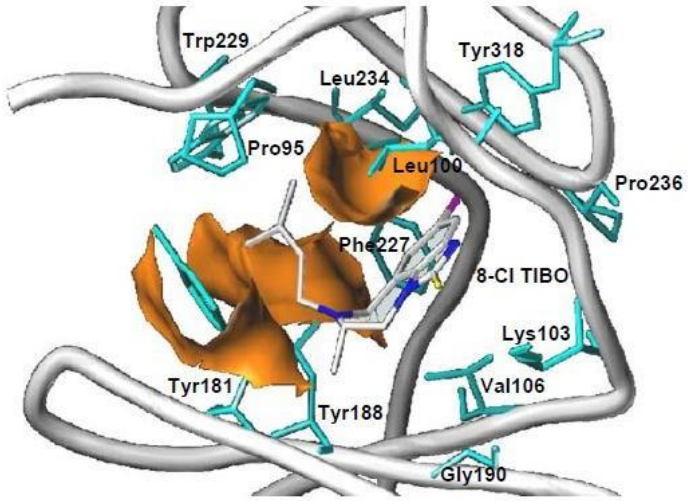
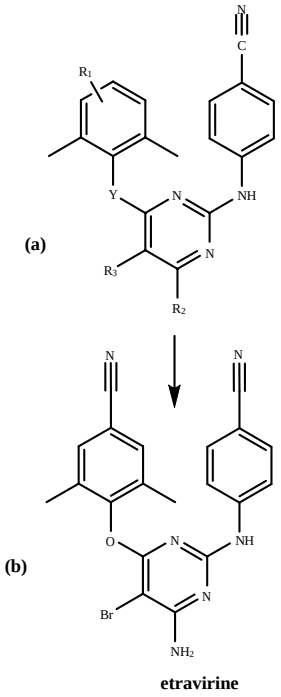


Fig.3 Amino acid residue of the NNIBP surrounding a bound NNRTI, 8-Cl TIBO (tivirapine). The illustrated portions of the NNIBP surface (orange) are modified by the common NNRTI-resistant mutations L100I, Y181C and Y188L.



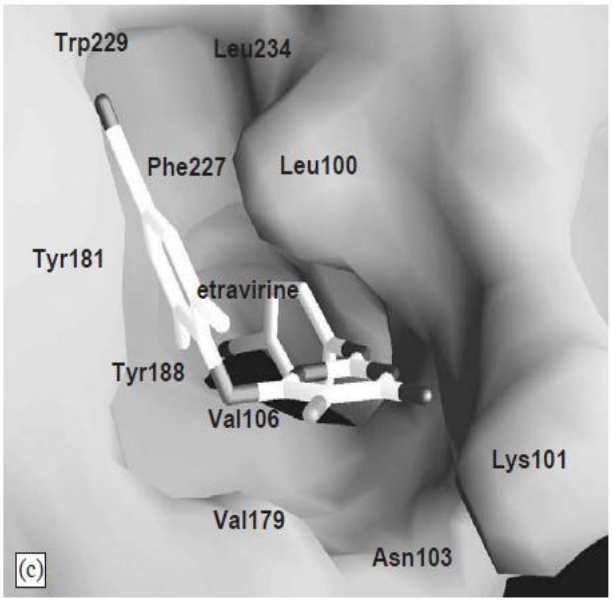
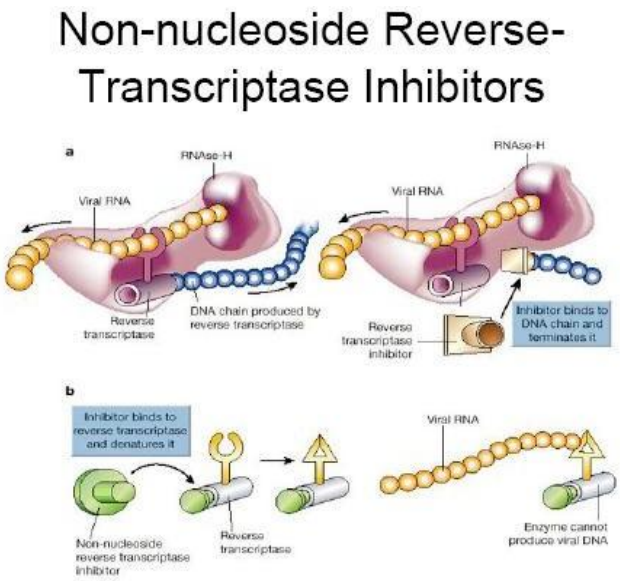
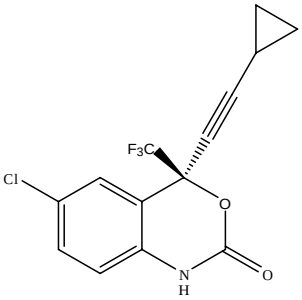
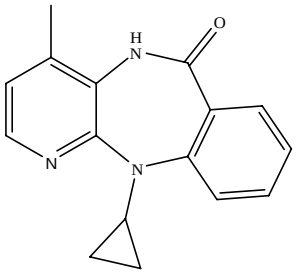


Fig.-4. Backbone of the potent DAPY series of compounds with which chemical substitutions were made to obtain the highly potent etravirine (b). (c) The principal mode of binding of etravirine to K103N mutant HIV-1 RT.

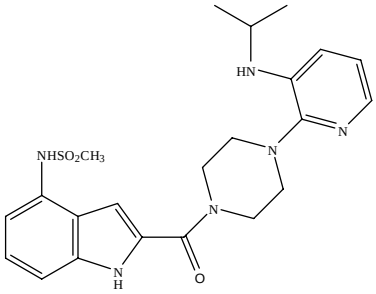




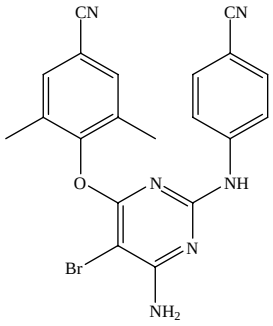
Efavirenz



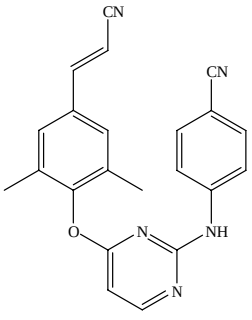
Nevirapine



Delavirdine

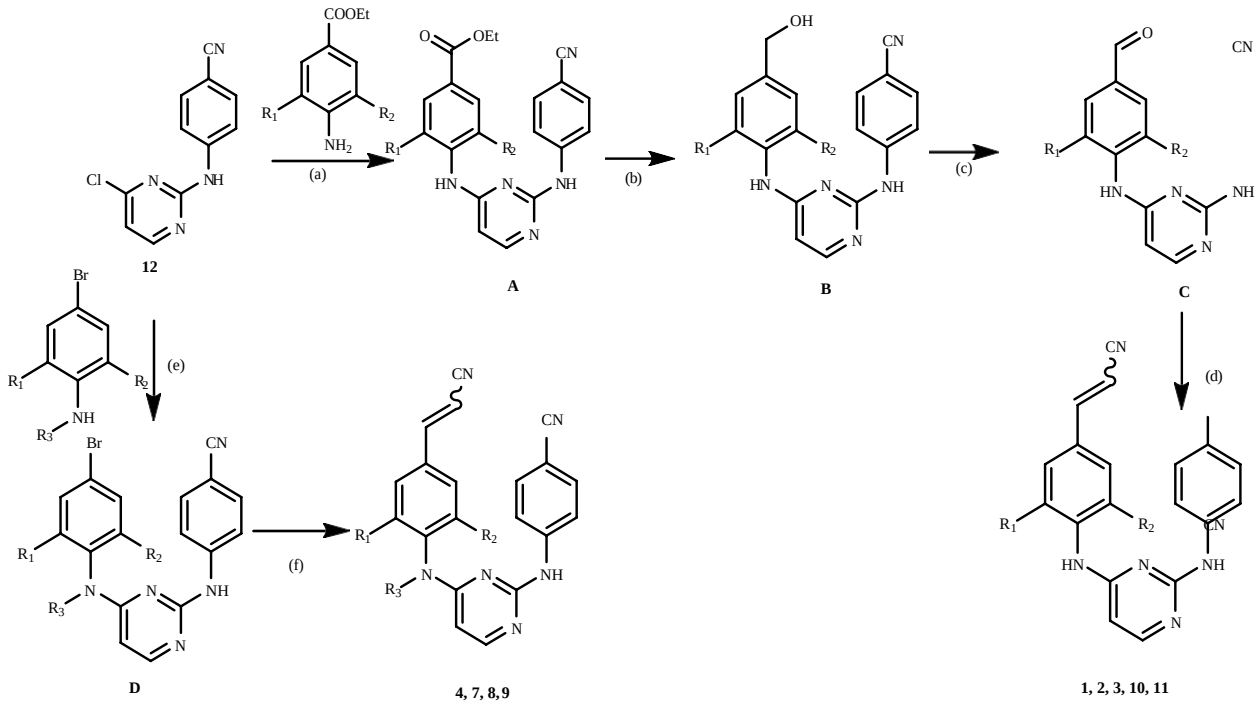


TMC 125

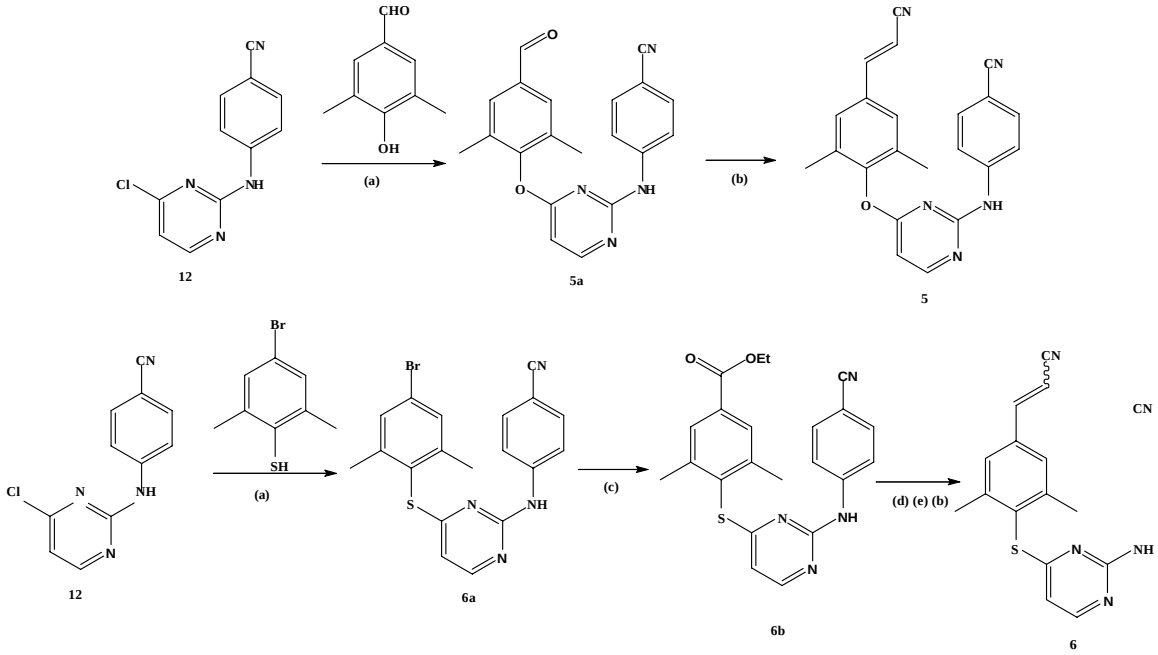


TMC 278, **1**

Structure of reference compounds, TMC 125, TMC 278



Scheme- 1. Synthetic routes via witting or heck reaction to 2,6-disubstituted rings. witting pathway:2,3: 150 0 C, h; 10,11: Hcl 3N, reflux,5-16h; (b) LiAlH4, THF,0.c to RT, 20 h;(c) Mno2, CH2Cl2/DMF,RT, 20 h; (d) (Eto)2P(O)CH2CN, t-buOK, THF, 0C to RT,4-20h. Heck pathway(e) 4,9: NMP, 140 C, 20 h; Hcl 3N, reflux, 20 h ; 8: heated neat;(f) acrylonitrile, Pd(oAc)2, p(o-Tol)3 , NEt3, CH3CN, 140C, 20h.



Scheme- 2. Synthesis of compounds 5 and 6. (a) Nah, NMP, dioxane, 150C, 12- 48 h; (b) (Et2O)2P(O)CH2CN, t-BuOK, THF, 0C to RT, 20h; (c)P(CO) 10 bar, Pd(OAc)2,PPh3, K2CO3, EtOH, DMF, 90C, 72 h; (d) LiAlH4, THF, 0 C to RT, 20 h; (e) MnO2, CH2Cl2, RT, 72 h.