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Current state-of-the-art in preclinical and clinical development of novel non-nucleoside HIV-1 reverse transcriptase inhibitors

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In spite of the tremendous advance in our understanding of the HIV-1 life cycle and of the molecular mechanisms underlying its pathogenesis, it is still not possible to fully control the HIV-1 infection for long periods of time, not to mention eradicate it. However, as it is still unclear whether an effective vaccine could be developed in a short time, therapeutic approaches based on the combination of different potent antiviral drugs are now thought to be the best option for the control of HIV-1 infection in patients. For this reason, development of novel molecules that are safer, more potent and less sensitive to drug resistance than available drugs is mandatory. Among the currently exploited anti-HIV drugs, non-nucleoside reverse transcriptase inhibitors play a major role. This review will summarise the most recent advances and evolutionary trends for this class of anti-HIV drugs.

Keywords: AIDS, HAART, HIV-1, non-nucleoside inhibitors, structure–activity relationships

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1. Introduction

1.1 The non-nucleoside HIV-1 reverse transcriptase inhibitors

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) of HIV-1 are promising drugs for the treatment of AIDS when used in combination with other anti-HIV drugs [1]. These inhibitors either emerged from large screening programmes for the discovery of new specific HIV-1 reverse transcriptase (RT) inhibitors, or as the result of comprehensive evaluation of molecules endowed with cellular HIV activity [2]. Both approaches led to the selection of the most active compounds as potential leads. Nevirapine (NVP) and delavirdine (DLV) are the only first-generation NNRTIs in clinical use. In spite of their very different structures, these compounds are absolutely specific for the HIV-1 RT, as they are not active against HIV-2. The molecular basis for their inhibitory activity, as well as for this absolute preference for the HIV-1 RT, has been fully elucidated only after the resolution of the crystallographic structures of different NNRTIs/RT complexes [3]. All of the known NNRTIs bind to a common allosteric site of RT, the non-nucleoside binding site (NNBS). The NNBS is close to, but distinct from, the enzymatic active site. Interaction of the NNRTIs with the NNBS causes a conformational change in the enzyme that impairs catalysis. The small differences in the amino acidic residues of this site between HIV-1 and HIV-2 RTs are responsible for the lack of inhibition of the latter enzyme. However, the first generation of NNRTIs have suffered from the rapid development of drug resistance. In fact, the high viral turnover and mutation rate cause the accumulation in the RT gene of drug resistance mutations that are readily selected during drug administration [4,5]. The rapid emergence of resistance mutations, coupled with the high level of cross-resistance, highlighted the urgent

need for novel NNRTIs with a broader spectrum of activity [6]. Such a goal has been pursued via two approaches: the improvement of already existing classes of drugs, or screening in search of novel lead structures [2]. In both cases, all the available information about resistance mutations (location in the RT structure, mechanism of action and interactions with the drug) have been incorporated into the drug-screening programmes. Such an implemented knowledge-based approach afforded more rational structure–activity relationship (SAR) studies, aimed at selecting compounds not only highly active against the wild-type virus, but also more sensitive to some of the known clinically relevant drug-resistance mutations. As a result of these efforts, the second-generation NNRTI efavirenz (EFV) has been introduced into clinical use. EFV showed improved activity with respect to the NNRTIs of first generation. However, it was ineffective against several NNRTI-resistant mutants [7,8].

1.2 Molecular basis for drug resistance to NNRTIs

The available structural and biochemical data made it clear that, despite being very different structurally, all of the known NNRTIs bind to a common site in the HIV-1 RT [9]. They all bind to a hydrophobic pocket, which is represented by a large (600 – 700 Å) solvent-accessible cavity, the inner surface of which is lined by a number of hydrophobic residues. Comparison of the structures of the HIV-1 RT complexed with different first-generation NNRTIs showed substantially similar binding modes [10]. These NNRTIs can be considered to consist of two hinged, six-membered rings. Within the binding site the two rings adopt a butterfly-like active conformation. Based on the butterfly-like model, the NNBS can be divided into two distinct regions, termed wing I and wing II, each occupied by one of the two ringed elements (or 'wings'). The wing I region is lined by the residues Y181, Y188 and W229, whereas the wing II region is defined by the residues V179, V106 and L100. Enzyme – inhibitor interaction is primarily stabilised through hydrophobic interactions with the Y181, Y188, L100 and V106 residues. In addition, drug-specific electrostatic interactions (charge and hydrogen bonds) contribute to the final strength of binding. The binding pocket communicates with the bulk solvent through a channel lined by the residues K101, V179 and E138 (p51), which might represent the entry site for the NNRTIs. Thus, it seems that all of the first-generation NNRTIs adopt a common binding mode within the NNBS and assume the butterfly-like conformation. In comparison, NNRTIs of the second generation are more flexible and can make new (and more) contact with the amino acid residues in the NNBS. The capability of second-generation NNRTIs to make contacts with chain residues that are unlikely to be disrupted by single acid mutations may explain their capacity to retain activity in the presence of mutations resistant to first-generation NNRTIs.

Comparison of the unliganded RT structure with the RT/NNRTIs complexes shows that, in the inhibitor-free structure, there is a small solvent-inaccessible cavity between Y181, Y183 and Y188. Upon inhibitor binding, these three residues rotate outwards, filling this cavity and opening up the larger pocket where NNRTIs bind. This movement involves extensive changes in the relative positions of the β4, β7 and β8 strands, which contain the catalytically essential residues D110, D185 and D186 [11]. Presteady-state kinetic analysis of the reaction catalysed by HIV-1 RT either in the absence or in the presence of NNRTIs [12,13] showed that the inhibition was due to a great reduction of the polymerisation rate (k_{pol}). The detailed crystallographic information available for NNRTIs can explain both the mechanism of resistance induced by the different mutations and the high level of cross-resistance characteristic of this class of compounds [14,15]. The first RT mutations shown to be associated with resistance to NNRTIs were the K103N and Y181C substitutions, which were selected in the presence of pyridinone derivatives, NVP and tetrahydroimidazobenzodiazepinone (TIBO) R82150. Indeed, the K103N and Y181C mutations have been observed with virtually all of the NNRTIs. Other drug-specific mutations include L100I, V106A and P236L. All of the amino acids mutated in the NNBS reduce the number of stabilising interactions with the drug. One noteworthy exception is the mutation K103N, which lines the proposed entry channel for the NNRTIs. Enzymological and crystallographic studies have shown that this mutation most likely imposes a thermodynamic barrier for the formation of the RT–inhibitor complex by creating a hydrogen bond not present in the wild-type enzyme [16-18].

1.3 Evolutionary trends

NNRTIs undoubtedly have several advantages over the nucleoside analogues (NRTIs), which can be summarised as follows: i) NNRTIs do not require metabolic activation, thus eliminating additional metabolic steps that, in turn, could limit their efficacy and/or increase their toxicity; ii) NNRTIs bind to the NNBS, which is unique to HIV-1 RT, resulting in an extremely high selectivity. Moreover, efficient interaction with NNBS can be achieved by a large number of different unrelated chemical structures. Differently, the NRTIs interact with the enzyme's active site. Consequently, the structural modifications that can be introduced are limited by the need to preserve the critical contacts with the catalytic amino acidic residues of the enzyme.

However, these advantages are coupled with a major disadvantage, that is the relatively high probability that single or double mutations in the NNRTI-binding pocket might confer resistance to different classes of molecules.

To overcome this major problem, two main approaches have been undertaken: i) improving existing NNRTIs through rationale drug design in order to find derivatives with higher potency against drug-resistant mutants; ii) discovering completely new inhibitors. These approaches are based on a range of different techniques, from structure-based molecular docking drug design to virtual screening of chemical libraries. As a matter of fact, the field of NNRTIs is still rapidly evolving, both in academic research laboratories and in the

pharma industry. A selection of the most recent advancements will be the topic of this review. However, before delving into the most recent publications and patents, it might be worth remembering that, in spite of many patent applications and primary publications describing novel NNRTIs, very few molecules are going to hit the market. This is mainly due to the high-risk and very expensive nature of the drug development process. Full development of a novel drug from chemical synthesis to the clinics has been estimated to take > 5 years and to cost > US$500 million. In addition, due to the unpredictable nature of complex biological systems, a molecule worth several hundreds of millions of dollars can fail at the latest stages of development, namely Phase II/III clinical trials. These facts fully justify the extremely rigid criteria that pharma industries apply to each drug candidate in the course of it's development process, as well as their tendency to focus on a single major hit, disregarding other potentially interesting candidates with only slightly less chance of successful development. Due to public funding, academic research could, in principle, more easily take up the high risks of drug development; however, for the very same reason, it cannot afford the enormous costs related to the clinical development process.

2. Potential novel drugs: from lead identification to preclinical development

2.1 Novel 8-substituted dipyridodiazepinones analogues of nevirapine (Boehringer Ingelheim)

NVP (**1**) was the first NNRTI licensed for clinical use in 1996 (**Figure 1**). Despite its good tolerability, NVP suffers from problems common to other NNRTIs, such as the rapid development of drug resistance due to drug-specific single amino acid mutation of the RT [19]. In addition, cross-resistance with other NNRTIs is often observed. Boehringer Ingelheim continued to develop novel classes of dipyridodiazepinones derivatives, based on the NVP skeleton [101]. As a result; 8-heteroarylthiomethyldipyridodiazepinone derivatives (e.g., compound **2**) were synthesised endowed with potent antiviral activity [20]. However, metabolic instability led to the abandonment of further development of this series. A further step was the synthesis of derivatives **3** and **4** endowed with excellent broad-spectrum activity against a panel of relevant RT mutants and with good biopharmaceutical properties [21,22]. Beginning with dipyridodiazepinones exemplified by compounds **3** and **4**, Boehringer Ingelheim synthesised new analogues that were revealed to be potent inhibitors of both wild-type and drug-resistant HIV-1 strains [23].

The most interesting compounds, **5** and **6**, showed similar overall potencies against a panel of mutant viruses, with differences against the Y188L mutation; in which compound **5** was six-fold more potent than compound **6**. Both compounds **5** and **6** showed similar profiles in protein binding with moderate three- and four-fold shifts, respectively, and were not potent inhibitors of any of the five major human cytochrome P450 (CYP450) isoforms. In pharmacokinetics studies in rat, compound **5** had an excellent half-life value (11 h) after intravenous administration at 1 mg/kg, and was very well adsorbed after oral gavage at 5 mg/kg (C_{max} = 6.5 μM). Furthermore, the pharmacokinetic characteristics were evaluated in dog and monkey. Compound **5** was well adsorbed in both species. The half-life was shortest in monkey (1.3 h), and high clearance (24 ml/kg/min) was observed after intravenous administration. In dog, compound **5** showed an intermediate half-life value (5.9 h) between those in rat and monkey, and showed excellent bioavailability. In summary, compound **5** showed good antiviral potency against both HIV-1 wild-type and the drug-resistant mutants, including those carrying the K103N mutation. Compound **5** also had a favourable pharmacokinetic profile and was selected as a lead compound for further development (**Table 1**).

2.2 DAPYs and rilpivirine (Janssen Pharmaceutica and Johnson & Johnson)

In 2005 Janssen Pharmaceutica published the discovery of R278474; TMC-278 as a result of a coordinated multidisciplinary effort started in 1987 with TIBO (**7**, **Figure 2**) [24]. Chemical modification of α-APA (**8**) led to the class of potent iminothiourea (ITU; **9**) NNRTIs. In an attempt to synthesise the corresponding imino-*N*-cyanoguanidine derivatives of ITU analogues, an unexpected ring closure occurred producing R106168 (**10**), the first compound of the diaryltriazine (DATA) class of NNRTIs. In 1996, molecular modelling studies suggested the replacement of the aminotriazine ring of DATA with a pyrimidine ring. Such a project led to the identification of the novel class diarylpirimidine (DAPY); of which dapivirine (TMC-120; **11**) and etravirine (**12**) are the representative members [25,26,102]. A further step was the synthesis of rilpivirine (**13**) that is the *E*-cyanovinyl analogue of TMC-120 [27]. Rilpivirine was synthesised in six high-yield reaction steps. The end product had < 0.5% of the *Z*-isomer [28]. The improved activity of compound **13** against HIV-1 wild-type and resistant mutants, with respect to compound **12**, may involve specific interaction of the cyano group in the wing I of the horseshoe-like conformation with the indole ring of Trp229. In addition, the flexible dihedral angle between the anilino ring and cyanovinyl moiety was also likely to contribute to the excellent resistance profile of compound **13**.

Rilpivirine was more active than NVP, EFV and dapivirine against wild-type HIV-1 and all single and double mutants tested (**Table 2**). Resolution of the geometric mixture into pure *E*- and *Z*-isomers revealed that the *E*-form was superior in terms of antiviral activity with respect to the corresponding *Z*-isomer. In particular, the stereoselectivity of rilpivirine was ~100-fold in favour of the *E*-isomer against the double mutant L100I/K103N, and 40-fold against the double mutant K103N/Y181C. The use of the *E*-rilpivirine in the

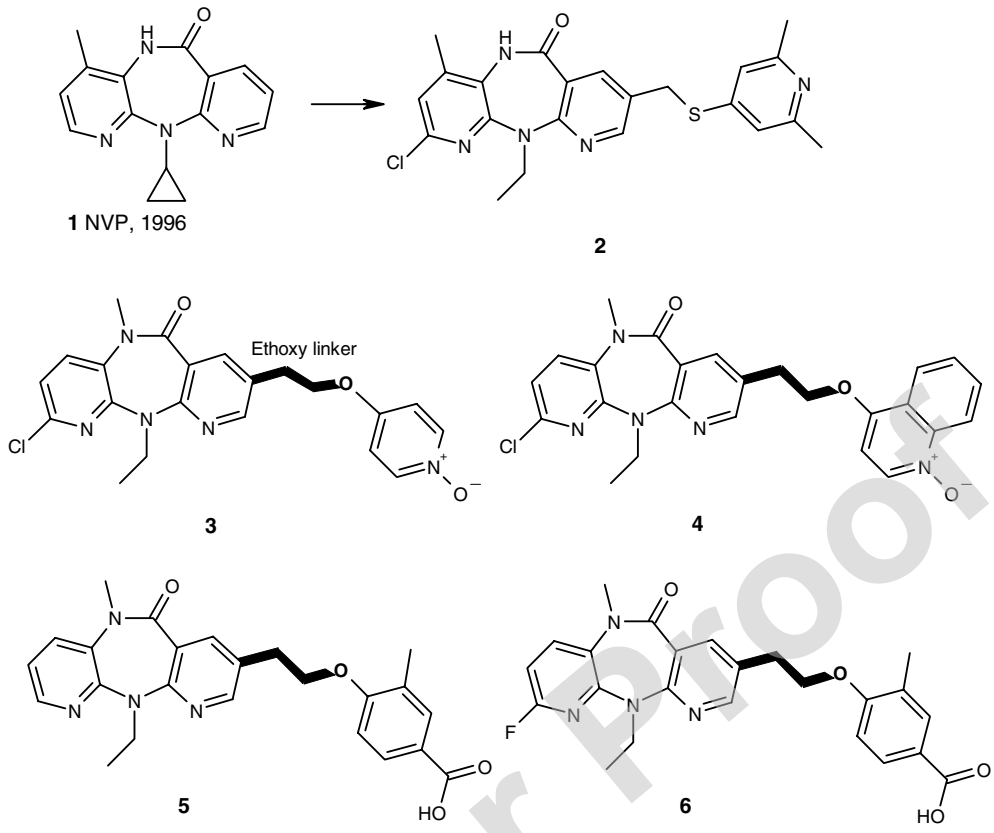


Figure 1.
NVP: Nevirapine.

Table 1. Antiviral activity of compounds 5 and 6 against wild-type HIV-1 and a panel of resistant mutants.

Compound	EC_{50} (nM)* §		IC_{50} (nM)‡§						
	Wild-type	K103N/ Y181C	Wild-type	K103N/ Y181C	V106A	K103N/ P225H	K103N/ V108I	K103N/ L100I	Y188L
5	1.6	15	17	221	36	5	5	4	172
6	1.5	14	15	150	27	2	4	3	27

*EC_{50}: Antiviral activity. The T lymphocyte cell line C8166 was infected (10-3 multiplicity) with wild-type and mutant RT viruses (HIV-1 p24 antigen method).
‡ IC_{50}: enzymatic activity (scintillation and luminescence counter method). §Each value represents the mean of at least three determinations.

Table 2. Antiviral activity of rilpivirine (13) against wild-type HIV-1 and a panel of resistant mutants.

				EC_{50} (nM)			
Compound	**wild-type**	**L100I**	**K103N**	**Y181C**	**Y188l**	**G190S**	**K103, Y181C**
Nevirapine	81.0	597.0	2879.0	10000.0	10000.0	1000.0	10000.0
Efavirenz	1.4	35.0	28.0	2.0	78.0	275.0	37.0
Dapivirine	1.2	11.0	2.0	7.0	37.0	2.0	54.0
Rilpivirine	0.4	0.4	0.3	1.3	2.0	0.1	1.0

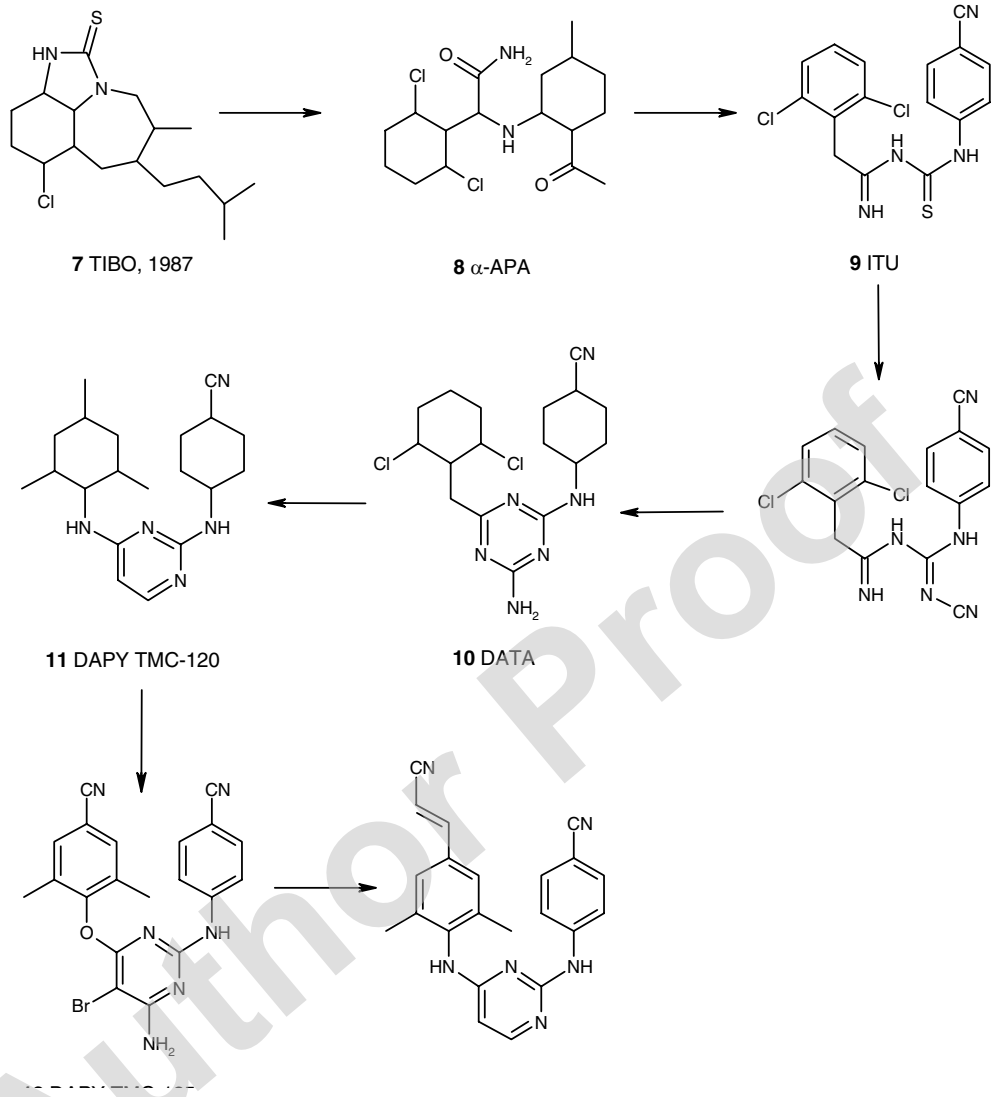


Figure 2.
DAPY: Diarylpirimidine; DATA: Diaryltriazine; ITU: Iminothiourea; TIBO: Tetrahydroimidazobenzodiazepinone.

context of highly-active antiviral therapy (HAART) has been the subject of a recent patent [103].

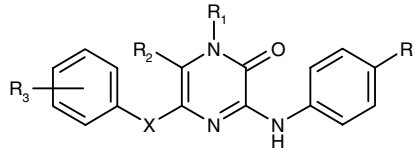
After oral administration of rilpivirine in PEG 400, the half-life ranged between 2.8 h in rat and 39 h in dog [27]. Oral biovailability was calculated to 32 and 31% in rat and dog, respectively. Rilpivirine was metabolised slowly in the hepatocytes of humans and of various species used in toxicological studies. Glutathione-dependent conjugative metabolism was the primary pathway observed in hepatocytes of rodents. Metabolic observations *in vitro* in hepatocytes indicated a slow clearance. *In vitro* studies showed a slow metabolic clearance for dog and a faster metabolic clearence for rat. Rilpivirine was not mutagenic in the Ames reverse mutation test up to bacteriotoxic concentrations. No relevant side effects were observed in the *in vitro* radioligand binding on various bioamine receptors. The compounds had low binding affinities for the sodium and calcium channels in human ether-a-go-go related gene (hERG). In addition, rilpivirine had no effect on cardiovascular, pulmonary, electrophysiological and behavioural parameters in dog.

Etravirine (TMC-125) and rilpivirine are now in Phase II/III clinical trials for the treatment of HIV infections (Section 3.1). Dapivirine is now being evaluated in Phase I/II trials as a microbicide to prevent sexual HIV-1 transmission.

2.3 Dihydropyrazinones (Janssen Pharmaceutica)

A molecular modelling approach based on evaluating the interaction energy in HIV-1 NNBS was used to predict the activity of a novel dihydropyrazinone series [29,30]. For this study the dihydropyrazinone molecule was dissected in three parts; the central dihydropyrazinone ring, the right anilino in

Table 3. Antiviral activity of dihydropyrazinones 14 – 22 against wild-type HIV-1 and a panel of resistant mutants.



						IC_{50} (μM)				
Compound	**R**	**R_1**	**R_2**	**R_3**	**X**	**LAI**	**L100I**	**K103N**	**Y181C**	**Y188L**
14	H	CH_3	H	2,4-$(CH_3)_2$	O	0.251	-	-	-	-
15	CN	CH_3	H	2,4-$(CH_3)_2$	O	< 0.001	0.316	0.032	0.159	0.398
16	CN	CH_3	CH_3	2,4-$(CH_3)_2$	O	0.005	0.398	0.398	2.511	0.611
17	CN	CH_3	H	2-CH_3	O	0.004	1.259	0.794	2.512	1.995
18	CN	H	CH_3	2-CH_3	S	0.040	5.012	0.631	5.012	0.631
19	CN	CH_3	H	2,4,6-$(CH_3)_3$	S	0.016	> 10	0.613	1.000	0.631
20	CN	CH_3	CH_3	2,4,6-$(CH_3)_3$	O	0.005	0.050	0.158	0.501	0.200
21	CN	CH_3	H	2,4,6-$(CH_3)_3$	SO_2	0.008	> 10	> 10	0.398	> 10
22	CN	CH_3	CH_3	2,4,6-$(CH_3)_3$	SO_2	0.005	0.158	3.162	0.032	1.995

the 3-position, and the left aryloxy(sulfanyl) wing in the 5-position. The cyano substituent in the 4-position of the aniline moiety was the optimal choice, as it was for the DATA/DAPY compounds. The presence of a 6-methyl group instead of hydrogen in the dihydropyrazinone ring did not increase significantly the activity against wild-type HIV-1, but there was a favourable effect on activity against mutants. Similar results were obtained by substitution of the methyl at position-1 with a hydrogen atom. Molecular modelling studies revealed that the 2-carbonyl moiety made a strong acceptor hydrogen bond with the NH group of K101, whereas the aniline NH acted as a strong hydrogen donor bond for the backbone C=O group of K101. Introduction of methyl substituents on the benzene ring on the left wing increased the activity against both HIV wild-type and drug-resistant mutants. The nature of the connecting group influenced the antiviral activity. Compounds with X = SO_2 were slightly superior to those with X = S, which was only confirmed for the Y181C mutation. Introduction of a methyl group at position-6 of the dihydropyrazinone ring significantly increased the activity against this mutation.

2.4 Benzophenones (GlaxoSmithKline)

By routine evaluation in an MT-4 cell assay, GlaxoSmithKline discovered three potent benzophenones (**23 – 25**). In a preliminary assay, these compounds proved to be much more potent than NVP and DLV, and equivalent to EFV against both wild-type HIV-1 and the Y181C mutant strain [31,104]. As far as the mechanism of action was concerned, compounds **23 – 25** proved to inhibit the HIV-1 RT, and the enzyme inhibition was consistent with the antiviral activity. Compounds **23 – 25** were found to be extremely potent against wild-type virus with IC_{50} values of ≤ 2 nM. A panel of 20 single and double mutant viruses was used to evaluate the inhibitory activity of compounds **23 – 25**. Against 16 of the 20 mutants, compounds **23 – 25** showed IC_{50} values of < 10 nM. Of particular significance was the activity of these compounds against the Y181C-, K103N- and K103N-containing double mutant NNRTI-resistant viruses, which account for a significant proportion of the clinical failure of the three currently marketed NNRTIs. The effect of protein binding was also evaluated. The presence of acid glycoprotein had limited effect on the antiviral activity of compounds **23 – 25** versus both wild-type HIV and the K103N resistant strain. In the presence of human serum, the fold change in IC_{50} value was slighty higher than that in acid glycoprotein.

The combination of acid glycoprotein and human serum did not have a dramatic effect on the antiviral activity. Pharmacokinetic studies indicated an acceptable metabolic fate and clearance of the drug from the body. The oral bioavailability was in the range of 27 – 58% when the drug was administered as a solution, and 3 – 20% when it was dosed as a suspension.

2.5 2-Quinolones (Wellcome and GlaxoSmithKline)

A structure-based approach was used to design a novel series of NNRTIs [32]. Detailed analysis of a wide range of crystal

Table 4. Antiviral activity of benzophenones 23 – 25 against wild-type HIV-1 and a panel of resistant mutants in comparison with EFV, NVP and DLV.*

	IC_{50} (nM)					
HIV-1 type	**23**	**24**	**25**	**EFV**	**NVP**	**DLV**
wild-type	1.4	1.4	2.4	0.9	100	200
K103N	1.9	2.0	3.3	27	6600	2700
V106A	10.0	30.0	16	1.5	9000	900
V106I	2.5	2.7	3.2	0.8	150	55
V108I	1.9	1.0	3.5	2.1	290	78
K101E	3.4	3.4	7.2	2.5	520	29
L100I	1.2	0.8	2.0	21	500	920
G190A	4.2	5.2	5.6	7	> 10000	35
P225H	0.7	0.5	1.8	1.7	1200	11
P236L	5.1	21	7.8	0.8	56	1600
Y181C	3.1	6.5	6.6	1.8	9400	2300
Y188C	0.4	0.3	0.6	1.4	2300	170
E138K	3.0	3.9	6.1	0.9	65	10
K103N/Y181C	3.6	8.1	7.6	46	> 10000	> 8400
K103N/P225H	1.5	3.0	7.9	150	9800	2300
K103N/G190A	16.0	26	15	490	> 10000	2700
K103N/L100I	3.0	4.0	6.6	1500	8000	> 8400
K103N/V108I	3.6	5.6	4.8	93	15000	3500
V106A/Y181C	16	28	21	3.1	> 10000	> 10000
V106I/Y181C	11	34	11	3.6	> 10000	4500
V108I/Y181C	3.4	4.1	6.2	3.1	> 10000	> 10000

*For experimental details see [40]. DLV: Delavirdine; EFV: Efavirenz; NVP: Nevirapine.

structures of viral RT/NNRTI complexes together with data on drug-resistance mutations afforded the identification of factors important for tight binding of inhibitors and resilience to mutations. Using this approach; a novel series of quinolones were designed and synthesised that proved to be potent and selective inhibitors of HIV-1 RT.

Compound **28**, with an IC_{50} value of 0.86 µM, was the most potent against the Y181C mutation, and the most potent inhibitor of the L100I mutant was compound **26** (IC_{50} = 1.36 µM). Compound **26** was the least affected by the resistance mutation (**Table 5**). The quinolones **26** – **29** were demonstrated to be potent inhibitors of wild-type (HIV-1 IIIB) and NVP-resistant strains in MT-4 cell assays. Crystal structure analysis of these compounds in complex with HIV-1 RT confirmed the predicted binding modes.

A series of new quinolones were designed on the basis of the previous work [32,33,105]. Molecular modelling studies suggested that, similar to EFV, the NH proton may form a hydrogen bond with the backbone carbonyl group of the K103 residue. Therefore, differences in the electron-withdrawing properties of the R substituents were expected to have an effect on the acidity of the NH proton and, thus, the strength of the hydrogen bond. As for the R_1 group, *iso*-propyl and *n*-propyl substituents were mainly introduced because they were viewed to be optimal from the design work. The compounds were initially assayed in a MT-4 infection model with wild-type HIV-1 and mutant strains of HIV-1. Some selected compounds are shown in **Table 6**. SAR analysis indicated that R = F; Cl as substituents were optimal for antiviral activity in MT-4 cells. On the contary, replacement of R with CH_3 and OCH_3; led to much less active compounds. For the R_1 group, variations between ethyl, *n*-propyl, *iso*-propyl and *iso*-butyl did not seem to have striking differences in antiviral activity.

Introduction of a cyclopropylethynyl group as the R_2 substituent led to the more potent compounds (**38** – **43**). When quinolone **41** was superimposed on EFV in the NNRTI binding pocket, the cyclopropylethynyl moiety for both compounds projected into a common subpocket defined by the Y181 and Y188 residues. Replacing the 6-fluoro atom of compound **41** with chlorine to give compound **42**, was expected to give a stronger hydrogen bond between the 1-NH

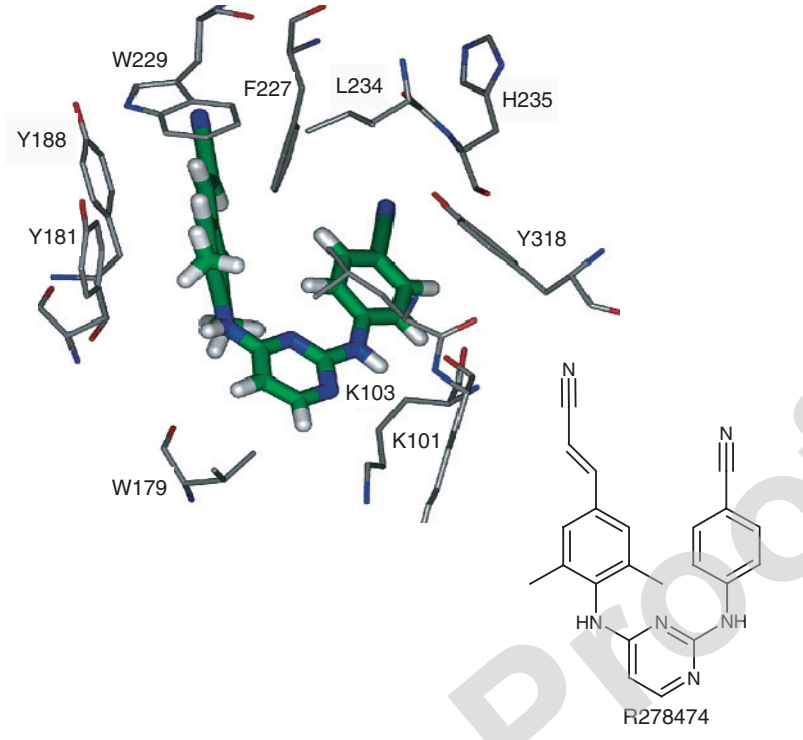


Figure 3. Rilpivirine in the NNRTI binding pocket. Reproduced with kind permission from JANSSEN PA, LEWI PJ, ARNOLD E *et al.*: In search of a novel anti-HIV drug: multidisciplinary coordination in the discovery of 4-[[4-[[4-[(1E)-2-cyanoethenyl]-2,6-dimethylphenyl]amino]-2- pyrimidinyl]amino]benzonitrile (R278474, rilpivirine). *J. Med. Chem.* (2005) **48**(6):1901-1909 [27].
NNRTI: Non-nucleoside reverse transcriptase inhibitor.

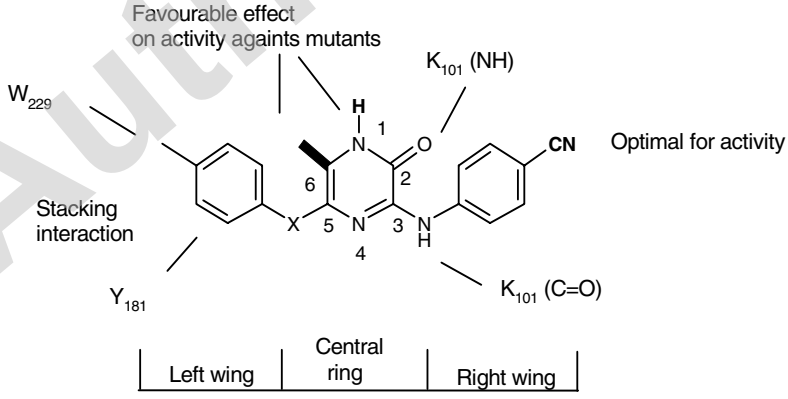
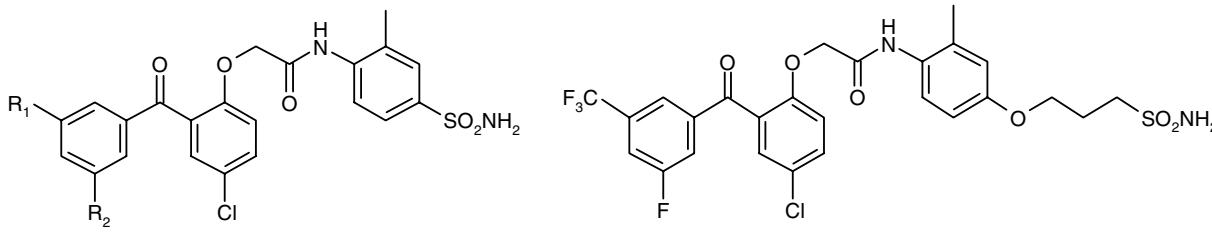


Figure 4. SAR of the novel pyraznone series.

proton and the backbone carbonyl of the K103 residue. The equipotent activity shown by compounds **41** and **42** demonstrated that the antiviral potency was not dependent on the strength of the hydrogen bond between 1-NH and the carbonyl of K103 residue.

The active compounds against the mutant strains came mainly from cyclopropylethynyl subgroup, as found in the MT-4 assay. Among them, compound **38** showed the broadest spectrum of antiviral activity. It was then followed by compounds **40** – **42**. However, in general, compounds **41** and **42** had higher potency.

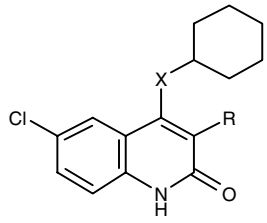
In conclusion, analogues **41** and **42** showed the broadest spectrum of activity and were more potent than NVP and DLV against mutant viruses that are generally insensitive to the currently known NNRTIs. The quinolone class of NNRTIs, as predicted by its design, has proven to be effective and potent against a wide range of diverse drug-resistant mutants, far more so than the first-generation NNRTIs.



23 GW4511 $R_1 = CF_3$, $R_2 = F$
24 GW4751 $R_1 = H$, $R_2 = CN$

25 GW3011

Figure 5.



Compound	R	X
26	*n*-Propyl	O
27	*iso*-Propyl	O
28	*n*-Propyl	S
29	*n*-Propyl	S=O

Figure 6.

Table 5. Antiviral activity in MT-4 cells (EC_{50}) and inhibition of wild-type and mutant HIV-1 RT (IC_{50}) by quinolone compounds 26 – 29.

	HIV-1IIIB	NVP-resistant HIV-1*	Wild-type RT	Cys181 RT	Ile100 RT
Compound	**EC_{50} (μM)**	**EC_{50} (μM)**	**IC_{50} (μM)**	**IC_{50} (μM)**	**IC_{50} (μM)**
26	0.035	0.607	0.71	1.13	1.36
27	0.051	0.710	0.63	2.50	1.96
28	0.100	0.368	0.24	0.86	1.61
29	0.242	2.210	0.38	1.26	2.38
NVP	HIV-1IIIB	NVP-resistant HIV-1*	0.30	> 100	> 100

*HIV-1 virus containing Tyr181Cys mutation in RT. NVP: Nevirapine; RT:Reverse transcriptase.

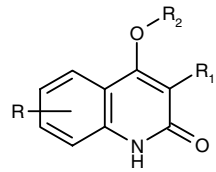
However, none of the analogues reported here showed improved potency upon EFV, particularly in comparison with EFV activity against the K103N strain.

2.6 TBZ (University of Messina [Italy])

Researchers at the University of Messina discovered thiazolobenzimidazole (TBZ, 45), a class of NNRTIs that displayed binding of the NNBS of the RT in a similar mode as other NNRTIs [34,106]. Molecular modelling studies suggested some chemical modifications aimed at improving the interactions of compound 45 with the amino acids of the NNBS. An opening of the thiazole nucleus, an introduction of chlorine atoms at position-5 or -6, (**46**, 47) or replacing the imidazolone ring with benzoxazolone (**48**) was a promising option (**Figure 7**) [35].

In the *in vitro* assay in MT-4 cells the position of the halogen substituent on the benzene ring of compounds **46** and 47 was crucial for the antiviral activity (**Table 8**). In fact, according to molecular modelling (**Figure 8**) the chlorine in the 6-position of compound **46** gave a favourable interaction profile that favoured hydrophobic contacts with the surrounding residues in the NNBS. As expected, the chlorine in the 5-position of compound 47 caused a decrease of inhibitory potency. The weaker activity shown by compound **48** (one order of magnitude less active than **46**) was also consistent with the importance of the hydrogen bond interaction of the NH group with the backbone carbonyl of Lys101. In CEM cell cultures against a panel of mutant virus strains, compound **46** kept activity against L100I and E138K RT HIV-1, but lost antiviral activity against K103N, Y181C and Y188H

Table 6. Antiviral activity of quinolinones 30 – 44 against wild-type HIV-1 and NVP-resistant strain in the MT-4 assay.



Compound	R	R_1	R_2	EC_{50} (nM)		
				HIV-1	NVP-resistant	CC_{50}/IC_{50}
30	6-F	*iso*-propyl	(*cyclo*-butyl)methyl	26	230	> 500
31	6-Cl	*iso*-propyl	(*cyclo*-butyl)methyl	29	230	> 500
32	6-Cl	*n*-propyl	*cyclo*-pentyl	96	1700	> 40000
33	6-Cl	ethyl	*cyclo*-pentyl	78	1020	> 40000
34	6-Cl	*iso*-butyl	*cyclo*-pentyl	52	1600	> 40000
35	6-Cl	*sec*-butyl	*cyclo*-pentyl	9	290	> 40000
36	6-CH_3	*n*-propyl	*cyclo*-pentyl	970	6400	> 40000
37	6-OCH_3	*n*-propyl	*cyclo*-pentyl	1010	24000	> 40000
38	6-F	ethyl	(*cyclo*-propyl)ethynyl	25	15	> 8000
39	6-F	*n*-propyl	(*cyclo*-propyl)ethynyl	72	81	> 8000
40	6-Cl	*n*-propyl	(*cyclo*-propyl)ethynyl	109	120	> 8000
41	6-F	*iso*-propyl	(*cyclo*-propyl)ethynyl	29	25	16000
42	6-Cl	*iso*-propyl	(*cyclo*-propyl)ethynyl	24	31	> 8000
43	6-F	*iso*-butyl	(*cyclo*-propyl)ethynyl	150	120	> 8000
44	6-F	*iso*-propyl	(*sec*-butyl)ethynyl	17	32	> 6500

NVP: Nevirapine.

RT HIV-1 strains (**Table 9**). Compound **46** was selected as a lead compound for further studies.

2.7 Indolyl aryl sulfone (University of Roma 'La Sapienza' [Italy])

Extensive SAR studies on diaryl sulfones at the University of Roma 'La Sapienza' led to the identification of potent NNRTIs, such as the pyrryl aryl sulfone (PAS **49**), and the indolyl aryl sulfones (IASs; **50** – **52**) related to L-737,126 (**53**; **Figure 9**) [36-38,107].

The indole derivatives bearing the 3-[(3,5-dimethylphenyl)sulfonyl] moiety (**52**) displayed high activity and selectivity not only against the HIV-1 wild-type strain, but also against the Y181C and K103N-Y181C viral variants and the EFV-resistant (EFV^R) mutant K103R-V179D-P225H [38]. Chemical modification of the 2-carboxyamido moiety of compound **53** improved the antiviral activity against both wild-type and mutant strains of HIV-1 [39]. Such a finding was a stimulus in designing new IASs utilising 3D quantitative structure-activity relationship (QSAR) models developed using a training set of 70 IASs derivatives. The structures of the training set were modelled on the structure of the related diaryl sulfone 739W94. The most predictive 3D QSAR model was then used to design the novel IAS derivatives **54** – **77** following the idea that introduction of carboxamide chain elongators containing hydrogen bond acceptors and donors would be beneficial for the interaction of the inhibitor with the NNBS [40,41].

The binding mode of IAS derivatives was investigated by means of docking studies into the HIV-1 NNBS using compound **53** as a representative member. Such a study led to identify a unique binding mode of compound **53** for all the used RTs. With the only uncertainty being of the carboxamide function, the other chemical features of compound **53** shared a common binding mode, namely: i) the indole NH makes a hydrogen bond with the Lys101 carbonyl; ii) the phenyl ring of the benzenesulfonyl moiety occupies a hydrophobic aromatic-rich pocket formed mainly by the side chains of Tyr181, Tyr188, Phe227 and Trp229; iii) the sulfonyl group fits in a little hydrophobic pocket made by the side chains of Val106, Lys103 (only α and β CH_2) and Val179; and iv) the 5-chlorine atom of indole makes favourable contacts with

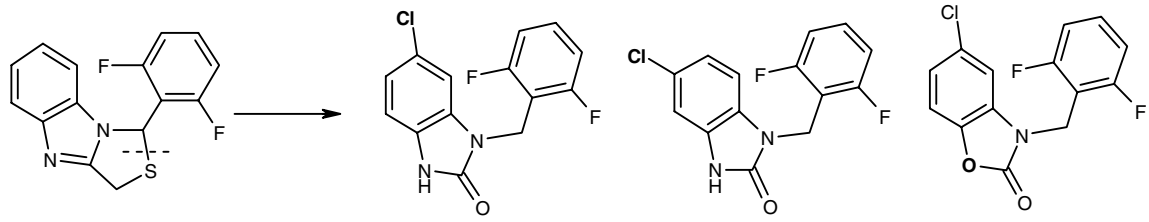


Figure 7.
TBZ: Thiazolobenzimidazole.

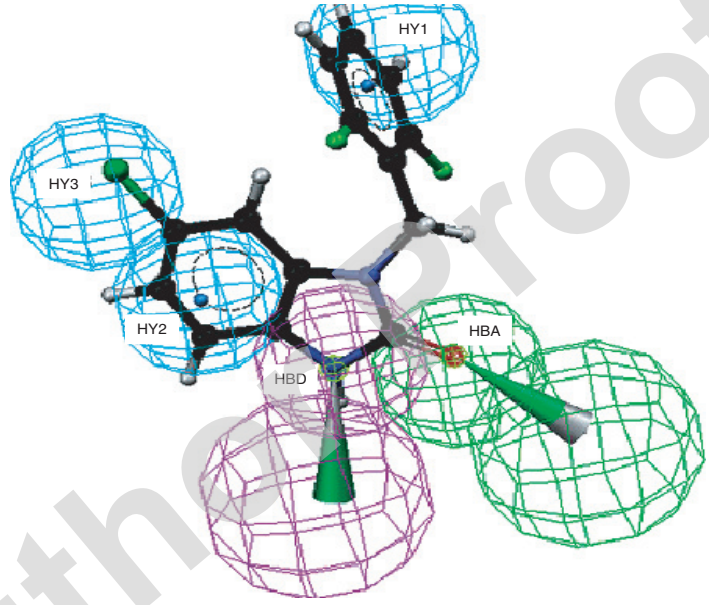


Figure 8. Three-dimensional common features pharmacophore model of NNRTIs aligned to compound 46. Reproduced with the kind permission from BARRECA ML, RAO A, DE LUCA L *et al.*: Computational strategies in discovering novel non-nucleoside inhibitors of HIV-1 RT. *J. Med. Chem.* (2005) **48**(9):3433-3437 [35].
NNRTI: Non-nucleoside reverse transcriptase inhibitor.

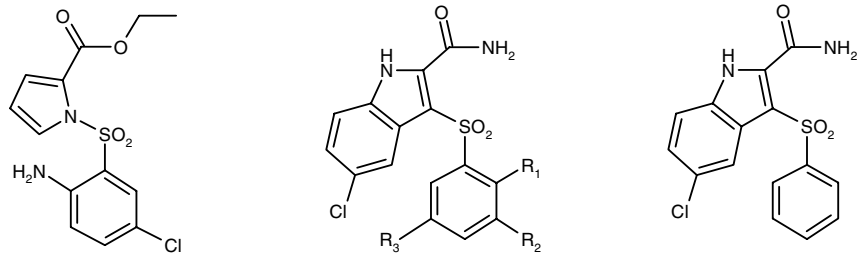


Figure 9.

Table 7. Antiviral activity of quinolones 38, 41 and 42 against wild-type HIV-1 and those containing NNRTI-resistance mutations in HeLa-CD4 MAGI assay.

Compound/strain	EC50 (nM)					
	38	**41**	**42**	**EFV**	**DLV**	**NVP**
wild-type RVA	25	10	16	1	159	89
K103N	420	400	320	23	2651	7100
V106A	340	150	57	1	870	10000
G190A	6	5	3	7	35	> 10000
Y188C	100	23	18	1	124	2200
V108I	60	26	36	1.4	97	340
Y181C	24	15	39	2	No data	> 10000
K103N/Y181C	300	620	620	43	1250	> 50000
K103N/G190A	68	100	52	552	8000	No data
V106A/Y181C	230	220	120	3	10000	> 10000
K103N/V108I	240	720	1200	74	3400	21000
V108I/Y181C	130	11	120	3	10000	> 10000

DLV: Delavirdine; EFV: Efavirenz; NNRTI: Non-nucleoside reverse transcriptase inhibitor; NVP: Nevirapine.

Table 8. Anti-RT and anti-HIV-1 activities, cytotoxicity and selectivity index in MT-4 cells of compounds 45 – 48.

Compound	IC50 (μM)	EC50 (μM)	CC50 (μM)	SI
45	24.57 ± 2	1.10 ± 0.32	50.0 ± 3.2	45
46	2.86 ± 0.1	0.24 ± 0.05	> 424	> 1766
47	60 ± 4	29.6 ± 2.1	> 424	> 14
48	20 ± 1.5	3.72 ± 0.47	> 382.2	> 91

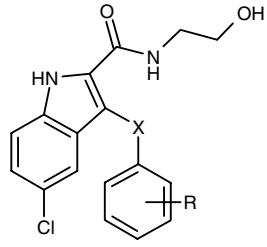
SI: Selectivity index: ratio CC_{50}/EC_{50}; RT:Reverse transcriptase.

Table 9. Anti-HIV-1 activity of compounds 45 and 46 against mutant HIV-1 strains in CEM cells.

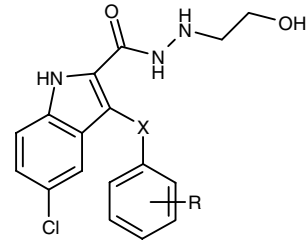
Compound	EC50 (μM)					
	HIV-1IIIB	**L100I**	**K103N**	**E138K**	**Y181C**	**Y188H**
45	1.39 ± 0.56	3.12 ± 0.94	15.6 ± 3.2	1.73 ± 1.02	13.8 ± 4.2	4.16 ± 1.12
46	0.88 ± 0.51	2.0 ± 1.4	> 20	2.9 ± 2.2	> 20	> 20

Pro236. A training set of 70 IASs derivatives was used to develop the 3D QSAR. The structures of the training set were modelled starting from the closely structurally related diaryl sulfone 739W94 extracted from the corresponding complex with the HIV-1 RT (pdb code 1jlq). On the basis of the SARs of the training set, 24 new IAS derivatives bearing 2-hydroxyethylaminocarbonyl and 2-hydroxyethylhydrazinocarbonyl moieties at position-2 of the indole nucleus were modelled and receptor-based aligned. Application of the 3D QSAR model allowed the pEC_{50} value prediction of the modelled derivatives (**Table 10**). Three compounds for each series (hydroxyethylamide and hydroxyethylhydrazide), for a total of six, were synthesised and tested for their anti-HIV-1 activity. Among predicted derivatives, the top four candidates (**64** and **65** for structure A, and **74** and **76** for structure B) and the bottom two candidates (**54** for structure A and **70** for structure B) predicted within the series were chosen for the synthesis. The experimental pEC_{50} values proved that the selected 3D QSAR model (model 3) was the most predictive (**Table 11**). Inspection of the docked conformations of derivatives **54**, **64**, **65**, **70**, **74** and **76** disclosed a different binding mode compared to that of the reference compound **53**. This change in the binding mode makes the 3D QSAR model slightly underpredictive for five out of six deivatives. The problem of this underpredictivity would certainly be resolved by using a wider training set that will include the newly

Table 10. pEC_{50} predictions of 2-hydroxyethylamide (54 – 65) and 2-hydroxyethylhydrazide (66 – 77) derivatives of IASs.



A



B

Compound	Structure	X	R	Predicted pEC50
54	A	S	H	5.93‡
55	A	S	2-CH_3	5.96
56	A	S	3-CH_3	6.00
57	A	S	4-CH_3	6.54
58	A	S	2,3-$(CH_3)_2$	5.97
59	A	S	3,5-$(CH_3)_2$	6.41
60	A	SO_2	H	6.76
61	A	SO_2	2-CH_3	6.32
62	A	SO_2	3-CH_3	7.05
63	A	SO_2	4-CH_3	7.11
64	A	SO_2	2,4-$(CH_3)_2$	7.13*
65	A	SO_2	3,5-$(CH_3)_2$	7.53*
66	B	S	H	6.49
67	B	S	2-CH_3	6.70
68	B	S	3-CH_3	6.12
69	B	S	4-CH_3	6.55
70	B	S	2,4-$(CH_3)_2$	6.16‡
71	B	S	3,5-$(CH_3)_2$	6.62
72	B	SO_2	H	7.06
73	B	SO_2	2-CH_3	7.10
74	B	SO_2	3-CH_3	7.36*
75	B	SO_2	4-CH_3	6.98
76	B	SO_2	2,4-$(CH_3)_2$	6.65
77	B	SO_2	3,5-$(CH_3)_2$	7.79*

*The four most active predicted compounds. ‡The two poorly active predicted compounds chosen for the synthesis.

synthesised IAS derivatives. In addition, resolution of the X-ray structure of the complex will give additional information.

2.8 Capravirine analogues (University of Southern Denmark [Denmark])

Capravirine (**78**), also known as S-1153 and AG-1549, is an imidazole NNRTI characterised by a favourable profile of resilience to many resistance mutations. Although capravirine development has been stopped, the synthesis of analogues is of current interest [42].

The anti-HIV-1 activity of capravirine analogues was evaluated in MT-4 cell cultures infected with wild-type strain IIIB strain. The activity of some selected analogues (**79** – **88**) is depicted in **Table 12**. Replacement of the ethyl in position-4

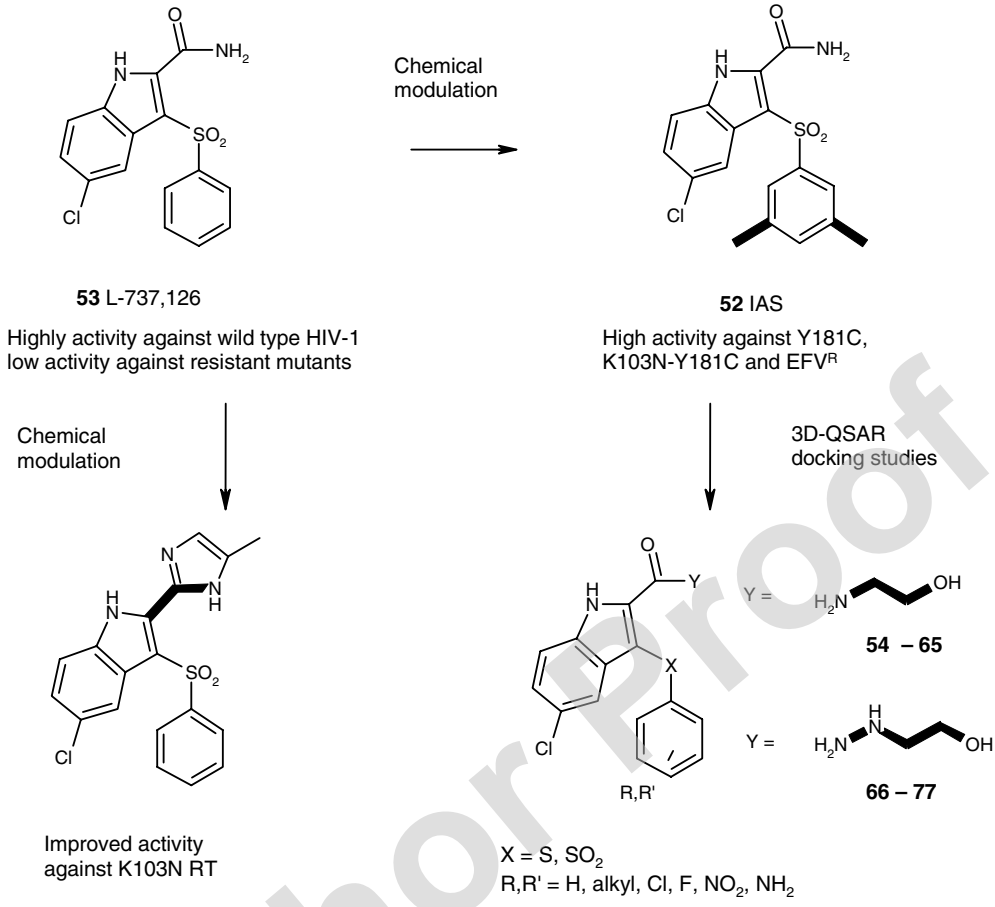


Figure 10.
EFV^R: Efavirenz; IAS: Indolyl aryl sulfones; RT: Reverse transcriptase; 3D-QSAR: 3D-quantitative structure-activity relationship.

Table 11. Predicted pEC_{50} and $SDEP_{pr}ed_{-set}$ values obtained with the models 1 – 6 after the prediction set.

Compound	pEC50						
	Experimental*	Predicted‡					
		M1	M2	M3	M4	M5	M6
54	6.89	5.16	5.78	5.93	5.86	6.09	5.25
64	8.00	6.85	6.17	7.13	5.69	7.08	5.81
65	9.00	6.54	4.10	7.53	4.36	6.51	4.09
70	5.64	7.38	4.37	6.16	4.87	7.25	4.36
74	8.00	5.54	6.97	7.36	5.92	5.91	6.27
76	9.00	6.59	6.37	7.79	7.00	6.69	6.41
$SDEP_{Pr}ed_{-set}$	-	1.55	2.90	1.00	2.82	1.38	2.96

*Experimental pEC_{50} (pEC_{50} = -LogEC_{50} (M)). ‡Predicted pEC_{50}: M = Model.

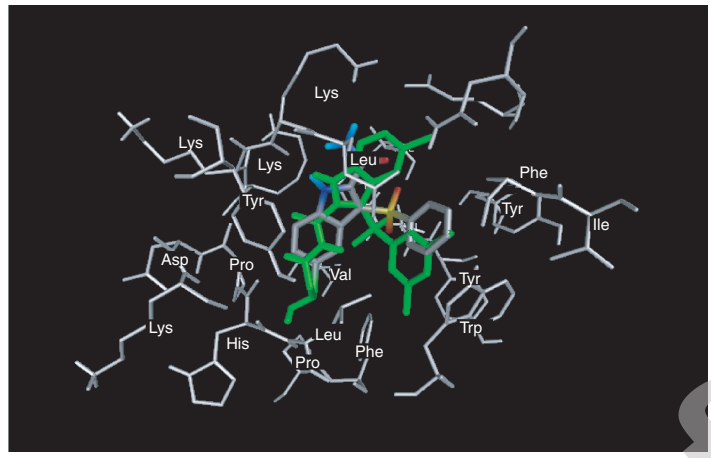
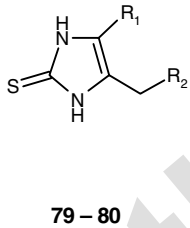
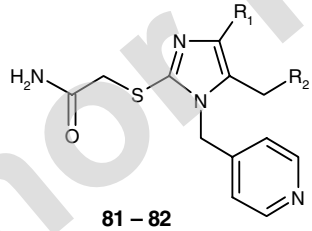


Figure 11. Superimposition of reference compound 53 and derivative 72 (coloured in green). A 5Å core of the NNBS is also included for comparison purposes. Nonpolar hydrogens are not displayed for sake of clarity. Colours used in the reference compound: red, oxygen; blue, nitrogen. Printed with permission from....
NNBS: Non-nucleoside binding site.

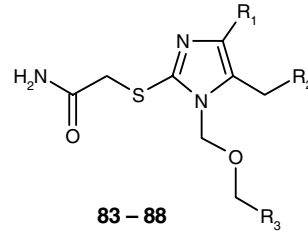
Table 12. Anti-HIV-1 activity of compounds 79 – 88 against HIV-1 in MT-4 cells



79 – 80



81 – 82

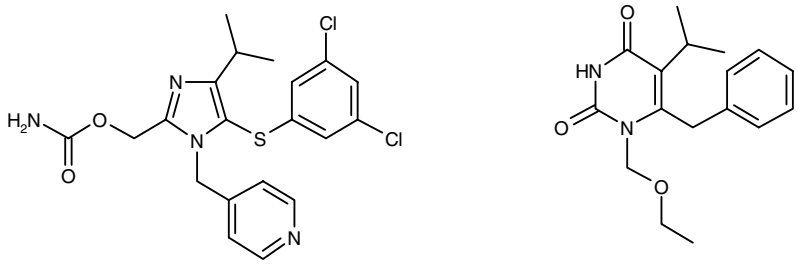


83 – 88

Compound	R1	R2	R3	CC50 (μM)	EC50 (μM)	SI
79	C_2H_5	3,5-$(CH_3)_2$Ph	-	> 100	4.46	> 22.4
80	*iso*-Pr	3,5-$(CH_3)_2$Ph	-	38.61	0.62	62.3
81	*iso*-Pr	3,5-$(CH_3)_2$Ph	-	64.04	0.31	206.5
82	*iso*-Pr	Cyclohexyl	-	> 100	3.75	> 26.6
83	C_2H_5	3,5-(CH3)2Ph	CH_3	> 100	1.33	> 75.2
84	C_2H_5	3,5-$(CH_3)_2$Ph	Ph	37.56	2.48	15.1
85	*iso*-Pr	3,5-(CH3)2Ph	CH_3	> 100	0.31	> 322.5
86	*iso*-Pr	3,5-$(CH_3)_2$Ph	Ph	29.43	0.15	196.2
87	*iso*-Pr	Cyclohexyl	CH_3	> 100	2.11	> 47.4
88	*iso*-Pr	Cyclohexyl	Ph	84.32	4.29	19.7
89	-	-	-	> 100	0.03	> 3333

SI: Selectivity Index CC50/IC50 ratio.

of the imidazole nucleus with an *iso*-propyl group led to more potent derivatives (compare **79** with **80**, **83** with **85**, and **84** with **86**). The activity of the compounds was also affected by the group at the 5-position of the imidazole ring in the order 3,5-$(CH_3)_2$-phenyl > cyclohexyl > phenyl (compare **81** with **82**, **85** with **87**, and **86** with **88**). The alkoxymethyl substituent at the 1-position of imidazole ring (compounds with an ethoxymethyl group and compounds with a benzyloxymethyl group) showed higher activity than compounds containing a 4-pyridylmethyl group at the same position (compare **81** and **82** with **85** – **88**). These new capravirine analogues proved to be active against the HIV-1, but in all cases



78 Capravirine

89 MKC

Figure 12.

they were less active than the uracil reference compound MKC-442 (**89**).

2.9 PETT analogues (University of Genova [Italy])

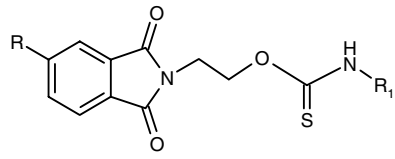
Ranise's research team synthesised several *O*-substituted *N*-acyl-*N*-arylthiocarbamates (ATCs) structurally related to *N*-phenethyl-*N*′-thiazolylthiourea (PETT) derivatives (**90**) [43]. Amongst the ATCs, the phthalimidoethyl-ATCs proved to be potent inhibitors of the multiplication of wild-type HIV-1. These compounds were found to be significantly active against the Y181C mutant, but ineffective against the K103R mutant. In molecular modelling studies compound **91**, one of the lead compounds of the ATC series, and some synthetic intermediates were superimposed to the X-ray structure of the PETT derivative **93**. Surprisingly, the intermediary thiocarbamate (TC) **94** more closely resembled the conformation of PETT **93**. Thus, compound **94** was selected for a preliminary screening. TC **94** proved to be able to target the vRT (IC_{50} = 0.6 μM) and to selectively inhibit the HIV-1-induced cytopathogenicity in MT-4 cells (CC_{50} > 100 μM, EC_{50} = 1.2 μM), even if it was threefold less potent than ATC **91** (EC_{50} = 0.4 μM). In order to explore the antiviral potential of the TC class, the authors planned the synthesis of analogues of the lead compound **94** [44]. Several ring-opened analogues (*O*-TCs) were concomitantly obtained from parallel synthesis along with their corresponding ring-closed congeners (*C*-TCs). As the overall conformation of enzyme-bound *O*-T*C* **95** approximated the X-ray structure of compound **93** within the NNBS, the *O*-TCs were also evaluated for anti-HIV-1 activity.

PETT isosteres that are not able to form an internal hydrogen bond are almost inactive, probably because such derivatives cannot assume the 'high-activity rigid conformation' (PETT **93**). This finding demonstrated a substantial difference between the TC and PETT series. Compound **94** was active in the low-micromolar range of concentration (**Table 13**). Replacement of the B1 ring of compound **94** with a cyclohexyl nucleus (**96**) led to a fourfold increase of the inhibitory potency, whereas introduction of the 1-naphthyl nucleus (**97**) caused a decrease of activity. The substitution pattern of the *N*-phenyl ring strongly affected the activity of TCs in the order *para* > > *meta* > *ortho*, as it is well exemplified by the positional isomers *ortho*- (**98**), *meta*- (**99**) and *para*-tolyl (**100**). In contrast, the electronic properties of the *para*-substituents did not affect the antiviral acitivity (compare **100** with **102**). Several *O*-TCs (compounds **95** and **103 – 109**) were selectively active against wild-type HIV-1. As in the case of the *C*-TCs, the potency of ring-opened congeners depended on the position of the substituent on the *N*-phenyl ring. Even though the general pattern of activity of the *O*-TCs mirrored that of the *C*-TCs (*para* > *meta* and *ortho*), overall the *para*-substituted *O*-TCs were less potent than the corresponding *para*-substituted *C*-TCs. The positional isomer **110**, carrying a methyl group at *para*-position of the phthalimide moiety, was sevenfold more potent than **102**. Shortening or extending the spacer group by only one methylene unit completely abolished the activity or caused a significant drop in the anti-HIV-1 potency. *C*-TC **101** and *O*-TC **108** were also tested in enzyme assays against recombinant wt RT (rRT) and rRTs carrying clinically relevant NNRTI resistance mutations (K103R, Y181C and K103N + Y181C; **Table 14**). Unlike EFV, compounds **101** and **108** and trovirdine were inactive against the double mutant. Conversely, against the Y181C mutant strain, compound **108** was threefold more active than trovirdine, whereas compound **101** was inactive. The different resistance profile of compounds **101** and **108** against the above single mutations was correlated with the preservation or opening of the imide ring. The Y181C mutant strain was significantly inhibited by *C*-TCs **100** and **102**. In contrast, the K103R and the K103N + Y181C double mutant strains turned out to be generally unsusceptible to the tested compounds with the exception of compound **102**, which showed a weak activity against the K103N mutant with an EC_{50} value of 20 μM.

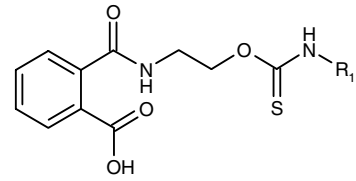
2.10 Pyridones (Johnson & Johnson)

Johnson & Johnson Pharmaceutical Research and Development started a research programme aimed at the synthesis of a new class of highly potent NNRTIs of HIV-1 [45]. To this end, the researchers optimised the anti-HIV activity of two lead compounds, the 4-benzyl and 4-benzoyl-3 dimethylaminopyridinones **111** and **112**, by modification of the alkyl substitutents at the C-5 and C-6-positions on the pyridinone ring and of the substitutents on the C-3 amino group.

Table 13. Cytotoxicity and anti-HIV-1 activity of C-TCs 94, 96 – 102, 110 and O-TCs 95, 103 – 109.*



94, 96-102, 110 *C*-TC



95, 103-109 *O*-TC

Compound	R1	R2	CC50 (μM)	EC50 (μM)	SI
94	Phenyl	H	> 100	1.2	> 83.3
95	Phenyl	-	> 100	3	> 33.3
96	Cyclohexyl	H	> 100	0.3	> 333
97	1-Naphthyl	H	> 100	6.5	> 15.4
98	2-Tolyl	H	> 100	3.7	> 27
99	3-Tolyl	H	> 100	1.5	> 66.7
100	4-Tolyl	H	> 100	0.02	> 5000
101	4-Iodophenyl	H	> 100	0.02	> 5000
102	4-Nitrophenyl	H	65	0.04	1625
103	2-Fluorophenyl	-	> 100	0.5	> 200
104	4-Tolyl	-	> 100	0.4	> 250
105	4-Cyanophenyl	-	> 100	0.1	> 1000
106	4-Chlorophenyl	-	> 100	0.1	> 1000
107	4-Bromophenyl	-	> 100	0.1	> 1000
108	4-Iodophenyl	-	> 100	0.13	> 769
109	4-Methoxyphenyl	-	> 100	0.2	> 500
110	4-Nitrophenyl	CH_3	80	0.01	8000
92	-	-	60	0.02	3000

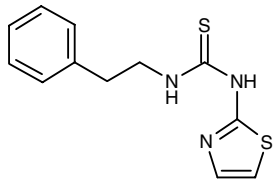
*Data represent mean values for three separate experiments.

Table 14. IC_{50} of C-TCs 101 and O-TC 108 against wild-type and single and double mutant recombinant RTs.

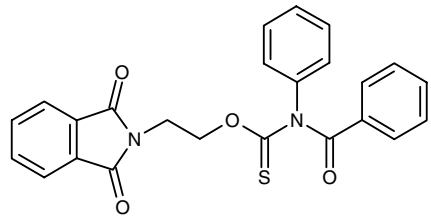
Compound	IC50 (μM)			
	Wild-type (IIIB)	**K103R**	**Y181C**	**K103N, Y181C**
101	0.09	2.3	> 20	> 20
108	1.7	> 20	4.8	> 20
EFV	0.014	2.1	0.02	0.5
Trovirdine	1.03	> 20	15	> 20

EFV: Efavirenz; RT: Reverse transcriptase.

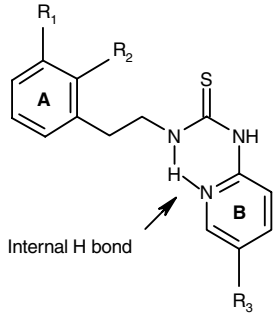
Compounds **111** and **112** were previously obtained by chemical modification of compounds of general formula **113**, and are in many respects hybrids of the HEPT analogue GCA-186 (**114**) and the Merck pyridinone L-697,661 (**115**; **Figure 13**). The presence of the 3-N$(CH_3)_2$ substitution in compounds **111** and **112**, as opposed to a non-functionalised amino group, was similarily inspired by the presence of an *iso*-Pr group at the corresponding position in the HEPT derivatives MKC-442 (**89**) and compound **114**. Among > 100 new analogues of the lead molecules **111** and **112**, the new lead compounds **116** and **117** in the 4-benzoylpyridinone series and compound **118** in the 4-benzylpyridinone



90 PETT LY73947

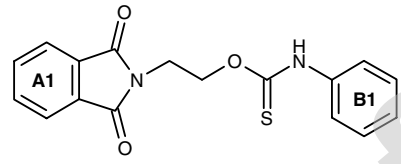


91 ATC

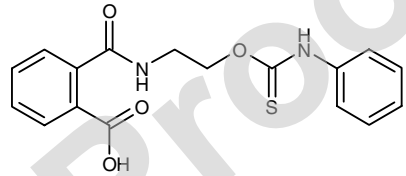


92 Trovirdine (R_1 = R_2 = H; R_3 = Br)

93 PETT (R_1 = F; R_2 = OEt; R_3 = CN)



94 C-TC



95 O-TC

Figure 13.

group were identified. These molecules were potent inhibitors of wild-type HIV-1, as well as of a large panel of HIV-1 mutant strains, which are responsible for the onset of resistance to the NNRTIs that are currently used in HAART therapy. Globally, the *in vitro* activity profile of these new molecules, and in particular of compound **118**, was better than that of EFV.

The next step of this SAR study involved looking closely at the influence of modifications at the C-5 and C-6-positions on the pyridinone ring and at the amino substitutent at C-3 on anti-HIV activity *in vitro*. Compound **119**, bearing a polar hydroxymethyl group at C-6, and the analogues **120** and **121** with extended nonpolar chains containing an ether function at C-5 and a C-6 methyl group, were highly active against the mutant strains. Several derviatives of 3-*N*,*N*-dialkylamino substituents displayed potent activity against wild-type HIV-1 and the three major mutant strains K103N, Y181C and Y188L. In particular, the *N*-methyl-*N*-(2-methoxyethyl)-substituted compounds **122** – **124**, as well as the doubly modified compounds **125** and **126** selected from the initial evaluation, were subsequently shown to be active at submicromolar concentrations against all the mutant strains except K103N + Y181C and F227L + V106A. A molecular modelling study was undertaken for compounds **111** and **124** using the HEPT-RT structure as a starting point. This study revealed two possible and essentially equivalent modes of binding of these compounds (orientations **1** and **2**, **Figure 14**), generated by rotation of the pyridinone ring about an axis defined by atoms N-1 and C-4. These orientations differ most importantly in the positioning of the extended amino side chain substituent. In orientation **2**; this side chain occupies the same space as the N-1 substituent in HEPT and HEPT analogues. The preferred mode of binding for compound **124**, corresponding to the predicted orientation **1**, was revealed in the X-ray crystal structure of the compound **124**–RT complex.

3. Drugs in advanced clinical development.

The final step in the development of a new drug is the clinical trial stage. Clinical trials are usually designated as Phase I, II or III, based on the type of questions that study is seeking to answer:

- In Phase I clinical trials, researchers test a new drug or treatment in a small group of people (20 – 80) for the first time to evaluate its safety, determine a safe dosage range and identify side effects.
- In Phase II clinical trials, the study drug or treatment is given to a larger group of people (100 – 300) to see if it is effective and to further evaluate its safety.
- In Phase III studies, the study drug or treatment is given to large groups of people (1000 – 3000) to confirm its effectiveness, monitor side effects, compare it with commonly used treatments and collect information that will allow the drug or treatment to be used safely.

Among the novel NNRTIs mentioned in the previous section, clinical trials information was available only for the DAPY class.

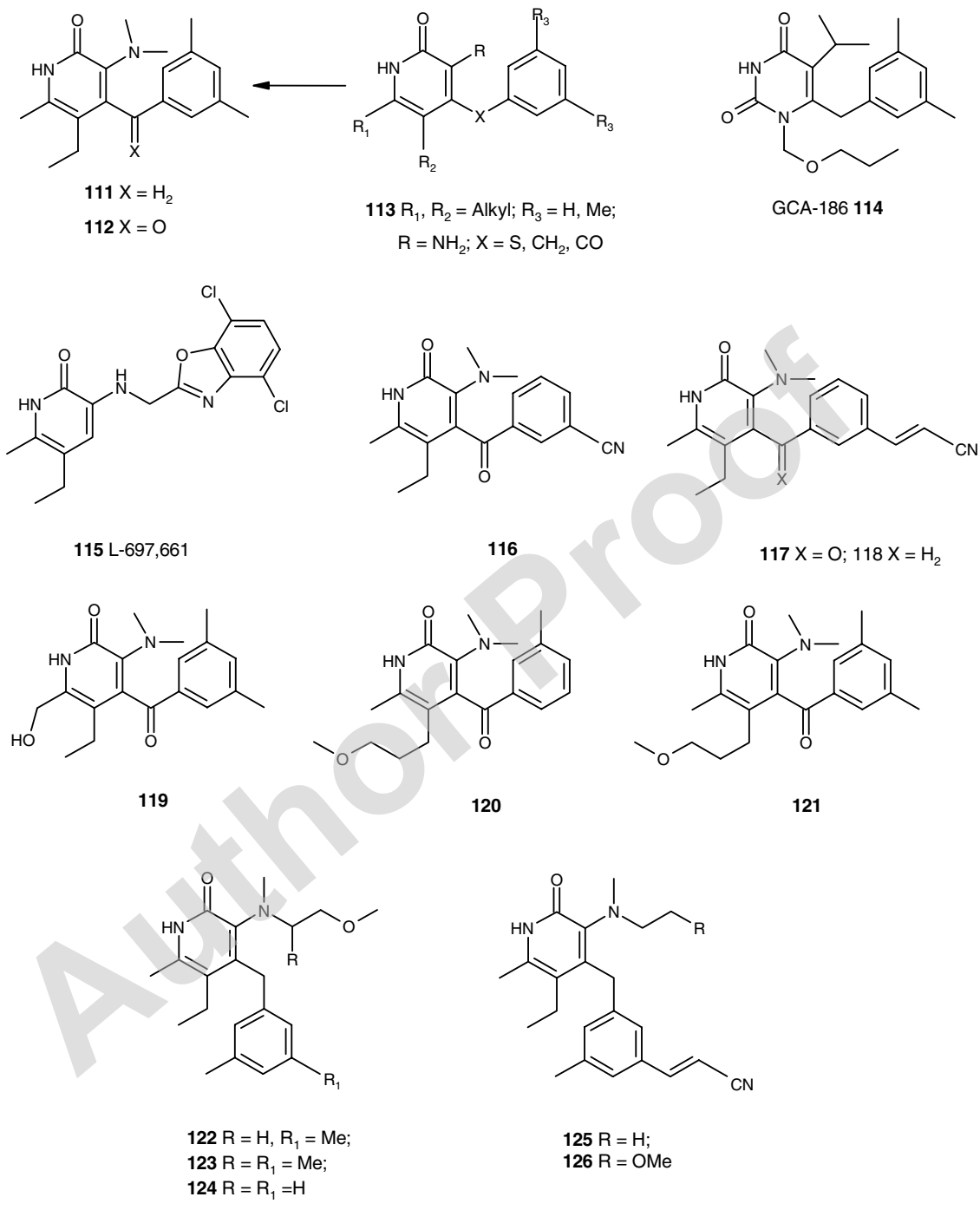
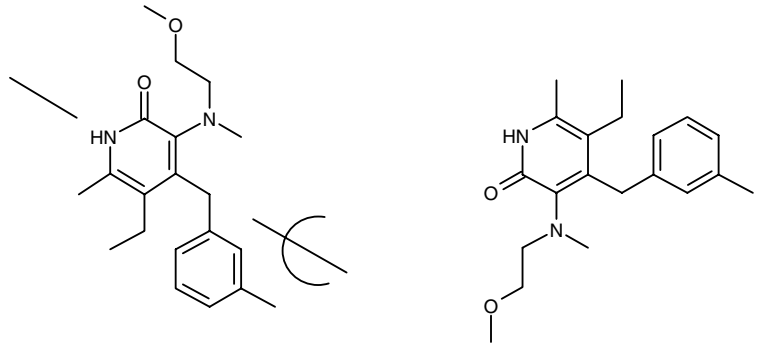


Figure 14.

3.1 Etravirine (TMC-125).

The diarylpyrimidine derivative etravirine was studied in HIV-1-infected patients with high-level phenotypic NNRTI resistance in an open-label Phase IIa trial. A total of 16 individuals receiving an NNRTI-containing anti-retroviral regimen (EFV or NVP) with an HIV-1 RNA viral load of > 2000 copies/ml and phenotypic resistance to NNRTIs, received TMC-125 for 7 days, as a substitute for their current NNRTI in their failing therapy. Etravirine proved to be well tolerated and demonstrated significant and rapid antiviral activity by a decrease in viral load in these patients with high levels of phenotypic NNRTI resistance to current NNRTIs. Over 7 days, the most significant adverse effects were diarrhoea (31%) and a mild headache (25%). In a recent study, monotherapy with etravirine in HAART-naive, HIV-1-infected individuals resulted in an impressive decline of viral load during one week of therapy [26,46-48]. At present, at least three different Phase II/III studies of etravirine are ongoing [201].



124 Orientation 1 **124** Orientation 2

Figure 15.

Table 15. Activity of compounds 111, 112 and 119 – 126 against wild-type HIV-1 and three relevant mutant strains.

Compound			EC50 (μM)*		
	LAI‡	SI*	K103N	Y181C	Y188L
111	0.008	12589	0.032	0.100	0.251
112	0.004	2512	0.010	0.063	0.158
119	0.001	12589	0.003	0.072	0.631
120	0.008	1259	0.126	0.160	0.158
121	0.002	12589	0.01	0.05	0.2
122	0.006	7943	0.079	0.398	0.251
123	0.002	19953	0.063	0.316	0.1
124	0.001	10000	0.079	0.794	0.398
126	0.001	10000	0.006	0.032	0.251
126	0.001	10000	0.02	0.04	0.316

*Wild-type virus (HIV-1 LAI). SI:Selectivity index: $CC_{50}{}^{d}/EC_{50}$ (ratio relative to LAI fold).

TMC-125-C227 is a Phase II randomised, active-controlled, open-label-trial to investigate the efficacy and tolerability of etravirine in HIV-1 infected subjects, who are protease inhibitor-naive, but with evidence of NNRTI resistance from previous NNRTI use. The aim of this study is evaluating the effect of etravirine on the viral load decrease on tolerance of the drug. The study will also investigate whether this new medication is well tolerated, and to further confirm that the medication is safe to be used. The study will enroll 120 patients for 48 weeks.

TMC-125-C211 is a Phase II/III, open-label, roll-over trial to evaluate the long-term safety and tolerability of etravirine, administered as part of an individually optimised anti-retroviral therapy, in HIV-1-infected subjects. In addition, the antiviral activity and immunological effect of etravirine as part of an anti-retroviral regimen over time, and the evolution of HIV phenotype and genotype will be evaluated. The trial, due to close in January 2007, involves 40 patients for a 48-week treatment period and a 4-week follow-up period.

TMC-125-C206/C216 is a Phase III, randomised, double-blind, placebo-controlled trial to evaluate the long-term efficacy, tolerability and safety of etravirine as part of an anti-retroviral therapy regimen containing TMC114/ritonavir and an investigator-selected optimised background in treatment-experienced HIV-1 infected patients. TMC114 is a protease inhibitor. In this trial, TMC114 will be given with a low dose of RTV, a PI commonly used with other, full dose protease inhibitors to improve activity. Additional assessment to be evaluated in this trial include changes in the HIV-1 genotype, drug susceptibility and the population pharmacokinetics of etravirine. The trial due to start in November 2005 will involve 600 patients with screening period of up to 6 weeks, a 48-week treatment period and a 4-week follow-up period.

3.2 Rilpivirine (TMC-278)

TMC-278-C204 is Phase II, randomised, partially blinded, dose-finding trial started on March 2005 with 320 patients and is expected to close in February 2008 [201]. The study will help determine a safe and effective dose of this new drug. Patients included in this study will be treatment-naive, which means they have not previously received treatment with anti-retroviral therapy or have been treated for a maximum of

Table 16. Activity of compounds 111, 112 and 119 – 126 against a panel of mutant strains.

Compound	EC50 (μM)					
	L100I	K101E	V106A	E138K	V179E	G190A
111	0.05	0.016	0.04	No data	No data	0.063
112	0.006	0.008	0.006	0.005	0.002	0.013
119	0.001	0.005	No data	0.004	0.001	0.003
120	0.020	0.032	0.040	0.020	0.008	0.016
121	0.008	No data	No data	No data	No data	No data
122	0.079	0.058	No data	0.017	0.009	0.078
123	0.003	0.010	No data	0.010	0.003	0.016
124	0.006	0.016	0.063	0.008	0.004	0.1
125	0.010	0.006	0.008	0.006	0.002	0.010
126	0.008	No data	No data	No data	No data	No data
NVP	0.316	0.316	5.012	0.050	0.195	7.943
EFV	0.04	0.006	0.04	0.002	0.005	0.01

EFV: Efavirenz; NVP: Nevirapine.

Table 17. Activity of compounds 111, 112 and 119 – 126 against a panel of mutant strains.

Compound	EC_{50} (μM)					
	G190S	F227C	L100I, K103N	K101E, K103N	K103N, Y181C	F227L, V106A
111	No data	No data	No data	No data	0.794	No data
112	0.004	0.631	0.04	0.013	0.158	0.398
119	0.002	0.234	0.020	0.007	0.149	0.794
120	0.032	0.631	0.063	0.158	0.200	0.040
121	No data	No data	No data	No data	No data	No data
122	0.062	0.869	1.000	0.362	8.680	10
123	0.042	0.339	0.121	0.069	3.344	2.512
124	0.039	0.79	0.794	0.1	10	3.981
125	0.125	0.794	0.2	0.158	0.199	3.162
126	No data	No data	0.05	No data	0.398	1
NVP	0.044	0.135	1.452	0.509	100	0.163
EFV	0.251	0.158	10	0.158	0.04	0.025

EFV: Efavirenz; NVP: Nevirapine.

2 weeks with licensed PIs or NRTIs. The study will last for 104 weeks. This includes a 4-week screening period, a 96-week treatment period and a 4-week follow-up period. Three different doses of rilpivirine will be compared to EFV. HIV-infected subjects will be randomly assigned with rilpivirine or to EFV in combination with two other anti-HIV drugs.

4. Expert opinion

NVP, DLV and EFV were discovered in late 1980s to early 1990s, were approved during 1996 – 1998 and have been introduced into clinical practice. By the time they were introduced in clinical practice, novel lead compounds were discovered (for example, capravirine, DPC083, etravirine) of which etravirine is now in advanced Phase III clinical trials. If this trend continues, it can be estimated that it takes ~ 5 – 6 years from lead identification to drug release for a novel NNRTI. Several hundred molecules are currently being exploited both in the academy and in the pharma industry, thus it is foreseeable that one or two novel NNRTIs with improved activity against drug-resistant mutants will hit the market in the next couple of years and more should come in 4 – 5 years. What is

most important is that these novel compounds will be the result of rational medicinal chemistry approaches and, thus, will incorporate the large body of information currently available regarding the interactions between NNRTIs and their molecular target. Why are novel NNRTIs needed? Current state-of-the-art combination therapy (HAART) offers excellent first-line treatment options for drug-naive HIV-1-infected patients. However, due to drug resistance and toxicity problems, HAART-failing patients are often left with few, if any, alternative therapeutic options. In addition, the recent finding of drug-resistant HIV-1 strains in treatment-naive patients further complicates the matter. The availability of novel drugs active against drug-resistant viral strains selected by first-line HAART will allow switching of therapy when failure occurs. In the most optimistic scenario, these novel drugs will select for specific and uncommon (i.e., different) drug resistance mutations, which will not be effective against former drugs. Thus, alternative cycles of first- and second-generation HAART regimens might allow effective viral suppression for substantially longer periods than those currently achievable.

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