Available online at www.sciencedirect.com
ScienceDirect
Antiviral Research 71 (2006) 90–95
Antiviral Research
www.elsevier.com/locate/antiviral

Mini-review

Rational design of polymerase inhibitors as antiviral drugs

Bo Öberg

Medivir AB and Karolinska Institute, Stockholm, Sweden

Received 24 February 2006; accepted 17 May 2006

Dedicated to Prof. Erik De Clercq on the occasion of reaching the status of Emeritus-Professor at the Katholieke Universiteit Leuven in September 2006.

Abstract

Almost all viruses have polymerases which are suitable targets for antiviral drugs. The development of selective polymerase inhibitors started with screening of compounds in virus-infected cell cultures and the mechanism of action was investigated once an inhibitor had been found. Especially nucleoside analogs were screened as their triphosphates were potential substrates for polymerases. However, the stepwise phosphorylation by cellular, and sometimes viral, kinases to the active triphosphate prevented a truly rational design of polymerase inhibitors.

Nucleotide analogs offers a type of compounds which could be designed in a more rational way than nucleoside analogs since the first, most selective, phosphorylation step is eliminated in the path to the active inhibitor.

The development of pyrophosphate analogs made rational design possible since these compounds act directly on the viral enzyme, but the room for structural variation was limited.

The non-nucleoside HIV reverse transcriptase inhibitors are direct inhibitors and can thus be designed in a truly rational way by use of structure information on the enzyme-inhibitor complex by use of X-ray and NMR. This rational design of allosteric inhibitors is also being used in the development of inhibitors to other viral polymerases.

Keywords: Rational design of an enzyme inhibitor and rational design of a drug; Types of polymerase inhibitors; Development of nucleoside analogs as polymerase inhibitors; Development of nucleotide analogs as polymerase inhibitors; Development of pyrophosphate analogs as polymerase inhibitors; Development of allosteric polymerase inhibitors

Contents

1. Introduction

The possibility to design antiviral drugs in a rational way and to selectively direct them against a viral function became possible by the discovery of virus-coded enzymes. The first viral enzyme to be reported was a viral polymerase, poxvirus DNA dependant RNA polymerase (Kates and McAuslan, 1967). Before that time the search for antiviral drugs was based on random screening.

Today many viral enzymes, especially polymerases and proteases, are used in the rational design of selective inhibitors and

E-mail address: bo.oberg@medivir.se.

0166-3542/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2006.05.012

Table 1
Types of viral polymerase inhibitors on the market

Type of inhibitor				Virus
Nucleoside (substrate) analog	Nucleotide (substrate) analog	Pyrophosphate (product) analog	Allosteric inhibitor	
Acyclovir				HSV, VZV
Penciclovir				HSV
Ganciclovir				HCMV
Brivudine				VZV
	Cidofovir			HCMV
		Foscarnet		HCMV, HSV
Zidovudine				HIV
Didanosine				HIV
Zalcitabine				HIV
Lamivudine				HIV, HBV
Stavudine				HIV
Abacavir				HIV
	Tenofovir			HIV
Emtricitabine				HIV
			Nevirapine	HIV
			Delavirdine	HIV
			Efavirenz	HIV
	Adefovir			HBV

known to be excellent targets for antiviral drugs. Some aspects on the rational design of polymerase inhibitors as antiviral drugs will be discussed.

2. Rational design of an enzyme inhibitor and rational design of a drug

Even if obvious to most researchers it might be worth pointing out the difference between the design of an enzyme inhibitor and the design of a drug intended for clinical use. Today a viral polymerase can be cloned, expressed and analyzed by X-ray, NMR and other methods to determine the structure and the exact binding of substrates, template and inhibitors. These tools to analyze the active site as well as knowledge about substrates and reaction products greatly facilitate the rational design of selective and potent inhibitors. Rapid identification of lead compounds and optimization of inhibitors is facilitated by large chemical libraries, combinatorial chemistry, high-throughput screening and computer aided *in silico* modelling. However, even if the tools have been developed, there is no guarantee that you are allowed to use them. Especially with the newly isolated viruses such as HCV, patenting of genes, enzymes, crystallographic coordinates and assays is making industrial research stressful. The finding of a solution to a problem (a patented drug) is sometimes made more difficult by patenting the problem (the virus).

However, in order to design a useful drug it is by no means enough to make a potent and selective enzyme inhibitor. Oral bioavailability, pharmacokinetics, metabolism, toxicity, cost of goods, stability, drug–drug interactions, etc should also fulfill predetermined criteria to make a drug clinically useful. These demands on drug properties are often more difficult to satisfy than the mere optimization of potency and selectivity against a viral enzyme.

In order to design a polymerase inhibitor useful as a drug it is, therefore, necessary to optimize with respect to several parameters in parallel. Otherwise it is all too easy to reach a dead end with an extremely potent inhibitor useful only in cell culture.

3. Types of polymerase inhibitors

Three types of polymerase inhibitors are recognized; substrate analogs (nucleoside and nucleotide analogs), product analogs (pyrophosphate analogs) and allosteric inhibitors (non-nucleoside reverse transcriptase inhibitors, NNRTIs). The possibility of rational design differs for these types as will be discussed. The resistance mutations are also different which makes combinations of polymerase inhibitors useful in reducing the rate of resistance development. Compounds intercalating or otherwise directly interacting with nucleic acids will not be discussed here.

The development of antiviral drugs was initially largely focused on polymerase inhibitors and this is reflected in the large number of such drugs on the market, but today several other targets are also important. The antiviral drugs, excluding their prodrugs, presently on the market directed against viral polymerases are shown in Table 1. It should be noted that no RNA polymerase inhibitor has yet been licensed as a drug, although this will probably change with the development of hepatitis C virus (HCV) RNA polymerase inhibitors, and that hepatitis B virus (HBV) DNA polymerase also acts as a reverse transcriptase (RT).

4. Development of nucleoside analogs as polymerase inhibitors

Many of the first antiviral drugs were nucleoside analogs. They were discovered by screening and serendipity aided by the efforts of many clever nucleoside chemists precariously guided by the inhibition of virus replication in cell culture. These efficacy results from cell cultures were of rather limited use for the

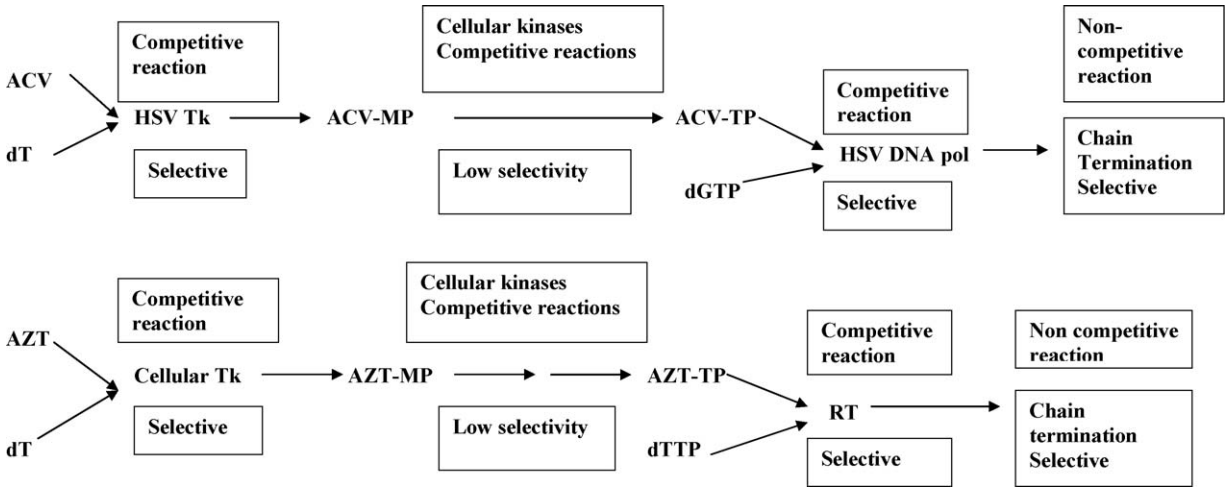

Fig. 1. Mechanism of action of acyclovir (ACV) and zidovudine (AZT) as competitive and chain terminating substrate analogs in nucleic acid synthesis.

medicinal chemist in designing the next compound to synthesize, since the mechanism of action was unclear in the beginning, and if it was due to an inhibition of a viral polymerase, there was a need for intracellular transformation to the active inhibitor, the triphosphate of the nucleoside analog. The triphosphates themselves have not been used as drugs as they are not easily taken up by cells and the phosphate groups are readily removed by extracellular enzymes.

A nucleoside analog acting as an inhibitor of a viral polymerase needs to be phosphorylated in three steps to the inhibitor, the triphosphate. This can then act as an inhibitor by competing with the natural nucleoside triphosphates and also be a terminator of the growing viral nucleic acid. The first step in this process is quite selective and can be due to viral or cellular kinases, the two ensuing phosphorylation steps much less selective and mostly carried out by cellular kinases. The last steps, inhibition of the viral polymerase, and in many cases chain termination, are also selective. This is illustrated for acyclovir and zidovudine in Fig. 1. All these reactions together determine the inhibition of viral replication in cell culture and *in vivo*.

Acyclovir owes part of its selectivity to the fact that the first phosphorylation is carried out by herpesvirus thymidine kinase (Elion et al., 1977). Zidovudine (Furman et al., 1986) and other nucleoside analogs active against HIV are phosphorylated by cellular kinases, the first step being the most discriminating and only allowing some nucleoside analogs to be phosphorylated.

The outcome of a cell culture assay to determine the antiviral effect of a nucleoside analog mainly depends on two different reactions, phosphorylation to a monophosphate and then inhibition of the polymerase by the triphosphate thus limiting the possibility of rational drug design as elaborated below.

Even if a rational design of nucleoside analogs as polymerase inhibitors is difficult some general principles have been used such as blocked 3′ hydroxyl groups, acyclic groups and various 5′ substituents in thymidine analogs.

What could be done in an optimization process is to analyze the first phosphorylation step by kinases and then the inhibition of the polymerase by triphosphates. However, this analysis has normally been performed in retrospect, when a good inhibitor has been identified by cell culture assays. Even if performed in an attempt to optimize the inhibitory effect of triphosphates of nucleoside analogs one has to remember that most enzyme assays determine the inhibition as a competitive (with normal nucleoside triphosphates) reaction and the most important function of an inhibitor could be to act as a chain terminator. Attempts to make monophosphate prodrugs to overcome the first hurdle have been made (Siddiqui et al., 1999) but not yet resulted in any licensed drugs. However, the use of phosphonates has been a useful approach to eliminate the first hurdle (see below).

Prediction of clinical effect from cell culture data has been especially difficult for nucleoside analogs and requires an understanding of relevant animal models (Johansson et al., 1986; Böttiger and Öberg, 2000). This is explained by the fact that several reaction steps are involved in the inhibition of the polymerase activity and that the competitive reactions depend on concentrations of normal nucleosides and nucleotides (Fig. 1). These concentrations could differ considerably when comparing cell cultures and infected tissues in patients. The thousand-fold difference in IC_{50} against HIV between AZT and ddI *in vitro* is not reflected in vivo where these compounds show similar potency, just to give one example.

5. Development of nucleotide analogs as polymerase inhibitors

The use of monophosphate nucleotide analogs (nucleoside phosphonates) as polymerase inhibitors avoids an important hurdle, the first phosphorylation step needed for activation to a "triphosphate", and thus facilitates rational design (De Clercq et al., 1986). Structure–activity relations can be better analyzed for nucleotide than nucleoside analogs and cell culture assays for inhibition of viral replication more directly reflect inhibition of a viral polymerase. As seen in Table 1 there are nucleotide analogs on the market against CMV, HIV and HBV. It seems reasonable that this approach to polymerase inhibitors could also be used for inhibition of viral RNA polymerases which might be of interest in the development of new drugs against influenza virus, HCV and SARS virus.

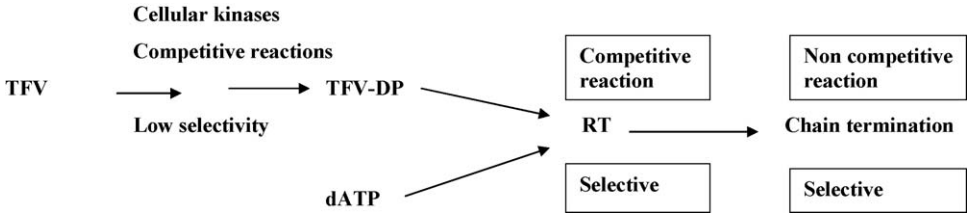

Fig. 2. Mechanism of action of tenofovir, a nucleoside analog phosphonate, by-passing the first phosphorylation step, required by nucleoside analogs, to a competing and chain terminating triphophate analog in nucleic acid synthesis.

The mechanism of action of the nucleotide analog tenofovir is shown in Fig. 2. Addition of two phosphate groups to a nucleoside phosphonate is not as restricted by the structure as the first phosphorylation of a nucleoside analog.

6. Development of pyrophosphate analogs as polymerase inhibitors

A simple byproduct of a polymerase reaction is pyrophosphate and analogs to pyrophosphate have been designed and evaluated as polymerase inhibitors (for review see Öberg, 1989). This evaluation was facilitated by the inhibitors direct action not involving any metabolic transformations and structure–activity relations could be established for various polymerases. Influenza virus RNA polymerase was initially found to be inhibited both by pyrophosphate (not surprising) and oxalic acid. Combining these two structures gave phosphonoformate which is an inhibitor of influenza virus RNA polymerase, herpesvirus DNA polymerases, HIV RT and HBV DNA polymerase by the reaction shown in Fig. 3. In parallel with this work phosphonoacetic acid was reported to inhibit herpesvirus DNA polymerases (Mao and Robishaw, 1975). However, the very simple structure of the product pyrophosphate was a limitation for further optimization and despite synthesis of a number of pyrophosphate analogs none was superior to phosphonoformate.

7. Development of allosteric polymerase inhibitors

With the allosteric polymerase inhibitors of HIV RT, the non-nucleoside reverse transcriptase inhibitors (NNRTIs), development of antiviral polymerase inhibitors has reached a stage where one could truly talk about rational design of polymerase inhibitors.

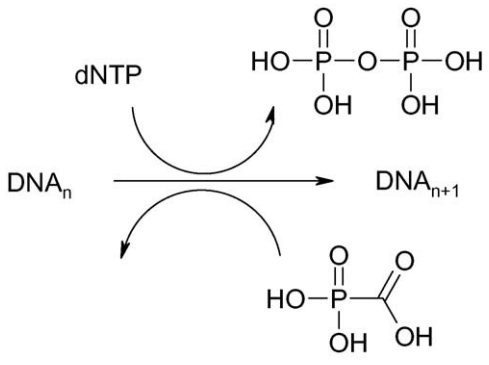

Fig. 3. Mechanism of action of foscarnet as a product analog of nucleic acid synthesis.

Screening of compound libraries against HIV RT rapidly resulted in several structurally different inhibitors as excellent starting points for rational design of potent and selective inhibitors (Miyasaka et al., 1989; Pauwels et al., 1990; Hargrave et al., 1991; Goldman et al., 1991; Romero et al., 1991; Bell et al., 1995). The direct action of these compounds at a hydrophobic site on the RT and X-ray crystallographic information on the binding and interaction with different amino acids in the RT made optimization of potency straightforward. It also helped in understanding resistance as this is directly seen in the binding pattern for different inhibitors to amino acids in RT. The mechanism of action is shown in Fig. 4. Fig. 5 gives one example of an

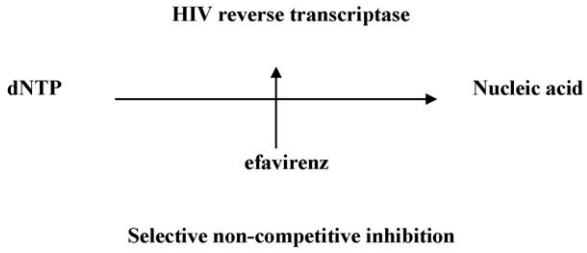

Fig. 4. Mechanism of action of the NNRTI efavirenz as an allosteric polymerase inhibitor.

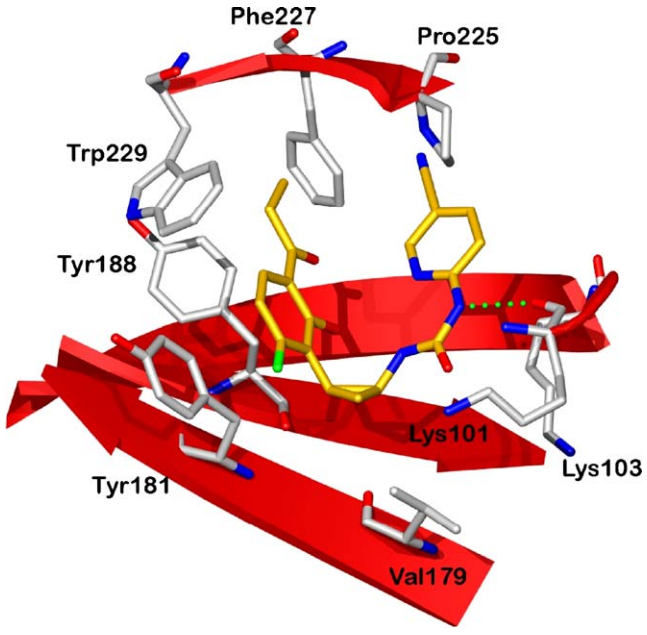

Fig. 5. Binding of an NNRTI (PETT compound) to HIV RT analyzed by X-ray crystallography reveals details on the interactions between the inhibitor and the enzyme (Courtesy: Dr Torsten Unge, BMC, University of Uppsala).

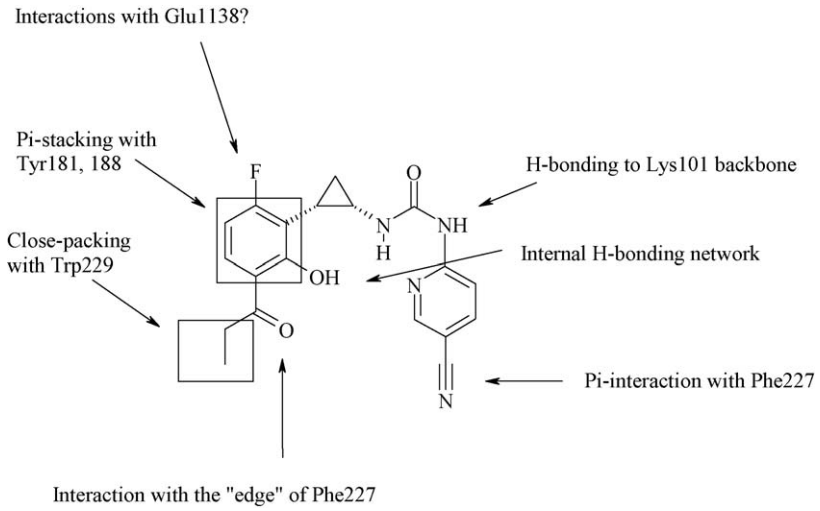

Fig. 6. Interaction between the NNRTI MIV-150 and various aminoacids in the HIV-RT. These results were derived from X-ray data after co-crystallization of HIV-RT and MIV-150 (Courtesy: Drs Charlotta Näslund and Christer Sahlberg).

NNRTI binding to HIV-1 RT thereby preventing the flexibility and movement of RT required for the elongation of the nucleic acid. A large number of RT structures with bound inhibitors have been published and many more are available at companies involved in the development of NNRTIs.

A drawback with the first NNRTIs was a rapid selection of resistant mutants. This has partly been overcome and detailed knowledge about the interaction between NNRTIs and RT as well as RT mutants makes it possible to design inhibitors less prone to resistance development and active against mutants selected by the first generation NNRTIs. The design and use of combinations of NNRTIs with different resistance patterns is clearly feasible, as the detailed interaction between inhibitors and aminoacids on the polymerase are known. One example of this is given in Fig. 6.

The direct action of NNRTIs on RT also makes prediction of clinical efficacy from cell culture data relevant once protein binding is taken into account, and there is also a good correspondence between enzyme inhibition and inhibition of virus replication in cell culture at least with compounds of a not too high molecular weight. A further consequence of the binding of NNRTIs to HIV RT is the advantage for use in vaginal microbicides both inactivating free virus and preventing replication in epithelial cells (D'Cruz and Uckun, 2006).

Development of NNRTIs against other viral polymerases, such as that of HCV, is ongoing and other polymerases are likely to have suitable sites for allosteric inhibitors. This work can be carried out in a rational way based on X-ray crystallography of wild type polymerase and polymerase with various resistance mutations.

8. Conclusions

Antiviral inhibitors can now be designed in a rational way whether they are polymerase or protease inhibitors or belong to some other type of enzyme inhibitors. However, the task to make a useful drug is complex since antiviral potency is only one of several parameters which have to be optimized in parallel. University researchers will typically not have all the resources to provide compounds ready to be assigned candidate drug status but rather to provide new ideas and lead compounds which could be developed by the pharmaceutical industry, having the resources to optimize with respect to several parameters in parallel.

These aspects on the rational design of polymerase inhibitors as antiviral drugs have not been written with the intention of providing a detailed overview of the field but rather to point out the progress from serendipity to rational design, a development which has gained so much by the industrious work of Prof. Erik De Clercq.

References

Bell, F.W., Cantrell, A.S., Hoegberg, M., Jaskunas, S.R., Johansson, N.G., Jordan, C.L., Kinnick, M.D., Lind, P., Morin Jr., J.M., Noreen, R., Oberg, B., Palkowitz, J.A., Parrish, C.A., Pranc, P., Sahlberg, C., Ternansky, R.J., Vasileff, R.T., Vrang, L., West, S.J., Zhang, H., Zhou, X.-X., 1995. Phenethylthiazolethiourea (PETT) Compounds, a new class of HIV-1 reverse transcriptase inhibitors. 1. Synthesis and basic structure–activity relationship studies of PETT analogs. J. Med. Chem. 38, 4929–4936.

Böttiger, D., Öberg, B., 2000. Predictive value of treatment effects in SIV/SHIV infections in monkeys. Curr. Opin. Anti-Infect. Invest. Drugs 2 (3), 255–264.

De Clercq, E., Holy, A., Rosenberg, I., Sakuma, T., Balzarini, J., Maudgal, P.C., 1986. A novel selective broad-spectrum anti DNA virus agent. Nature 323, 464–467.

D'Cruz, O.J., Uckun, F.M., 2006. Dawn of non-nucleoside inhibitor based anti-HIV microbicides. J. Antimicrob. Chemother. 57, 411–423.

Elion, G.B., Furman, P.A., Fyfe, J.A., de Miranda, P., Beauchamp, L., Schaeffer, H.J., 1977. Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl) guanine. Proc. Natl. Acad. Sci. U.S.A. 74, 5716–5720.

Furman, P., Fyfe, J., St.Clair, M., Weinhold, K., Rideout, J., Freeman, G., Nusinoff-Lehrman, S., Bolognesi, D., Broder, S., Mitsuya, H., Barry, D., 1986. Phosphorylation of 3′-azido-3′deoxythymidine and selective interaction of 5′-triphosphate with human immunodeficiency virus reverse transcriptase. Proc. Soc. Natl. Acad. Sci. U.S.A. 83, 8333–8337.

Goldman, M.E., Nunberg, J.H., O'Brien, J.A., Quintero, J.C., Schleif, W.A., Freund, K.F., Gaul, S.L., Saari, W.S., Wai, J.S., Hoffman, J.M., Anderson,

P.S., Hupe, D.J., Emini, E.A., Stern, A.M., 1991. Pyridinone derivatives: specific human immunodeficiency virus type 1 reverse transcriptase inhibitors with antiviral activity. Proc. Natl. Acad. Sci. U.S.A. 88, 6863–6867.

Hargrave, K.D., Proudfoot, J.R., Grozinger, K.G., Cullen, E., Kapadia, S.R., Patel, U.R., Fuchs, V.U., Mauldin, S.C., Vitous, J., Behnke, M.L., Klunder, J.M., Pal, K., Skiles, J.W., McNeil, D.W., Rose, J.M., Chow, G.C., Skoog, M.T., Wu, J.C., Schmidt, G., Engel, W.W., Eberlein, W.G., Saboe, T.D., Campbell, S.J., Rosenthal, A.S., Adams, J., 1991. Novel non-nucleoside inhibitors of HIV-1 reverse transcriptase. J. Med. Chem. 34, 2231–2241.

Johansson, N.G., Datema, R., Eklind, K., Gotthammar, B., Hagberg, C.-E., Kovacs, S., Larsson, A., Lindborg, B., Noren, J.O., Stening, G., Öberg, B., 1986. Design of selective antiviral compounds. Innovative Approaches Drug Res., 135–154.

Kates, J.R., McAuslan, B.R., 1967. Poxvirus DNA-dependent RNA polymerase. Proc. Soc. Natl. Acad. Sci. U.S.A. 58, 134–141.

Mao, J.C.-H., Robishaw, E.E., 1975. Mode of inhibition of herpes simplex virus DNA polymerase by phosphonoacetate. Biochemistry 14, 5475–5479.

Miyasaka, T., Tanaka, H., Baba, M., Hayakawa, H., Walker, R.T., Balzarini, J., De Clercq, E., 1989. A novel lead for specific anti-HIV-1 agents. J. Med. Chem. 32, 2507–2509.

Öberg, B., 1989. Antiviral effects of phosphonoformate (PFA, foscarnet sodium). Pharmacol. Ther. 40 (2), 213–285.

Pauwels, R., Andries, K., Desmyter, J., Schols, D., Kukla, M.J., Breslin, H.J., Raeymaeckers, A., Van Gelder, J., Woestenborghs, R., Heykants, J., Schellekens, K., Janssen, M.A.C., De Clercq, E., Janssen, P.A.J., 1990. Potent and selective inhibition of HIV-1 replication in vitro by a novel series of TIBO derivatives. Nature 343, 470–474.

Romero, D.L., Busso, M., Tan, C., Reusser, F., Palmer, J.R., Poppe, S.M., Aristoff, P.A., Downey, K.M., So, A.G., Resnick, L., Tarpley, W.G., 1991. Non-nucleoside reverse transcriptase inhibitors that potently and specifically block human immunodeficiency virus type 1 replication. Proc. Natl. Acad. Sci. U.S.A. 88, 8806–8810.

Siddiqui, A.Q., McGuigan, C., Ballatore, C., Zuccotto, F., Gilbert, I.H., De Clercq, E., Balzarini, J., 1999. Design and synthesis of lipophilic phosphoramidate d4T-MP prodrugs expressing high potency against HIV in cell culture: structure determinants for in vitro activity and QSAR. J. Med. Chem. 42, 4122–4128.