Drugs of the Future 2003, 28(3): 257-265

CCC: 0377-8282/2003

Review Article

Helicase-primase inhibitors

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CONTENTS

Abstract

The protein family of helicases separate or unwind duplex nucleic acids such as oligonucleotides, RNA or DNA into single strands. This enzymatic activity is essential for metabolic processes of DNA and RNA replication, transcription *etc.* and are often associated in enzyme complexes. To multiply, many pathogens encode their own helicase and/or primase to synthesize DNA. Inhibition of this enzymatic activity abrogates microbial propagation and can lead to the development of potent drugs for treating and curing infection. Recently, helicase-primase inhibitors were reported for the treatment of herpes simplex disease. Almost 25 years after the launch of the milestone antiherpes drug aciclovir (Zovirax®), these drug candidates represent the first compound class which outperforms the only available nucleosidic inhibitors of the herpes TK/DNA-polymerase with respect to all preclinical pharmacological parameters. The helicase-primase enzyme complex appears to be the Achilles heel of herpes simplex viruses and is a prime target of antiinfective drug discovery. The antibiotic ciprofloxacin inhibits the bacterial topoisomerase II (DNA-gyrase) and IV and thus interferes with supercoiling of DNA. Drugs that inhibit supercoiling or unwinding of DNA seem to be the most effective antimicrobial agents. The mechanism of action of these drugs is based not only on the inhibition of a target but also on a mode of action that triggers subsequent molecular events, cascades and pathways which prevent propagation and even survival of the pathogen.

Introduction

Since the first publication in 1976 (1, 2), numerous helicases have been identified in eukaryotes, prokaryotes and viruses and many more enzymes of the universally present protein family were predicted based on sequence homologies (www.ebi.ac.uk, www.ncbi.nlm.nih.gov, www.ddbj.nig.ac.jp). Helicases are involved in fundamental metabolic processes of DNA and RNA including replication, recombination and repair as well as transcription, RNA-splicing and translation (1-10).

DNA replication is the core biological function of all life forms (3, 4, 11). Helicases (5-10), primases (12) and topoisomerases (13-15) are essential enzymes performing the first or prerequisite steps of these complex, multistep processes. Loss of function results in disease, premature ageing and death (*e.g.*, Werner syndrome, Bloom syndrome, xeroderma pigmentosa *etc.*) (16, 17).

Selective inhibition of microbial topoisomerases is one mechanism of action of antibiotics in treating bacterial infections (18-26), whereas inhibition of helicase or helicase-primase is the mechanism of action of investigational drugs treating herpes simplex disease (27-34). Fluoroquinolones bind to the bacterial topoisomerase-DNA complex and stabilize DNA strand breaks created by DNA gyrase and topoisomerase IV. Ternary complexes of the drug, enzyme and DNA block progress of the replication fork resulting in direct inhibition of bacterial DNA synthesis (19-26). The indispensable nature of gyrase in the bacterial cell and the absence of a direct counterpart in mammalian cells make DNA gyrase an ideal drug target (24-26).

In contrast to bacteria, viruses are obligate intracellular parasites which exploit the resources and many enzymatic functions of the host cell for their propagation. Hence, less targets are provided by the pathogen for chemotherapy. While vaccinia virus and poxviruses encode their own topoisomerase, such enzymatic activity has not yet been demonstrated for herpesviruses. However, many viruses encode their own helicase enzyme such as herpes simplex virus (HSV) UL5 (11, 35-40), hepatitis C virus (HCV) NS3 (41) and human papilloma virus (HPV) E1 (42). Again, the viral helicases are essential for viral propagation and are not homologous to enzymes of the host. The NS3 helicase of HCV (41, 43-46) and the helicase-primase of HSV have already been selected for drug discovery (27-34). The proven concept

of chemotherapy based on the inhibition of enzymes involved in modifying the topology of DNA is now expanding from topoisomerases to helicases (30) and thus into new indications.

Structure and function of helicases

Helicases are enzymes that catalyze the separation of 2 complementary strands of duplex nucleic acids into single strands in an energy-dependent reaction (5-10). Unwinding of duplexes, either in 3' to 5' or 5' to 3' direction, is powered by the NTPase activity of the enzyme transducing chemical energy provided by hydrolysis of nucleoside 5'-triphosphate (NTP) into a nucleic acid strand separation and displacement activity. Helicase proteins share short conserved amino acid sequences or motifs (7, 8). All helicases bind ATP via the classical Walker A (phosphate-binding loop or P-loop) and B (Mg^{2+}-binding aspartic acid) motifs. Based on 7 homologous motifs the helicases have been grouped into 4 superfamilies designated SF1 to SF4 (7). Helicases from SF1 and SF2 contain 7 helicase signature motifs (I, Ia, II-VI), whereas helicases from SF3 and SF4 contain 3 (A-C) and 5 motifs (1, 1a, 2-4), respectively. The herpes simplex DNA helicase-primase (unwinding of DNA in the 5' to 3' direction) is assigned to SF1 and the NS3 RNA helicase (exhibits a 3' to 5' directionality with respect to the template strand) from HCV belongs to SF2. The X-ray crystal structures of several helicases are now available and allow for discussing proposed enzymatic mechanisms, sequence and structural alignments and molecular modeling (5-7).

Drugs, therapy and investigational compounds

A search of the Physicians' Desk Reference (www.pdr.net) revealed that the mechanism of action of launched drugs with respect to helicases and topoisomerases is only documented for anticancer drugs. These drugs, together with marketed antibiotics targeting topoisomerases and investigational antiviral agents and drug candidates inhibiting the HSV helicase, are listed in Table I.

The success story of treating bacterial infections with quinolone antibiotics led to attempts to transfer the wealth of knowledge from bacteriology to the field of virology (47-56). Vaccinia virus or poxviruses, for example, encode a viral topoisomerase but such enzymatic activity has not yet been shown for herpesviruses (11). Consequently, while selective antiviral properties of quinolone derivatives or analogues were reported for vaccinia viurs (48), antiviral activity against topoisomerase-deficient viruses is inconsistent within the literature and is probably based on an unspecific or cytostatic effect, since a selectivity or therapeutic index could not be demonstrated and thus clinical trials were never initiated.

The cancer drugs listed in Table I are intercalators with a more or less unspecific mode of action (57). Consequently, some agents such as the anthracycline anticancer drugs doxorubicin, epirubicin, daunomycin and nogalamycin and the chemically related mitoxantrone are also active against HCV (43). However, due to the lack of specificity, high cytotoxicity, a small therapeutic index, adverse reactions and side effects are common.

First-line treatment of HCV infections and future therapeutic options have been reviewed elsewhere (44, 45). HCV infections are currently treated with interferon (IFN)-α or a combination of IFNs and ribavirin. Efficacy, especially the response rate, and tolerability are low. Currently, the therapeutic standard for treating HCV infection is low and a need for new drugs is evident. Non-IFN-based therapeutics are still at the research level, including protease and helicase inhibitors, ribozymes, antisense and cytokine-based therapies and T-cell-based therapeutic vaccines.

Helicase activity may be blocked by competitive inhibitors of the NTP binding site, allosteric modulation of the NTPase or RNA binding site, uncoupling of the NTPase and unwinding reaction, competitive inhibition of the RNA binding site and blockage of translocation of the helicase along the polynucleotide chain (43).

Several inhibitors of the HCV NS3 NTPase/helicase activity have been discovered *in vitro*. However, although a wide range of competitive inhibitors like ribavirin-5'-triphosphate (RTP), ribavirin-5'-diphosphate (RDP), adenosine-5'-γ-thiotriphosphate, (ATP-γ-S) or ADP inhibit the NTPase of the enzyme in the low μM range, helicase is still capable of unwinding RNA/DNA. In addition, paclitaxel, a compound unrelated to NTPs, was able to block the ATP-binding site of the NS3 protein and consequently inhibit NTPase. However, once again, the activity of helicase was not abrogated. The noncompetitive compound trifluoperazine is only active at concentrations greater than 100 μM. There are several macromolecules that can uncouple NTPase activity from helicase activity, such as modified polynucleotides and positively or negatively charged polymers (*e.g.*, dextrane sulfate, heparin and polylysine of defined length), that inhibit helicase activity without affecting NTPase activity. Adaptamers, piperidine and heterocyclic substituted carboxamide as well as benzimidazoles and benzoxazoles linked by a spacer and/or attached directly or via a spacer to amino acid moieties, inhibit binding of RNA to the NS3 protein in a competitive manner (43).

Despite the intense focus of research on NS3 of NTPase/helicase as an antiviral target (40, 43, 58, 59), no *in vivo* data have been reported to date. One reason is the lack of cellular and/or animal models to evaluate compounds in a high-throughput manner. While the development of NS3 enzyme inhibitors to treat HCV infections is still in its infancy, a breakthrough has been reported for the treatment of herpes simplex infections in animal models (28-31).

Table I: Drugs and investigational compounds targeting helicases and topoisomerases.

Drug Name	Source	Mechanism of Action/Target
Launched antibacterial drugs (www.pdr.net)		
Moxifloxacin HCl	Bayer	Bacterial type II topoisomerases (topoisomerase II (DNA gyrase) and topoisomerase IV)
Ciprofloxacin	Bayer	Bacterial type II topoisomerases (topoisomerase II (DNA gyrase) and topoisomerase IV)
Norfloxacin	Merck	DNA gyrase
Ofloxacin	Ortho-McNeil	DNA gyrase
Levofloxacin	Ortho-McNeil	Bacterial type II topoisomerases (topoisomerase II (DNA gyrase) and topoisomerase IV)
Gatifloxacin	Bristol-Myers Squibb	Bacterial type II topoisomerases (topoisomerase II (DNA gyrase) and topoisomerase IV)
Trovafloxacin mesylate	Pfizer	Bacterial type II topoisomerases (topoisomerase II (DNA gyrase) and topoisomerase IV)
Unspecific topoisomerase and/or helicase inhibitors (www.pdr.net)		
Epirubicin HCl	Pharmacia & Upjohn	Intercalation triggers DNA cleavage by topoisomerase II, also inhibits DNA helicase activity
Doxorubicin HCl	Pharmacia & Upjohn	Intercalator, interaction of doxorubicin with topoisomerase II to form DNA-cleavable complexes; intercalation inhibits nucleotide replication and action of DNA and RNA polymerases
Idarubicin HCl	Pharmacia & Upjohn	Interacts with the enzyme topoisomerase II
Topotecan HCl	GlaxoSmithKline	Uncompetitive topoisomerase I inhibitor, binds to the topoisomerase I-DNA complex and prevents religation of these single strand breaks
Irinotecan HCl	Pharmacia & Upjohn	Topoisomerase I inhibitor
Daunorubicin HCl	Bedford	Inhibits topoisomerase II activity by stabilizing the DNA-topoisomerase II complex
Fludarabine phosphate	Berlex	Metabolite 2-fluoro-ara-ATP inhibits DNA polymerase alpha, ribonucleotide reductase and DNA primase
Mitoxantrone	Immunex	Potent inhibitor of topoisomerase II, intercalates into DNA and causes crosslinks and strand breaks
Valrubicin	Celltech	Interferes with the normal DNA breaking-resealing action of DNA topoisomerase II
Helicase-primase inhibitors (27-34)		
Bay 57-1293	Bayer	HSV helicase-primase (UL5 and UL52)
BILS-179 BS	Boehringer/Biomega	HSV helicase-primase (UL5)
T-157602	Tularik	HSV helicase-primase (UL5)
ER622	Merck	Not disclosed in publication but obviously helicase-primase UL5

Herpes simplex virus (HSV) infections and therapy

The omnipresence of oral and genital herpes is caused by HSVs serotype 1 and 2. The incidence rate is high (~1.6 million new cases of HSV-2 are predicted per year in the U.S.) and the prevalence ranges from 50-95% (HSV-1) and 6-50% (HSV-2) depending on age, race, sex, marital and social status, number of sexual partners and geographical location of the host but not on seasonal variations. The mucocutaneous infections interfere with everyday activities and, especially in the immunocompromised patient population, the disease can be sight-impairing (keratitis, keratoconjunctivitis) or even life-threatening (herpes encephalitis, visceral HSV infections [hepatitis], neonatal disseminated herpetic disease). Disease symptoms of infection result from cell death and an associated immune response. After primary or initial infection, the virus persists for life in a latent form in neurons of the host and periodically reactivates with diverse internal and external stimuli. The recurrent disease often results in significant psychosocial distress for the patient (11, 60).

No vaccine is available and only one compound class of nucleosidic inhibitors of viral DNA polymerase is available to treat infections. The drugs (aciclovir, valaciclovir, penciclovir and famciclovir) decrease or abrogate disease symptoms but do not cure the disease (61-66). Due to the increasing resistance seen in the immunocompromised population, the unsolved problem of latent infection and recurrent disease, the morbidity and mortality associated with serious herpetic disease and the pandemic mucocutaneous lesions interfering with social contacts, the medical need to develop new therapeutic options is clear.

A review summarizing the current therapeutic options has recently been published, providing a contemporary interpretation of the challenge to establish novel

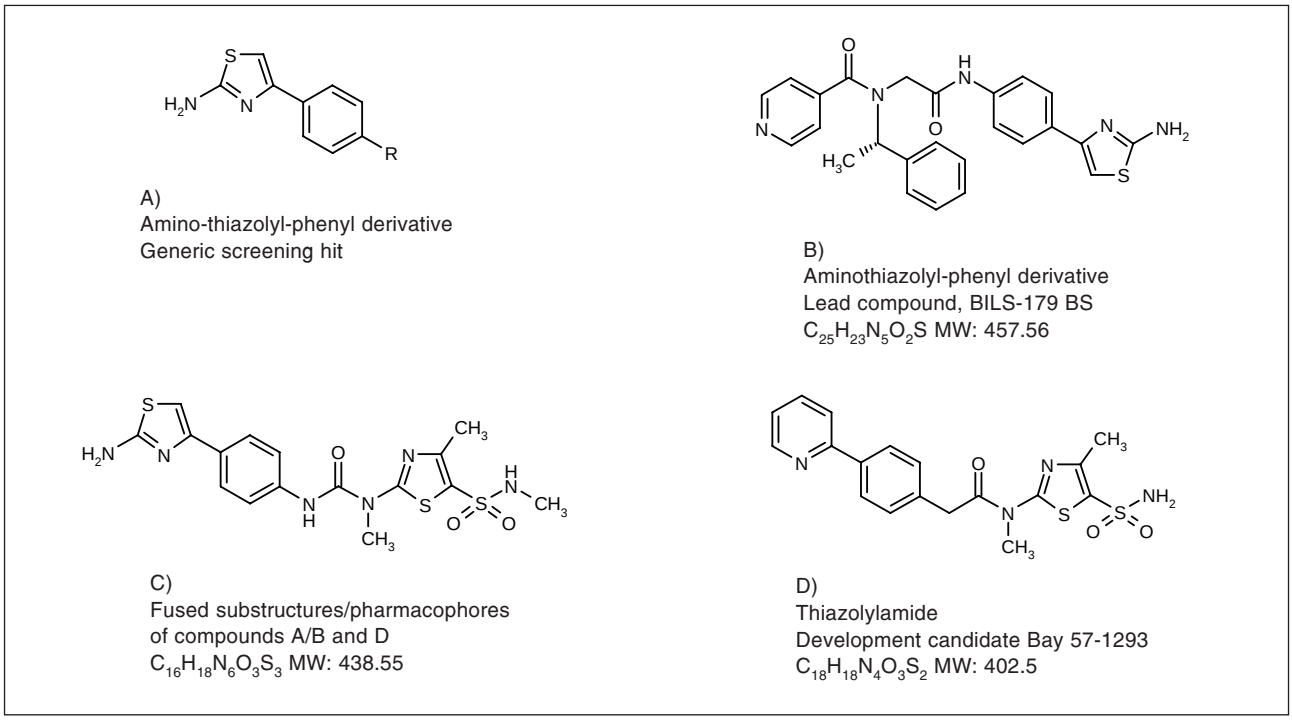

Fig. 1. Helicase-primase inhibitors. A) Generic compound discovered by screening with various test systems: Boehringer/Biomega (28, 32), Merck (ER622, R=Br) (67), Tularik (T-157602, R=Cl) (27, 33), Bayer (R=H, unpublished results, 34). B) Optimized compound with *in vivo* antiviral activity (Boehringer/Biomega). C) Compound with fused pharmacophores reported in A/B and D. D) Development candidate (Bayer).

treatments for herpes simplex disease (60). The discovery of the viral helicase-primase inhibitors and the preclinical profile of these drug candidates is reviewed below.

Drug discovery of HSV helicase primase inhibitors

Soon after the genome of the HSV was published (www.ebi.ac.uk), the enzymatic activity of a viral helicase-primase (38, 39) and subsequently the first simple enzyme inhibitors were reported (67-69).

Due to the fact that amino-thiazolyl-phenyl inhibitors of herpes helicase are present in all diversified compound libraries (27, 28, 32-34, 70) and these agents can be detected using enzymatic NTPase and helicase test systems (27, 28) and cell-based viral replication assays (29), initial hits were discovered at several pharmaceutical companies including Boehringer/Biomega (28, 32), Merck (67), Tularik (27, 33) and Bayer (unpublished results, 34) (Fig. 1). Merck apparently focussed on herpes protease inhibitors and Tularik and Boehringer/Biomega discontinued the projects before the compounds could be optimized to become development candidates, especially in regard to oral bioavailability and pharmacokinetic and safety profiles. However, even for a not fully optimized lead compound such as BILS-179 BS, the overall profile is remarkable. In a parallel effort at Bayer, novel thiazolylamide inhibitors of the HSV helicase-primase were identified with an innovative test system (29). The novel assay enables discovery and profiling of compounds at high-throughput with simultaneous examination of efficacy and tolerability. This assay led not only to the discovery of the thiazolylamide inhibitors but also sped up the process of evaluation of approximately 3,500 program compounds synthesized during subsequent lead structure optimization to single out candidates for pharmacokinetics, animal models and preclinical toxicology. Thus, this novel assay is optimal for drug discovery.

Surprisingly, the aminothiazolylphenyl compounds discovered by several investigators and the *N*-[5-(aminosulfonyl)-4-methyl-1,3-thiazol-2-yl]-*N*-methylacetamide substructure of the thiazolylamide Bay 57-1293 published by Bayer AG, can be fused to a potent antiherpes simplex inhibitor (34). Structural formulas are shown in Figure 1. However, the activity of these compounds of fused substructures *in vivo* did not yet justify further evaluation. X-ray crystal structures or NMR studies of a helicase/thiazoleamide inhibitor complex are needed to clarify the situation at the molecular level.

Pharmacological profiles of anti-HSV drugs and candidates

The preclinical pharmacological profiles of standard nucleosidic drugs (aciclovir, valciclovir, penciclovir and

Table II: Preclinical pharmacological profile of HSV helicase-primase inhibitors compared to thymidine kinase (TK)/DNA polymerase inhibitors (28-34, 60-66, 74, M. Liuzzi personal communication).

	ACV	VACV	PCV	FCV	Bay 57-1293	Bils-179
In vitro (target)						
TK aff. [μM]	172	see ACV	1.5	see PCV		
Pol K_i [μM]	0.07-0.08	see ACV	8.5	see PCV		
Helicase IC_{50} [μM]						1.3
Primase IC_{50} [μM]						0.15
ATPase IC_{50} [μM]					0.03	0.43
Cell culture						
IC_{50} [μM]	0.5-1	see ACV	4	see PCV	**0.01-0.02**	**0.027-0.1**
SI (selectivity index)	~250	see ACV	~200	see PCV	**> 2000**	**> 2000**
$T_{1/2}$ [h] in cells	0.7-1 ACV-TP	see ACV	10-20 PCV-TP	see PCV		
Mouse lethal challenge model [t.i.d]						
ED_{50} [mg/kg]						
HSV-1/HSV-2	22/16	17/14	see FCV	18/26	**0.5/0.5**	24/35
Pharmacokinetics						
$T_{1/2}$ [h]	2-3 man	~2.9 ACV man	~2.0	2.0 PCV man	**6** mice	0.9 mice
T_{max} [h]	1.5-2.5 man	0.5-1 VACV		0.5-1 FCV	2.0-3.0	0.4-0.5
C_{max}	200 mg ACV	500 mg VACV	see FCV	125 mg FCV		
Dose [mg/kg]	2.7 man	6.7 man		1.7 man	1 mice	25 mice
[mg/l]	0.58	3.28 ACV		0.8 PCV	1.8	14.4
[μM]	2.6	14.6 ACV		3.2 PCV	4.4	31.5
Bioavailability	10-25%	54%	low	77%	> 60%	~50%
Protein binding	9-33%	see ACV	< 20%	see PCV	> 95%	> 95%

ACV, aciclovir; VACV, valaciclovir; PCV, penciclovir; FCV, famciclovir

famciclovir) and herpes helicase-primase inhibitors (Bay 57-1293 and BILS-179 BS) are compared in Table II (28, 29, 60-66, 74). While the binding constants or IC_{50} values of all drugs and candidates at the target level are in the same order of magnitude, the helicase-primase inhibitors are approximately 2 orders of magnitude more potent than the nucleosidic drugs in blocking viral replication in cell culture experiments. This potency also results in a 10-fold higher selectivity index of > 2,000 compared to 200-300 for the nucleosidic drugs. While the antiviral activity of the nucleosidic drugs and BILS-179 BS are comparable *in vivo*, the potency of Bay 57-1293 is outstanding and at least 20-fold superior to the other drugs listed in Table II. The pharmacokinetic profile of Bay 57-1293 includes high exposure, low clearance, a long half-life, high oral bioavailability with no evidence of potential for drug interactions. The potency of Bay 57-1293 combined with a favorable pharmacokinetic profile results in superior efficacy in terms of survival rate in HSV-infected mice, rats and guinea pigs, time to healing, rebound of disease after cessation of treatment and, most importantly, reduction of frequency and severity of recurrent disease (29, 31). Finally, the resistance rate is 1 order of magnitude lower for helicase-primase inhibitors as compared to nucleosidic drugs (*e.g.*, 1 x 10^{-3} to 1 x 10^{-4} for aciclovir *vs.* 0.5-4.5 x 10^{-6} for helicase-primase drugs) (29, 71).

Conclusions

Helicases and topoisomerases are essential enzymes for all life forms (1-17). They catalyze prerequisite steps of fundamental biological processes such as DNA replication by unwinding and changing the topology (supercoiling) of duplex nucleic acids, respectively. Consequently, many intercalating agents of nucleic acids can inhibit the activity of these enzymes (Table I). This mechanistic effect is exploited in cancer therapy (57); however, for treatment of infections caused by human pathogens, this mechanism of action is not specific enough for development of potent, efficacious and especially well tolerated antimicrobial drugs.

The discovery of specific topoisomerase inhibitors led to discovery of quinolone antibiotics such as ciprofloxacin (18-26). The launched nucleosidic antiherpes drugs aciclovir, valaciclovir, penciclovir and famciclovir are prodrugs which, after phosphorylation by the viral thymidine kinase (TK) in infected cells and subsequent phosphorylation by cellular kinases, inhibit viral DNA polymerase (60-66). The recently published drug candidates to treat HSV disease target the helicase-primase enzyme of viruses of the herpes group (27-34) and mark a breakthrough in drug discovery regarding helicases (Fig. 2).

Although the aminothiazolylphenyl (27, 28, 32, 33) and the thiazoleamide compounds (29, 34) are similar

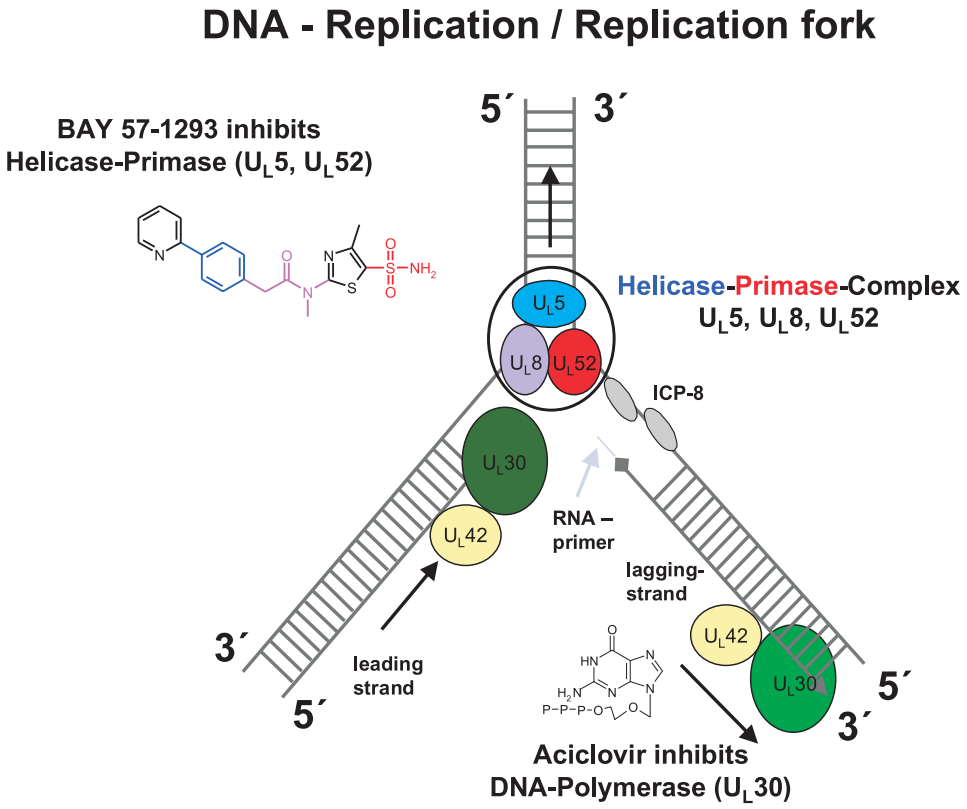

Fig. 2. Mechanism of DNA synthesis inhibition by aciclovir and the new helicase-primase drug candidates. Simplified illustration of HSV DNA synthesis (30, 36). The helicase-primase complex, consisting of viral proteins U_L5 (helicase), U_L8 and U_L52 (primase), unwinds HSV DNA at the replication fork and primes both lagging strand and leading strand DNA synthesis. The single-stranded DNA binding protein, ICP8 (gray), binds to single-stranded template DNA. HSV DNA polymerase and its accessory protein, U_L42, promote leading and lagging strand DNA synthesis. The arrows indicate the direction of movement of the DNA replication proteins. The new drugs target the helicase-primase complex. Amino-thiazolyl-phenyl-containing compounds, thiazole urea and thiazole amide derivatives enhance binding of the U_L5 and U_L52 subunits of the helicase-primase complex to both leading and lagging strand DNA resulting in inhibition of helicase activity, primase activity and viral DNA synthesis. Aciclovir (triphosphate) binds to HSV DNA polymerase, inhibiting polymerase activity and synthesis of both leading and lagging DNA strands.

(both share the phenyl and the carbonyl moiety), they were discovered independently using different screening assays. In accordance with the corresponding pharmacophores, the pattern of resistance conferring mutations within the HSV helicase gene UL5 matches to some extent. However, mutations in the primase gene UL52 that confer resistance were only reported for the thiazoleamide compounds (29; Bay 54-6322 two resistant mutants UL52 A897T [missing data, print error in ref. 29] and UL5 K356N). So far, one may speculate that the aminothiazolephenyl compounds (27, 28) bind only to the helicase subunit (encoded by the UL5 gene) of the helicase-primase complex (UL5, UL8, UL52) while the thiazoleamide compounds target both the helicase and primase subunit simultaneously (29). Interestingly, the activity of outstanding antimicrobial drugs is based on the interaction with 2 targets. For example, ciprofloxacin and moxifloxacin inhibit bacterial DNA gyrase and topoisomerase IV (19-23), the prodrug aciclovir is converted by the viral TK and subsequently by cellular kinases to the active drug that inhibits and inactivates viral DNA polymerase (60-66) and the herpes simplex helicase-primase inhibitors bind to the UL5 and UL52 gene product of the viral helicase-primase enzyme complex (29). The dual drug target concept mimics combination therapy with a single drug. One may conclude that current antiinfective chemotherapy is not just about MIC, IC_{50} and K_i values, but rather the consequences for the pathogen which are caused by binding of the drug to multiple targets, avidity effects, dead end enzyme complexes and subsequently initiated cascades or pathways that interfere at least with replication if not survival of the pathogen.

The resistance rate of the helicase-primase inhibitors in cell culture experiments is at least 1 order of magnitude lower as compared to nucleoside drugs (28, 29, 61, 62, 71). However, although many *in vitro* selected aciclovir-

resistant mutants are not pathogenic in animal models (72), aciclovir-resistant pathogenic viruses are a growing problem in the immunocompromised patient population. To date, no comparative *in vivo* data with respect to viruses resistant to helicase-primase or nucleosidic drugs exist. Resistance monitoring in animals and humans will provide the missing data and end various speculations regarding development of resistance under treatment.

Although many helicase and topoisomerase inhibitors other than the above mentioned positive examples are very potent and effective, only a minority of the analogues or congeners matches the preclinical profile with respect to safety and tolerability as well as for development and launch. Thus, tolerability and safety distinguish between success and failure in the clinic (*e.g.,* trovafloxacin) (73); due to a high therapeutic standard in many indications efficacy is expected.

A fully established preclinical screening cascade is crucial for successful drug discovery and a test system that enables discovery and profiling of compounds regarding efficacy and tolerability simultaneously at high-throughput is crucial (29). This strategy may avoid or at least reduce failures and serious complications during drug development and after launch.

One of the major hurdles in drug discovery is to single out the development candidate which is, besides the enormous technical progress in the field, still based on empirical knowledge of the investigator. Simultaneously monitoring several drug parameters in cell culture in one test system simplifies decision making. Once a prime target has been identified for drug discovery, it stimulates extended research in the field as in the case of topoisomerase inhibitors.

Only one compound class (nucleoside inhibitors of the TK/DNA Pol) is launched for the treatment of herpes simplex disease. A lack of therapeutic options to treat HSV infections does not match the medical standard in developed countries. Growing resistance to nucleosidic drugs, especially in the immunocompromised patient population, and the mortality and morbidity still associated with life-threatening herpes encephalitis or sight-impairing keratitis or conjunctivitis as well as the unsolved problem of recurrent HSV disease emphasize the existing medical need for effective anti-HSV agents.

Even at once-daily dosing, the preclinical profile of helicase-primase inhibitors is superior to nucleosidic drugs (29). Bay 57-1293 may further reduce mortality and morbidity for life-threatening HSV disease as well as decrease the establishment of latency and subsequent relapse. Reduced time to healing of herpetic lesions, prevention of rebound of disease, reduction of frequency and severity of recurrent disease, suppression of viral shedding and, based on the novel mechanism of action, efficacy against herpes simplex strains resistant to current medication has been demonstrated *in vitro* and in diverse animal models (28, 29, 31). Clinical trials will show whether this profile translates into the launch of a new chemical entity.

After decades of intensive research in academia and industry, helicase-primase inhibitors represent the only compound class that is superior to the nucleosidic drugs with regard to relevant preclinical profiles (60). Thus, it appears that the activity of the helicase-primase enzyme complex of HSV is the Achilles heel for viral replication and is a suitable target for antiinfective drug discovery.

Acknowledgements

The team of researchers (ref. 29) for their contribution to the project. Especially Dr. Wolfgang Bender for collecting and providing the compound library called Bender Pool. Dr. Rüdiger Fischer for his confidence in the core team and for chemical synthesis of the majority of the relevant compounds. Dr. Olaf Weber for his belief in the compound class and compiling the first *in vivo* data. Dr. Udo Schneider for funneling compounds into the project. Central research for their cooperation across institute and site barriers. Dr. Helga Rübsamen-Waigmann, Dr. Pol Bamelis and Dr. Lockhoff for their continuous support. Special thanks to my laboratory assistants Manuela Hucke, Kirsten Ostertag-Palm and Esteban Clemente. Bayer AG for giving me the opportunity to work in the research facility that has been built in honor of Dr. Gerhard Domagk. Ciprofloxacin originated from this building and central research. I wish to express my respect to H.J. Zeiler, K.G. Metzger, P. Schacht and K. Grohe, who made ciprofloxacin and BAY Y-3118. The author thanks the reviewers for their contribution toward improving the manuscript and Wolfgang Kosten for continuous support in database access.

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