CHEMICAL REVIEWS

REVIEW

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L-Pentoses in Biological and Medicinal Applications

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CONTENTS

1. INTRODUCTION

Carbohydrates form a major class of biomolecules, yet in the past they have often been overshadowed by other biopolymers, including oligonucleotides and proteins. Recent advances in glycochemistry and glycobiology have enabled the isolation and purification of carbohydrates from natural sources in larger scale resulting in better understanding of their roles in biological processes and diseases.[1] Consequently, such development has renewed the interest in carbohydrate chemistry, particularly in the utilization of carbohydrates in medicinal applications. The past decades have also witnessed an increase especially in the medicinal applications of the rare L-carbohydrates, both synthetic and semisynthetic derivatives of which have been prepared and utilized as antiviral[2] and antibacterial[3] agents. Furthermore, L-monosaccharides have been shown to possess antineoplastic properties, being useful for all major forms of cancer therapy.[4] The present review focuses on the pharmaceutical applications of sugar derivatives containing a specific subclass of L-carbohydrates, namely, the L-pentoses and mimics thereof. This group of compounds ranges from complex structures such as the antibiotic everninomicin[5] to simple monosaccharides including L-arabinose, L-lyxose, L-ribose, and L-xylose. L-Arabinose is a natural sugar present in a variety of plant carbohydrates such as hemicelluloses, while L-lyxose and L-xylose are rare in nature and hence mostly prepared by synthetic methods. Among the L-pentoses, L-ribose is the only one not obtained from natural sources. The references in this review have been drawn from the electronic version of the Drug Data Report (MDL/ISIS database). The scope is limited to derivatives containing L-aldoses having a five-carbon backbone and one endocyclic heteroatom, hence excluding the carbasugars and *C*-glycosides. Furthermore, only pentoses containing a minimum of two heteroatom substituents are covered to exclude other mono- and dideoxy compounds. All compounds covered in the present review have, as a minimum, undergone biological testing for pharmaceutical use. The chapters have been arranged according to pharmaceutical activities of the compounds presented.

2. BIOLOGICALLY ACTIVE COMPOUNDS CONTAINING L-PENTOSES

2.1. Compounds with Antiviral Activity

Viral infection is a common cause of disease resulting in high mortality rates in some cases. In general, vaccination is the most effective way to prevent the emergence and spreading of viral infections. However, several disease causing viruses, exemplified by the human immunodeficiency virus (HIV), exist against which vaccines are not available. One of the major obstacles in the search for antiviral drugs is that, in contrast to other microorganisms, viruses need only a small number of enzymes of their own for reproduction, hence leaving a limited number of targets for selective inhibition of the viral reproduction.

2.1.1. Anti-HIV Agents. Viral resistance emerging during antiviral therapy is a major challenge requiring new drugs for the control of HIV infection. Results from clinical trials have shown that drug combinations have greater antiviral efficacy compared with single drugs.[6] The main target for HIV therapy is the virally encoded reverse transcriptase (HIV-RT). Currently, there are two major classes of HIV-RT inhibitors consisting of nucleoside analogues and structurally unrelated nonnucleoside inhibitors.

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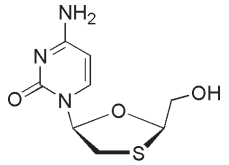
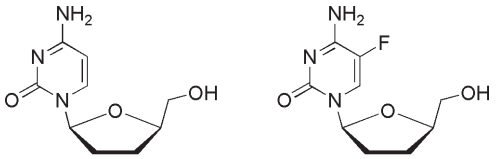


Lamivudine
(3TC)

Figure 1. First antiviral L-nucleoside approved by FDA.



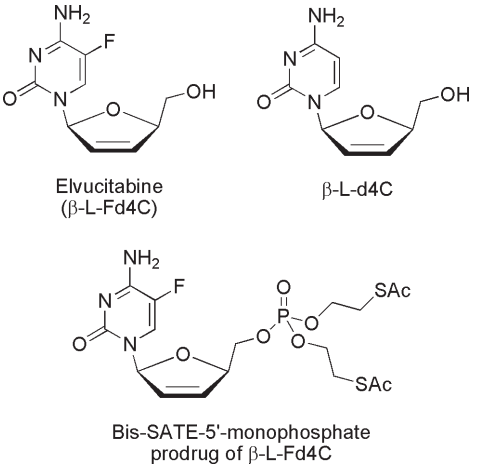
β-L-ddC β-L-FddC

Figure 2. Anti-HIV active 2′,3′-dideoxycytidine analogues.

The first FDA approved nucleoside analogue showing antiviral activity, and possessing the unnatural L-configuration is the β-L-2′,3′-dideoxy-3′-thiacytidine (3TC, lamivudine, Figure 1).[7]

In 1991 Mansuri and co-workers reported the synthesis of the L-isomer of the anti-HIV agent 2′,3′-dideoxycytidine (zalcitabine, ddC).[8] The β-L-ddC (Figure 2) was tested against HIV in CEM cells but showed only moderate activity (ID_{50} = 0.66 μM) compared to ddC (ID_{50} = 0.04 μM). However, long-term usage of ddC has been associated with severe peripheral neurophathy, which is believed to be caused by the depletion of mitochondrial DNA in the host cells. In contrast, β-L-ddC showed no inhibition against mitochondrial DNA synthesis at up to 100 μM concentration. Three years later, β-L-ddC was tested in vitro against hepatitis B virus (HBV) by another research group,[9] and was reported to inhibit the growth of HBV by more than 90% in comparison with ddC which did not show any inhibition. Gosselin and co-workers prepared the 5-fluoro analogue of β-L-ddC (Figure 2) from L-xylose and studied its anti-HIV activity in different cell culture systems.[10]

β-L-FddC demonstrated in vitro inhibitory effects on the replication of HIV-1 LAI infected CEM-SS cells and was shown to be 10 times more active than β-L-ddC. Interestingly, both β-L-ddC and β-L-FddC inhibited the replication of HIV-1 N119, a virus strain resistant against the selective nonnucleoside anti-HIV-1 agent nevirapine. Both β-L-enantiomers also showed activity against a 3′-azido-3′-deoxythymidine (AZT)-resistant virus strain (HIV-1 G 910-6).[11] The active forms of both β-L-ddC and β-L-FddC are the 5′-triphosphates. Sommadossi and co-workers demonstrated that β-L-ddCTP and β-L-FddCTP were not substrates for HIV-1 reverse transcriptase (RT) but in contrast acted as potent chain-terminating agents toward HIV-1 RT.[12] While β-L-ddC and β-L-FddC did not show cytotoxicities in a series of in vitro biological assays reported by Van Draanen and co-workers,[13] Yuen et al. reported that in vivo administration of β-L-FddC into mice resulted in considerable increase in the CD_4^+ CD_8^+ double positive circulating T cells.[14] One possible explanation for this observation could be that the β-L-FddC treated mice may have a malfunction in the T cell maturation process, leading to the release of the CD_4^+ CD_8^+ T cells into the circulation. Due to the short duration of Yuen's experiment it was not possible to conclude whether the drug-treated mice experienced any autoimmune disorders resulting from the treatment.



Elvucitabine
(β-L-Fd4C)

β-L-d4C

Bis-SATE-5'-monophosphate
prodrug of β-L-Fd4C

Figure 3. Anti-HIV active 2′,3′-dideoxy-2′,3′-didehydro-β-L-cytidine analogues and prodrug thereof.

The observation that the 2′,3′-dideoxy-2′,3′-didehydro-β-D-cytidine (β-D-d4C) showed almost as potent anti-HIV activity as β-L-ddC inspired Cheng and co-workers to prepare the corresponding L-enantiomer (β-L-d4C, Figure 3) and its 5-fluoro analogue (β-L-Fd4C, Figure 3), the antiviral activities of which were evaluated.[15]

The activity of β-L-d4C against HIV (EC_{50} ~ 1.0 μM in MT-2 cells) was similar to the previously synthesized D-enantiomer (EC_{50} = 0.7 μM) while β-L-Fd4C showed significantly improved anti-HIV activity (EC_{50} = 0.09 μM) in comparison with the cytidine analogues lacking the 5-fluoro substituent. Cytotoxicities of the L-nucleosides were, however, significantly reduced, showing ED_{50} values of >20 and >100 μM (mitochondrial DNA content in CEM cells) for β-L-d4C and β-L-Fd4C, respectively, when compared to β-D-d4C (ED_{50} = 2 μM). Since reverse transcriptase activity is required for both HIV and hepatitis B virus (HBV) replication, β-L-Fd4C was tested for activity against HBV as well.[16] Both in vitro and in vivo studies of β-L-Fd4C demonstrated potent anti-HBV activity with an EC_{50} value of 8 nM in HepG2 cells and suppression of HBV reverse transcription in both duck[17] and woodchuck[18] models. Furthermore, Chen and co-workers were able to improve the anti-HBV activity by preparing the *S*-acyl-2-thioethyl (SATE)-bearing 5′-monophosphate prodrug of β-L-Fd4C (Figure 3), which showed an EC_{50} value more than 8-fold lower than the parent nucleoside.[19] It was also observed that the intracellular half-life of the phosphorylated metabolites of β-L-Fd4C was approximately 5 times longer than that of the corresponding metabolites of the approved anti-HIV agent lamivudien.[20] A two-drug combination study with β-L-Fd4C and the D-nucleoside analogue stavudine (d4T) or zidovudine (AZT) showed synergistic anti-HIV activity in MT-2 cells.[21] Additionally, β-L-Fd4C was shown, at least in part, to protect cells from mitochondrial toxicity induced by d4T. In 2004 a 21 day, open label phase II clinical trial of β-L-Fd4C (elvucitabine) in combination with the nonnucleoside inhibitor formulation Kaletra was initiated.[22] Results from the trial demonstrated that the drug combination was able to reduce the viral load by approximately 98%. Furthermore, it was decided that a dose of 10 mg once daily would be used in upcoming clinical trials.

All of the nucleoside analogues approved by the FDA for the treatment of AIDS can be considered as 2′,3′-dideoxy-nucleosides. Gumina and co-workers have synthesized and evaluated

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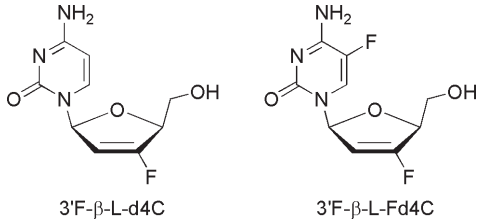

Figure 4. Anti-HIV active 2′,3′-didehydro-2′,3′-dideoxy-3′-fluorocytidine analogues.

the biological activity of 2′,3′-didehydro-2′,3′-dideoxy-3′-fluorocytidine (3′F-β-L-d4C, Figure 4).[23]

Preliminary biological evaluation of 3′F-β-L-d4C, with synthesis starting from L-xylose, showed potent anti-HIV activity (EC_{50} = 0.03 μM in PBM cells) with little or no toxicity (IC_{50} = 86.9 μM in PBM cells and IC_{50} > 100 μM in CEM cells). Further studies proved the antiviral activity against the wild-type HIV-1 strain (xxBRU) showing an EC_{90} value of 0.14 μM.[24] Additionally, 3′F-β-L-d4C showed potent activity against HBV in HepAD38 cells (EC_{90} = 0.25 μM). However, 3′F-β-L-d4C showed significantly decreased antiviral activity against the clinically important lamivudine-resistant strain HIV-$1_{M184\ V}$ (EC_{90} = 82.0 μM), the 5-fluoro analogue of 3′F-β-L-d4C (Figure 4) studied in parallel showing a similar trend with almost 500-fold increase in EC_{90} for HIV-$1_{M184\ V}$. Molecular modeling studies demonstrated that the 3′-fluoro atom of the L-3′-fluoro-2′,3′-unsaturated nucleosides is within a hydrogen bonding distance from the amide backbone of Asp185 of the wild-type HIV-1 RT, thus favoring the binding of the triphosphate of the nucleoside.[25] However, in the lamivudine-resistant strain HIV-$1_{M184\ V}$ this favorable binding mode cannot be obtained due to the bulky side chain of Val184 occupying the space required for the nucleoside triphosphate at the active site. These results also explained the difference in the fold increase (FI = EC_{50} HIV-$1_{M184\ V}$/EC_{50} HIV-1_{xxBRU}), 104 for 3′F-β-L-d4C compared to 17 for the nonfluorinated analogue β-L-2′,3′-didehydro-2′,3′-dideoxycytidine. Nevertheless, a chemical and enzymatic stability study demonstrated that the 3′-F substitution stabilized the glycosyl bond. A pharmacokinetic study in three rhesus monkeys following single-dose oral administration of 3′F-β-L-d4C showed a mean maximum plasma concentration (C_{max}) = 4.4 μg/mL and time of maximum concentration (T_{max}) = 1.67 h.[26] The concentration of 3′F-β-L-d4C in the plasma of rhesus monkeys remained higher than the EC_{90} value against wild-type HIV-1 12 h after the oral doses. However, a large variation was observed in the oral bioavailability ranging from 15 to 31%, suggesting that development of a prodrug to improve its oral absorption might be needed.

2.1.2. Anti-HBV Agents. Hepatitis B is a potentially life-threatening liver infection caused by the hepatitis B virus (HBV). Worldwide, about 2 billion people have been infected by HBV, and more than 350 million live with chronic infection. About 25% of the adults chronically infected die from liver cancer or cirrhosis.[27] Chronic hepatitis B is normally treated with interferon alpha and a nucleos(t)ide reverse transcriptase inhibitor (NRTI) such as lamivudine. However, a significant drawback of lamivudine therapy is the selection of resistant viral mutants;[28] hence new anti-HBV agents are needed to overcome these limitations.[29]

On the basis of the discovery that the antiherpes virus active 2′-fluoro-5-methyl-β-D-arabinofuranosyluracil (D-FMAU) showed neurotoxicity,[30] hence limiting its usefulness as a clinically effective antiviral agent, and reports[31] showing that some of the unnatural L-nucleosides are more potent antiviral agents than the corresponding

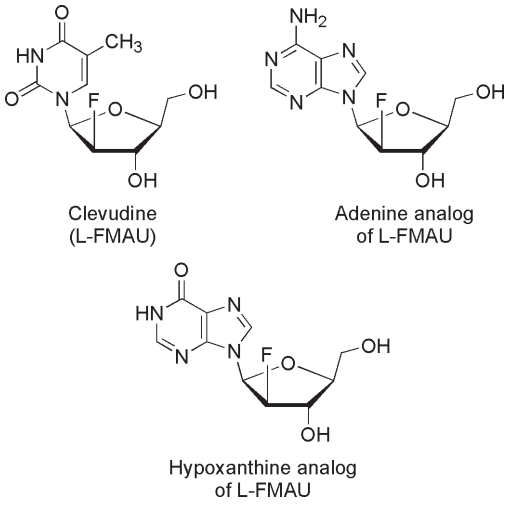

Figure 5. Anti-HBV active 2′-fluoro-L-arabinofuranosyl nucleosides.

D-nucleosides, Chu and co-workers prepared the L-analogue of D-FMAU (L-FMAU, Figure 5).[32]

L-FMAU was found to be a potent anti-HBV agent (EC_{50} = 0.1 μM in 2.2.15 cells), but interestingly L-FMAU had no significant antiherpes simplex virus activity although D-FMAU exhibited extremely potent activity against herpes simplex virus. The L-analogue did not show significant anti-HIV activity when evaluated against HIV-1 in MT2 cells, although nucleosides with anti-HBV activity often show anti-HIV activity. Additionally, the toxicity of L-FMAU was evaluated in bone marrow precursor cells with no toxicity detected at up to 100 μM concentration. Moreover, L-FMAU showed potent activity as a selective inhibitor of Epstein−Barr virus (EBV) replication, being the first L-nucleoside analogue described in literature with anti-EBV activity.[33] The pharmacokinetics of L-FMAU in rats following intravenous administration was independent of dose over the dosage range of 10−50 mg/kg.[34] Interestingly, L-FMAU was the first nucleoside analogue displaying double peaks in the plasma concentration after oral administration to rats.[35] Since woodchuck hepatitis virus (WHV) holds many similarities with HBV, the pharmacokinetics was also studied in woodchucks with parameters obtained consistent with those observed in rats.[36] Being the first thymine derivative with anti-HBV activity and the only 2′-deoxy nucleoside with a 3′-OH group, the mode of action of L-FMAU is likely to be different from other cytosine derivatives acting as chain terminators.[37] An in vitro study by Cheng and co-workers in 2.2.15 and HepG2 cells showed that L-FMAU is metabolized to its mono-, di-, and triphosphates.[38] Furthermore, it was found that the 5′-triphosphate of L-FMAU is the active compound acting as a potent inhibitor of HBV DNA polymerase with K_i = 0.12 μM and that L-FMAU not only acts as a substrate for thymidine kinase but for deoxycytidine kinase as well.[39] The potent anti-HBV activity of L-FMAU was further evaluated in vivo by oral administration to experimentally infected ducklings. The results displayed a strong inhibitory effect on hepadnavirus replication.[40] The phase III clinical trials of L-FMAU (clevudine) as a therapy for chronic HBV infection were recently stopped. The drug, which is already approved in some Asian countries, was discontinued due to safety concerns, in particular myopathy.[41]

A set of purine nucleosides having the same 2-deoxy-2-fluoro-β-L-arabinosyl moiety as L-FMAU have been reported to possess

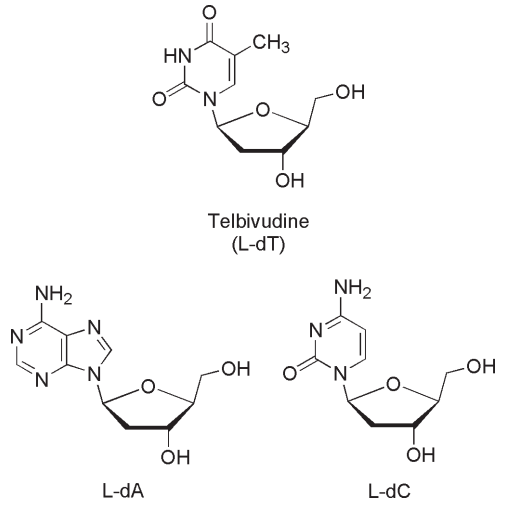

Figure 6. β-L-2′-Deoxynucleosides with anti-HBV activity.

potent anti-HBV activities by Chu and co-workers.[42] Especially the adenine and hypoxanthine derivatives (Figure 5) exhibited good in vitro anti-HBV activity with EC_{50} = 1.5 and 8 μM in 2.2.15 cells, respectively.

Although Šmejkal and Šorm reported the synthesis of L-thymidine (L-dT, Figure 6) from L-arabinose already in 1964,[43] as late as in 1992 Spadari and co-workers showed that L-dT is a substrate for herpes simplex virus type 1 (HSV1) thymidine kinase.[44]

The anti-HSV1 activity of L-dT was tested in different HeLa cells and shown to act as a competitive inhibitor markedly reducing HSV1 replication. It was found that the 5′-triphosphate of L-dT did not interfere with HIV-RT; however, this compound turned out to be a strong inhibitor of the HBV and DHBV DNA polymerases (IC_{50} = 0.46 and 1.0 μM).[45] A few years later Bryant and co-workers reported that L-dT and some other β-L-2′-deoxynucleosides (L-dC and L-dA, Figure 6) possess a selective and specific antiviral activity against HBV replication (anti-HBV activity of L-dT EC_{50} = 0.19 μM in the 2.2.15 cell line containing HBV ayw strain genome derived from human hepatoblastoma HepG2 cells).[46] Structure−activity analysis showed that the 3′-of the β-L-2′-deoxynucleosides conferred the specific antihepadnavirus activity as reported earlier for L-FMAU as well. It was also demonstrated that the stereochemistry of the 3′-C is crucial for the anti-HBV activity. Changing the sugar unit of L-dT from 2-deoxy-β-L-ribose to 2-deoxy-β-L-xylose, which are identical except for the 3′-OH which is in the opposite orientation, resulted in loss of anti-HBV activity. An in vivo study on woodchucks chronically infected by WHV showed that L-dT reduced the viral load by as much as 10^8 genome equivalents/(mL of serum) and no drug-related toxicity was observed in the animals. L-dT was further tested against a wide range of viruses (HIV-1, HSV-1, HSV-2, EBV, and others) without showing activity (EC_{50} > 100 μM).[47] In vitro studies in 2.2.15 and human peripheral blood mononuclear (PBM) cells indicated that L-dT did not exhibit cellular or mitochondrial toxicities and did not inhibit human cellular DNA polymerases at concentrations as high as 100 μM. Pharmacokinetic studies with HepG2 cells demonstrated that the 5′-triphosphate of L-dT, the predominant metabolite, exhibited an extended half-life of 15 h and the concentration of the triphosphate remained above the EC_{50} for HBV in 2.2.15 cells for 24 h.[48] In a randomized double-blind clinical phase IIb trial

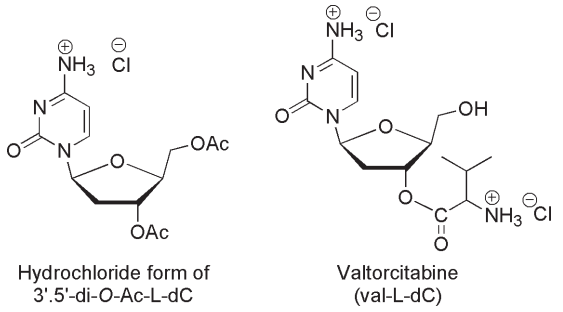

Figure 7. Prodrugs of L-dC.

the efficiency and safety of L-dT (telbivudine) was compared with lamivudine in 104 hepatitis B e-antigen (HBeAg) positive adults.[49] The 1 year telbivudine treatment reduced HBV in blood to less than 200 particles/mL in 61% of the patients, compared to only 32% achieving this result with lamivudine. Additionally, the alanine aminotransferase levels, a marker of HBV-related liver inflammation, were normalized in 86% of the telbivudine-treated patients compared with 63% for lamivudine. In 2005 "the GLOBE study", an international phase III clinical trial with telbivudine including 1370 patients took place. The patients were randomly assigned to receive 600 mg of telbivudine or 100 mg of lamivudine once daily.[50] Among the patients with HBeAg-positive chronic hepatitis B, telbivudine provided superior response on all evaluated virologic markers compared to lamivudine. In October 2006 FDA approved telbivudine as a new treatment for patients with chronic hepatitis B.

Parallel to the development of telbivudine, Gosselin and others studied the anti-HBV activity of 2′-deoxy-β-L-cytidine (L-dC), another β-L-2′-deoxynucleoside.[46−48] The in vitro and in vivo anti-HBV activity and toxicity of L-dC was comparable to the results obtained with telbivudine (EC_{50} = 0.24 and 0.19 μM in 2.2.15 cells, respectively). In the pharmacokinetic studies with HepG2 cells the 5′-triphosphorylated L-dC was the major metabolite as also found for telbivudine. Interestingly, the 5′-phosphorylated deaminated metabolites of L-dC were also detected, of which L-dUTP was the major one.[48] This observation was of particular significance as the administration of one nucleoside yields two distinct pharmacologically active 5′-phosphorylated derivatives. Additionally, exposure of HepG2 cells with L-dC in combination with telbivudine did not hinder this phosphorylation, suggesting that combination antiviral therapy could be possible. Further, both in vitro and in vivo studies in the woodchuck model of chronic HBV infection suggested that the two drugs have potent antiviral synergy.[51] However, pharmacokinetic studies indicated that the oral bioavailability of L-dC was poor, 9 and 16% in woodchucks and monkeys, respectively.[52] On the basis of these results, Gosselin and co-workers searched for potential prodrugs with more favorable oral absorption profiles, first by acetylation of either the sugar hydroxyl groups or the cytosine exocyclic amino group.[53] The acetylated compounds showed anti-HBV activity with EC_{50} values comparable to that of the parent nucleoside in 2.2.15 cells being noncytotoxic in HepG2 cells at >200 μM. The most promising compound was the hydrochloride form of 3′,5′-di-*O*-acetyl-β-L-dC (Figure 7) for which a saturated solution in water has the concentration of 3.3 mol/L compared to 1.03 mol/L for L-dC.

In addition to its excellent solubility, 3′,5′-di-*O*-acetyl-β-L-dC showed a good stability profile at acidic pHs. Pharmacokinetic parameters in cynomolgus monkeys following oral administration of 3′,5′-di-*O*-acetyl-β-L-dC showed that the time to maximum plasma concentration was 1.0 h with a C_{max} of 5.6 μM as

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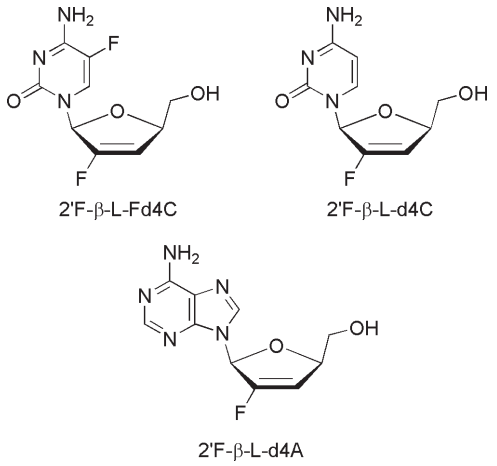


Figure 8. 2′-Fluoro-2′,3′-unsaturated anti-HBV active L-nucleosides.

compared to 2.3 h and 3.4 μM for L-dC, respectively. These results warranted further evaluation of other mono- and diacylated derivatives of L-dC as prodrug candidates. A second set of ester prodrugs of L-dC, mono- and divalinyl esters, were synthesized.[54] The C_{max} and AUC (area under the plasma concentration time curve) for L-dC achieved with oral administration of these prodrugs were 2.5−5-fold higher than estimated for an equivalent dose of L-dC. The prodrugs are rapidly and completely converted to L-dC which in turn is phosphorylated to the 5′-triphosphate of L-dC reaching levels up to 100 times the EC_{90} of HBV with a half-life of 15 h. The hydrochloric form of 3′-*O*-valinyl-β-L-dC (val-L-dC, valtorcitabine, Figure 7) entered phase IIb clinical trial in late 2004 as a fixed-dose combination with telbivudine.

With 2′,3′-dideoxy-2′,3′-didehyro-β-D-cytidine (β-D-d4C) showing equipotent HIV activity to that of β-L-ddC, Cheng and co-workers synthesized the L-enantiomer of β-L-d4C and its corresponding 5-fluoroanalogue (β-L-Fd4C) and evaluated their antiviral activity.[15] The inhibitory activity of the compounds against extracellular circular and intracellular replicating HBV DNA were tested in vitro using 2.2.15 cells. Both compounds demonstrated significant anti-HBV activity, ED_{50} = 0.008 and 0.002 μM for β-L-d4C and β-L-Fd4C, respectively. In particular, the anti-HBV activity of β-L-Fd4C was exceptional, being 10−20 times more active than the approved antiviral drug lamivudine. In addition, both compounds showed potent anti-HIV activity at a 10 μM concentration but no effect on the mitochondrial DNA content of CEM cells at the same concentration. It is well-established that 2′,3′-dideoxy nucleosides are unstable in acidic media, resulting in cleavage of the glycosidic bond. Chen and co-workers have prepared 2′-fluoro-2′,3′-unsaturated 5-fluorocytocine (2′F-β-L-Fd4C, Figure 8), the 2′-fluorinated analogue of β-L-Fd4C.[55] The fluorine at 2′-position provides acid stability, and due to its similar size to hydrogen it is also attractive as an isosteric replacement of hydrogen.

Incorporation of the fluorine atom at the 2′-position improved the in vitro cytotoxity in CEM cell lines by 10-fold for 2′F-β-L-Fd4C (ID_{50} = >50 μM) compared to β-L-Fd4C (ID_{50} = 7 μM), although the anti-HBV activity (EC_{50} = >0.03 μM in CEM cells) was not enhanced. In a parallel study the 2′-fluoro-2′,3′-unsaturated analogue of cytidine (2′F-β-L-d4C, Figure 8) and adenine (2′F-β-L-d4A, Figure 8) were synthesized and evaluated for their antiviral activities against HIV-1 in PBM cells and HBV in 2.2.15 cells,

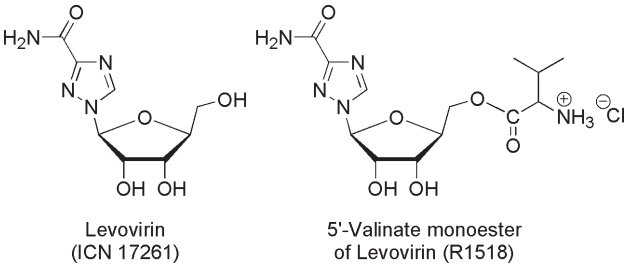


Figure 9. First L-nucleoside with immunomodulatory activity and a prodrug thereof.

showing moderate to potent anti-HIV (EC_{50} 0.17, 0.51, and 1.5 μM) and anti-HBV (EC_{50} 0.225, 0.18, and 1.7 μM) activities.[56] The anti-HBV activity of 2′F-β-L-d4C was evaluated in vivo in HBV-carrying transgenic mice, and its pharmacokinetics was assessed in rhesus monkeys.[57] 2′F-β-L-d4C demonstrated efficacy comparable to lamivudine in the short-term treatment of HBV-transgenic mice. A favorable pharmacokinetic profile and acceptable oral bioavailability in rhesus macaques were observed as well.

2.1.3. Anti-HCV Agents. Hepatitis C virus (HCV) is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma worldwide. It is estimated that approximately 170 million people are chronically infected with HCV.[58] In several countries, HCV-related end-stage liver disease is the most common cause of liver transplantation.[59] Currently, the most effective therapy for the treatment of HCV is the combination of pegylated interferon and ribavirin, being however associated with significant side effects such as hemolytic anemia and neuropsychiatric symptoms.[60]

Ribavirin is a nucleoside analogue with demonstrated efficacy in treating HCV as combination therapy with interferon alpha.[61] The activity of ribavirin is not only a result from direct inhibition of viral replication but also due to its ability to enhance T cell-mediated immunity.[62] Ramsamy and co-workers investigated whether the immunomodulatory properties of ribavirin were limited to nucleosides with D-configuration or if L-nucleoside derivatives of ribavirin would possess similar activity. Among the compounds prepared, 1-β-L-ribofuranosyl-1,2,4-triazole-3-carboxamide (ICN 17261, Figure 9), the L-enantiomer of ribavirin, was found as the most potent compound.[63]

ICN 17261 showed the same enhancement of antiviral type 1 cytokines and suppression of type 2 cytokines in human T cells as ribavirin and was the first L-nucleoside reported to possess immunomodulatory activity. Structural manipulations of the furanose moiety, including conversion of 2′-OH or 3′-OH to a hydrogen or inversion of the stereochemistry at 3′-C, resulted in reduced or total loss of type 1 cytokine activity. Even if both in vitro and in vivo studies with ICN 17261 and ribavirin showed similar induction of type 1 cytokine, the former showed no in vitro antiviral activity against HIV, influenza A or B, and respiratory syncytial virus (RSV) (EC_{50} > 600, > 200, and > 1000 μM, respectively) compared to the antiviral activity of ribavirin (EC_{50} = 40, 5.0, 2.6, and 40 μM, respectively).[64] An in vivo study in rats by oral gavage of ICN 17261 (180 mg/kg) for 4 weeks did not generate any observable clinical pathology, whereas ribavirin in equivalent doses induced a significant anemia and leucopenia. Phase I clinical trials with ICN 17261 (levovirin) were initiated in 2001. Levovirin was supposed to improve the treatment of HCV due to its better tolerability profile compared to that of ribavirin. However, results from phase I/II clinical trials showed that combination of levovirin

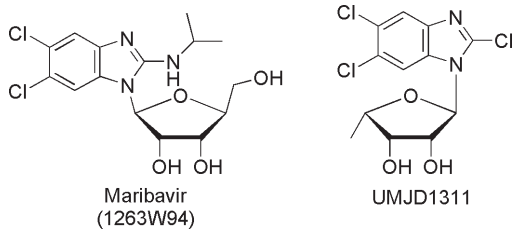


Figure 10. Anti-HCMV activie L-nucleoside analogues.

and pegylated interferon alfa-2a failed to generate a virological response comparable to ribavirin and peginterferon alfa-2a in patients with chronic hepatitis C, hence leading to discontinuation of the development of levovirin.[65] Clinical phase I trials were also conducted in order to investigate the pharmacokinetics of the hydrochloride form of the 5′-valinate monoester of levovirin (R1518), a prodrug of levovirin (Figure 9). The peak plasma concentration of levovirin was rapidly reached following single oral doses of R1518 (T_{max}(mean) = 2.8 h) with a mean maximum plasma concentration (C_{max}) = 34.48 μg/mL compared to results obtained following oral administration of levovirin (T_{max}(mean) = 3.8 h and C_{max} = 2.68 μg/mL).[66] The valine monoester prodrug significantly improved the bioavailability of levovirin.

2.1.4. Anti-HCMV Agents. Infections by herpes viruses are easily transmitted and hence one of the major viral diseases in man. Human cytomegalovirus (HCMV) is one of the eight human herpes viruses that cause widespread infections. Normally, it establishes a lifelong, persistent, but asymptomatic, infection in healthy individuals, while able to cause substantial morbidity and mortality in immunocompromised patients such as transplant recipients and those with AIDS.[67]

In 1996 Drach, Townsend, and co-workers reported that the L-nucleoside analogue β-L-ribofuranosyl-2-isopropylamino-5,6-dichlorobenzimidazole (1263W94, Figure 10) is one of the most potent members of a new class of compounds that selectively inhibit HCMV.[68]

1263W94 showed significant anti-HCMV activity in vitro against 10 clinical HCMV isolates, with IC_{50}s from 0.03 to 0.13 μM, compared to 0.15 to 1.10 μM for the FDA approved anti-HCMV active D-nucleoside analogue ganciclovir.[69] In contrast to other nucleoside analogues, phosphorylated metabolites of 1263W94 were observed in neither uninfected nor HCMV-infected cells. Furthermore, significant inhibition of HCMV DNA polymerase was not observed at concentrations of 1263W94 up to 100 μM. These findings suggest that the antiviral activity of 1263W94 results from a novel mechanism which is different from those reported earlier. A preclinical 1 month oral toxicology study in rats and monkeys did not show any adverse pharmacological effects, having no-observed-effect levels (NOEL) of 100 and 180 (mg/kg)/day in rats and monkeys, respectively.[70] Oral doses of 10 mg/kg demonstrated excellent bioavailability in both species, resulting in concentrations in plasma that were 24- to 114-fold greater than the mean IC_{50} reported for the 10 clinical HCMV isolates. Since HCMV infections are a major concern in patients with AIDS, 1263W94 was tested in combination with the most commonly used anti-HIV agents to investigate whether they antagonize the inhibition of HCMV by 1263W94.[71] The nucleoside reverse transcriptase inhibitors did not show any effect on the anti-HCMV activity of 1263W94. These results were encouraging, indicating that the treatment of HCMV infection with 1263W94 in HIV-infected patients would not be compromised by concurrent HIV therapy. An initial 28 day phase I clinical study in HIV-infected men with asymptomatic CMV shedding showed that single and multiple oral doses (300−1200 mg/day) of 1263W94 (maribavir) were well-tolerated and were associated with antiviral activity.[72] Unexpectedly, phase III clinical trials of maribavir were discontinued with primary analysis showing no statistical difference between maribavir and placebo in reducing the rate of CMV replication in bone marrow transplant patients.

α-L-5-Deoxylyxofuranosyl-2,5,6-trichlorobenzimidazole (UMJD-1311, Figure 10) is another new benzimidazole derivative showing potent anti-HCMV activity with IC_{50} value of 0.2 μM in HFF cells.[73] The inhibition of HCMV replication by UMJD-1311 was demonstrated to be different from other viral DNA polymerase inhibitors as is the case with maribavir. Surprisingly, however, UMJD-1311 was active against maribavir-resistant viruses, hence indicating that UMJD-1311 acts via a third distinct mode of action for inhibition.[74]

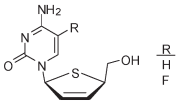
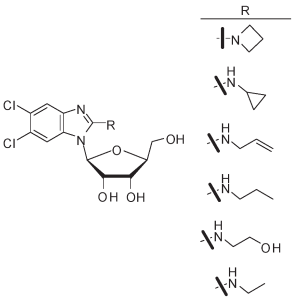
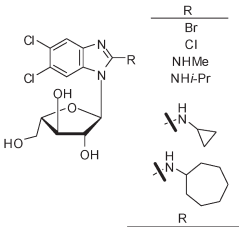
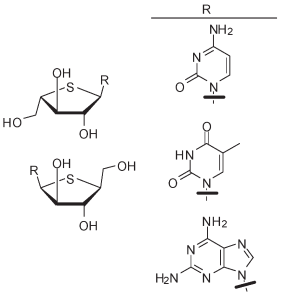
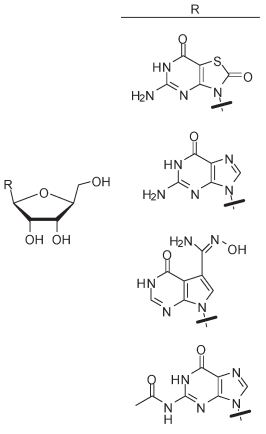
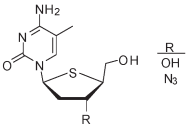
2.1.5. Antiviral Agents Reported in the Patent Literature Only. A number of pharmaceutically active compounds containing L-pentoses appear in the patent literature. Due to the free online patent databases, this material should be readily available to most researchers. Antiviral agents of interest appearing exclusively in the patent literature have been collected in Table 1 providing also references to the original source in which information on the pharmaceutical activity can be retrieved.[75−80]

2.2. Antibacterial and Antifungal Compounds

Antibacterial compounds are the largest group of drugs used for treatment of infections. The first antibiotic, penicillin, was isolated in 1928, launching a new era in medicinal research. Since the initial discoveries, thousands of compounds with antibiotic properties have been prepared and isolated. For several decades, bacterial infections were largely considered to be under control. Since the 1980s, however, the widespread and unrestricted use of various antiobiotics has resulted in the emergence of multidrug resistance in bacteria. The rapid increase in resistant organisms has in turn contributed to a growing demand for novel drugs and the improved usage of the already available agents. Most of the antibacterial drugs act by inhibition of protein or nucleic acid synthesis or by impairing the construction of the cell wall. In contrast to bacteria, both fungi and humans are eukaryotes, turning the discovery and design of drugs targeting the fungi without affecting the human cells into a highly challenging task.

2.2.1. Aminoglycoside Antibiotics. The aminoglycoside antibiotics were the first natural product based drugs discovered by systematic screening for antimicrobial activity. Most of the aminoglycosides are naturally occurring compounds which are isolated from actinomycetes of either genus *Streptomyces* (labeled -mycin) or *Micromonospora* (labeled -micin).[81] All natural aminoglycoside antibiotics share certain specific structural features. Besides being constructed from a variety of aminosugars, as the name suggests, they all contain a nonsugar scaffold, streptamine or 2-deoxystreptamine, to which the sugar substituents are connected from positions 4, 5, and/or 6.[82] Aminoglycoside antibiotics act by binding to the decoding region (A-site) within the bacterial 16S rRNA of the 30S subunit.[83] Structural studies on three-dimensional ribosome−aminoglycoside antibiotic complexes have shown that the 2-deoxystreptamine scaffold plays a key role in the anchoring of antibiotics to the decoding site.[84] Due to the natural role of the ribosome in providing an environment for protein production, the effect of the antibiotic binding is expressed in mistranslation of mRNA or premature

Table 1. Antiviral Compounds Containing L-Pentoses Reported in the Patent Literature Only

Molecular structure	Phase	Activity	Ref.
	Biological testing	Antiviral pyrimidine L-nucleoside	75
	Biological testing	Antiviral benzimidazole L-nucleoside	76
	Biological testing	Antiviral L-arabinofuranosyl benzimidazoles	77
	Biological testing	Antiviral L-4′-thio-arabinofuranosyl nuclosides	78
	Biological testing	Immunomodulatory purine L-nucleosides	79
	Biological testing	Antiviral β-L-5-methylcytosine nucleosides	80

termination of protein synthesis. It was shown that the mistranslated proteins are incorporated into the cell membrane increasing the permeability of the membrane and hence the aminoglycoside uptake, leading to cell death.[85]

Streptomycin (Figure 11) was the first aminoglycoside antibiotic discovered, isolated in 1943 from the actinobacterium *Streptomyces griseus* by Waksman and co-workers and was the first antibiotic that could be used against *Mycobacterium tuberculosis*.[86]

Streptomycin is the only aminoglycoside in clinical use that has a streptamine, or more precisely a streptidine, moiety as the aminocyclitol backbone. Further, the structure includes a disaccharide, an *N*-methylated L-glucosamine α-1,2-linked to L-streptose

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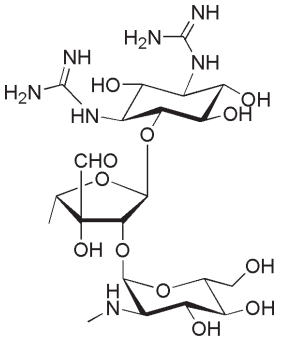
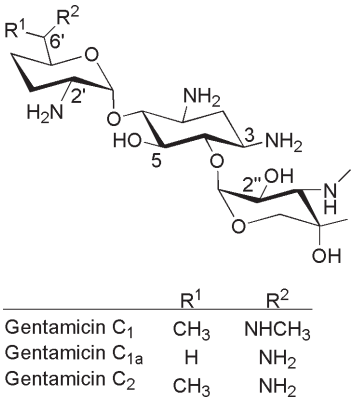


Streptomycin

Figure 11. First aminoglycoside antibiotic discovered.



	R^1	R^2
Gentamicin C_1	CH_3	$NHCH_3$
Gentamicin C_{1a}	H	NH_2
Gentamicin C_2	CH_3	NH_2

Figure 12. Gentamicin aminoglycosides.

(5-deoxy-3-C-formyl-L-lyxose), attached to the 4-position of streptidine. Streptomycin showed in vitro activity against a variety of gram-negative bacteria when first introduced into clinical practice. The polar structure of streptomycin, and all other aminoglycosides, leads to poor oral absorption, making parenteral routes of administration necessary.[87] However, the use of streptomycin as monotherapy soon gave rise to bacteria resistant to the aminoglycoside. The drug resistance in combination with side effects such as nephrotoxicity and ototoxicity, and above all the availability of more efficient drugs contributed to the diminished usage of streptomycin in the industrialized countries in the 1960s.[88] One of the main reasons for streptomycin resistance in bacteria are mutations in the gene encoding the ribosomal protein S12[89] and in the 16S rRNA gene,[90] although about one-third of the clinical isolates resistant to streptomycin lack mutations in these genes. Recently, Zaha and co-workers found that the efflux system also, in addition to enzymatic modifications, could be involved with a low level of streptomycin resistance.[91] To reduce the emergence of resistant organisms, various multidrug chemotherapy regimens have been adopted. Combination therapy with a cell−wall−active β-lactam or vancomycin enhances the streptomycin uptake resulting in improved antimicrobial activity.[92] This synergy has also been reported to be strain- or species-specific.[93] A streptomycin−rifampin combination has proven to be highly efficacious against *Mycobacterium uclerans*,[94] and combination with teicoplanin was shown by Nicolau and co-workers to provide bactericidal effects on vancomycin-resistant *Enterococcus faecalis* strains.[95] The increasing number of multidrug−resistant strains of *M. tuberculosis* has become a growing public health problem in many regions.[96] However, tuberculosis treatment with internationally approved regimens has resulted in a very high cure rate. These regimens of combination therapy have lifted streptomycin back as a first line treatment for tuberculosis.[97]

Gentamicin (Figure 12) was first isolated in 1963 from a gram-positive bacteria belonging to the genus *Micromonospora*[98] and had its clinical breakthrough during the 1970s due to the rapid appearance of new streptomycin-resistant strains.

However, already in 1979 high-level plasmid-mediated gentamicin resistance among clinical isolates of *Enterococcus faecalis* was reported, indicating further problems caused by drug-resistant bacteria.[99] The clinically used gentamicin C is a broad-spectrum antibiotic that consists of three closely related aminoglycosides, gentamicin C_1 (25−50%), C_{1a} (20−35%), and C_2 (25−50%) with similar antibacterial activity, and is the aminoglycoside antibiotic used most often due to its low cost and reliable activity against both gram-negative and gram-positive bacteria. Gentamicins belong to the largest group of clinically important aminoglycoside antibiotics, the ones possessing a 4,6-disubstituted 2-deoxystreptamine scaffold. All members of the gentamicin C group have a 3-deoxy-4-C-methyl-3-(methylamino)-L-arabinopyranose attached to the 6-position of 2-deoxystreptamine, while the substituent at position 4 is a 2,6-diamino-2,3,4,6-tetradeoxy-D-erythrohexose with a varying methylation pattern at C-6 and NH_2-6. Yet today, almost 50 years after gentamicin was first isolated, it continues to be the antibiotic of choice to treat hospital-acquired enterobacteriaceae and *Pseudomonas aeruginosa* infections. Typically, gentamicin is used in combination with a β-lactam. Such a combinatorial regime has shown synergistic bactericidal effects against *Streptococcus pneumoniae* isolates with high-level penicillin and streptomycin resistance.[100] An interesting phenomenon was observed when gentamicin and β-lactams, such as carbenicillin, were combined and allowed to stand. Under these circumstances, the aminoglycoside was inactivated by acylation of its amino groups by the β-lactam function of carbenicillin.[101] The mechanisms for bacterial resistance mentioned earlier for streptomycin are causing resistance against gentamicin as well. For example, mutations in the ribosomal protein L6 of *Escherichia coli* causing gentamicin resistance were reported relatively early.[102] However, by far the most important mechanism for gentamicin resistance in clinical isolates of both gram-negative and gram-positive bacteria are enzymatic modifications of the amino and hydroxyl groups of the aminoglycoside antibiotic. Aminoglycoside-modifying enzymes belong to three classes: (1) aminoglycoside nucleotidyltransferases (ANTs), (2) aminoglycoside phosphotransferases (APHs) that utilize ATP to regiospecifically modify the hydroxyl groups in the drugs by transferring AMP or the γ-phosphate of ATP, respectively, and finally (3) aminoglycoside acetyltransferases (AACs) that utilize acetyl-CoA as a donor for *N*-acetylation of amino groups.[103] The drugs modified by the enzymes do not bind properly to the ribosome, allowing bacteria to survive in the presence of the drug. A strain of *Pseudomonas aeruginosa* that inactivated gentamicin by acetylation of the 3-amino group of 2-deoxystreptamine was discovered by Davies and co-workers in 1972.[104] Since then, several aminoglycoside-modifying enzymes, e.g., APH(2″) initially discovered in *Enterococcus gallinarum* isolate, have been reported to mediate high-level gentamicin resistance.[105] However, the AAC(6′) enzymes that confer resistance to most of the naturally occurring aminoglycosides are sensitive to the clinically used gentamicin, since approximately one-third of the drug content is gentamicin C_1 that has a methyl group at N-6′ making it less susceptible to AAC(6′) enzymes. Among all aminoglycoside-modifying enzymes in enterococci, the bifunctional AAC(6′)-I-APH(2″) has, nevertheless, the greatest clinical importance due to its

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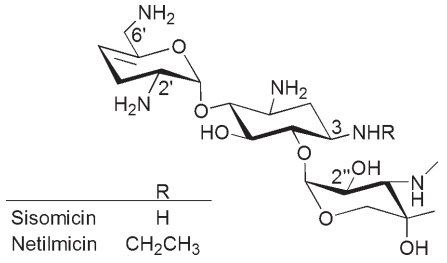


Figure 13. 4′,5′-Unsaturated aminoglycoside antibiotics.

generation of resistance toward nearly all aminoglycosides except streptomycin.[106] The discovery of these new aminoglycoside-modifying enzymes in enterococci, which are able to inactivate most of the clinically used aminoglycoside antibiotics, including gentamicin, is of great concern and requires reevaluation of the current strategy for predicting synergistic combinations with aminoglycosides. While aminoglycosides are mainly administered intramuscularly or intravenously, certain alternative drug delivery approaches are used under specific circumstances in order to increase the concentration of the antibiotic at the site of infection or to reduce the risk of nephrotoxicity and ototoxicity. An example is provided by inhalation of aerosolized gentamicin solution, used for the treatment of serious respiratory tract infections, including those occurring in cystic fibrosis patients.[107] Another approach of special interest is the development and use of liposome-encapsulated gentamicin, used for both local applications and intravenous administration.[108] Yet another signifcant application is antibiotic-loaded bone cement used as an effective drug delivery system to prevent bone and soft tissue infections in joint arthroplasty, e.g., hip replacements, in clinical orthopedics.[109] Recently, some interesting prodrug approaches for prolonging the time of renal clearance for gentamicin have been reported. Reversibly pegylated prodrug derivatives of gentamicin were found to be capable of releasing gentamicin for prolonged periods in vivo.[110] Promising results were reported by linking (2-sulfo)-9-fluorenylmethoxycarbonyl to three amino groups of gentamicin C_1.[111]

Sisomicin (Figure 13) was isolated as a new kind of aminoglycoside antibiotic from *Micromonospora inyoensis* in 1970. The novelty of sisomicin arose from its structure containing an unsaturated sugar unit, previously unencountered in any aminoglycoside antibiotic, being a 4′,5′-unsaturated analogue of gentamicin C_{1a}.[112]

The activity of sisomicin against gram-negative isolates was similar to that of gentamicin.[113] However, sisomicin was shown to be twice as active as gentamicin against *Pseudomonas* strains but the equal susceptibility to enzymatic inactivation reduces the therapeutic advantage over gentamicin.[114] To overcome the problems induced by strains producing aminoglycoside-modifying enzymes, new semisynthetic aminoglycosides were prepared. A highly successful strategy utilized in the development of new compounds was based on alkylation of the N-1 amino function. This approach yielded a large number of drug candidates from which only one has been clinically developed, netilmicin (Figure 13), which is the 1-*N*-ethyl derivative of sisomicin.[115] Netilmicin was found to have potency similar to gentamicin against aminoglycoside-susceptible gram-negative bacteria in both in vitro and in vivo tests.[116] Regardless of the marginal improvements in terms of antibacterial activity, clinical studies have demonstrated that netilmicin is significantly less nephrotoxic than gentamicin.[117] Similar to other aminoglycoside antibiotics, netilmicin exhibits in vitro synergistic effects against a wide range of organisms in combination with other antibacterial drugs. Of the aminoglycoside-modifying enzymes found

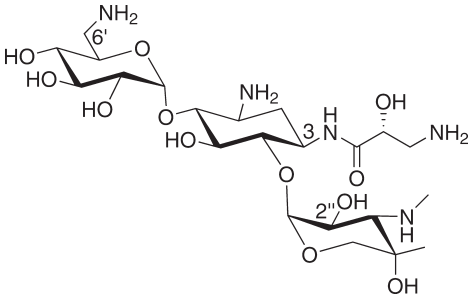


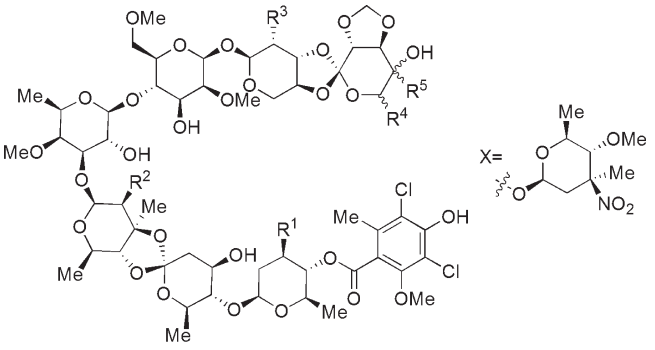
Figure 14. Semisynthetic aminoglycoside isepamicin.

in gentamicin-resistant strains only three, AAC(2′), AAC(6′), and AAC(3), significantly reduce the activity of netilmicin,[118] while the ethyl group on N-1 successfully protects it against ANT(2″)[119] and the lack of hydroxyl groups at C-3′ and C-4′ obviously protects it against enzymes modifying these positions, such as APH(3′) and ANT(4′). Being launched into the U.S. market in the early 1980s, netilmicin has been widely used worldwide against infections originating from gram-negative bacteria. Netilmicin has demonstrated good results in the treatment of complicated urinary tract infections and in combination with β-lactams in cystic fibrosis patients with *Pseudomonas aeruginosa*.[120] Further, netilmicin has proven to be effective as once-daily therapy in neonates and continues to play a valuable role in the treatment of pediatric infections.[121] More recently, netilmicin has been established as a first-line topical antimicrobial drug for treatment of bacterial ocular infections, as an ophthalmic single-drug solution,[122] or as a fixed combination with the synthetic corticosteroid dexamethasone.[123]

Another clinically developed semisynthetic aminoglycoside is isepamicin (Figure 14), first synthesized in 1978,[124] largely used in Japan and now also available in Europe. Isepamicin, which is a gentamicin B derivative having an (*S*)-3-amino-2-hydroxypropionyl substituent at N-1 of the 2-deoxystrptamine backbone, is protected against AAC(3) enzymes but also against ANT(2″) enzymes, probably due to sterical hindrance. Further, isepamicin is resistant to many of the AAC(6′) enzymes, possibly due to methylation of the amino function at position 3″ which hinders proper binding to the AAC(6′) enzyme of type II.[125]

Despite their potential nephrotoxicity and ototoxicity and the continuous reports on resistance development, the aminoglycoside antibiotics remain important, in some cases even irreplaceable, for the treatment of serious gram-negative and gram-positive pathogens.

2.2.2. Orthosomycin Antibiotics. The orthosomycin antibiotics are a class of natural products produced by a wide variety of *Actinomycetes* of the genera *Streptomyces* and *Micromonspora*.[126] This class of compounds is characterized by two features: First, the compounds are oligosaccharides composed of three to eight monosaccharide residues, and, second, at least one glycoside bond is replaced by an orthoester linkage. The orthosomycins can be divided into two groups on the basis of additional structural features, namely, (1) compounds containing an aminocyclitol residue and (2) esters of dichlorisoeverninic acid, of which only the latter group is covered in this review. Of the orthosomycins belonging to this group, the avilamycins,[127] curamycins,[128] the everninomicins,[129] and flambamycin[130] were first isolated in the 1960s and 1970s and were found to possess strong activities against gram-positive bacteria. The above-mentioned orthosomycins all share a common nonreducing 2,6-di-*O*-methyl-β-D-mannopyranosyl-(1↔1)-α-L-lyxopyranoside fragment in which the L-lyxose can have an alkyl or acyl group on



	R^1	R^2	R^3	R^4	R^5
Avilamycin-A	OH	H	OCO*i*-Pr	Me	COMe
Curamycin-A	OH	H	OCOMe	Me	COMe
Flambamycin	OH	OH	OCO*i*-Pr	Me	COMe
Everninomicin-B	X	OH	OMe	H	CH(OMe)Me
Everninomicin-C	X	H	OMe	H	H
Everninomicin-D	X	H	OMe	H	CH(OMe)Me
Everninomicin-2	OH	H	OMe	H	CH(OMe)Me

Figure 15. Dichlorisoeverninic acid containing orthosomycin antibiotics.

OH-2 and is bound to the following monosaccharide unit via an orthoester linkage (structures in Figure 15).

The everninomicin complex of antibiotics isolated from *Micromonspora carbonaceae* by Wagman and co-workers consisted of at least five components,[129] of which everninomicin-B,[131] -C,[132] -D,[133] and -2 were characterized.[134] While all of the early isolates demonstrated in vitro activity against a wide range of gram-positive organisms, most studies focused on the pharmacological properties of the main component everninomicin-D, showing antimicrobial activity against certain penicillin G-resistant strains as well. Preliminary toxicity studies in mice showed low toxicity (LD_{50} = 3750 mg/kg) of the antibiotic when given orally. Intravenous administration, however, indicated a significantly higher degree of toxicity (LD_{50} = 125 mg/kg) later explained by the poor absorption via the gastrointestinal tract.[135] Pharmacokinetic studies in dogs showed, similar to mice, poor absorption also via intramuscular administration. The serum levels obtained were, nevertheless, sufficiently high for exerting an antimicrobial effect against gram-positive bacteria. Despite the promising preliminary results from the pharmacological studies, everninomicin-D was never considered for clinical use due to adverse effects such as nephrotoxicity.

More recently, a new everninomicin component, everninomicin 13,384-1 (SCH27899), was isolated from the fermentation broth of *Micromonspora carbonacea* var. Africana (Figure 16).[136]

The new compound demonstrated superior antimicrobial activity against 428 clinical strains of bacteria (MIC_{90} = 0.25 μg/mL) in comparison to vancomycin (MIC_{90} = 2 μg/mL), which has emerged as the number one therapy against gram-positive pathogens. Further, everninomicin 13,384-1 showed excellent in vitro activity against fluoroquinolone and vancomycin resistant strains (MIC_{90} = 0.25 and $\leq$ 4 μg/mL, respectively). These findings are highly significant considering the growing numbers of gram-positive strains reported to display multiresistance against β-lactams, macrolides, tetracycline, aminoglycosides, and fluoroquinolones, hence making the development of new antibiotics of special importance.[138] The antimicrobial activity of everninomicin 13,384-1 was shown to result from selective binding to the 50S ribosomal subunit leading to inhibition of the protein synthesis.[139] Pharmacokinetic studies in mice, rats, rabbits, and cynomolgus monkeys following intravenous administration of everninomicin 13,384-1 as a β-cyclodextrin complex, used to overcome toxicity problems,[140] showed significant variations in body clearance between the different species.[141] Everninomicin 13,384-1, however, showed excellent in vivo activities against penicillin-resistant pneumococci in mice, following a single intravenous injection at a dose of 60 mg/(kg of body weight).[142] The half-life of everninomicin 13,384-1 was found to be 6 times longer than that for vancomycin (2.3 h versus 0.36 h in the bloodstream and 3 h versus 0.45 h in lung tissues) which contributes to its exceptional in vivo activities. A number of chemical modifications have been performed on everninomicin 13,384-1 in order to improve its pharmacokinetic properties. The new analogues did not, however, prove to be better than the parent compound.[143] Evrninomicin 13,384-1 entered clinical trials under the trade name of Ziracin and reached phase III clinical trials as an intravenous antibiotic for treatment of gram-positive nosocomial infections. Unexpectedly, however, the manufacturer announced the voluntary discontinuation of clinical development of evrninomicin 13,384-1 in May 2000 on the basis of results from completed phase II and phase III clinical studies, which failed to show sufficient advantage of evrninomicin 13,384-1 when efficacy and clinical safety profiles were balanced in comparison with products already approved.

The orthosomycin antibiotic avilamycin, a complex comprised of 16 components isolated from cultures of *Streptomyces viridochromogenes*,[127] is one out of four antimicrobial agents approved for use as growth promoter for food animals in the European Union. There are several proposed mechanisms for the growth promotion in chicken, ranging from an increase in volatile fatty acid production and nitrogen retention to more antibacterial properties such as reduction of *Clostridium perfringens* in the intestinal tract of the broiler.[144] It has also been reported that avilamycin reduces stress-induced diarrhea in newly weaned piglets.[145] Already in 1973, Wolf showed that avilamycin binds to the 30S subunit in the ribosome and is hence interfering with the protein synthesis.[146] However, resistance against avilamycin has been reported to occur frequently in *Enterococcus faecium* isolates from broiler.[147] This resistance was found by Aarestrup and Jensen to be associated with mutations in the ribosomal protein L16,[148] which earlier had been reported by Adrian and co-workers to give rise to everninomicin resistance as well.[149] It was found that the avilamycin binding site overlaps the binding site of everninomicin on the 50S subunit, leading to everninomicin cross-resistance in avilamycin-resistant enterococcal isolates.[150] The development of Ziracin for human use was supposed to terminate the use of avilamycin as a growth promoter in food animals in order to prevent further development of cross-resistant bacteria. Avilamycin, however, remained in use due to the discontinuation of the clinical trials of Ziracin.

2.2.3. Pradimicin Antibiotics. The pradimicins represent a fairly new class of antifungal antibiotics having in common the dihydrobenzo[α]naphthacenequinone scaffold substituted with 1−3 sugar moieties and a D-amino acid. The pradimicins were first isolated in 1988 by Oki and co-workers from the fermentation broth of *Actinomadura hibisca* and was found to exhibit in vitro activity against a wide variety of fungi and yeasts.[151] This family of antibiotics was also reported to possess in vivo activity against fungal infections caused by *Candida albicans*, *Aspergillus fumigatus* , and *Cryptococcus neoformans* in mice. The main component pradimicin A has further been shown to possess some in vitro anti-HIV activity.[152] More recently, two new members of this family, pradimicin T1 and T2 (Figure 17), have been produced by an actinomycete strain AA3798.[153]

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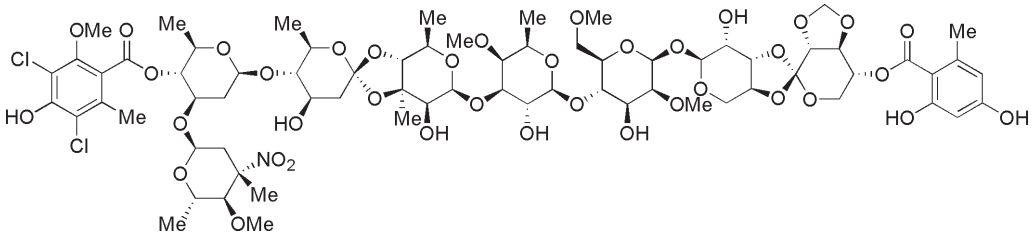

Figure 16. New everninomicin component, everninomicin 13,384-1.

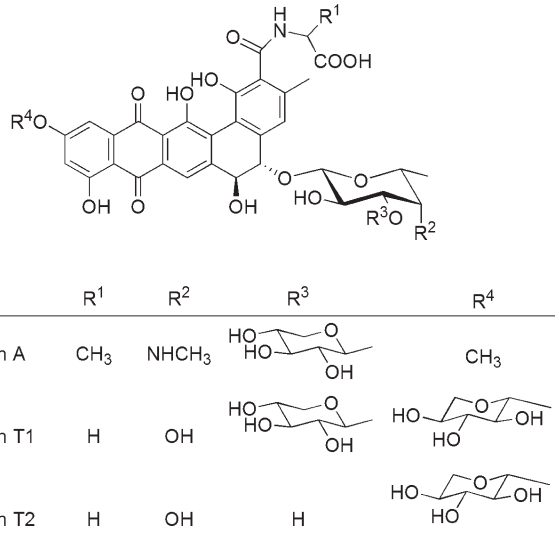

Figure 17. L-Xylopyranose containing antifungal pardimicin antibiotics.

These new antibiotics contain a β-L-xylopyranosyl bound to the hydroxyl group at C-11 of the dihydrobenzo[α]naphthacenequinone scaffold.[154] The pradimicins are activated by calcium, and the active complex formed has been shown to recognize and bind specific sugars on the fungal cell surface in a lectine-like manner, resulting in induction of potassium leakage.[155] Pradimicin T1 was reported by Oki and others to provide MICs ranging from 1.6 to 25 μg/mL when tested against 12 fungi, which is similar to earlier results obtained for pradimicin A (Figure 17).[151] The activity profile of pradimicin T2 was, on the other hand, more narrow.[153] However, only in the case of the *Candida albicans* strain ATCC 38247 did pradimicin T1 and T2 show activity superior to the antifungal drug amphotericin B (MIC = 1.6, 3.1, and 12.5 μg/mL, respectively).

2.2.4. Antibacterial and Antifungal Compounds Reported in the Patent Literature Only. Antibacterial and antifungal agents of interest reported in the patent literature only have been collected in Table 2 providing also references to the original source from which information on the pharmaceutical activity can be retrieved.[156−158]

2.3. Antineoplastic Compounds

The relatively few differences between cancer cells and normal cells makes the development of new antineoplastic drugs even more challenging than in the case of antiviral and antibacterial therapies. The most significant difference between these cells is in the length of their cell cycles. Accordingly, the selectivity of most antineoplastic drugs used today is based on the rapid cell division in cancer. Unfortunately, due to their cytotoxic properties, such drugs also act on cells dividing rapidly under normal circumstances, including cells in the bone marrow, digestive tract, and hair follicles, being responsible for most of the side effects associated with cancer therapies.

2.3.1. Alkylating Agents. Alkylating agents are a group of cytostatic drugs that act by forming covalent bonds to vital targets in the cell, particularly DNA, and hence impairing cell functions. The activity of this class of compounds was discovered during a military operation in World War II when a group of Allied soldiers and Italian civilians were accidentally exposed to mustard gas and were later found to suffer from leucopenia (low white blood cell counts). On the basis of these findings, Louis Goodman and colleagues reasoned that an agent damaging the rapidly growing white blood cells might have a similar effect on cancer. In the early 1940s, several patients diagnosed with Hodgkin's disease, lymphosarcoma, leukemia, or other types of cancer were treated with nitrogen mustard, *N*-methylbis(2-chloroethyl)amine hydrochloride (later known as chloromethine), showing remarkable, albeit temporary, improvement.[159] Another class of DNA alkylating agents widely utilized in cancer therapy consists of *N*-alkyl-*N*-nitrosoureas. In 1968 it was discovered that the antibiotic compound streptozotocin (*N*-(methylnitrosocarbamoyl)-α-D-glucosamine) possessed excellent activity against a malignant islet-cell tumor.[160] During the next decade, the syntheses of several new glycosylated nitrosourea derivatives were reported.[161] More recently, Gorbacheva and co-workers, inspired by the reduced myelosuppressive toxicity associated with glycosylated nitrosoureas,[162] reported excellent in vivo cytostatic properties of a new nitrosourea, 3-α-L-arabinopyranosyl-1-methyl-1-nitrosourea (aranoza, Figure 18), in L1210 leukemia cells in mice.[163]

In comparison with the clinically approved antitumor agent MNU (*N*-methyl-*N*-nitrosourea), aranoza showed a more pronounced inhibition of DNA synthesis and prolonged inhibition of RNA synthesis in L1210 leukemia cells. In addition, a marked decrease in the carbamoylating activity, believed to play a major role in bone marrow toxicity, was observed in comparison with MNU. Clinical phase I−II trials including 337 patients with different types of malignant tumors confirmed the antitumor activity of aranoza, providing good response rates (24.8, 66.6, 50, 36.4, and 40%) in melanoma, uterine cancer, breast cancer, Hodgkin's disease, and lymphosarcoma, respectively, producing only mild side effects.[164] One of the limiting factors in the use of *N*-nitrosoureas in clinical practice is the rapid development of tumor resistance. Goodtzova and co-workers studied the repair mechanism of DNA damage induced by aranoza in sensitive and resistant leukemia cells in mice and found a significant increase in the O^6-methylguanine-DNA methyltransferase (MGT) activity in the resistant cells.[165] O^6-Methylguanin (O^6-MG) has been shown to be the major cytotoxic lesion induced by this class of alkylating agents.[166] It was found that the aranoza-resistant cells had an MGT activity 9 times higher than the sensitive ones. Further, the study demonstrated that the aranoza treatment only resulted in a rapid decline of MGT activity in the resistant cells

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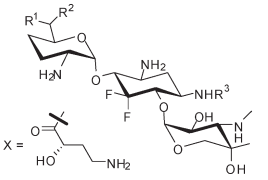
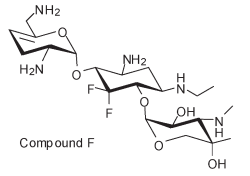
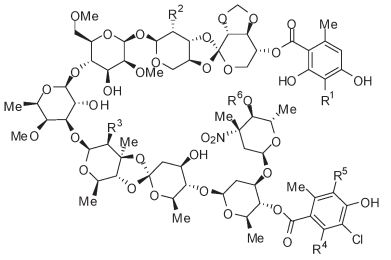
Table 2. Antibacterial and Antifungal Compounds Containing L-Pentoses Reported in the Patent Literature Only

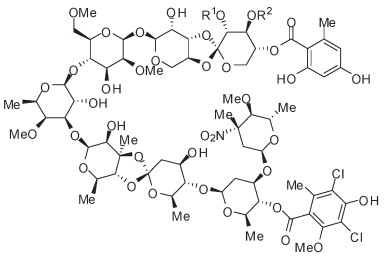
Molecular structure	Phase	Activity	Ref.
	Biological testing	Antibacterial aminoglycoside derivatives	156

	R^1	R^2	R^3
Compound A	CH_3	$NHCH_3$	H
Compound B	CH_3	$NHCH_3$	X
Compound C	CH_3	NH_2	H
Compound D	CH_3	NH_2	X
Compound E	H	NH_2	H

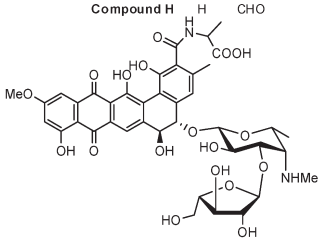


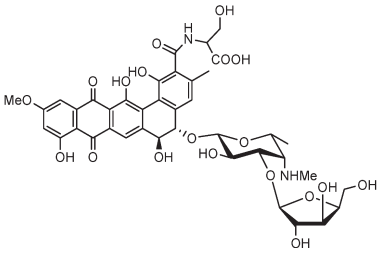
	Biological testing	Antibacterial orthosomycins	157

	R^1	R^2	R^3	R^4	R^5	R^6
Compound A	Cl	OH	OH	OMe	Cl	Me
Compound B	H	OH	=O	OMe	Cl	Me
Compound D	H	OMe	OH	OMe	Cl	Me
Compound E	H	OH	OH	OH	H	Me
Compound F	H	OH	OH	OMe	H	Me
Compound G	H	OH	OH	OMe	Cl	H



	R^1	R^2
Compound C	Me	Me
Compound H	H	CHO

	Biological testing	Antifungal pardimicin derivatives	158



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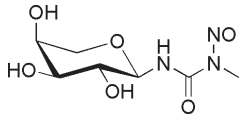


Figure 18. Structure of aranoza a L-arabinopyranosyl nitrosourea derivative.

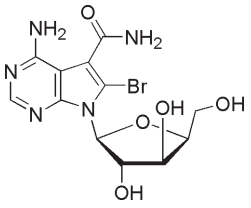
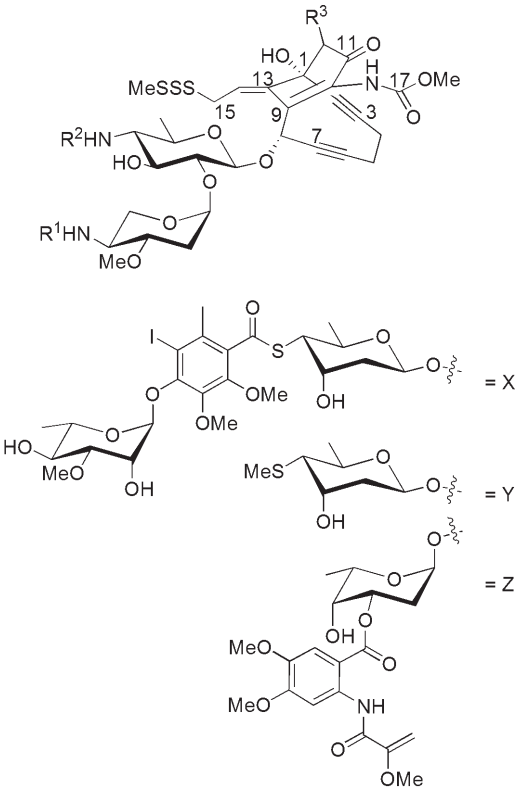


Figure 19. Protein kinase inhibitor xylocydine.

which however was recovered after 72 h while in the sensitive cells the treatment resulted in a 2-fold decrease of activity which remained at this level for 72 h. Preliminary experimental data demonstrated a synergistic effect of aranoza in combination with cisplatin in the treatment of disseminated malignant melanoma (response rate, 56.3%). A randomized phase III clinical study further confirmed the combination of aranoza and cisplatin to be sufficiently well-tolerated and relatively effective.[167] Additionally, aranoza was tested in combined chemotherapy together with interferon alpha, although this combination appeared unable to improve the response. Aranoza has been approved by the Russian medical authority for clinical use in patients with advanced malignant skin melanoma.[168] Further clinical trials are in progress to evaluate new aranoza-containing combinations in patients with melanoma and small cell lung carcinoma.

2.3.2. Protein Kinase Inhibitors. Protein kinase inhibitors are enzyme inhibitors that specifically block the action of one or more protein kinases. Serine/threonine protein kinases such as the cycline-dependent kinases (CDKs) play an important role in the proliferation and differentiation of eukaryotic cells by phosphorylating target proteins which are crucial for the cell cycle progression. CDKs are considered as potential targets for anticancer therapy since up-regulation of the activity of these enzymes is occurring frequently in cancer cells due to loss or low expression of CDK inhibitors. In an attempt to search for selective CDK inhibitors, Chun, Lee, and others synthesized xylocydine (Figure 19),[169] a brominated L-xylose analogue of the naturally occurring antitumor active nucleoside analogue antibiotic sangivamycin.[170]

Preliminary results showed that xylocydine exhibited potent inhibitory activity against the cycline-dependent protein kinase Cdc2 (IC_{50} = 1.6 μM) as compared to other reported Cdc2 inhibitors including olomoucin and butyrolactone-1 (IC_{50} = 7.0 and 0.6 μM, respectively). More recently, it was shown by in vitro kinase assays that xylocydine selectively blocks the activity of CDK1 and CDK2/cyclin A (IC_{50} = 1.4 and 61 nM) while exhibiting only minor inhibition of other serine/threonine protein kinases tested (IC_{50} = 21−86 μM).[171] Furthermore, xylocydine showed a dose-dependent growth inhibition on a human hepatocellular carcinoma (HCC) cell line when 2−50 μM doses were used. In vivo studies demonstrated that xylocydine significantly suppressed HCC cell growth (82%) in nude mice carrying human HCC cells when administered intraperitoneally (IP; 100 (mg/kg)/day for 3 weeks).[172] Moreover, xylocydine did not give rise to any toxic effects on the liver or kidney and did not induce loss in body weight of mice when treated with the novel CDK inhibitor with 200 (mg/kg)/day doses for 18 days.



	R^1	R^2	R^3
Esperamicin A_1	*i*-Pr	Y	Z
Calicheamicin	Et	X	H

Figure 20. Ten-membered enediyne anticancer antibiotics esperamicin A_1 and calicheamicin.

2.3.3. Anticancer Antibiotics. This structurally diverse class of antitumor active agents consists of naturally occurring DNA-damaging secondary metabolites, typically isolated from several *Streptomyces* and *Micromonospora* species.[173] In the present review, the coverage is limited to the "ten-membered enediyne anticancer antibiotics" only. Such compounds are structurally characterized by two distinct regions: (1) an aglycon part, consisting of an unsaturated 10-membered, bridged ring structure containing two acetylenic groups conjugated with a double bond, and (2) an oligosaccharide fragment consisting of two to four monosaccharide units including a 4-(alkylamino)-2,4-dideoxy-3-*O*-methyl-α-L-threopentopyranose residue.

Esperamicin (Figure 20) was the first member of this new group of anticancer antibiotics to be discovered, being first isolated in 1985 from an *Actinomadura verrucosospora* strain.[174]

During the next 3 decades, the isolation of three new glycosylated 10-membered enediynes was reported: calicheamicin (Figure 20) from *Micromonospora echinospora* in 1987,[175] namenamicin (Figure 21) from *Polysyncraton lithostrotum* in 1996,[176] and, most recently, shishijimicin (Figure 21) from *Didemnum proliferum* as late as in 2003.[177]

The discovery of esperamicin received considerable interest due to its unique core structure and antitumor properties.[178] The extreme cytotoxicity of esperamicin, with IC_{50} values ranging from 0.3 to 8.3 ng/mL against various murine and human tumor

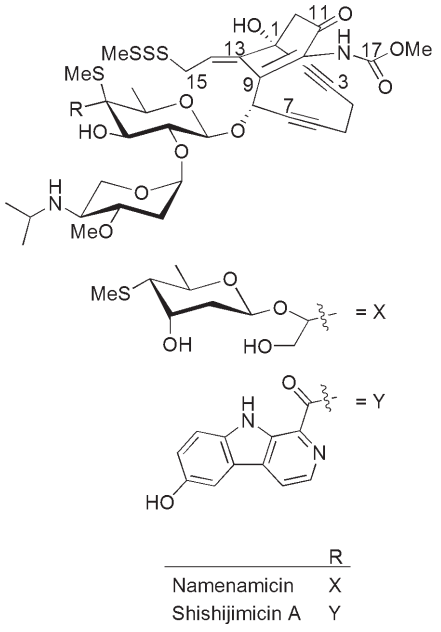


Figure 21. Structures of the anticancer antibiotics namenamicin and shishijimicin.

cell lines, was investigated and found to be related to its ability to form single-stranded and double-stranded DNA breaks.[179] The unique mechanism by which esperamicin produced the observed DNA lesions was proposed to be triggered by the reduction of the methyl trisulfide group to a thiolate anion followed by intramolecular addition of the anion to the adjacent α,β-unsaturated ketone. This converts the bridgehead to a tetragonal center, inducing a great deal of strain to the 10-membered ring which is released by the enediyne undergoing a Bergman rearrangement forming a phenylene diradical.[180] The diradical is the active form of the drug abstracting hydrogens from the deoxyribose of DNA leading to DNA breakage. Radical formation, and hence the DNA cleavage by esperamicin, was demonstrated to be significantly accelerated by thiol compounds such as cysteine and glutathione. Furthermore, the trisaccharide side chain of esperamicin was shown to play an important role in the binding of the drug to the minor groove of DNA, where it preferentially attacks sequences including 5′-CTC-3′, 5′-TTC-3′, and 5′-TTT-3′.[181] In vivo evaluations of the antitumor activity of esperamicin, by IP or IV administration, were performed using a variety of leukemia, melanoma, and lung and colon carcinomas xenografted into nude mice. Esperamicin was active against all IP implanted tumors when administered intraperitoneally. Additionally, it showed activity against IV implanted murine leukemias when administered intravenously, demonstrating that esperamicin is active against tumors located distal to the point of drug administration.[182]

Calicheamicin, the most prominent member of the 10-membered enediynes, showed excellent in vivo activity against murine tumors being approximately 1000-fold more active than the anthracycline antibiotic adriamycin at doses of 0.5−1.5 μg/(kg of body weight).[183] Chalicheamicin was shown to possess a similar mechanism for the antitumor activity as esperamicin.[184] However, in contrast to esperamicin, calicheamicin displayed high specificity for sequences such as 5′-TCCT-3′ and 5′-TTTT-3′, efficiently cutting the double-stranded DNA at •T and G•C-rich sequences.[185] This ability to specifically recognize both A•T and G•C-rich sequences makes calicheamicin an unusual minor-groove binder. Despite the remarkable cytotoxicities of enediynes, clinical applications of these compounds have been limited due to relatively unspecific mode of action. To overcome the limitations, much research has been focused on the development of enediyne derivatives with improved selectivity toward tumor cells. A promising approach for achieving more selective treatment is based on the use of monoclonal antibodies (mAbs) for targeting the cytotoxic agent toward the cancer cell.[186] The antibody targeted strategy is based on the expression of unique and tumor-specific antigens on the surface of cancer cells which some of the mAbs are able to recognize and specifically bind. These tumor-associated antigens thus enable a tumor specific mode of therapy.[187] The calicheamicin-based antibody−drug conjugate (ADC) gemtuzumab ozogamicin (GO) is the first anticancer agent linked to a mAb that has been used in clinical practice (Figure 22).[188]

The drug is comprised of the humanized anti-CD33 antibody (produced from a mammalian myeloma cell line) covalently bound to *N*-acetyl-γ-calicheamicin dimethyl hydrazide via a acid-labile 4-(4′-acetylphenoxy) butanoic acid linker which releases calicheamicin at the lower pH of the intercellular compartment following internalization (endocytosis) of the ADC.[189] CD33 is found on leukemic lymphoblasts from the majority of patients with acute myeloid leukemia (AML) but not on normal hematopoietic stem cells, thus being an attractive target for mAb-based AML therapy. GO was approved by the FDA in 2000 under the name Mylotarg for use in patients over 60 years of age suffering from relapsed AML and has been widely utilized in CD33-positive patients since then.[190] GO alone provides, as a standard dose 9 mg/m^2 by IV infusion, a well-tolerated treatment of CD33-positive patients with an overall remission rate of 28%.[191] However, as with most drugs in clinical use, the increasing use of GO has also been shadowed by the growing numbers of reports on patients showing enediyne resistance due to overexpression of multidrug-resistance proteins (MRPs) belonging to the adenosine triphosphate (ATP)−binding cassette (ABC) transporters,[192] and severe adverse drug effects such as development of hepatic veno-occlusive disease.[193] The problems associated with the MRP expression have been significantly reduced by the use of GO in combination with the multidrug resistance modifier cyclosporine. A phase II clinical study showed increased overall remission rate (34%) when cyclosporine was included into GO-based regimens.[194] Preliminary clinical results from a study using GO-based combination regimens on younger people with AML demonstrated a high complete response rate (90%) and favorable safety profile, suggesting that GO-based combination regimens could be used as a first-line therapy for newly diagnosed CD33-positive AML patients younger than 65 years as well.[195] The successful use of GO as a target-specific chemotherapy has led to many attempts to expand the ADC strategy to provide tumor-specific treatment options for other cancers. One of the attempts led to the development of CMC-544 (Figure 22), a CD22-targeted immunoconjugate of *N*-acetyl-γ-calicheamicin dimethyl hydrazide.[196] CD22 is an antigen expressed on B-lymphocytes, thus being an attractive target in the treatment of B-lymphoid malignancies. CMC-544 bound CD22 with sub-nanomolar affinity and caused a potent growth inhibition of human B-cell lymphoma xenografted into mice. Unfortunately, but not unexpectedly, a recent report demonstrated that CMC-544 significantly reduced activity on MDR expressing cell lines.[197] Yet another application where the same bifunctional

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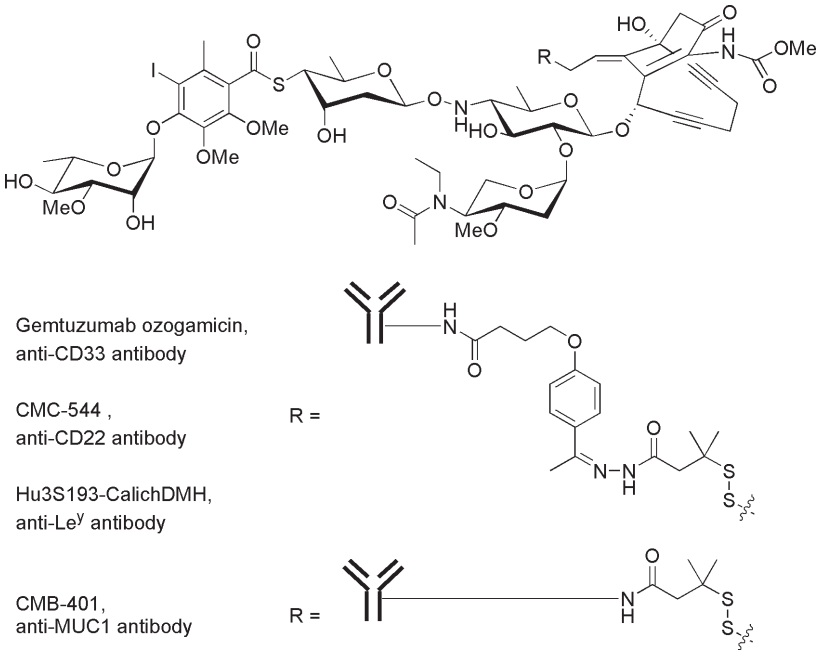

Figure 22. *N*-Acetyl-γ-calicheamicin-based antibody-drug conjugates.

acid labile linker as in GO and CMC-544 has been utilized is the development of a calicheamicin conjugate of hu3S193 (Hu3S193-CalichDMH, Figure 22), a humanized antibody recognizing the Lewis^y antigen which is highly expressed on various carcinomas.[198] In contrast to CMC-544 and GO which are active against one specific tumor, the hu3S193-calicheamicin conjugate showed in vivo growth inhibition of xenografted human gastric, colon, and prostate carcinomas.

Theories have been presented indicating that the linker technology based on the pH-dependent release mechanism being utilized in the ADCs mentioned above possibly in part bears the responsibility for the reduced activity in MRP expressing tumors. Since these ABC transporters actively pump the detached calicheamicin out of the cell, the intracellular concentration of the agent and the activity will decrease. These hypotheses were supported by in vitro and in vivo studies comparing the cytotoxic activity of an *N*-acetyl-γ-calicheamicin conjugate of CTM01, a murine mAb targeted against the MUC1 antigen expressed on a number of epithelial cancer cells, having an amide linker stable to hydrolysis with a corresponding conjugate containing the acid labile hydrazone linkage.[199] The results demonstrated that the amide conjugate was equivalent or even superior to the hydrazone conjugate in all preclinical models that were examined. The amide conjugate was further developed using a fully humanized version of the antibody, hCTM01, having an immunoaffinity approximately 30% higher than the murine antibody. This conjugate, referred to as CMB-401 (Figure 22), showed good results in mice xenografted with human breast carcinoma giving complete regression by either IP or IV administration. In clinical trials, however, CMB-401 showed only limited evidence of activity for ovarian cancer as well as for lung cancer.[200]

2.3.4. Plant Saponins. In 1992, Sashida, and co-workers reported the isolation of a new class of cholestane glycosides from the bulbs of *Ornithogalum saundersiae*, a member of the lily family cultivated in Africa as a garden plant.[201] These saponins share the same 16β,17α-dihydroxycholest-22-one steroidal scaffold containing an acylated β-D-xylopyranosyl-(1→3)-α-L-arabinopyranosyl substituent at the C-16 hydroxyl group. All members of these new saponins

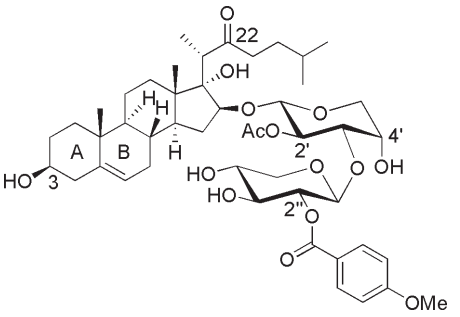

Figure 23. Structure of the anticancer active steroidal saponin OSW-1.

possessed outstanding cytotoxicity against leukemia HL-60 cells with IC_{50} values ranging from 0.1 to 0.3 nM.[202] The major constituent OSW-1 (Figure 23), having an acetyl group at the O-2′ and a *p*-methoxybenzoyl group at the O-2″, was the most potent, showing strong cytostatic activities against various malignant tumor cell lines being 10−100 times more active than the clinically applied anticancer agents mitomycin C, adriamycin, cisplatin, camptothecin, and taxol.

Furthermore, in vivo evaluation showed that OSW-1 prolonged the life span of mice infected with P388 leukemia by 59% with a single administration of 0.01 mg/kg. Regardless of the exceptional cytotoxicity against different human tumors, OSW-1 showed surprisingly low toxicity against normal human pulmonary cells (IC_{50} = 1500 nM). When tested against the NCI (U.S. National Cancer Institute) 60 cell lines, OSW-1 showed a mean IC_{50} of 0.78 nM with a cytotoxicity profile similar to that of cephalostatins. The similarity in cytotoxicity profile suggests that these two compound classes could share a related mechanism of action. Fuchs and co-workers proposed that an oxocarbenium ion formed at the 22-ketone might be the intermediate responsible for the anticancer activity of OSW-1 and cephalostatins.[203] Various analogues of OSW-1 have been prepared and tested for cytotoxicity, providing a preliminary set of structure−activity relationship information.

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Modifications of the carbohydrate part have shown that the disaccharide moiety is crucial for the antitumor activity.[204] In particular, removal of the acyl groups at the 2′ and 2″ positions or the OH group at the 4′-position drastically decreases the cytotoxicity of OSW-1.[205] Since the formation of C22-oxo-carbenium ion was suggested to be involved in the mechanism of action, a number of modifications in the C-17 side chain have been performed. It was found that the side chain could tolerate certain modifications without affecting the antitumor potency, for example, replacement of the ketone group by an ester group provided the 23-oxa analogue of OSW-1 with activity similar to that of the parent compound.[206] Surprisingly, the antitumor activity of OSW-1 was found to be independent of the 22-one function, excluding the possibility of a common mechanism of action with the cephalostatins.[207] Furthermore, Yu and co-workers showed that the intact steroid ring is not required for the biological activity and that OSW-1 analogues where the steroidal A,B-ring was removed retained considerable inhibitory activity against the growth of HeLa and Jurkat cells (IC_{50} = 0.8 to 21.1 μM).[208] While several groups have reported the total synthesis of OSW-1,[209] the limited availability, due to the complex chemical synthesis of the compound, constrains its biological testing. Quite recently, however, it has been shown that OSW-1 acts by a mechanism which damages the mitochondrial membrane of human leukemia and pancreatic cancer cells, triggering the activation of the Ca^{2+}-dependent apoptosis pathway.[210] This mechanism differs from those of all other anticancer agents examined to date which makes OSW-1 an attractive drug candidate as it might have potential in treating cancers that are resistant to currently available drugs.

In addition to OSW-1, steroidal saponins showing cytostatic activity on HL-60 cells (IC_{50}'s from 1.3 to 9.7 μg/mL) have been isolated from the bark[211] and leaves[212] of *Dracaena draco* (Agavaceae). Furthermore, steroidal saponins from the roots and rhizomes of *Dracaena angustifolia* were shown to possess antiproliferative activity against human HT-1080 fibrosacroma, having IC_{50} values ranging from 0.2 to 0.6 μg/mL.[213]

Recently, several acacic acid-based saponins isolated from different *Acacia* and *Albizia* species have been reported to show marked cytotoxic activity against human fibrosacroma,[214] hepatoma,[215] leukemia,[216] and ovarian cancer cells.[217] The avicins, isolated from the pods of *Acacia victoriae* Benth. (Leguminosae) in 2001, are probably the most thoroughly studied anticancer active acacic acid-based triterpenoid saponins.[218]

The main fraction obtained from the extraction of these saponins showed high activity against Jurkat cells with an IC_{50} value of 0.2 μg/mL.[219] Additionally, the same fraction inhibited the growth of a number of cancer cell lines with IC_{50}s in the range of 0.72−6.5 μg/mL. However, normal cells (mouse fibroblasts and immortalized breast epithelial) were not significantly affected, showing an IC_{50} > 25 μg/mL. From the main fraction, the two most potent compounds were further separated and purified, namely, avicin D and avicin G (Figure 24). Avicin G, being the most potent, showed significantly higher growth inhibitory activities (IC_{50} 0.12−1.49 μg/mL) than avicin D and the main fraction. The structures of avicins D and G were reported to be an acacic acid scaffold with a trisaccharide unit, β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-β-D-2-acetamido-2-deoxyglucopyranosyl, and a tetrasaccharide unit, α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl, bound to OH-3 and COOH-28, respectively.[220] Furthermore, both contain an ester-linked side

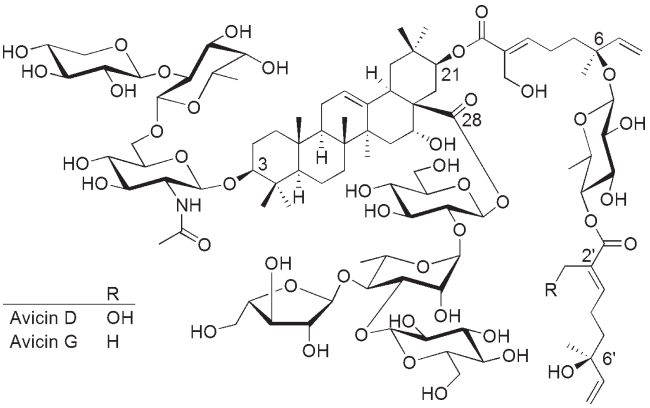

Figure 24. Acacic acid-based saponins avicin D and avicin G.

chain at C-21 composed of two monoterpene units separated by a β-D-quinovopyranosyl residue. The only structural difference between avicin D and G is that avicin D has a hydroxymethyl group at the 2′-position of the outer monoterpene while avicin G has a methyl group at the same position. The avicins D and G are diastereoisomers of the previously reported anticancer active triterpenoid saponins, isolated from *Archidendron ellipticum*, eliptoside E and A, respectively, having opposite stereochemistry at C-6 and C-6′ of the monoterpene units.[221] The mechanism by which the avicins act was found to be induction of cancer cell apoptosis by direct perturbation of the mitochondria.[219] The avicins were further shown to suppress the development of dimethylbenz[α]anthracene (DMBA)-induced skin carcinogenesis in mice by 70% and exhibit an antimutagenic and antioxidant effect.[222] Furthermore, the avicins have been reported to inhibit the tumor necrosis factor (TNF)-induced activation of nuclear transcription factor-κB (NF-κB) in Jurkat cells. It was proposed that this inhibition is due to the interaction of the α,β-unsaturated esters on the monoterpene side chain with a cystein thiol group on NF-κB via Michael-type addition.[223] More recent studies have, however, demonstrated that the avicins suppress NF-κB in a dithiothreitol (DTT)-reversible manner and that the Michael acceptor sites are apparently not involved, but rather the electronegative carbonyl groups which undergo transesterification with the cysteine thiol group of NF-κB.[224] Gutterman and co-workers also demonstrated that the mechanism by which the avicins act on the mitochondria is by increasing the permeability of the outer mitochondrial membrane to cytochrome *c*, leading to inhibition of respiration and increased sensitivity of cells to oxidative stress.[225] The inhibition of respiration was further demonstrated to be mediated by the closure of a voltage-dependent anion channel (VDAC) which slows down the cellular metabolism, thereby preparing the cells for apoptosis.[226] The induced hypersensitivity of the mitochondria to oxidative stress could have great synergistic potential if avicins would be used in combination with anticancer drugs which increase generation of reactive oxygen species (ROS) in tumor cells, such as cisplatin.

Besides the acacic acid-based saponins, triterpene saponins based on oleanolic acid or hederagenin have demonstrated potent cytotoxic or cytostatic activity. Kingston and co-workers have reported the isolation and structure determination of several triterpenoid saponins from *Albizia subdimidata* (Splitg.) Barneby & J. W. Grimes (Fabaceae),[227] and *Acacia tenuifolia* (L.)

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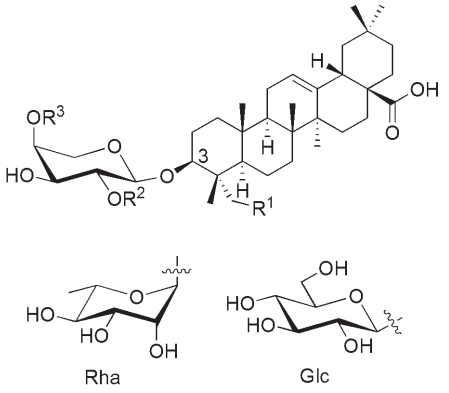

	R^1	R^2	R^3
Hederacolchiside A	OH	Rha	Glc
Hederacolchiside A_1	H	Rha	Glc
α-Hederin	OH	Rha	H
β-Hederin	H	Rha	H
δ-Hederin	OH	H	H

Figure 25. Structures of the oleanolic acid- and hederagenin-based saponins hederacolchiside A and A_1 and α-, *β*-, and *δ*-hederin.

Willd. (Mimosaceae)[228] showing significant cytotoxicity against mammalian cell lines. Furthermore, anticancer active triterpenoid saponins with similar scaffolds have been isolated from *Acantophyllum squarrosum* Boiss. (Caryophyllaceae),[229] and *Trevesia palmata* (Roxb. Ex Lindl.) Vis. (Araliaceae),[230] the latter showing antiproliferative activity against J774 murine monocytemacrophage, WEHI-164 murine fibrosarcoma, and HEK-293 human epithelial kidney cell lines with IC_{50}'s ranging from 0.06 to 0.52 *μ*M. The hederacolchisides and hederins isolated from a variety of higher plants belonging to the families Araliaceae and Ranunculaceae have demonstrated potent antitumor activities against a number of cancer cells. For example, Sashida and co-workers reported that hederacolchiside A and A_1 and α- and *β*-hederin, isolated from *Pulsatilla chinensis* (Bunge) Regel, showed cytotoxic activity against HL-60 human leukemia cells with IC_{50} values of 3.8, 2.3, 7.1, and 4.4 *μ*g/mL, respectively (Figure 25).[231]

Danloy et al. isolated α-hederin (hederagenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside) from *Hedera helix* and showed in vitro that the saponin-inhibited proliferation of mouse B16 melanoma cells and induced membrane alteration leading to cell death at rather low concentrations (<5 *μ*g/mL).[232] However, α-hederin showed no specificity for cancer cells but inhibited the cell division of noncancer mouse 3T3 fibroblasts as well. Hederacolchiside A (hederagenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→4)]-α-L-arabinopyranoside) has demonstrated in vivo anticancer activity inhibiting the rate of tumor growth in mice xenografted with human lung carcinoma by 82.1% at a dose of 6.4 mg/(kg of body weight).[233] Hederacolchiside A_1 has in addition to *Pulsatilla chinensis* been isolated from *Hedera colchica* K. Koch and was demonstrated to exhibit significant in vitro antiproliferative activity against six human cancers (colon, ovarin, lung, breast, prostatic, and skin cancer) with IC_{50} values ranging from 4.5 to 12 *μ*M, thus being more potent than hederacolchiside A and α- and *β*-hederin.[234] In several studies, the cytotoxicities of various hederacochisides and hederins have been compared to gain information about structure−activity relationships. The results obtained suggest that the characteristic oligosaccharide, particularly the disaccharide *O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside, at C-3 of the triterpenoid scaffold is crucial for the activity.[231,234] These findings are further supported by the fact that *δ*-hederin (hederagenin 3-*O*-α-L-arabinoside) having a monosaccharide residue at C-3 only is noncytotoxic.[235] In addition to its tumoricidal activity, hederacolchiside A_1 has been shown to inhibit matrigel-induced angiogenesis at micromolar concentrations (<3 *μ*g/mL) which is below the IC_{50} value for inhibition of melanoma cell profileration (3.75−4.5 *μ*g/mL).[236] These results raise the possibility that hederacolchiside A_1 might slow down cancer proliferation and metastatis in vivo by inhibiting neoangiogenesis. In addition to their anticancer properties, hederacolchisides and hederins have been reported to possess antiinflammatory,[237] antioxidant,[238] and neuroprotective activity.[239]

Despite the significant antitumor activities of the steroidal and triterpenoid saponins, many of these have been reported to induce hemolysis of red blood cells, which is a major drawback for this type of abundant natural products.[235b,240] The correlation between the hemolytic and antitumor activity of this class of compounds must be explored before saponins can be clinically developed as antitumor agents.

A quillaic acid-based saponin fraction QS-21 (Figure 26) purified from bark extracts of the South American tree *Quillaja saponaria* Molina has found a different application in the cancer therapy as it demonstrated potent adjuvant activity but low toxicity.[241]

Adjuvants are substances that significantly enhance the immunogenicity of vaccines.[242] The success of antigene-based anticancer vaccines often requires the use of an adjuvant to boost the immune response. QS-21 showed antibody stimulation effect in mice when used as an adjuvant for antigen doses ranging from 1 to 125 *μ*g.[243] At all tested doses of antigen, a 5 *μ*g dose of QS-21 was required for a 100-fold increase in antibody titers. The adjuvant activity was nicely demonstrated by a combination of 1 *μ*g of antigen with 5 *μ*g of QS-21 inducing a higher antibody titer than that induced by 125 *μ*g of antigen in the absence of adjuvant. The adjuvant active triterpenoid fraction QS-21 comprises two structural isomers (in 2:1 ratio) that share the quillaic acid scaffold with a branched trisaccharide attached to 3-OH and a linear tetrasaccharide linked to the carboxyl group at C-17. Additionally, both isomers contain a dimeric acyl chain attached to the first sugar of the tetrasaccharide with L-arabinofuranosyl moiety at the end. The two isomers differ only in the structure of the terminal sugar of the tetrasaccharide segment, the major isomer incorporating a *β*-D-apiose residue while the minor one is capped with *β*-D-xylose.[244] Jacobsen and co-workers showed that during vaccine formulation the major constituent of QS-21 undergoes isomerization at neutral or alkaline pH via an intramolecular transesterification shifting the acyl chain from OH-4 to OH-3 of the D-fucose moiety in the tetrasaccharide.[245] Kinetic analysis of the isomerization and hydrolysis of the acyl chain showed that the pH of maximum stability of QS-21 was pH 5.5.[246] Furthermore, it was shown that the critical micellar concentration of QS-21 was 50 *μ*g/mL and that the adjuvant is more stable in micellar form. Hence the best formulation of QS-21 was in 20 mM succinate-buffered saline, pH 5.5, at a concentration of 500 *μ*g/mL. Helling et al. reported that a conjugate of G_{M2}, a cell surface ganglioside overexpressed in malignant melanoma, and keyhole limpet hemocyanin (KLH) with QS-21 was an effective vaccine inducing not only a long-lasting high-titer IgM G_{M2} antibody response but also a consistent high-titer IgG response in melanoma patients.[247] The

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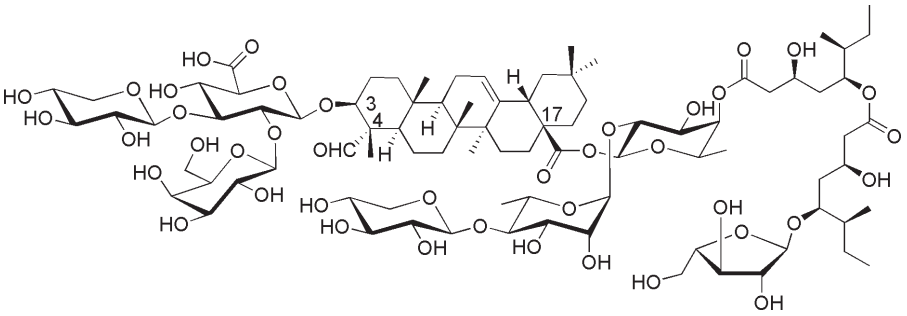
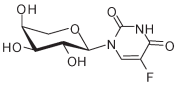
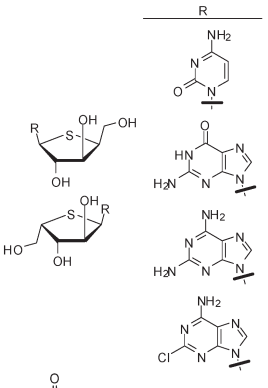
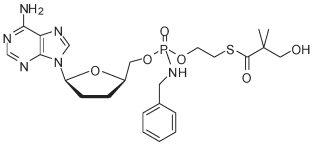


Figure 26. Quillaic acid-based saponin adjuvant QS-21.

Table 3. Antineoplastic Compounds Containing L-Pentoses Reported in the Patent Literature Only

Molecular structure	Phase	Activity	Ref.
	Biological testing	Pyrimidine-based antimetabolite	259
	Biological testing	Antineoplastic L-4′-thio-xylofuranosyl nuclosides	260
	Preclinical	Anticancer active nucleoside analog dimer	261
	Biological testing	Anticancer active phosphoroamidate prodrug	262

same group previously showed that mice immunized with a KLH conjugate of G_{D3}, another ganglioside expressed in malignant melanoma, and QS-21 demonstrated a long-lasting high-titer IgM response and consistent IgG antibodies.[248] Initial clinical studies showed that out of 6 melanoma patients immunized with a G_{D3}-lactone-KLH conjugate, a derivative of G_{D3}-KLH lactonized by acetic acid containing 30 μg of ganglioside plus 100 μg of QS-21, 4 patients showed IgG titers while all patients showed IgM titers against G_{D3}-L and G_{D3}.[249] A lactonized KLH conjugated to a third ganglioside abundantly expressed in melanomas, sarcomas, and neuroblastomas, G_{D2}, was also shown to induce a long-lasting antibody response in patients with melanoma when used with QS-21 as adjuvant.[250] Further, QS-21 has successfully been used as adjuvant with a fully synthetic globo H antigen vaccine in prostate cancer patients resulting in production of significant IgG and IgM antibody titers.[251] The same globo H-KLH conjugate vaccine plus QS-21 entered initial clinical trial with patients having methastatic breast cancer.[252] The vaccine was well-tolerated and demonstrated generation of IgM antibody titers in most patients. Small cell lung cancer patients have also been reported to produce IgM antibodies against polysialic acid (polySA) when vaccinated with 30 μg of *N*-propionylated (NP)-polySA conjugated KLH mixed with 100 μg of QS-21.[253] Some of the patients produced IgG antibodies to NP-polySA, but these did not cross-react with polySA. In addition to cancer vaccines, QS-21 has been used as adjuvant in numerous clinical trials of vaccines against infectious diseases such as malaria,[254] hepatitis B,[255] and HIV.[256] Despite the wide applications of QS-21 as a vaccine adjuvant, relatively little is known about the mechanism by which it enhances the immune

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response or the minimum critical structure of QS-21 for these adjuvant activities. Structure−activity studies have shown that the aldehyde group at C-4 is critical for the adjuvant effect, and it has been suggested that the aldehyde of QS-21 might interact with T-cell surface receptors forming a Schiff base with a free amino group.[257] Moreover, it has been demonstrated that the dimeric acyl chain is important for the activity as hydrolysis of the ester linkage significantly reduces the adjuvant activity.[246] Recently, Livingston, Ragupathi, Gin, and others reported the synthesis and evaluation of chemically stable QS-21 analogues.[258] To overcome the hydrolytic instability of the ester linkage of the side chain, they replaced it with a more robust amide linkage. Remarkably, these new amide-modified analogues, some of which contain a considerably simplified acyl chain, enhanced the immunogenicity of the G_{D3}-KLH melanoma conjugate vaccine in mice in a manner similar to QS-21, still exhibiting lower overall toxicity effects. This finding, that simpler, hydrolytically stable semisynthetic saponin derivatives possess similar or even superior adjuvant activity than QS-21 should trigger the research of their, this far, unknown mechanism of action.

2.3.5. Anticancer Agents Reported in the Patent Literature Only. Antineoplastic agents of interest appearing only in the patent literature have been collected in Table 3, providing also references to the original source from which information on the pharmaceutical activity can be retrieved.[259−262]

3. CONCLUSIONS

As shown in the present review, L-pentoses are found in a wide variety of pharmaceutically active compounds with a range of potential applications, especially in the fields of antiviral, antibacterial, and anticancer therapy. Further, even if not covered within the present review, L-pentoses are additionally found in natural products with potential applications in pharmacological treatment of obesity,[263] neurodegenerative disorders[264] and inflammatory diseases such as rheumatoid arthritis.[265] Additionally, these kinds of compounds have been reported to possess potent thrombin receptor antagonist activity and hence could have applications in the treatment and prevention of atherothrombotic events.[266] Due to the growing demand for effective, selective, and nontoxic drug candidates in pharmaceutical research, new synthetic and semisynthetic compounds as well as compounds from natural sources are constantly screened for pharmaceutical activity. The L-nucleosides have drawn great attention during the past several , and significant progress has been made in the battle against viral infections and cancer using these mimics. In spite of the development of new potential synthetic drugs, natural compounds acquired from microbial and plant sources remain important in pharmaceutical applications. Ongoing biological studies are unveiling the mechanism of action and biological targets of these complex secondary metabolites, providing a better understanding of their interaction with the body at the molecular level. Together with the increasing knowledge on the various steps associated with drug uptake and disposition, the understanding of the substrate−receptor interactions can be used for effective design and evaluation of new analogues of these potent drug candidates.

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Jonas J. Forsman, born in 1978, studied organic chemistry at Åbo Akademi University, obtaining his M.Sc. in 2004 with biochemistry and pharmaceutical chemistry as minor subjects and Ph.D. in 2011 under the guidance of Professor Reko Leino at the Laboratory of Organic Chemistry at Åbo Akademi University. His thesis research focused on the utilization of rare sugars in the synthesis of pharmaceutically active ingredients. He recently joined the PCAS Finland Oy fine chemical company and currently works on the development and manufacturing of active pharmaceutical ingredients for the pharmaceutical industry.

Reko Leino was born in Turku 1969. He studied chemical engineering at Åbo Akademi University where he joined the research group of late professor Jan H. Näsman (1955-2000) in 1993 and graduated with M.Sc. in chemical technology in 1994 and Dr.Sc. in technology in 1998 with the doctoral thesis "*Siloxy Substituted Metallocene Catalysts*". Following a postdoctoral year at Stanford University with Prof. Robert M. Waymouth, working on stereocontrol in syndiospecific propylene polymerization, he briefly returned to Åbo Akademi and then in 2001 joined the biopharmaceutical company Carbion Ltd. (later merged with Biotie Therapies Corp.), getting introduced to the development of carbohydrate-based pharmaceuticals. Since 2003 he has been professor of Synthetic Organic Chemistry at Åbo Akademi University and the Head of the Laboratory of Organic Chemistry

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