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Review Article

Milestones in camptothecin research

Hans-Georg Lerchen
Bayer AG, BHC PH-R-EU, Building Q18 51368 Leverkusen, Germany

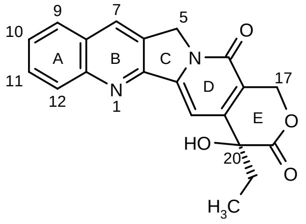
CONTENTS

Abstract

The discovery of the natural product camptothecin in the early 1960s provided the lead structure for a novel, highly promising class of antitumor agents. Significant progress involving the understanding of the mechanism of action, the drug metabolism, chemistry driven analoging programs and clinical research for dose and schedule optimization led to major breakthroughs in the field. Today, with two successful compounds in clinical practice (irinotecen and topotecan) and more than 10 clinical development programs, the campothecins have reached a very high clinical relevance.

Introduction

Camptothecin **1** is a pentacyclic alkaloid isolated by Wall *et al.* in the early 1960s from the Chinese tree, *Camptotheca acuminata* (1). The compound aroused immediate interest as a potential cancer chemotherapeutic agent due to its impressive activity against leukemias and a variety of solid tumors in experimental systems (Fig. 1).



(**1**)

Fig. 1. Structure of 20(*S*)-camptothecin.

Almost 40 years of experience and intense research efforts involving this class of agents reflect an interplay of enthusiasm and setbacks. Significant insights in understanding the mechanism of action resulted in major progress in the field. Today, with two successful compounds in clinical practice (irinotecan and topotecan) and 11 compounds in clinical development, the camptothecins have become a class of anticancer compounds of very high clinical relevance (Table I).

This review highlights several important milestones in the field such as the discovery of camptothecin, the elucidation of the mechanism of action and metabolism, the approval and clinical success of irinotecan and topotecan, and the different approaches and rationales for the development of second- and third-generation analogs currently in clinical development.

Discovery of camptothecin and early analoging activities

Camptothecin

In the early 1960s Wall and coworkers isolated 20(*S*)-camptothecin **1** from the Chinese tree, *Camptotheca acuminata* (1). Initial investigations indicated a high potency of the compound in a number of preclinical leukemia and solid tumor models. To overcome the poor solubility, the sodium salt was synthesized by hydrolysis of the lactone ring. This sodium salt forms an equilibrium with the ring-closed lactone form. As its sodium salt, camptothecin was moved to clinical trials in 1970. Promising activity was initially observed (2); however, severe side effects and drug-related toxicities finally led to discontinuation of the clinical program (3, 4).

Stimulated by the challenging structure and its very interesting biological activity, initial synthetic approaches to camptothecin were developed (5, 6). Today, a number of total syntheses of camptothecin and analogues have been described and reviewed extensively (7-9). Almost two decades after its discovery, structure-activity relationships were established in order to elucidate the mechanism of action of camptothecin. During semisynthetic or total synthetic chemistry programs the particular importance of the lactone ring and the 20(*S*)-configuration for

Table I: Camptothecins currently in clinical practice or in clinical development.

Drug Name	Delivery System	Source	Status
Irinotecan HCl (CPT-11)	Water-soluble	Pharmacia/Aventis	Launched
Topotecan HCl	Water-soluble	GlaxoSmithKline	Launched
Rubitecan (9-NC)	Lipophilic	SuperGen	Phase III
Exatecan Mesilate (DX-8951-f)	Water-soluble	Daiichi	Phase III
Lurtotecan (OSI-211)	Liposomal formulation	OSI Pharm.	Phase II
CKD-602	Water-soluble	Chong Kun Dang	Phase II
Diflomotecan (BN-80915)	Homocamptothecin	Beaufour-Ipsen	Phase II
Afeletecan HCl (Bay-38-3441)	Water-soluble prodrug	Bayer	Phase II
Prothecan®	PEG-conjugate	Enzon	Phase II
BNP-1350 (karenitecin)	Lipophilic	Bionumerik	Phase I
Gimatecan (ST-1481)	Lipophilic	Sigma-Tau	Phase I
DE-310	Polymeric conjugate	Daiichi	Phase I
Camptothecin Polyglutamate	Polymeric conjugate	Cell Therapeutics	Phase I

(**2**) R = NH_2 9-Aminocamptothecin
(**3**) R = NO_2 9-Nitrocamptothecin (Rubitecan)

(**4**) 10,11-Methylenedioxy-camptothecin

Fig. 2. A-ring substituted camptothecin analogs.

activity was recognized. In contrast, modifications in the A-ring and B-ring, particularly in the 9, 10 and 11 positions, were tolerated and led to improved analogues (10-12).

9-Aminocamptothecin and 9-nitrocamptothecin

From a series of A-ring modified early analogues of camptothecin, particularly 9-aminocamptothecin **2**, 9-nitrocamptothecin **3** and 10,11-methylenedioxy-camptothecin **4** were identified as the most potent analogues (13) (Fig. 2).

Both 9-aminocamptothecin and 9-nitrocamptothecin moved to clinical development. Development of 9-aminocamptothecin was terminated in 1999 by IDEC after extensive clinical trials.

However, 9-nitrocamptothecin (rubitecan, SuperGen), which is converted to 9-aminocamptothecin *in vivo*, is currently undergoing phase III clinical trials in pancreatic cancer (14). Three phase III studies have compared orally administered rubitecan with gemcitabine (first-line treatment), with 5-FU (prior treatment with gemcitabine) and with other chemotherapies in third-line treatment. Previously, rubitecan had been investigated by Stehlin Foundation (15, 16) and moved into clinical trials (17). An NDA for rubitecan (Orathecin^{TM}) is expected to be filed in 2002.

Mechanism of action

Molecular target

Almost 20 years after discovery of the camptothecins, the molecular target has been shown to be topoisomerase I (18). Topoisomerase I relaxes DNA by forming a covalent bond of its tyrosine 723 residue with the 3'-phosphate of the DNA backbone at the cleavage site. Camptothecin binds to and stabilizes the cleavable complex between topoisomerase I and DNA which inhibits the religation step and causes DNA double-strand breaks during replication. The cytotoxicity of camptothecin derivatives could be correlated with an accumulation of the cleavable complex (19, 20). This, rather than the total inhibition of topoisomerase I, might be the critical event leading to cytotoxicity. A better understanding of the interactions was provided by an x-ray crystal structure of the human topoisomerase I-DNA cleavable complex (21). Co-crystals were obtained from models of both the covalent and the noncovalent complex of topoisomerase I and DNA. Based on this work and also on results from mutagenesis and cross-linking studies, different structure-based models have been developed to explain the *in vitro* results on topoisomerase I inhibition by different camptothecin derivatives (22-24). These models and the

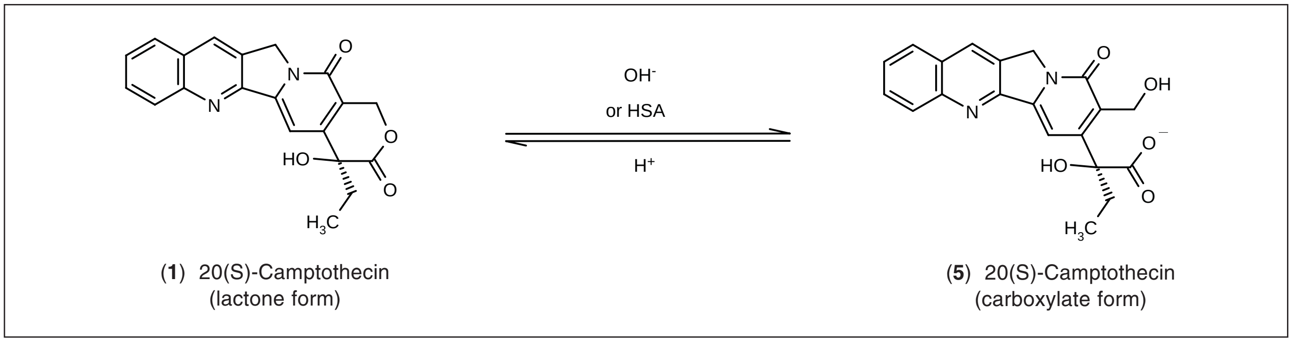


Fig. 3. pH-Dependent equilibrium of lactone and carboxylate form of 20(*S*)-camptothecin.

understanding of relevant interactions and sterical parameters might contribute to the design of highly potent analogues.

The understanding of the mechanism of action implies that camptothecins are specific to the S-phase of the cell cycle. Therefore, only a small portion of tumor cells is susceptible to camptothecins. This provides the rational for extending the duration of therapy and for slow-release drug formulations to achieve a sustained drug exposure and to maximize efficacy (25).

Lactone ring stability issues

Early structure-activity relationships already indicated the importance of the intact lactone E-ring of camptothecin for activity. Particularly, the ring opened carboxylate form of several camptothecin derivatives has been shown to be significantly less active (20, 26). The camptothecin lactone ring is easily hydrolized and forms an equilibrium with its ring opened carboxylate form with the carboxylate predominating at a pH of 7 or above (27) (Fig. 3). The clinical relevance of this equilibrium became obvious when it was found that human serum albumin preferentially binds the ring opened carboxylate form and thus drives the equilibrium to the inactive carboxylate form in human serum (28). In mouse serum the lactone form is much more stable. These findings might provide an explanation why the high activity observed in mouse models often was not predictive of the same level of clinical activity for camptothecins (Fig. 3).

Subsequently, the opening of the lactone ring particularly in human serum has been extensively investigated. A number of factors such as substitution pattern of camptothecin derivatives, lipophilicity, formulation, chemical modifications and environment have been shown to have an impact on lactone stability and biological activity (29).

Pharmacokinetic and pharmacodynamic investigations during clinical trials were very challenging. Efficient and highly sensitive HPLC-based methods for the separation and simultaneous detection of lactone and carboxylate forms of a variety of camptothecin derivatives starting from sample preparation directly at the bedside have been developed and extensively reviewed (30-33). Activity of camptothecins in the clinic to a certain extent has been shown to positively correlate with the levels of intact lactone.

Therefore, the development of second- and third-generation camptothecins clearly focused on both an improvement of DNA binding and a stabilization of the lactone ring. Successful approaches include water-soluble and lipophilic camptothecins with particular substitution patterns, homocamptothecins, prodrugs, delivery systems and liposomal formulations. These approaches are discussed in the following sections.

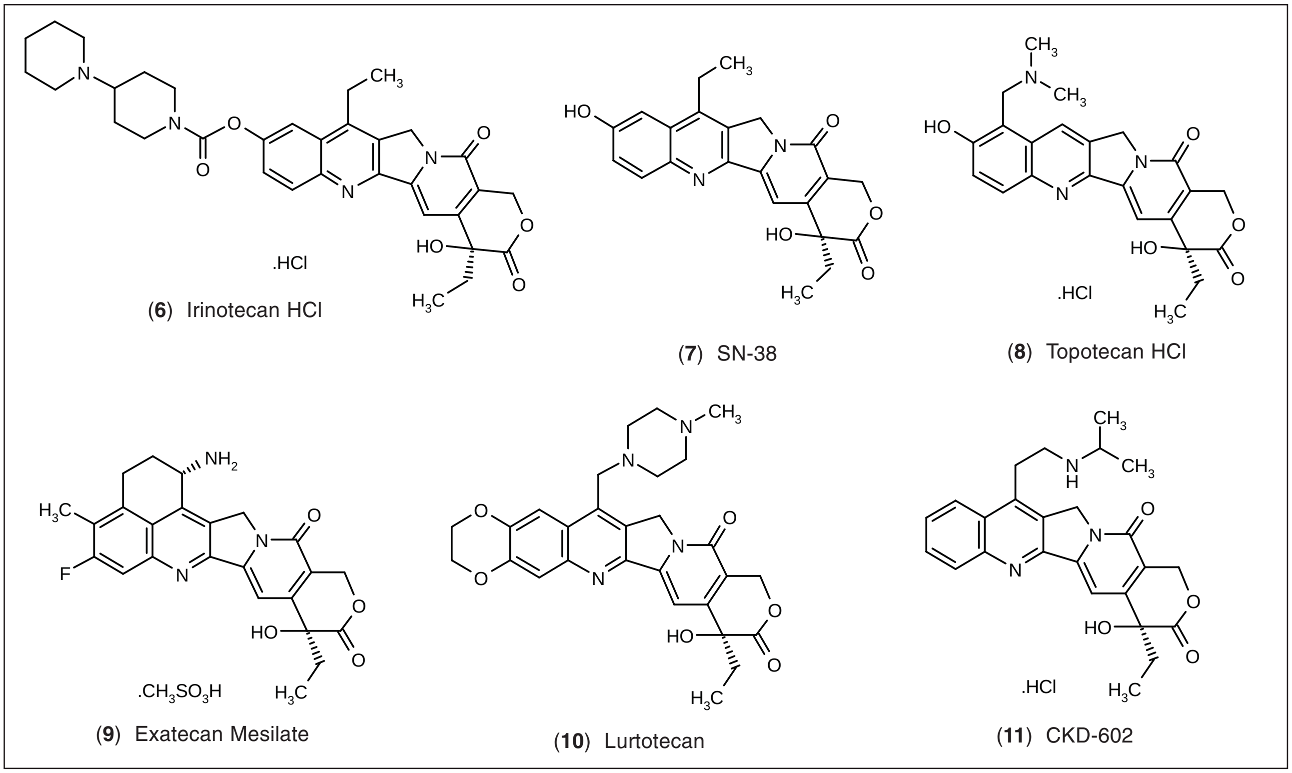
Water-soluble camptothecin analogues

Second-generation camptothecin derivatives have been optimized for improved water solubility to facilate intravenous drug administration. Highlights resulting from various programs at different companies and institutions are irinotecan **6** and topotecan **8**, two compounds which are successfully used in clinical practice, and exatecan **9**, liposomal lurtotecan **10** (OSI-211) and CKD-602 **11**, which are in advanced stages of clinical development (Fig. 4).

Irinotecan

Irinotecan **6** was discovered at Yakult Honsha and was first approved in Japan in 1994 (Camptotesin®) for lung, cervical and ovarian cancer (34). Today it is marketed in the U.S. by Pharmacia (Camptosar®) and by Aventis in Europe (Campto®). Irinotecan **6** is a prodrug which is cleaved *in vivo* by carboxylic esterases, particularly by hCE-2, to release the active metabolite SN-38 **7** (35, 36).

Several major milestones were involved in making irinotecan a successful anticancer agent. For example, progress in preclinical investigations provided insights in pharmacology, metabolism (enzymes involved in activation, detoxification and intestinal toxicities) and resistance mechanisms (37-41). Method development and determination of clinical pharmacokinetics and metabolism and its impact on administration schedules and combination therapies (33, 42) were also important, as well as



(**6**) Irinotecan HCl

(**7**) SN-38

(**8**) Topotecan HCl

(**9**) Exatecan Mesilate

(**10**) Lurtotecan

(**11**) CKD-602

Fig. 4. Water-soluble-second generation camptothecin derivatives.

evaluation of a variety of schedules demonstrating that diarrhea and short-lasting neutropenia are the major side effects. Large phase III studies established irinotecan as second-line therapy in colorectal cancer after 5-FU and, in 2000, the compound was approved as first-line treatment of metatstatic colorectal cancer in combination with 5-FU/leucovorin. Irinotecan has also been investigated in other malignancies such as gastrointestinial cancers, small cell lung cancer and non-small cell lung cancer (41, 43, 44).

Topotecan

Topotecan (Hycamtin®; GlaxoSmithKline) **8** is another semisynthetic camptothecin derivative that was launched in 1996 for the second-line treatment of metastatic ovarian cancer. In contrast to irinotecan which is a prodrug, topotecan **8** has been selected for intrinsic activity against the enzyme (45).

Both preclinical and clinical data suggest that a prolonged exposure to the drug is favorable for activity. Therefore, most clinical data are obtained with a daily infusion through 5 days (46, 47). In a randomized phase III trial in ovarian cancer patients, topotecan showed similar activity as paclitaxel (48). In 1998 topotecan was approved for second-line treatment of refractory small cell lung cancer (49). More recently, a number of studies have focused on orally administered topotecan (50). The drug has also shown good potential in several combination regimens together with other cytotoxic agents such as cisplatin, carboplatin, doxorubicin and etoposide (51).

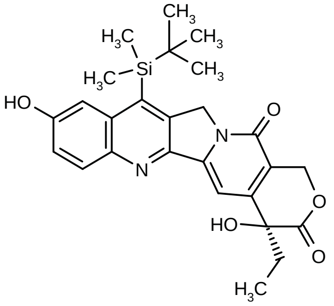
Exatecan

Exatecan mesilate (DX-8951-f) **9** is a total synthetic camptothecin derivative developed by Daiichi and is currently in phase III. Exatecan has proven to be a highly potent inhibitor of topoisomerase I and of tumor growth in several preclinical models, including those resistant to irinotecan and other camptothecins (52, 53).

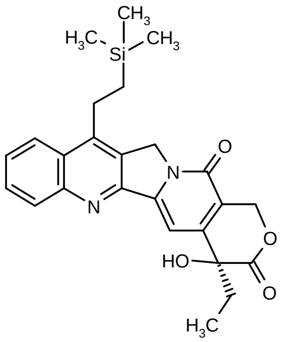
In the clinic, five different therapy schedules have been investigated in phase I. Similar to topotecan, the daily x 5 every 3 weeks schedule has been favored and the maximum tolerated dose was 0.3-0.5 mg/m^2/day (54). In phase II studies, activity has been observed in pancreatic cancer, non-small cell lung cancer and in advanced ovarian cancer, whereas no significant activity could be demonstrated in advanced colorectal cancer (55). Further phase II studies and a phase III trial in pancreatic cancer are ongoing.

Lurtotecan

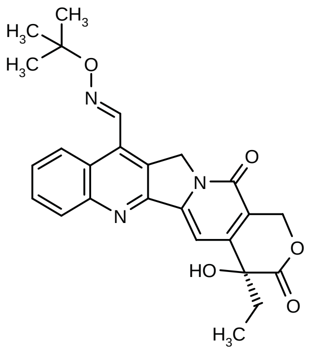
Lurtotecan **10** was originally discovered at Glaxo as a result of an optimization program stimulated by the extraordinary high potency of 10,11-methylenedioxy-20(*S*)-



(**12**) DB-67



(**13**) BNP-1350



(**14**) Gimatecan

Fig. 5. Lipophilic camptothecin derivatives.

camptothecin (56). After disappointing results in phase II trials, clinical development was discontinued by Glaxo.

A license on the compound was granted to NeXstar. In an effort to improve the pharmacokinetic profile and particularly the lactone ring stability, a liposomal formulation (NX-211) was developed, which has been shown to be more effective and less toxic compared to lurtotecan in preclinical models (57, 58). In 2001, OSI Pharmaceuticals received worldwide development and commercialization rights from Gilead, having merged with NeXstar in 1999. Currently, OSI-211 (formerly NX-211) is in phase II trials in patients with ovarian cancer and in patients with recurrent small cell lung cancer who are treated on day 1 and 8 every 21 days.

CKD-602

CKD-602 **11** is the lead compound from a series of synthetic camptothecin analogs which incorporate a side chain with basic nitrogen in the 7-position and has been developed at Chong Kun Dang (59). Structure and therapy schedules are similar to topotecan. In phase I trials, partial responses were observed in patients with stomach and ovarian cancer. Currently it is in phase II clinical trials (60, 61).

Lipophilic camptothecin analogues

A better understanding of the mechanism of action of camptothecins suggest that an improvement of various factors such as topoisomerase I inhibition, lipophilicity, lactone ring stability and a longer lasting stabilization of the cleavable complex may create a therapeutic advantage. Approaches by several groups to achieve these goals resulted in a number of promising semisynthetic or total synthetic camptothecin derivatives modified in the 7-position with lipophilic substituents, such as DB-67 **12**, BNP-1350 **13** and gimatecan **14** (62-64). BNP-1350 and gimatecan are currently in clinical trials (Fig. 5).

Karenitecins

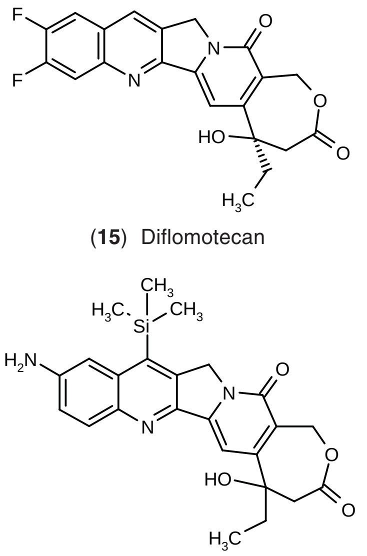
BNP-1350 **13** (Bionumerik) is the most advanced compound from the karenitecins, a class of semisynthetic, highly lipophilic, 7-modified camptothecin derivatives. Supported by computational models of the key interactions of camptothecin with topoisomerase I and DNA, the compound has been designed for increased potency, lactone ring stability, oral bioavailability and insensitivity to Pgp/MRP/LRP drug resistance. In preclinical models including multidrug-resistant ones, the compound has shown at least comparable activity to SN-38 (63). Despite its potential for oral administration in initial phase I studies, daily infusions during 5 days have been investigated and the recommended dose for phase II is 1 mg/m^2.

Gimatecan

Gimatecan (ST-1481) **14** is the lead compound from a series of 7-oxyiminomethyl derivatives developed at Sigma-Tau in collaboration with the Italian National Cancer Institute. Preclinical data suggest an enhanced therapeutic index, favorable biodistribution and good potency, particularly when administered p.o. daily at low doses (65). Phase I clinical trials were recently begun.

Homocamptothecins

Although earlier SARs of the camptothecins suggested the importance of the lactone E-ring for activity (26),



(**15**) Diflomotecan

(**16**) DB-38

Fig. 6. Homocamptothecins.

Lavergne *et al.* in 1997 came up with an interesting class of camptothecin derivatives with an expanded β-hydroxy lactone ring (66). Topoisomerase I poisoning activity was retained as shown in both cell-free assays and living HT-29 cells. Furthermore, such derivatives are less electrophilic which resulted in an improved stability of the lactone ring translating to a slow and irreversible hydrolysis in human plasma. Based on these findings, which also may lead to greater efficacy in humans, an optimization program was initiated and resulted in the selection of diflomotecan **15** for clinical trials.

Subsequent efforts utilizing the cascade radical annulation approach to combine appropriate lipophilic substituents in the 7-position with a 7-membered lactone ring provided homosilatecans such as **16**, which are the most stable camptothecins thus far (67) (Fig. 6).

Diflomotecan

The most advanced representative of the homocamptothecins is diflomotecan (BN-80915) **15** from Beaufour-Ipsen, which currently is in phase II clinical trials (68, 69). Preclinically, **15** has shown good activity, particularly with long-term oral treatment (14-42 days), and has outperformed the clinically relevant benchmarks in the class. Oral bioavailability of 30% and 59% demonstrated preclinically in rats and dogs, respectively, has also been confirmed in patients.

A number of phase I studies have investigated single-dose i.v. administration every 3 weeks, oral treatment daily for 5 consecutive days, as well as weekly and daily times 5 schedules.

Prodrugs and delivery systems

Due to their unique potency, camptothecin and its derivatives have also been employed as the toxophore moiety in a variety of prodrugs and delivery systems. These include low-molecular-weight drug conjugates such as esters of the 20-hydroxy group (70) and 20-*O*-modified glycoconjugates (71) to improve solubility and lactone ring stability, or polymeric conjugates such as polyglutamates (72), polyethylene glycols (73) and polysaccharides (carboxymethyl-dextrans). The rationale for polymeric delivery systems is an enhanced permeation and retention of polymers in tumor tissue (EPR-effect) because of leaky blood vessels and a less efficacious lymphatic drainage (74). The therapeutic efficacy of drugs might be improved when utilizing polymeric delivery systems because of reduced systemic toxicity, longer retention time within the body and an altered biodistribution.

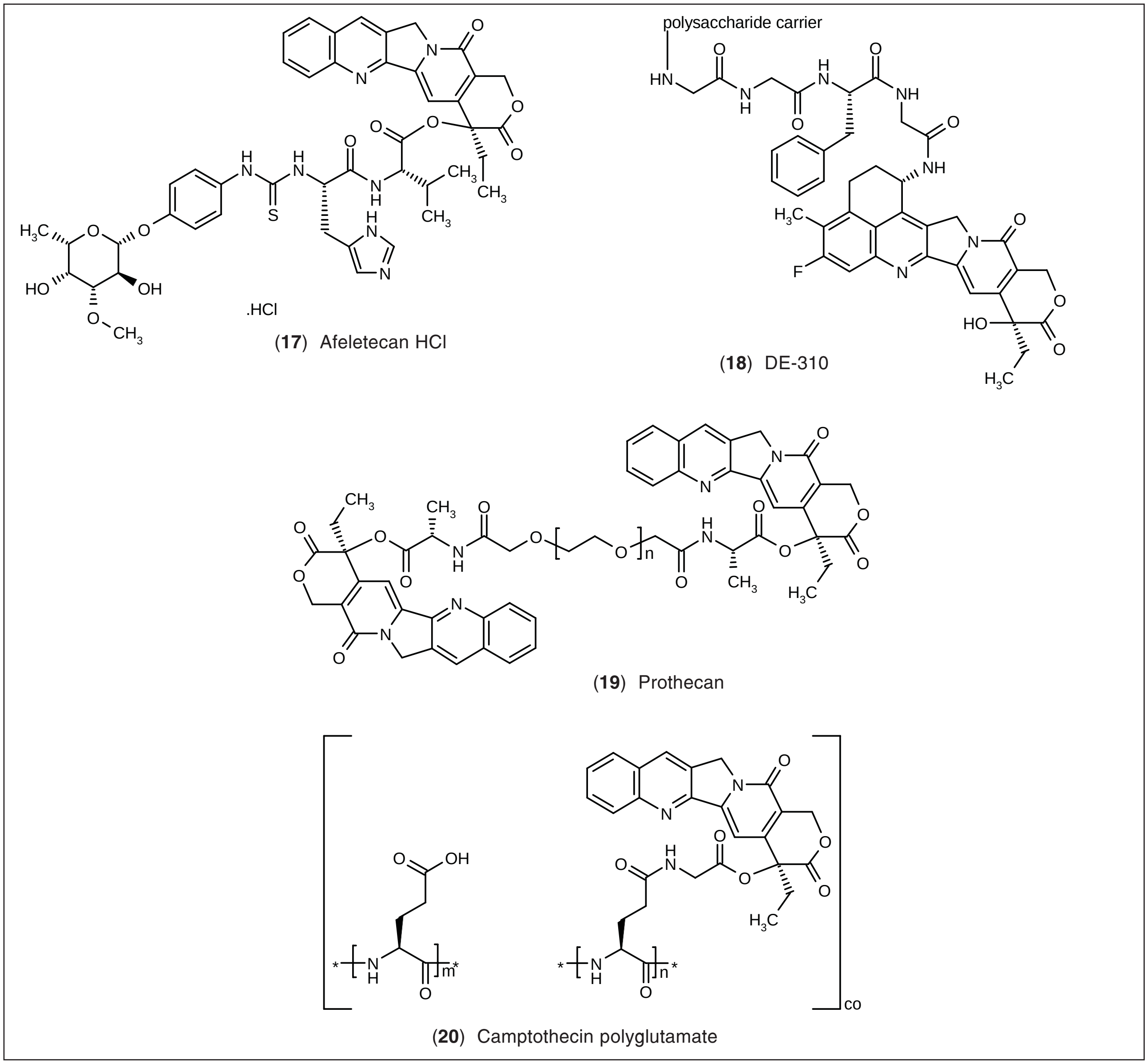
These approaches have proven promising in preclinical investigations and a number of compounds are in clinical development now (Fig. 7).

Afeletecan

Bayer's afeletecan (Bay-38-3441) **17** is a 20-*O*-linked glycoconjugate of camptothecin carrying a modified fucoside residue. The glycopeptide carrier system has been designed and optimized for an improved biodistribution due to lectin interaction and cellular uptake into tumor cells by an active transport mechanism. Preclinically, the water-soluble conjugate has shown high lactone ring stability, sufficient hydrolytic and proteolytic stability and excellent activity in xenograft models (75). It has been proven to be well tolerated using different schedules in phase I. Phase II studies investigating i.v. administration daily times 3 every 3 weeks have begun.

DE-310

DE-310 **18** (Daiichi) is a conjugate of exatecan with a biodegradable macromolecular carbohydrate carrier which is attached to the amino group of exatecan via the tetrapeptide Gly-Gly-Phe-Gly (drug-load ~7%). Preclinically, a drastic prolonged half-life could be demonstrated (~2 days for DE-310 compared to 0.3 h for exatecan (76). The AUC of free exatecan in Meth A tumor tissue was found to be 30 times higher than that in plasma. In correlation with these data, a single i.v. dose of the conjugate demonstrated at least comparable or better tumor inhibitions as prolonged therapy schedules of exatecan with the same overall dose (77). Activity of the conjugate was already observed at doses of 1/4 the maximum tolerated dose. The polymer releases both the glycine conjugate and exatecan by different enzymatic cleavage processes in tissue homogenates from Meth A tumors. DE-310 is currently in phase I clinical trials.



(**17**) Afeletecan HCl

(**18**) DE-310

(**19**) Prothecan

(**20**) Camptothecin polyglutamate

Fig. 7. Camptothecin prodrugs and delivery systems currently in clinical trials.

Similar conjugates of camptothecin derivatives with carboxymethyl dextran have been synthesized by Harada *et al.* (78).

Prothecan

To improve solubility and delivery to tumor tissue, Greenwald *et al.* have coupled camptothecin to nonimmunogenic 40 kDa polyethylene glycol (PEG) (79). Intense investigations of a number of linker groups between polymer and camptothecin have been investigated with respect to release properties, half-life in plasma and the impact on antitumor activity both *in vitro* and *in vivo* (80). Finally, Prothecan **19** (Enzon) with an alanine spacer between camptothecin and PEG was selected for clinical trials because of the prolonged half-life, lower toxicity and high efficacy. In phase I trials, single doses of 7000 mg/m^2 have been reached with myelosuppression being the principal dose-limiting toxicity.

Camptothecin polyglutamate

Polyglutamic acid has already proven to be an appropriate biodegradable macromolecular carrier when conjugated with paclitaxel, providing CT-2103 (Cell Therapeutics), which is currently in phase III clinical trials. Now camptothecin has been attached to the carboxy side chains of polyglutamate via glycine spacers, which enabled a higher drug load. Efficacy has been correlated with the size and the drug load of respective conjugates. Water-soluble drug conjugates **20** of 49 kD molecular weight and 37% camptothecin load have shown the best activity in preclinical models and have been selected for clinical trials (81).

Conclusions

The discovery of the natural product camptothecin in the early 1960s provided the lead structure for a novel, highly promising class of antitumor agents. The development of efficient isolation procedures as well as total- or semisynthetic approaches created the basis for a variety of chemistry driven optimization programs. Initial shortcomings have been solved and highly potent, water-soluble analogues such as irinotecan and topotecan have been identified and moved into clinical trials. Both compounds have now been proven to be quite successful in clinical practice, particularly in the treatment of colon, ovarian and lung cancers.

An important milestone in the field was the elucidation of the mechanism of action of camptothecins and the identification of topoisomerase I, which from a pharmacological point of view is an exciting molecular target of a ubiquitous nature. These insights provided novel rationales for the design of improved analogues. However, for a number of camptothecin derivatives the high efficacy observed in mouse xenograft models did not translate to the same level of activity in the clinic. This has been attributed to the instability of the lactone ring, which is essential for activity, in the presence of human serum albumin. Lactone ring stabilization became an important optimization criterium and has been achieved by different means, *e.g.*, in lipophilic analogues modified in the 7-position, with innovative formulations or in 20-*O*-acylated prodrugs and delivery systems.

Due to these issues, the determination of pharmacokinetic and pharmacodynamic parameters of camptothecins is extraordinarily complex and at the same time crucial to understanding the efficacy and safety profiles of the compounds and to provide a rationale for administration schedules and combination therapies. Much progress has been made in optimizing therapy schedules and managing side effects in the clinic.

More than 10 clinical programs on analogues and delivery systems have addressed these issues and are ongoing. Significant progress may be expected with respect to efficacy, drug safety, better compatibility in combination regimens with other agents and ease of use because of less frequent intravenous application schedules or oral formulations.

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