Recent Advances in Topoisomerase I-Targeting Agents, Camptothecin Analogues

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Abstract: The present review concentrates on camptothecin (CPT) analogues, the most extensively studied topoisomerase I (topo I) inhibitors, and provides concise information on the structural features of human topo I enzyme, mechanisms of interaction of CPT with topo I, structure−activity relationship study of CPT analogues including the influence of lactone stability on antitumor activity, and recent updates of valuable CPT analogues.

INTRODUCTION

DNA topoisomerases are ubiquitous enzymes, which play essential roles for the survival of prokaryotic and eukaryotic organisms. They participate in many critical cellular processes associated with separation of DNA strands such as replication, transcription, recombination, and repair. Topoisomerases can also act as DNA strand transferases and catalyze recombination and transposition reactions. The enzymes affect DNA topology by regulating supercoiling, catenation/decatenation and unknotting of the nucleic acid [1−3]. Topoisomerases are classified into two classes, types I and II, based primarily on their modes of cleaving DNA. Type I DNA topoisomerase (topo I) is monomeric and known to act by making a transient nick on a single-strand of duplex DNA, passing another strand through the nick and changing the DNA linking number by one unit [4]. On the other hand, type II topoisomerase (topo II) is dimeric and catalyzes a similar process by transiently nicking both strands of the DNA, passing another double-stranded DNA segment through the gap and changing the linking number by two [1,5]. In their catalytic actions, the common intermediates are enzyme-linked DNA breaks, which are usually referred to as the 'cleavable complex'. Especially, topo I is present throughout the cell cycle, and its activity varies less than topo II during cell cycle [6,7]. In addition, intracellular levels of topo I are elevated in a variety of human solid tumors relative to the respective normal tissues, which makes topo I an attractive target for developing selective cancer chemotherapy [8,9]. Topo I inhibitors can be divided into two main classes, topo I poisons and suppressors, and both inhibit catalytic activity (DNA relaxation). Topo I poisons trap and stabilize cleavable complexes, while topo I suppressors inhibit or reverse the formation of cleavable complexes by acting directly on the enzyme. Over the recent years, there has been an increasing interest in this field of topoisomerase inhibitors. Indeed, numerous classes of compounds have been demonstrated to interfere with DNA topoisomerases, and the diversity of topoisomerase inhibitors has been discussed in recent reviews [10−12].

Among the various topoisomerase inhibitors reported to date, the present review will concentrate only on camptothecin (CPT) analogues since they are one of the most potent and extensively studied topo I poisons as anticancer agents. We will briefly discuss here the structural features of human topo I, mechanism of interaction of CPT with topo I, and the structure−activity relationship of CPT analogues. Moreover, clinically important CPT analogues will be briefly highlighted in this review.

CAMPTOTHECINS AS TOPO I INHIBITORS

CPT (**1**), a pentacyclic alkaloid isolated by Wall in 1966 from the Chinese tree *Camptotheca acuminata* (Nyssaceae), was reported to possess potent antitumor activity [13]. Early clinical trials of the water-soluble sodium salt of CPT, now known to be an inactive form of the drug, were discontinued in the early 1970s because of severe and unpredictable toxic side effects [14,15]. In 1985, however, it was reported that the molecular target of CPT was the nuclear enzyme DNA topo I [16,17], and this discovery of unique mechanism of action revived interest in CPT and its analogues as anticancer agents. Over the last ten years, a large number of CPT analogues have been emerged as a prominent class of anticancer agents, and they are indeed the first specific topo I inhibitors to reach clinical application. Because of their novel mechanism of action, potent antiproliferative activity on a wide spectrum of cancer cells including multidrug-resistant lines and impressive activity in xenograft models, CPT analogues have been extensively investigated, and their recent advances have been covered by many review articles [10−12, 18−21]. Numerous research efforts have been focused on water-soluble derivatives to overcome the intrinsic poor solubility of CPT in aqueous system. As results, irinotecan (CPT-11, Camptosar or Campto, **3**; Fig. (**1**)), which is also known as the prodrug of SN-38 (**2**), and topotecan (Hycamtin, **4**) were approved as anticancer agents in 1997, and three other water-soluble analogues, exatecan (DX-8951f, **5**), lurtotecan (GG-211, GW-211or GI-147211,

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6) and CKD602 (**7**), are currently under clinical evaluations. Moreover, preclinical and clinical studies of non-water-soluble CPT analogues, rubitecan (9-nitrocamptothecin, 9-NC or RFS-2000, **8**), 9-aminocamptothecin (9-AC, **9**), DB-67 (**10**), BNP-1350 (**11**) and SK 2134 (**12**), are also being investigated. Whereas most CPT analogues share the highly electrophilic and unstable 6-membered α-hydroxylactone ring, the newly emerging homocamptothecin (hCPT) derivatives, BN-80915 (**13**), BN-80927 (**14**) and homosilatecans (**15**), contain a stabilized 7-membered β-hydroxylactone ring, and two hCPT analogues, BN-80915 and BN-80927, are currently undergoing clinical trials as well. Since irinotecan and topotecan, available in the clinic, have been subjected to numerous review articles [22−25], we will focus only on the relatively new and important CPT drugs under either preclinical or clinical evaluations and briefly summarize their status of development.

STRUCTURAL FEATURES OF HUMAN TOPO I

Human topo I is a monomeric protein of 765 amino acids. Based on sequence similarity with other eukaryotic topo I and on limited proteolysis analysis data, the human enzyme has been divided into four domains, the amino-terminal, the core, a linker, and the active site-containing carboxy-terminal domain [26,27]. The relatively disorganized, protease-sensitive and non-conserved amino-terminal domain ends at approximately residue 214 and is known to be dispensable for relaxation activity. The highly conserved core domain (residues 215−635) contains most of the amino acid residues responsible for binding DNA and catalytic activity. The linker, a second unconserved and protease-sensitive domain, spans from residues 636 to 712 and connects the core domain to the conserved carboxy-terminal domain (residues 713−765) which is not necessary for enzyme activity in vitro. The solution of the crystal structure of a truncated form of human topo I in 1998 has been the major breakthrough in the research field of topo I inhibitors, finally allowing an accurate examination of the enzyme active site in its complex with DNA [28, 29]. The crystal structures of reconstituted human topo I comprising the core and carboxyl-terminal domains in covalent and noncovalent complexes with 22-bp DNA duplexes reveal an enzyme that "clamps" around essentially B-form DNA. The core domain and the first eight residues of the carboxyl-terminal domain of the enzyme, including the active-site nucleophile tyrosine^{723}, which forms a phophoester bond with the 3' phosphate at the site of cleavage of the scissile strand of the substrate DNA, share significant structural similarity with the bacteriophage family of DNA integrases. The main features of these structures are the presence of a central DNA-binding pore of 15−20 Å of diameter, composed primarily of positively charged residues, with the catalytic Tyr^{273} group contained within "channel region" [28]. In addition, the three-dimensional structure of a 70-kDa amino terminally truncated form of human topo I in complex with a 22-bp duplex oligonucleotide has also revealed all of the structural elements of the enzyme that contact DNA. The linker region that connects the central core of the enzyme to the carboxyl-terminal domain assumes a coiled-coil configuration and protrudes away from the remainder of the enzyme. The positively charged DNA-proximal surface of the linker makes only a few contacts with the DNA downstream of the cleavage site. In combination with the crystal structures of the reconstituted human topo I before and after DNA cleavage, this information is suggestive of which amino acid residues are involved in catalyzing phosphodiester bond breakage and religation. The authors also suggested that these domains interact with the rotating double helix through a mechanism termed "controlled rotation": the topoisomerization reaction happens as a sequence of controlled rotations, driven by the torsional stress of supercoiled DNA and controlled by the ionic interactions between DNA, two long helices of the core domain (cone helices) and the protruding linker domain. After relaxation occurred, the enzyme reseals the broken strand, releasing DNA with a lower linking number [29].

MECHANISMS OF ACTION OF CAMPTOTHECIN

The primary cellular DNA lesion induced by CPT has been established to be the reversible human DNA topo I−CPT−DNA covalent complexes, the cleavable complexes [30,31]. Extensive in vitro biological studies on the properties of these complexes have suggested that CPT binds at the interface between topo I and DNA, and inhibit specifically the religation step in the cleavage/religation reaction [31,32]. The molecular mechanism of inhibition appears to be rather intriguing and uncompetitive, because CPT does not interact with topo I alone, nor does it bind to DNA, but interacts with the enzyme−DNA complexes to form a reversible nonproductive complexes [33,34]. It is generally believed that CPT exerts its major cytotoxic effects by binding and stabilizing the cleavable complex, a transient species where the hydroxyl group on tyrosine 723 of topo I binds covalently to DNA via its phosphodiester backbone and causes a single-strand break. The formation of a stable ternary complex between CPT, topo I, and the cleaved DNA leads to the S-phase specific arrest of replication at the single strand level, causing irreversible DNA damage and eventually cell death [35,36]. This mechanism of S-phase specific cytotoxicity has been studied extensively, and a replication of fork collision model has been established [37]. The reversible topo I−CPT−DNA cleavable complexes are not sufficient for cell killing. Upon their collisions with the advancing replication forks, however, cell death ensues. The collision is known to be potentially lethal only if the cleavable complex is formed on the strand complementary to the leading strand of DNA synthesis [36]. Contrary to these reports, recent studies have suggested that CPT analogues may interact directly with double-stranded DNA prior to the action of topo I, and the DNA-associated drugs are likely to be involved in the subsequent formation of a ternary complex [38]. Although the molecular details of the interactions remain uncertain in the absence of the exact crystal structure of the ternary complex, this mechanism accounts for the good correlation found between the ability to induce stabilized cleavable complexes and the cytotoxicity of various CPT analogues [39,40]. A second, S-phase independent, activity of CPT at higher doses has been recently proposed, leading to cytotoxicity in human colon cancer cell lines [41]. S-phase independent cytotoxicity of

CPT could be responsible for the apoptosis of non-dividing postmitotic neurons, and involvement of transcription has been suggested [42]. More detailed mechanisms of interaction of CPT with topo I have been discussed in recent reviews [43−45].

STRUCTURE−ACTIVITY RELATIONSHIPS INCLUDING LACTONE STABILITY

CPT analogues are planar molecules with an arc shape, but they do not intercalate into DNA in spite of this planarity. Structure−activity relationship studies in biochemical systems with purified topo I and blood components, as well as in tissue culture and in animal models, have been accumulated [12,18,44], and the results can be summarized as follows. (1) Substitutions at positions 7 and 9 do not generally affect topo I inhibitory activity, suggesting the absence of tight interaction with the receptor site and the regions of the A and B rings of CPT around positions 7 and 9. In fact, the introduction of an ethyl group at 7 position in SN-38 resulted in the remarkable potency of SN-38 as a topo I inhibitor [46]. More recently, a systemic study has revealed that the 7 position of CPT is a favorable site for the introduction of a lipophilic group, since the antitumor activity is maintained or improved with proper substituents [47]. (2) Addition of small substituents at position 10 generally increase topo I inhibition [48]. Substitution at 10 position with a hydroxyl group contributes to the increased activity of SN-38 and topotecan. (3) Bulky substitutions at position 11 and even small substituents at position 12 deactivate CPT derivatives, indicating a tight interaction between the topo I cleavage complex and the concave of the drug. In contrast, addition of an ethylenedioxy (or methylenedioxy) ring at positions 10 and 11 increases activity. (4) Intact lactone ring with natural 20-*S* configuration is critical for antitumor activity of CPT analogues. The fact that 20-*R* CPT isomer is inactive [40], suggests that the 20-hydroxyl group must interact closely, possibly through hydrogen bonding with the topo I cleavage complex. Any changes, such as replacement of the lactone by a lactam group, reduction of the lactone, removal of the carbonyl oxygen, or removal of the 20-hydroxyl, inactivate the molecule [49], which is indicative that CPT forms a covalent intermediate with a nucleophile from topo I or DNA [50,51]. Later on, a hypothetical binding mode for CPT has been proposed on the basis of chemical and biochemical information combined with the three-dimensional crystal structure of the covalent topo I−DNA complex [28,45,52]. This model is considered to account fairly well for many interactions between human topo I−DNA complex and CPT.

Based primarily on the reactivity of the E-ring lactone with nucleophiles, CPT has been believed to function as an alkylating agent by forming a labile covalent bond between the lactone and the enzyme [53]. The α-hydroxyl configuration (20-*S*) was presumed essential to enhance the reactivity of the lactone by either intramolecular hydrogen bonding or interaction with the enzyme. Recently, however, the hypothesis of covalent interaction between the E-ring lactone and the topo I−DNA complex has been first challenged by the unexpectedly potent activity of homocamptothecin (hCPT) [54,55]. In this molecule, a methylene moiety is introduced between the lactone and the 20-hydroxyl group in CPT, as a result of which the reactivity of the 7-membered lactone is diminished. Fortunately, the extraordinary activity of hCPT can be rationalized in a manner that preserves the hypothesis of covalent interaction [44]. First, the 20-*S* stereospecificity in CPT suggests that the hydroxyl group interacts with topo I enzyme. Then, intramolecular hydrogen bonding between the hydroxyl and the lactone carbonyl would not only activate the lactone but also diminish the interaction with the enzyme. On the other hand, the β-hydroxyl in hCPT is free to interact optimally with topo I although the lactone has relatively low intrinsic reactivity. Therefore, the reactivity of the lactone in hCPT could be facilitated in the topo I complex. Second, low reactivity of the lactone in hCPT would retard both the formation and the reversal of the covalent bond between drug and enzyme. The net effect could be to maintain, or even increase, the steady-state level of topo I−DNA cleavage complexes. Moreover, longer persistence of cleavage complexes could increase their cytotoxic potential. Another recent finding concerning the E-ring lactone was the retained activity (albeit lower potency) of CPT derivatives where the 20-hydroxy was replaced with Cl or Br [56]. This surprising result, therefore, contradicted the presumed essentiality of the 20-hydroxyl group that had been deduced from the inactivity of deoxy derivative. It was reasoned that the polarizability of the halogen might allow an interaction sufficient to stabilize the complexes with the enzyme.

It has been well illustrated that CPTs exhibit unique dynamics and in vivo reactivity with respect to both drug hydrolysis and blood protein interactions [57]. These important factors on the E-ring lactone stability have been shown to confound their pharmaceutical development and clinical implementation. Regarding hydrolysis of the drug, the 6-membered α-hydroxylactone pharmacophore of CPT is highly reactive and readily converts to the ring opened carboxylate form at pH 7 and above, which is known to be inactive [40,49]. Thus, CPT analogues exist in an equilibrium consisting of two distinct species: (1) the biologically active lactone form in which the lactone ring is closed; and (2) a biologically inactive carboxylate form generated upon the hydrolysis of the lactone ring of the parent drug [16,40]. In human blood and tissues, the CPT equilibrium of active lactone versus inactive carboxylate form can be greatly affected by the presence of human serum albumin (HSA). Direct information on the different nature of interactions with HSA has been obtained from time-resolved fluorescence spectroscopic measurements taken on the intensely fluorescent CPT lactone and carboxylate species [58]. These data suggest that the lactone form binds to HSA with moderate affinity, yet the carboxylate form binds tightly to HSA, displaying a 150-fold enhancement in its affinity for highly abundant serum protein. Therefore, when the lactone form of CPT is added to a solution containing HSA, the preferential binding of the carboxylate form to HSA drives the chemical equilibrium to the right, resulting in the lactone ring hydrolyzing more rapidly and completely than when CPT is in aqueous solution without HSA. Furthermore, it has been also reported that the stabilities of

CPTs in human plasma and blood could be strongly modulated through A,B-ring substitutions, although their anti-topo I activities are frequently retained with modification of A,B-rings. For example, greater than 99.5% of CPT and 9-AC convert to the corresponding carboxylate in phosphate-buffered saline (pH 7.4) at 37 ºC in the presence of human plasma, whereas the plasma stabilities of topotecan, CPT-11, and SN-38 are vastly improved (with lactone levels enhanced by 10-folds or more) under the identical conditions [58,59]. In a similar fashion, less than 0.5% of 9-AC and 1 % of CPT remain as the lactone form at equilibrium in whole blood [60]. Topotecan (12%), CPT-11 (21%), and SN-38 (20%) all display markedly improved human blood stabilities compared to CPT. It has been pointed out that these high variations of two species in human plasma or whole blood may explain in part why 9-AC was highly effective against human tumors in xenograft models [61] but has shown poor results in human trials [62,63]. Therefore, these dynamic processes seem to present a major obstacle to achieving successful chemotherapy of cancer in the development of CPT analogues.

It has been clearly demonstrated from bioanalytical measurements that these dual substitutions at 7, 10 positions (where the 10 substituent is a hydroxyl group) result in greatly improved human blood stabilities of CPT analogues [64]. SN-38 is one example with this dual 7-alkyl-10-hydroxy substitution pattern, and these structural changes were shown to block SN-38 from associating with the high affinity CPT carboxylate-binding pocket of HSA, thus ensuring high potency of the drug. A new agent with the dual substitution is BD-67 (**10**; Fig. (**1**)), and its design was based on the following two considerations: (1) dual 7,10-substitution patterns can eliminate the highly specific binding of the carboxylate form over the lactone form by HSA [65,66], and (2) lactone ring stabilization is further promoted by enhanced lipophilicity or lipid partitioning due to the bulky *tert*-butyldimethylsilyl group [67,68]. Indeed, DB-67 not only displayed superior stability in human blood (30% of lactone present at equilibrium) but also showed comparable in vitro cytotoxicity, compared to CPT, topotecan and CPT-11 [69]. Another milestone for enhancing lactone ring stability has been achieved by replacing the 6-membered α-hydroxylactone with a 7-membered β-hydroxylactone. These E-ring expanded CPT analogues (hCPTs) have been shown to exhibit enhanced plasma stability and high anti-topo I activity, because of the dramatically more stable β-hydroxylactone ring [70,71]. The newly emerging agents, homosilatecans (**15**; Fig. (**1**)), designed by combining E-ring expansion of hCPT and dual 7,10 substitution, are the most blood-stable CPT analogues showing greater than 80% lactone levels after 3 h of incubation in human blood [72]. This accumulated knowledge on structure−stability−activity relationships will provide a good guideline for the rational design of potent CPT analogues displaying markedly improved human blood stability.

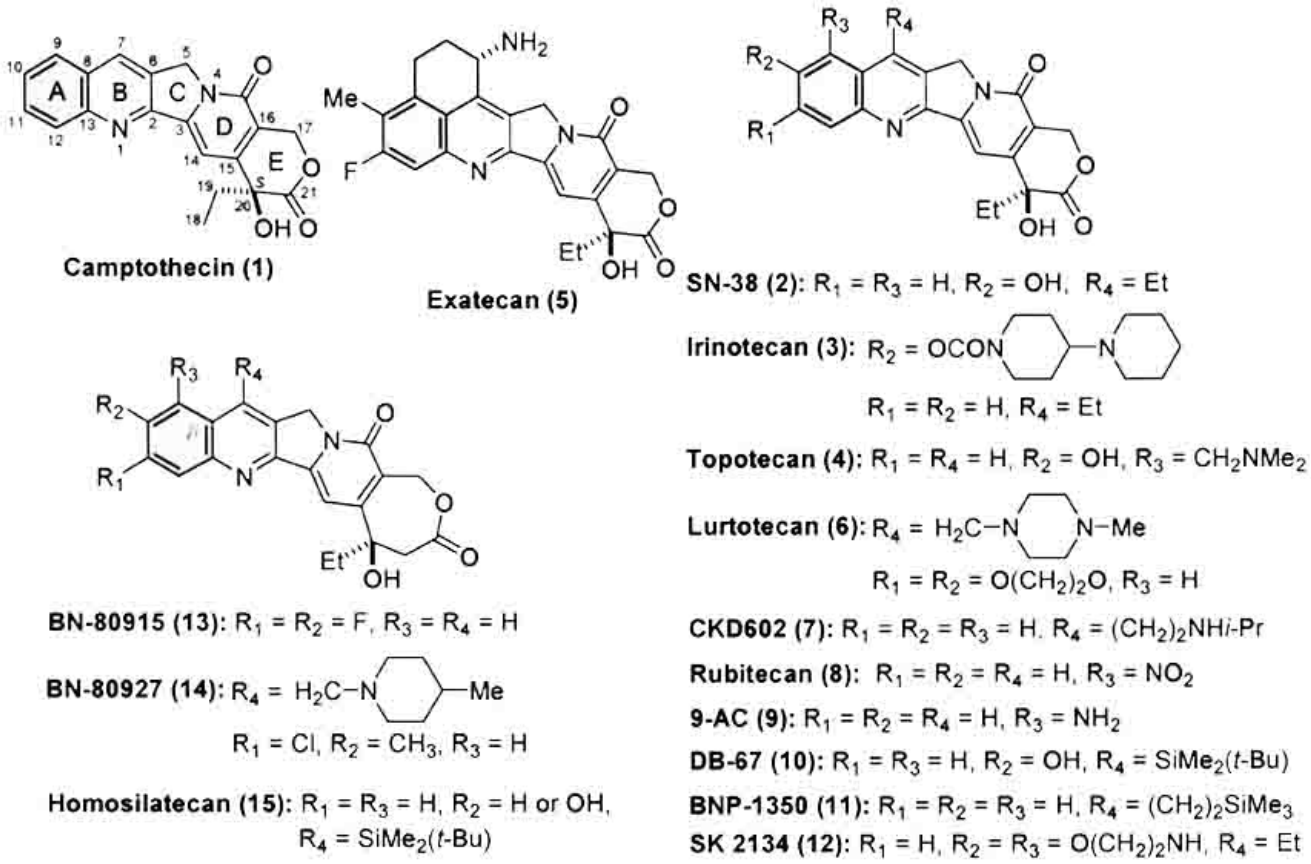

Fig (1). Chemical Structures of Camptothecin Analogues.

RECENT ADVANCES IN CAMPTOTHECIN ANALOGUES

Exatecan

Exatecan mesylate dihydrate is known as a new water-soluble hexacyclic camptothecin analogue with favorable attributes compared to topotecan and CPT-11, including greater potency against topo I, broad antitumor activity and low cross-resistance against MDR-1 overexpressing tumors. In preclinical studies, it demonstrated a favorable toxicology profile with hematologic dose-limiting toxicity and moderate gastrointestinal toxicity, linear pharmacokinetics, P450 hepatic metabolism (CYP3A4 and CYP1A2), and predominately fecal excretion. The results of phase I clinical trials indicated that the toxicity profile was similar for all schedules of administration. Hematologic toxicity was dose-dependent and reversible. Neutropenia was dose-limiting in minimally pretreated patients, whereas neutropenia and thrombocytopenia were dose-limiting in heavily pretreated patients. Non-hematologic toxicities included moderate gastrointestinal toxicity (nausea, vomiting, diarrhea), transient elevation of hepatic transaminases, asthenia, and alopecia. Two cases of acute pancreatitis not predicted by preclinical toxicology were also observed. Antitumor activity was seen in several solid tumor types including non-small-cell lung cancer, extrapulmonary small-cell cancer, colorectal cancer, hepatocellular cancer, sarcoma and as well as in CPT-11 and topotecan-resistant tumors. The daily ×5, every 3-week schedule with the drug administered as a 30-min intravenous infusion was selected for future phase II clinical trials based on its superior antitumor activity [73,74].

Lurtotecan

Lurtotecan, a 10,11-ethylenedioxy substituted analogue of CPT, was brought into clinical development because of its higher water solubility and greater potency as compared to topotecan. Between 1995 and 1996, 67 eligible patients with pretreated breast cancer, chemo-naive colorectal and non-small-cell lung cancers were entered into three multi-centered and non-randomized phase II clinical trials. Treatment schedule consisted of intravenous lurtotecan administered at a dose of 1.2 mg/m^2/day for five consecutive days every three weeks. Hematological toxicity was common with grade 3−4 neutropenia in 54% of patients, and neutropenic fever together or not associated with infection in 14.5% of patients. Grade 3−4 thrombocytopenia and grade 2−4 anemia were observed in 20% and in 68% of patients, respectively. Non-hematological toxicity was generally mild to moderate and consisted mainly of gastrointestinal toxicity, asthenia and alopecia. The antitumor activity of lurtotecan was moderate in breast cancer patients (response rate (RR) = 13%) and minimal in non-small-cell lung cancer patients (RR = 9%). No objective responses were obtained in colorectal patients. Lurtotecan, at the doses and schedules employed in phase II trial studies, showed an acceptable safety profile but a modest antitumor activity in the examined tumor types [75]. Lurtotecan has been formulated into a low-clearance unilamellar liposome, NX 211, and interest has been renewed in the clinical development of lurtotecan by preclinical data with the liposomal formulation showing an increased therapeutic index. Comparative studies between free drug and NX 211 have been performed assessing pharmacokinetics in nude mice, tissue distribution in tumor-bearing mice, and antitumor efficacy in xenografts. Compared with lurtotecan, NX 211 demonstrated a significant increase in plasma residence time and a subsequent 1500-fold increase in the plasma area under the drug concentration curve [76].

CKD-602

CKD-602, 7-[2-(*N*-isopropylamino) ethyl]-20(*S*)-camptothecin, is a novel water-soluble antitumor agent developed by Chong Kun Dang Pharm. (Korea), and is in phase II clinical trials. It is a potent topo I inhibitor that overcomes the poor aqueous solubility and toxicity profile of CPT. In a phase I clinical trial in 15 cancer patients, the maximum tolerated dose was determined to be 0.7 mg/m^2/day when CKD-602 was administrated according to the daily ×5, every 3-week schedule. Dose-limiting toxicities were shown to be grade 3−4 neutropenia, and moderate thrombocytopenia, diarrhea and vomiting occurred infrequently. Partial responses were observed in patients with stomach and ovarian cancer [77]. In 2000, CKD-602 was licensed to Alza for co-development in the US

9-AC

9-AC is a non-water-soluble topo I inhibitor under development by IDEC. Because of its potent in vitro cytotoxicity and promising preclinical antitumor activity in a colorectal cancer animal model, it entered a clinical trial as a 72-h intravenous infusion in 1993. Predictable myelosuppression was the major dose-limiting toxicity, and pharmacokinetic studies showed a relatively short plasma half-life of the active lactone form. Unfortunately, phase II clinical studies using this schedule showed minimal or no activity in tumors such as colorectal and lung cancer. Only modest activity was observed in ovarian cancer and in refractory lymphomas. Efforts to improve systemic drug exposure by utilizing alternative schedules of administration of 9-AC such as prolonged, continuous intravenous infusions have also been tested. However, phase II clinical studies of 120-h weekly infusions of 9-AC have not shown improved activity against solid tumors such as colorectal cancer. Although final judgment regarding the fate of 9-AC must await the results of these phase II clinical trials that are not yet fully available, it is generally accepted that further development of intravenously administered 9-AC for the treatment of colorectal cancer is not promising. This experience with 9-AC raises important questions concerning how to best select new topo I-targeting drugs for future clinical development [78].

Rubitecan

Orally active rubitecan is a water-insoluble topo I inhibitor with a broad antitumor activity, and it is being developed by SuperGen for the treatment of solid tumors

including breast, lung, ovarian, colorectal and pancreatic cancers, and melanoma. It is in a pivotal phase III clinical trial in pancreatic cancer at more than 130 sites, as well as in phase II clinical testing for the treatment of 11 additional tumor types. In a phase II clinical trial in pancreatic cancer patients (including 45 with metastasis and 29 who had failed conventional treatment) given >2 courses of 9-NC p.o., 33% responded with median survival of 16.2 months, 30% were stable, and 37% did not responded [79,80]. Recently, orphan drug status has been granted for the treatment of pancreatic cancer. In 1999, rubitecan was licensed to Abbot for co-promotion in the US and for exclusive distribution and promotion outside the US.

DB-67

DB-67 is under preclinical study, and has been reported to display superior stability in human blood when compared with clinically relevant camptothecin analogues. It was prepared by using the radical cascade approach developed by Curran and his co-workers [57,81,82]. In human blood, DB-67 displayed a half-life of 130 min and a percent lactone value of 30% at equilibrium in phosphate-buffered saline (pH 7.4) at 37 ºC. The *tert*-butyldimethylsilyl group renders the new agent 25-times more lipophilic than CPT, and DB-67 is readily incorporated, as its active lactone form, into cellular and liposomal bilayers. In addition, the dual 7-alkylsilyl and 10-hydroxy substitution in DB-67 enhances drug stability in the presence of HSA. Thus, the net lipophilicity and the altered HSA interactions together function to promote the enhanced blood stability. In vitro cytotoxicity assays using multiple different cell lines derived from eight distinct tumor types indicate that DB-67 is of comparable potency to CPT, topotecan and CPT-11. In addition, cell-free cleavage assays reveal that DB-67 is highly active and forms more stable top1 cleavage complexes than camptothecin or SN-38. Because of impressive blood stability and cytotoxicity profiles, DB-67 has been selected as an excellent candidate for additional in vivo pharmacological and efficacy studies.

BNP-1350

BNP-1350 is a novel semi-synthetic, highly lipophilic, silicon-containing CPT analogue and is undergoing a phase II clinical trial [83]. It has been designed by supercomputer for superior oral bioavailability, superior lactone stability, broad antitumor activity, increased potency and insensitivity to Pgp/MRP/LRP drug resistance. The in vitro assays for anti-proliferative capacity in five colon cancer cell lines indicated that BNP-1350 was similarly effective or slightly more potent than SN-38. Growth inhibition of >50% was obtained for BNP-1350 given i.p. in six out of the seven human tumor xenografts studied. BNP-1350 was similarly effective when given i.p. or p.o., and oral bioavailability was presumed to be 67%. In a phase I clinical trial in 14 patients with advanced solid tumors including pancreatic and colorectal cancers, 0.15−2.4 mg/m^2/day given by infusion for 5 days on a 21-day schedule resulted in stable disease in 3 patients for >2 cycles. Dose-limiting toxicities comprising reversible grade 4 neutropenia and thrombocytopenia occurred at 1.2 and 2.4 mg/m^2/day. The i.v. dose of 1.0 mg/m^2/day has been recommended for phase II clinical trials.

SK 2134

SK 2134 is a novel hexacyclic CPT analogue, in which the 9, 10 positions of the A-ring were modified by introducing a 1,4-oxazine ring. It was developed in our laboratory (SK Chemicals Co., Korea) and can be readily prepared from SN-38 by using a semi-synthetic approach. In vitro cytotoxicity assays demonstrated that SK 2134 is about 2-fold more potent than topotecan and as potent as CPT toward human cancer cell lines A549, H128, WiDr, MKN45, SK-OV-3 and SK-BR-3, although slightly less potent than SN-38. Its lactone stability in human plasma is much higher than that of CPT and similar to that of topotecan, but lower than that of SN-38. In vivo antitumor activity of SK 2134 was measured against human tumor xenograft (WiDr) in nude mice, and the inhibition rate (IR) values at maximum tolerated doses (MTD) were 98.6% and 98.2% in SK 2134- and SN-38-treated groups, respectively, on the day 28. In addition, the other parameters used to evaluate the in vivo antitumor activity were also similar between SK 2134 and SN-38 treated group, although the administered dose of SK 2134 was 10-fold lower than that of SN-38. Since SK 2134 exhibited particularly potent in vivo antitumor activity, it has been selected for further development [84].

BN-80915 and BN-80927

BN-80915 and BN-80927 are two leading compounds in a series of novel hCPT analogues under development by Beaufour Ipsen (France). Recently, it is widely accepted that this E-ring modification fully conserves the topo I inhibitory activity. A key feature of hCPT is the slow and irreversible hydrolytic E-ring opening, which could give reduced toxicity by providing higher plasma concentrations of the active lactone form. BN-80915, a difluoro-hCPT, exhibited high antiproliferative activity on a panel of tumor cell lines, including those with cross-resistance, and was found to be active at very low doses in a variety of human tumor xenografts when administered orally. It was claimed that the potentials of BN-80915 for the higher efficacy and reduced adverse effects would be beneficial to patients. In Europe, BN-80915 is currently undergoing clinical evaluations in both oral and i.v. formulations under the name of Diflomotecan [85−87].

BN-80927 is another novel and highly stable hCPT that shows the unprecedented dual inhibitory activities for both topo I and topo II enzymes. It is more potent than SN-38 and topotecan on a variety of human tumor cell lines, and has higher cytotoxic effects on cells exhibiting multidrug-resistant phenotypes. The combination of topo I and topo II inhibitory activities along with the effect of BN-80927 on resting and chemo-resistant cells may explain its outstanding in vivo antitumor activity in xenograft models compared to clinically available CPT analogues. Therefore, BN-80927 is being developed as a promising anticancer agent with unique

features in terms of biological profile and plasma stability [88,89].

Homosilatecans

Homosilatecans are hCPT derivatives containing a very lipophilic *tert*-butyldimethylsilyl group at 7 position, which was prepared by the cascade radical annulation approach. They displayed markedly enhanced human blood stabilities compared to clinically relevant CPT drugs, and they are indeed the most blood-stable analogues yet to be identified that exhibit intrinsic potency against topo I target. In addition, the new homosilatecans do not show any significant interspecies variations in blood stabilities previously noted for CPT and 9-AC. The IC_{50} cytotoxic values of the homosilatecans against MDA-MB-435 tumorigenic metastatic human breast cancer cells, following 72-h exposure, were in the 20- to 100-nM range. It has been claimed that successful treatment strategies achieved in animal models with homosilatecans might be more readily translated to a clinical development [57,90].

In conclusion, research activities in this field of topo I-targeting agents continue to grow exponentially, resulting in a wealth of new information on the functional role, the biochemical and structural properties of the enzyme, key interactions between the enzyme and drugs, and essential structural requirements of the CPT analogues. Accumulations of this valuable knowledge, either available now or in the future, further stimulate the rational design of novel potent topo I-targeting agents as clinically important anticancer drugs.

REFERENCES

[1] Wang, J. C. *Annu. Rev. Biochem.* **1996**, *65*, 635.

[2] Nitiss, J. L. *Biochim. Biophys. Acta.* **1998**, *1400*, 63.

[3] Rybenkov, C. U.; Vologodskii, A. V.; Cozzarelli, N. R. *Science* **1997**, *277*, 690.

[4] Gupta, M.; Fujimori, A.; Pommier, Y. *Biochim. Biophys. Acta.* **1995**, *1262*, 1.

[5] Froelich-Ammon, S. J.; Osheroff, N. *J. Biol. Chem.* **1995**, *270*, 21429.

[6] Heck, M. M.; Hittelman, W. N.; Earnshaw, W. C. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 1086.

[7] Romig H.; Richter, A. *Biochim. Biophys. Acta.* **1990**, *1048*, 274.

[8] Husain, I.; Mohler, J. L.; Seigler, H. F.; Besterman, J. M. *Cancer Res.* **1994**, *54*, 539.

[9] Lima, C. D.; Wang, J. C.; Mondragon, A. *Nature* **1994**, *367*, 138

[10] Bailly, C. *Curr. Med. Chem.* **2000**, *7*, 39.

[11] Gatto, B.; Capranico, G.; Palumbo, M. *Curr. Pharm. Des.* **1999**, *5*, 195.

[12] Pommier, Y. *Biochimie* **1998**, *80*, 255.

[13] Wall, M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; McPhail, A. T.; Sim, G. A. *J. Am. Chem. Soc.* **1966**, *88*, 3888.

[14] Gottlieb, J. A.; Luce, J. K. *Cancer Chemother. Rep.* **1972**, *56*, 103.

[15] Moertel, C. G.; Schutt, A. J.; Reitemeier, R. J.; Hahn, R. G. *Cancer Chemother. Rep.* **1972**, *56*, 95.

[16] Hsiang, Y. H.; Hertzberg, R.; Hecht, S.; Liu, L. F. *J. Biol. Chem.* **1985**, *260*, 14873.

[17] Hsiang, Y. H.; Liu, L. F. *Cancer Res.* **1988**, *48*, 1722.

[18] Kawato, Y.; Terasawa, H. *Prog. Med. Chem.* **1997**, *34*, 69.

[19] Rothenberg, M. L. *Ann. Oncol.* **1997**, *8*, 837.

[20] Arbuck, S. G.; Takimoto, C. H. *Semin. Hematol.* **1998**, *35*, 3.

[21] Rothenberg, M. L.; Blanke, C. D. *Semin. Oncol.* **1999**, *26*, 632.

[22] Rothenberg, M. L. *Oncologist* **2001**, *6*, 66.

[23] Bleiberg H. *Eur. J. Cancer* **1999**, *35*, 371.

[24] Kollmannsberger, C.; Mross, K.; Jakob, A.; Kanz, L.; Bokemeyer, C. *Oncology* **1999**, *56*, 1.

[25] Burris III, H. A. *Semin. Hematol.* **1999**, *36*, 26.

[26] Stewart, L.; Ireton, G. C.; Champoux, J. J. *J. Biol. Chem.* **1996**, *271*, 7602.

[27] Champoux, J. J. *Prog. Nucl. Acid. Res. Mol. Biol.* **1998**, *60*, 111.

[28] Redinbo, M. R.; Stewart, L.; Kuhn, P.; Champoux, J. J.; Hol, W. G. *Science* **1998**, *279*, 1504.

[29] Stewart, L.; Redinbo, M. R.; Qiu, X.; Hol, W. G.; Champoux, J. J. *Science* **1998**, *279*, 1534.

[30] Gatto, B.; Liu, L. F. In *Advances in DNA Sequence-Specific Agents*; Palumbo M., Ed.; CRC Press Inc.: Boca Raton, **1998**; Vol. *8*, pp. 39−66.

[31] Hsiang, Y. H.; Hertzberg, R.; Hecht, S.; Liu, L. F. *J. Biol. Chem.* **1985**, *260*, 14873.

[32] Svejstrup, J. Q.; Christiansen, K.; Gromova, I. I.; Andersen, A. H.; Westergaard, O. *J. Mol. Biol.* **1991**, *222*, 669.

[33] Hertzberg, R. P.; Caranfa, M. J.; Hecht, S. M. *Biochemistry* **1989**, *28*, 4629.

[34] Holm, C.; Covey, J. M.; Kerrigan, D.; Pommier, Y. *Cancer Res.* **1989**, *49*, 6365.

[35] Tsao, Y. P.; D'Arpa, P.; Liu, L. F. *Cancer Res.* **1992**, *52*, 1823.

[36] Tsao, Y. P.; Russo, A.; Nyamuswa, G.; Silber, R.; Liu, L. F. *Cancer Res.* **1993**, *53*, 5908.

[37] Zhang, H.; D'Arpa, P.; Liu, L. F. *Cancer Cells* **1990**, *2*, 23.

[38] Yang, D.; Strode. J. T.; Spielmann, H. P.; Wang, A. H.-J.; Burke, G. *J. Am. Chem. Soc.* **1998**, *120*, 2979.

[39] Fan, Y.; Weinstein, J. N.; Kohn, K. W.; Shi, L. M.; Pommier, Y. *J. Med. Chem.* **1998**, *41*, 2216.

[40] Jaxel, C.; Kohn, K. W.; Wani, M. C.; Wall, M. E.; Pommier, Y. *Cancer Res.* **1989**, *49*, 1465.

[41] Borovitskaya, A. E.; D'Arpa, P. *Oncol. Res.* **1998**, *10*, 271.

[42] Morris, E. J.; Geller, H. M. *J. Cell Biol.* **1996**, *134*, 757.

[43] Liu, L. F.; Desai, S. D.; Li, T.; Mao, Y.; Sun, M.; Sim, S. *Ann. N. Y. Acad. Sci.* **2000**, *922*, 1.

[44] Kohn, K. W.; Pommier, Y. *Ann. N. Y. Acad. Sci.* **2000**, *922*, 11.

[45] Champoux, J. J. *Ann. N. Y. Acad. Sci.* **2000**, *922*, 56.

[46] Tanizawa, A.; Kohn, K. W.; Kohlhagen, G.; Leteurtre, F.; Pommier, Y. *Biochemistry*, **1995**, *43*, 7200.

[47] Dallavalle, S.; Delsoldato, T.; Ferrari, A.; Merlini, L.; Pence, S.; Carenini, N.; Perego, P.; De Cesare, M.; Pratesi, G.; Zunino, F. *J. Med. Chem.* **2000**, *43*, 3963.

[48] Hsiang, Y. H.; Liu, L. F.; Wall, M. E.; Wani, M. C.; Nicholas, Q. W.; Manikumar, G.; Kirschenbaum. S.; Sliber, R.; Potmesil, M. *Cancer Res.* **1989**, *49*, 4385.

[49] Hertzberg, R. P.; Caranfa, M. F.; Holden, K. G.; Jakas, D. R.; Gallagher, G.; Mattern, M. R.; Mong, S. M.; Bartus, J. O.; Johnson R. K.; Kingsbury, W. D. *J. Med. Chem.* **1989**, *32*, 715.

[50] Pommier, Y.; Tanizawa, A.; Kohn, K. W. *Adv. Pharmacol.* **1994**, *29B*, 73.

[51] Crow, R. T.; Crothers, D. M. *J. Med. Chem.* **1994**, *37*, 3191.

[52] Pommier, Y.; Kohlhagen, G.; Kohn, K. W.; Leteurtre, F.; Wani, M. C.; Wall, M. E. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 8861.

[53] Wall, M. E.; Wani, M. C. *Ann. Rev. Pharmacol. Toxicol.* **1977**, *17*, 117.

[54] Lesueur-Ginot, L.; Demarquay, D.; Kiss, R.; Kasprzyk, P. G.; Dassonneville, L.; Bailly, C.; Camara, J.; Lavergne, O.; Bigg, D. C. *Cancer Res.* **1999**, *59*, 2939.

[55] Bailly, C.; Lansiaux, A.; Dassonneville, L.; Demarquay, D.; Coulomb, H.; Huchet, M.; Lavergne, O.; Bigg, D. C. *Biochemistry*, **1999**, *38*, 15556.

[56] Wang, X.; Zhou, X.; Hecht, S. M. *Biochemistry*, **1999**, *38*, 4374.

[57] Burke, T. G.; Bom, D. *Ann. N. Y. Acad. Sci.* **2000**, *922*, 36.

[58] Mi, Z.; Burke, T. G. *Biochemistry*, **1994**, *33*, 10325.

[59] Burke, T. G.; Mi, Z. *J. Med. Chem.* **1994**, *37*, 40.

[60] Burke, T. G.; Mi, Z. *Anal. Biochem.* **1993**, *212*, 285.

[61] Giovanella, B. C.; Stehlin, J. S; Wall, M. E.; Wani, M. C.; Nicholas, A. W.; Liu, L. F.; Silber, R.; Potmesil, M. *Science* **1989**, *246*, 1046.

[62] Hochberg, F.; Grossman, S. A.; Mikkelsen, T.; Calabresi, P.; Fisher, J.; Piantadosi, S. *Proc. Am. Soc. Clin. Oncol.* **1998**, *17*, 388.

[63] Takimoto, C. H.; Dahut, W.; Harold, N.; Nakashima, H.; Lieberman, R.; Liang, M. D.; Arbuck, S. G.; Chen, A. P.; Hamilton, J. M.; Cantilena, L. R.; Allegra, C. J.; Grem, J. L. *Ann. N. Y. Acad. Sci.* **1996**, *803*, 324.

[64] Burke, T. G.; Mi, Z. *J. Med. Chem.* **1993**, *36*, 2580.

[65] Burke, T. G.; Munshi, C. B.; Mi, Z.; Jiang, Y. *J. Pharm. Sci.* **1995**, *84*, 518.

[66] Mi, Z.; Burke, T. G. *Biochemistry* **1994**, *33*, 12540.

[67] Burke, T. G.; Staubus, A. E.; Mishra, A. K. *J. Am. Chem. Soc.* **1992**, *114*, 8318.

[68] Burke, T. G.; Mishra, A. K.; Wani, M. C.; Wall, M. E. *Biochemistry* **1993**, *32*, 5352.

[69] Bom, D.; Curran, D. P.; Kruszewski, S.; Zimmer, S. G.; Strode, J. T.; Kohlhagen, G.; Du, W.; Chavan, A. J.; Fraley, K. A.; Bingcang, A. L.; Latus, L. J.; Pommier, Y.; Burke, T. G. *J. Med. Chem.* **2000**, *43*, 3970.

[70] Lavergne, O.; Lesueur-Ginot, L.; Pla Rodas, F.; Bigg, D. C. H. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2235.

[71] Lavergne, O.; Lesueur-Ginot, L.; Pla Rodas, F.; Kasprzyk, P. G.; Pommier, J.; Demarquay, D.; Prévost, G.; Ulibarri, G.; Rolland, A.; Schiano-Liberatore, A.; Harnett, J.; Pons, D.; Camara, J.; Bigg, D. C. H. *J. Med. Chem.* **1998**, *41*, 5410.

[72] Bom, D.; Curran, D. P.; Chavan, A. J.; Kruszewski, S.; Zimmer, S. G.; Fraley, K. A.; Burke, T. G. *J. Med. Chem.* **1999**, *42*, 3018.

[73] Royce, M. E.; Hoff, P. M.; Dumas, P.; Lassere, Y.; Lee, J. J.; Coyle, J.; Ducharme, M. P.; De Jager, R.; Pazdur, R. *J. Clin. Oncol.* **2001**, *19*, 1493.

[74] De Jager, R.; Cheverton, P.; Tamanoi, K.; Coyle, J.; Ducharme, M.; Sakamoto, N.; Satomi, M.; Suzuki, M. *Ann. N. Y. Acad. Sci.* **2000**, *992*, 260.

[75] Gamucci, T.; Paridaens, R.; Heinrich, B.; Schellens, J. H.; Pavlidis, N.; Verweij, J.; Sessa, C.; Kaye, S.; Roelvink, M.; Wanders, J.; Hanauske, A. *Ann. Oncol.* **2000**, *11*, 793.

[76] Emerson, D. L.; Bendele, R.; Brown, E.; Chiang, S.; Desjardins, J. P.; Dihel, L. C.; Gill, S. C.; Hamilton, M.; LeRay, J. D.; Moon-McDermott, L.; Moynihan, K.; Richardson, F. C.; Tomkinson, B.; Luzzio, M. J.; Baccanari, D. *Clin. Cancer Res.* **2000**, *6*, 2903.

[77] Lee, J. H.; Lee, J. M.; Lim, K. H.; Kim, J. K.; Ahn, S. K.; Bang, Y. J.; Hong, C. I. *Ann. N. Y. Acad. Sci.* **2000**, *992*, 324.

[78] Takimoto, C. H.; Thomas, R. *Ann. N. Y. Acad. Sci.* **2000**, *992*, 224.

[79] Stehlin, J. S.; Giovanella, B. C.; Natelson, E. A.; De Ipolyi, P. D.; Coil, D.; Davis, B.; Wolk, D.; Wallace, P.; Trojacek, A. *Int. J. Oncol.* **1999**, *14*, 821.

[80] Verschraegen, C. F.; Gupta, E.; Loyer, E.; Kavanagh, J. J.; Kudelka, A. P.; Freedman, R. S.; Edwards, C. L.; Harris, N.; Steger, M.; Steltz, V.; Giovanella, B. C.; Stehlin, J. S. *Anticancer Drugs* **1999**, *10*, 375.

[81] Bom, D.; Curran, D. P.; Kruszewski, S.; Zimmer, S. G.; Thompson Strode, J.; Kohlhagen, G.; Du, W.; Chavan, A. J.; Fraley, K. A.; Bingcang, A. L.; Latus, L. J.; Pommier, Y.; Burke, T. G. *J. Med. Chem.* **2000**, *43*, 3970.

[82] Josien, H.; Ko, S. B.; Bom, D.; Curran, D. P. *Chem. Eur. J.* **1998**, *4*, 67.

[83] Van Hattum, A. H.; Pinedo, H. M.; Schluper, H. M.; Hausheer, F. H.; Boven, E. *Int. J. Cancer* **2000** *88*, 260.

[84] Kim, D.-K.; Ryu, D. H.; Lee, J. Y.; Lee, N.; Kim, Y.-W.; Kim, J.-S.; Chang, K.; Im, G.-J.; Kim, T.-K.; Choi, W.-S. *J. Med. Chem.* **2001**, *44*, 1594.

[85] Demarquay, D.; Huchet, M.; Coulomb, H.; Lesueur-Ginot, L.; Lavergne, O.; Kasprzyk, P. G.; Bailly, C.; Camara, J.; Bigg, D. C. *Anticancer Drugs* **2001**, *12*, 9.

[86] Lavergne, O.; Demarquay, D.; Kasprzyk, P. G.; Bigg, D. C. *Ann. N. Y. Acad. Sci.* **2000**, *992*, 100.

[87] Lavergne, O.; Demarquay, D.; Bailly, C.; Lanco, C.; Rolland, A.; Huchet, M.; Coulomb, H.; Muller, N.; Baroggi, N.; Camara, J.; Le Breton, C.; Manginot, E.; Cazaux, J. B.; Bigg, D. C. H. *J. Med. Chem.* **2000**, *43*, 2285.

[88] Demarquay, D.; Coulomb, H.; Huchet, M.; Lesueur-Ginot, L.; Camara, J.; Lavergne, O.; Bigg, D. *Ann. N. Y. Acad. Sci.* **2000**, *992*, 301.

[89] Huchet, M.; Demarquay, D.; Coulomb, H.; Kasprzyk, P.; Carlson, M.; Lauer, J.; Lavergne, O.; Bigg, D. *Ann. N. Y. Acad. Sci.* **2000**, *992*, 303.

[90] Bom, D.; Curran, D. P.; Chavan, A. J.; Kruszewski, S.; Zimmer, S. G.; Fraley, K. A.; Burke, T. G. *J. Med. Chem.* **1999**, *42*, 3018.