Cancer Chemother Pharmacol (2010) 65:363–371
DOI 10.1007/s00280-009-1042-5

ORIGINAL ARTICLE

A water soluble prodrug of a novel camptothecin analog is efficacious against breast cancer resistance protein-expressing tumor xenografts

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Received: 22 February 2009 / Accepted: 19 May 2009 / Published online: 4 June 2009

Abstract
Purpose Identification of a novel topoisomerase I inhibitor which shows superior efficacy and less individual variation than irinotecan hydrochloride (CPT-11).
Methods A novel camptothecin analog that is effective against breast cancer resistance protein (BCRP)-positive cells was screened, and a water soluble prodrug was generated. Antitumor activity of the prodrug was examined in BCRP-positive and -negative xenografts both as a single agent and in combination with other anti-cancer drugs.
Results A novel camptothecin analog, CH0793076, was discovered. Because CH0793076 was found to be highly lipophilic, a water soluble prodrug (TP300) was generated. TP300 is stable in an acidic solution but is rapidly converted to CH0793076 under physiological pH conditions such as in sera. This efficient prodrug activation would minimize interpatient differences in pharmacokinetic and toxicity profiles. Unlike CPT-11, TP300 does not exhibit cholinergic interaction or cause acute diarrhea at effective doses. In mouse xenograft models, TP300 showed antitumor activity against both BCRP-positive and -negative xenografts, whereas CPT-11 was less active against BCRP-positive xenografts. In addition, the effective dose range (MTD/ED_{50}) for TP300 was wider than for CPT-11 and TP300 showed additive or synergistic antitumor effects in combination with other anti-cancer drugs such as capecitabine, oxaliplatin, cisplatin, bevacizumab and cetuximab.
Conclusion It is therefore expected that TP300 will provide an additional treatment option for patients who will undergo chemotherapy with camptothecins.

Keywords Camptothecin · Breast cancer resistance protein · Prodrug · CPT-11

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Introduction

Two well-characterized DNA topoisomerase I inhibitors, irinotecan hydrochloride (CPT-11) and topotecan, are currently used in clinical. CPT-11 is a water-soluble prodrug which is converted to the active form SN-38 by a carboxylesterase [1]. Topotecan is a water-soluble semisynthetic derivative of camptothecin. CPT-11 has been used mainly for treatment of advanced and/or metastatic colorectal cancer (CRC) [2, 3], whilst topotecan is used mainly for treatment of late-stage ovarian cancer and small cell lung cancer (SCLC) [4, 5]. Although CPT-11 has been prescribed more frequently than topotecan, the efficacy of CPT-11 is limited

for several reasons. First, due to the low efficiency of the enzymatic conversion from CPT-11 to SN-38, there is wide inter- and intra-patient variability with respect to pharmacokinetics which leads to considerable individual variation in toxicity [6–8]. Secondly, due to acetylcholinesterase (AChE) inhibition by the prodrug moiety of CPT-11, acute cholinergic diarrhea occurs during and shortly after drug administration [9, 10]. Thirdly, patients with single-nucleotide polymorphisms (SNPs) in the UDP-glucuronosyltransferase 1 (UGT1A1) gene coding for the enzyme that forms SN-38-glucuronide (SN-38G) may suffer severe toxicity [11–16]. Fourthly, SN-38G deconjugated to SN-38 by the β-glucuronidase of the intestinal microflora results in severe delayed diarrhea [17]. Finally, BCRP, which is an ABC transporter and can efflux various anti-cancer drugs from cells, has been implicated in cellular resistance to camptothecin agents such as SN-38 and topotecan [18–21]. This ABC half-transporter requires dimerization to form a functional transporter and probably works as a homodimer [22, 23]. Indeed, the expression of BCRP protein was observed in more than 20% of tumor in various types of cancer including CRCs [24]. Therefore, a novel topoisomerase I inhibitor, which shows superior efficacy and less individual variation than drugs such as CPT-11, will be beneficial to cancer patients.

Here, we report the antitumor activity of a novel hexacyclic camptothecin analog, CH0793076. CH0793076 was active even against cells expressing BCRP. Because CH0793076 is highly lipophilic and poorly soluble in water, a water-soluble prodrug of CH0793076, TP300, was generated for intravenous formulation. TP300 is stable in aqueous solutions at acidic pH levels but is rapidly converted to the active form (CH0793076) under neutral pH conditions such as in sera [25]. TP300 did not inhibit AChE and thereby did not induce acute diarrhea at the effective dose range. Furthermore, it showed strong antitumor activity against BCRP-positive tumor xenografts and also in combination with other anti-cancer drugs. Thus, TP300 is a potential new option for treatment using camptothecin agents.

Materials and methods

Chemicals and antibodies

TP300, CH0793076 and SN-38 were synthesized at Chugai Pharmaceutical Research Center (Kamakura, Japan). CPT-11 was purchased from Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan). Capecitabine and bevacizumab were provided from Hoffmann-La Roche (Basel, Switzerland). Other drugs were purchased from the following suppliers: oxaliplatin from Sanofi-Aventis (Paris, France), cisplatin from Nippon Kayaku Co., Ltd. (Tokyo, Japan) and cetuximab from Bristol-Myers Squibb (Princeton, NJ, USA).

Assay for DNA topoisomerase I

DNA relaxation activity from human DNA topoisomerase I (Topo-I) was measured using a Topoisomerase I Drug Screening Kit (TopoGEN, Inc., Port Orange, FL, USA). Supercoiled plasmid DNA and human Topo-I (ProteinOne, Bethesda, MD, USA) were incubated at 37°C for 30 min in the presence and absence of the test compounds. After separation of the supercoiled DNA (Sc-DNA) and relaxed DNA (Re-DNA) by agarose gel electrophoresis, ratio of the Sc-DNA and Re-DNA was quantified by using a VersaDoc Imaging System 5000 (Bio-Rad Laboratories, Hercules, CA, USA), and the IC_{50} values for each compound were calculated.

Human cancer cells

The human cancer cell lines were purchased from the following suppliers: HCT116 and COLO 201 from Dainippon Pharmaceutical Co., Ltd. (Tokyo, Japan), AsPC-1, NCI-H460, HT-29, WiDr, HCT-15, HCT-8 and Calu-6 from American Type Culture Collection (Rockville, MD, USA), and PC-6 from Immuno-Biological Laboratories (Gunma, Japan). The MCF-7/WT and MCF-7/TPT300 cells were gifted by Dr. Liau (National Taiwan University Hospital, Republic of China) [21]. The A2780 cells were gifted by Dr. Onishi (Kagoshima City Hospital, Japan). The cells were cultured either in McCoy's 5a containing 2 mM L-glutamine and 10% FBS (for HCT116 and HT-29), RPMI1640 containing 10% FBS (for COLO201, NCI-H460, PC-6 and A2780), RPMI1640 containing 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g/L glucose and 20% FBS (for ASPC-1), RPMI1640 containing 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g/L glucose and 10% FBS (for HCT-15 and HCT-8), EMEM containing 1 mM sodium pyruvate, 0.1 mM non-essential amino acid and 10% FBS (for WiDr and Calu-6) or Dulbecco's modified Eagle medium containing 10% FBS (for MCF-7/WT and MCF-7/TPT300), in a 5% CO_2 incubator at 37°C.

Generation of the SN-38-resistant cell line and the BCRP-transfected cell line

A2780/SN75 cells, an SN-38-resistant A2780 human ovarian tumor cell line, were obtained as a result of stepwise, continuous exposure to SN-38. The cells were first treated with SN-38 at 1.56 nM and maintained with 75 nM of SN-38. To create cells that constitutively express BCRP, full-length human BCRP cDNA was amplified by PCR with a set of primers, $^{5'}$CGCGAATTCTCGAGACTGTA

GGCACT$^{3'}$, and $^{5'}$CGCGAATTCTCACCTTAACACACC GG$^{3'}$, and human liver mRNA as the template. The resulting cDNA was cloned at the *Eco*RI site of pRC/CMV, generating pRC/CMV-BCRP. The plasmid carrying the human BCRP cDNA (pRC/CMV-BCRP) or vector (pRC/CMV) was introduced into PC-6 cells using Lipofectamine™ (Life Technologies, Bethesda, MD, USA) and the cells were selected in the presence of 1 mg/mL of G418. One of the clones that grew in the presence of 1 mg/mL of G418 was designated PC-6/BCRP and used for the experiments. A clone carrying the pRC/CMV was also collected and designated PC-6/pRC.

In vitro antiproliferative assay

The single cell suspension was seeded onto 96-well plates. The cells were cultured in the presence of TP300, CH0793076 or SN-38 for 4–7 days at 37°C. On the last day of the culture, absorbance at 450 and 630 nm was measured in each well by a microplate reader after the addition of Cell Counting Kit-8 (Dojindo Laboratories, Japan). The experiments were carried out in triplicate and the mean IC_{50} values calculated.

Western blotting

Western blotting to detect the 140 kDa BCRP dimer was performed according to the method of Kage et al. [23]. Cells were lysed with extraction buffer containing 10 mM Tris–HCl (pH 8.0), 0.1% Triton X-100, 10 mM $MgSO_4$ and 2 mM $CaCl_2$. The proteins in the cell lysate were separated on a 5–20% SDS-polyacrylamide gel and transferred onto nitrocellulose membranes. The membrane was incubated with the mouse anti-BCRP monoclonal antibody BXP-21 (Signet laboratories Inc., Dedham, MA, USA) and then with horseradish peroxidase-linked anti-mouse IgG (Amersham Biosciences, Tokyo, Japan). BCRP protein was detected by ECL plus Western Blotting Detection System (Amersham Biosciences, Tokyo, Japan) using VersaDoc Imaging System 5000 (Bio-Rad Laboratories, Hercules, CA, USA).

Acetylcholinesterase (AChE) assay

Acetylcholinesterase (AChE) activity was assayed with a human recombinant AChE (0.075 U/mL, Sigma-Aldrich Co. St. Louis, MO, USA) in the presence or absence of the test compounds in phosphate buffer (pH 7.4) containing 1.728 mmol/L acetylthiocholine iodide, 0.3 mmol/L 5,5′-Dithiobis(2-nitrobenzoic acid), 5% DMSO at 37°C for 10 min. After the reaction was terminated by the addition of *N*,*N*-dimethylformamide, absorbance at 412 nm was measured using a microplate reader and the IC_{50} values for each compound were calculated.

Xenograft mouse models

Five-week-old male athymic nude mice (CAnN.Cg-*Foxn1*^{nu}/CrlCrlj) were purchased from Charles River Japan, Inc. (Yokohama, Japan). The single cell suspensions were inoculated subcutaneously into the right flank of each mouse. Tumor size and body weight were measured twice a week. The tumor volume was estimated by using the formula $ab^2/2$, where a and b represent tumor length and width, respectively. Administration of the drugs was initiated on the day when the mean tumor volume reached 200–300 mm^3. All animal experiments were conducted in accordance with the Guideline for Accommodation and Care of Laboratory Animals promulgated by Chugai Pharmaceutical.

Determination of the antitumor effects of TP300 and CPT-11

TP300 dissolved in saline containing 1 mM citric acid monohydrate and CPT-11 dissolved in saline were each administered by intravenous (IV) bolus once per week for 3 weeks. Tumor growth inhibition (%) was calculated by the formula $(1 - T/C) \times 100$ (%), where T and C represent the mean tumor volume change in the treated and control group, respectively, between the first administration day (day 0) and day 21. The dose limiting toxicity (DLT) was defined as survival or body weight loss. The dose was regarded as toxic if two or more of a group of at least six mice died, or the mean relative body weight for the group was less than 80%. The maximum tolerated dose (MTD) was defined as the highest dose which did not cause toxic effects. Effective dose ranges were defined as the range from the lowest effective dose which resulted in 50% inhibition of tumor growth (ED_{50}) to MTD, calculated from the tumor growth inhibition on day 21.

To determine the antitumor activity of TP300 in combination with other anti-cancer drugs, drugs were dissolved and diluted in solutions as follows: capecitabine in 0.5% carboxymethyl cellulose sodium salt solution (0.5% CMC), oxaliplatin in 5% glucose solution, and cisplatin and bevacizumab in saline. TP300 was administered as an IV bolus once per week for 6 weeks or for 3 weeks in combination with cetuximab. Capecitabine was orally administered for two cycles of daily dosing for 14 days followed by a 7-day rest period. Platinum agents were administered as an IV bolus every other week (oxaliplatin) or every 3 weeks (cisplatin) for 6 weeks. Bevacizumab and cetuximab were administered as an IV bolus twice a week for 6 and 3 weeks, respectively.

Statistics

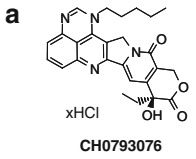
Comparative statistical analysis was performed using Wilcoxon's test. When the probability (P) value was less

than 0.05 ($P < 0.05$), the difference between the two groups was considered significant.

Results

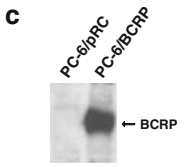
Water soluble prodrug of a new camptothecin analog with antiproliferative activity against BCRP expressing cells

The activity of SN-38 (the active form of CPT-11) was shown to be affected by BCRP expression [18–21]. Therefore, we synthesized new camptothecin derivatives and found a new hexacyclic derivative of camptothecin, CH0793076 (Fig. 1a). Inhibition of human DNA topoisomerase I (Topo-I) activity by CH0793076 was approximately equal to that of SN-38; the IC_{50} value for inhibition of the DNA relaxation activity by Topo-I was 2.3 μmol/L for CH0793076 and 5.5 μmol/L for SN-38 (Fig. 1b). To confirm the effect of BCRP on the antiproliferative activity of CH0793076, we established cells which constitutively overexpressed BCRP. The PC-6/BCRP cells carrying pRC/CMV-BCRP expressed significant amounts of the 140 kDa BCRP protein, but PC-6/pRC cells bearing the control vector did not (Fig. 1c). The IC_{50} value of SN-38 for the PC-6/BCRP cells was 12 times greater than that of the PC-6/pRC cells, whereas the IC_{50} value of CH0793076 for the PC-6/BCRP cells was only twice that of the PC-6/pRC cells (Fig. 1d). Although we did not determine the intracellular concentrations of CH0793076, the increase in the IC_{50} value of CH0793076 against PC-6/BCRP suggests that CH0793076 has a low binding affinity to BCRP and is only slightly exported by BCRP.



b

IC_{50} (μmol/L)	
SN-38	CH0793076
5.5	2.3



d

Cell line	IC_{50} (nmol/L) [Mean ± SE]	
	SN-38	CH0793076
PC-6/BCRP	5.1 ±1.8	0.35 ±0.04
PC-6/pRC	0.43 ±0.07	0.18 ±0.01
IC_{50} ratio	12	2

Fig. 1 Inhibition of the growth of BCRP-expressing cell lines by CH0793076. **a** Chemical structure of CH0793076. **b** Inhibition of human DNA topoisomerase-I by CH0793076 and SN-38. CH0793076 and SN-38 were incubated with 250 ng of supercoiled plasmid DNA and human topoisomerase-I at 37°C for 30 min. The IC_{50} values for CH0793076 and SN-38 are shown. **c** Expression of the BCRP protein in the PC-6 cells carrying BCRP cDNA (PC-6/BCRP) and carrying only the vector (PC-6/pRC). The BCRP protein was detected by western blotting with mouse anti-BCRP monoclonal antibody BXP-21. The position of the BCRP protein is indicated by an *arrow*. **d** Antiproliferative activity against PC-6/BCRP and PC-6/pRC cells. The cells were exposed to the compounds for 6 days at 37°C. The experiments were carried out in triplicate and the mean of the IC_{50} values are shown

Effect of TP300 on acetylcholinesterase (AChE) activity

Because CH0793076 is hardly soluble in water, we generated TP300, a water-soluble prodrug of CH0793076, for intravenous formulation (Fig. 2a). TP300 is stable in an aqueous solution at low pH but is rapidly (within 3 min) converted to the active form in neutral pH conditions and

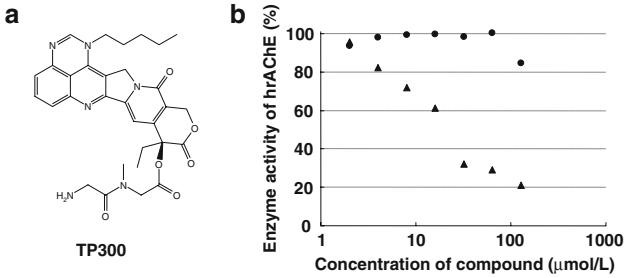
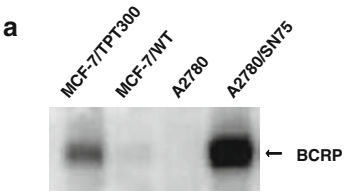


Fig. 2 Effect of TP300 on the human acetylcholinesterase activity. **a** Chemical structure of TP300. **b** Human recombinant acetylcholinesterase (rhAChE, 0.075 U/mL) was incubated with 2–128 μM of TP300 (*closed circle*) or CPT-11 (*closed triangle*) at 37°C for 10 min; percentages of inhibition are shown



b

Cell line	IC_{50} (nM) [Mean ± SE]		
	SN-38	CH0793076	TP300
MCF7/TP300	60	2.4	nd
MCF7/WT	0.89	0.32	nd
IC_{50} ratio	67	7.5	-
A2780/SN75	99	nd	9.4
A2780	1.8	nd	1.1
IC_{50} ratio	55	-	8.5

Fig. 3 Antiproliferative activities of CH0793076 and TP300 against camptothecin-resistant cells. **a** Expression of the BCRP protein in MCF-7, topotecan-resistant MCF-7 (MCF-7/TPT300), A2780 and the SN-38-resistant A2780 (A2780/SN75) cells. BCPR in the cell extract was analyzed by western blotting with the mouse anti-BCRP monoclonal antibody BXP-21. The position of the BCRP protein is indicated by an *arrow*. **b** The cells were cultured in the presence of CPT-11, CH0793076 or TP300 for 7 days (for MCF-7 and MCF-7/TPT300) or 3 days (for A2780 and A2780/SN75), and the IC_{50} values are shown. *nd* not done

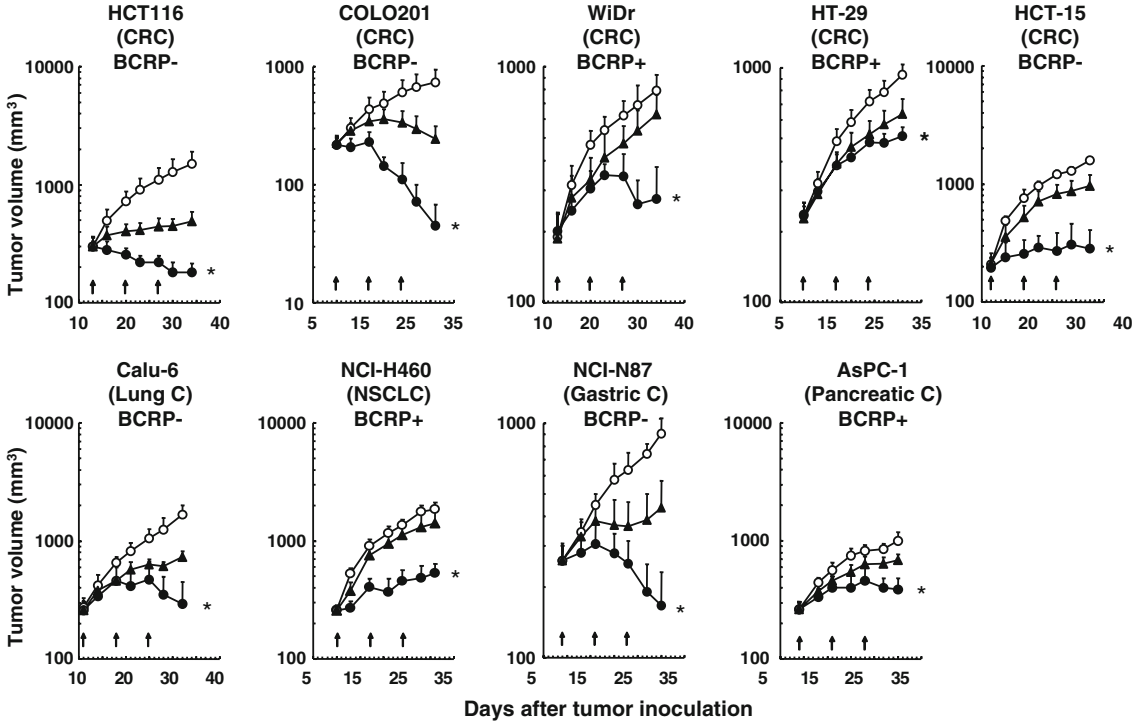


Fig. 4 Antitumor effect of TP300 and CPT-11 in human cancer xenograft models. TP300 and CPT-11 were administered at the maximum tolerated dose (MTD) by bolus intravenous injection once per week for 3 weeks, for a total of three doses. The MTD was 47 mg/kg for TP300 and 100 mg/kg for CPT-11. Each group consisted of six or seven mice. Mean values of tumor volume along with standard deviations are indicated. *Open circle* vehicle, *closed circle* TP300, *closed triangle* CPT-11. The BCRP protein was detected by western blotting. *Asterisk* statistically significant difference between mice treated with TP300 and mice treated with CPT-11 ($P < 0.05$)

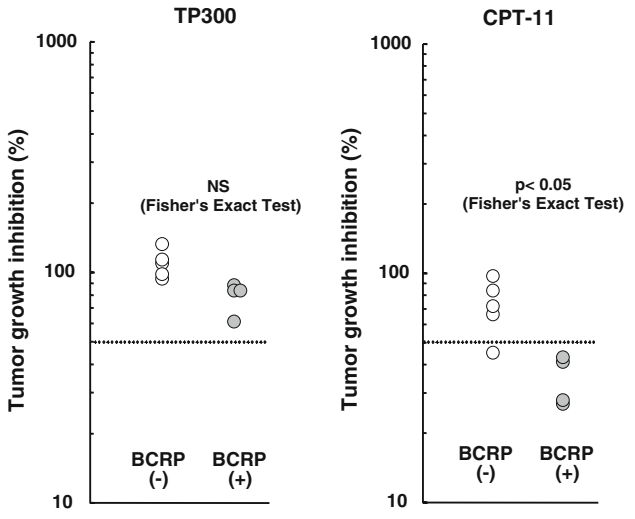


Fig. 5 Correlation between the efficacy of TP300 and CPT-11 in various human cancer xenograft models. Tumor growth inhibition (%) by TP300 or CPT-11, shown in Fig. 4, was plotted separately for the BCRP-positive and -negative tumor xenografts. *Dotted lines* indicate 50% of tumor growth inhibition. Statistical analysis of the antitumor effect of each drug and BCRP expression was calculated using Fisher's exact test. *NS* Not significant

also in human sera [25]. CPT-11 has been reported to inhibit AChE, which results in acute diarrhea [9, 10]. Therefore, we asked whether TP300 affects the activity of AChE. CPT-11 inhibited recombinant human AChE (rhAChE) activity in a concentration-dependent manner with an IC_{50} of 22 μM, but TP300 only slightly affected rhAChE activity at the high concentration of 128 μM (Fig. 2b). It is also of note that CH0793076, the active drug and major metabolite of TP300, did not inhibit acetylcholine esterase activities even at higher concentrations (up to 128 μM) (data not shown).

Antiproliferative activity of TP300 against camptothecin-resistant cell lines

Next, we asked whether TP300 is also effective against naturally emerging camptothecin-resistant cell lines, topotecan-resistant human breast cancer cells (MCF-7/TPT300) [21] and SN-38-resistant human ovarian cancer cells (A2780/SN75). BCRP was expressed in both cells but not in the parental cells (Fig. 3a). The antiproliferative activities of TP300 and CH0793076, compared with SN-38, were much less affected by the expression of BCRP. The ratio of IC_{50} for SN-38 between MCF-7/TPT300 and MCF-7/WT was 67 and that for CH0793076 was 7.5. Similarly, the ratio of IC_{50} for SN-38 between A2780/SN75 and A2780 was 55 and that for TP300 was 8.5 (Fig. 3b). The results confirm that BCRP has significant impact on the

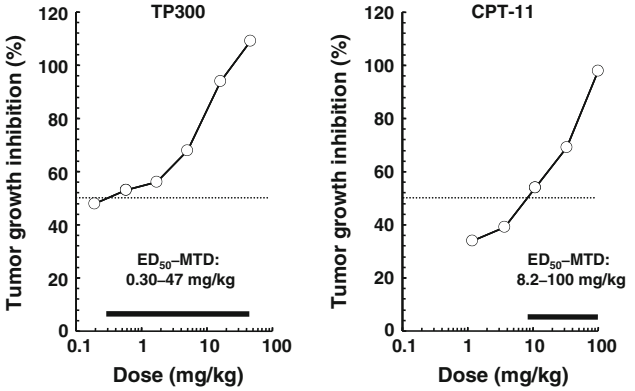


Fig. 6 Effective dose ranges for TP300 and CPT-11 in the HCT116 human CRC xenograft model. The various doses of TP300 and CPT-11 were administered to mice bearing the HCT116 xenograft by bolus intravenous injection once per week for 3 weeks, for a total of three doses. The doses of TP300 and CPT-11 were plotted logarithmically on the *x*-axis and the tumor growth inhibition (%) was plotted on the *y*-axis. Each group consisted of seven mice. The toxic dose was 63 mg/kg for TP300 and 133 mg/kg for CPT-11. The therapeutic window of each compound is the range between ED_{50} and MTD

antiproliferative activity of SN-38 and also demonstrate that TP300 is much less affected by BCRP as compared to SN-38. In addition, the antiproliferative activity of TP300 was not significantly affected by expression of MDR1, whereas MDR1-overexpressing cells were highly resistant to paclitaxel (not shown).

Superior efficacy and wider therapeutic index over CPT-11

Because the antiproliferative activity of TP300 was not drastically affected by BCRP, TP300 is expected to be efficacious regardless of BCRP expression, thereby showing a wider antitumor spectrum than CPT-11. We examined the antitumor activity of TP300 in nine human cancer xenograft models, among which four (WiDr, HT-29, NCI-H460 and AsPC-1) were BCRP-positive and five (HCT116, COLO 201, HCT-15, Calu-6 and NCI-N87) were BCRP-negative as judged by western blotting. CPT-11 showed more than 50% tumor growth inhibition in four out of five BCRP-negative models, but tumor growth inhibition by CPT-11 was less than 50% in all four BCRP-positive models. In contrast, TP300 showed more than 50% of tumor growth inhibition in all nine models, regardless of the expression of BCRP (Figs. 4, 5). There was no marked body weight loss during the dosing period. Antitumor activity of TP300 was slightly weaker against BCRP-positive xenografts than that against BCRP-negative

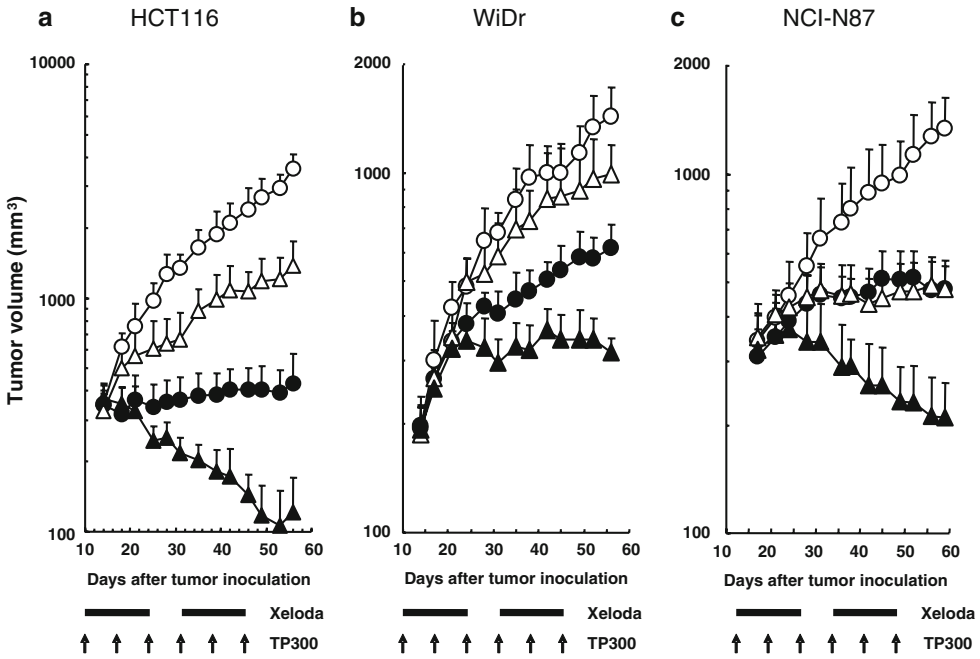


Fig. 7 Antitumor effect of TP300 in combination with capecitabine. Tumor growth inhibition by TP300 in combination with capecitabine was examined in the HCT116 human colon cancer (**a**), WiDr human colon cancer (**b**) and NCI-N87 human gastric cancer (**c**) xenograft models. TP300 was administered by bolus intravenous injection once per week for 6 weeks. Capecitabine was orally administered for two cycles of daily dosing for 14 days followed by a 7-day rest period. Control mice were administered the vehicle of the compound. Mean values of tumor volume with standard deviations are indicated. Each group consisted of 5–7 mice. **a** *Open circle* vehicle, *closed circle* TP300 at 24 mg/kg (1/2 MTD), *open triangle* capecitabine at 180 mg/kg (1/2 MTD), *closed triangle* TP300 + capecitabine. **b** *open circle* vehicle, *closed circle* TP300 at 16 mg/kg (1/3 MTD), *open triangle* capecitabine at 270 mg/kg (MTD), *closed triangle* TP300 + capecitabine. **c** *Open circle* vehicle, *closed circle* TP300 at 16 mg/kg (1/3 MTD), *open triangle* capecitabine at 135 mg/kg (1/4 MTD), *closed triangle* TP300 + capecitabine

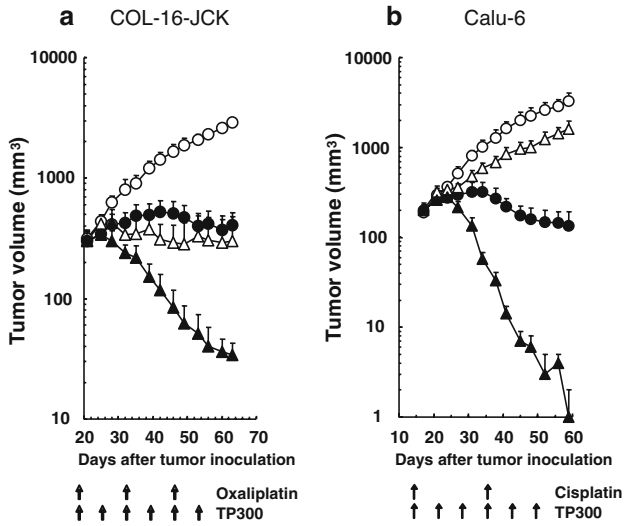


Fig. 8 Antitumor effect of TP300 in combination with oxaliplatin or cisplatin. Tumor growth inhibition by TP300 in combination with oxaliplatin (**a**) or cisplatin (**b**) was examined in the COL-16-JCK human rectal cancer (**a**) and Calu-6 human lung cancer (**b**) xenograft models, respectively. TP300 was administered by bolus intravenous injection once per week for 6 weeks. Platinum agents were administered by bolus intravenous injection every other week (oxaliplatin) or every 3 weeks (cisplatin) for 6 weeks. Control mice were administered the vehicle of the compound. Mean values of tumor volume along with standard deviations are indicated. Each group consisted of seven mice. **a** *Open circle* vehicle, *closed circle* TP300 at 24 mg/kg (1/2 MTD), *open triangle* oxaliplatin at 7.5 mg/kg (1/2 MTD), *closed triangle* TP300 + oxaliplatin. **b** *Open circle* vehicle, *closed circle* TP300 at 24 mg/kg (1/2 MTD), *open triangle* cisplatin at 5 mg/kg (1/2 MTD), *closed triangle* TP300 + cisplatin

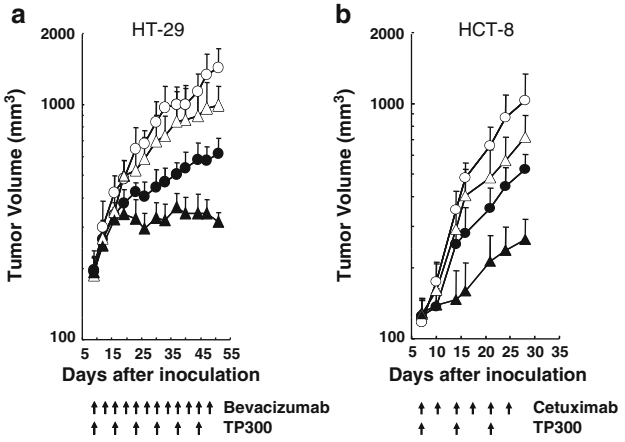


Fig. 9 Antitumor effect of TP300 in combination with bevacizumab or cetuximab. Tumor growth inhibition by TP300 in combination with bevacizumab (**a**) or cetuximab (**b**) was examined in the HT-29 (**a**) and HCT-8 (**b**) human colon cancer xenograft models, respectively. TP300 was administered by bolus intravenous injection once per week for 6 weeks to the HT-29 model and for 3 weeks to the HCT-8 model. Bevacizumab at 2.5 mg/kg and cetuximab at 40 mg/kg were intraperitoneally administered twice a week for 6 and 3 weeks, respectively. Control mice were administered the vehicle. Mean values of tumor volume along with standard deviations are indicated. Each group consisted of 6 or 7 mice. **a** *Open circle* vehicle, *closed circle* TP300 at 45 mg/kg (MTD), *open triangle* bevacizumab at 2.5 mg/kg, *closed triangle* TP300 + bevacizumab. **b** *Open circle* vehicle, *closed circle* TP300 at 12 mg/kg (1/4 MTD), *open triangle* cetuximab at 40 mg/kg, *closed triangle* TP300 + cetuximab

xenografts, and as mentioned, presumably due to the recognition and export of CH0793076 by BCRP, although the binding affinity of CH0793076 to BCRP is much weaker than that of SN-38.

We also compared the effective dose ranges of TP300 and CPT-11 in the BCRP-negative HCT116 xenograft model. Using MTD/ED_{50} as the measure of therapeutic index, the effective dose range of TP300 was between 0.30 and 47 mg/kg (MTD/ED_{50} = 157) and that of CPT-11 was between 8.2 and 100 mg/kg (MTD/ED_{50} = 12) (Fig. 6). The toxic dose was 63 mg/kg for TP300 and 133 mg/kg for CPT-11.

Additive to synergistic antitumor effects of TP300 in combination with other anti-cancer drugs

In order to further demonstrate the potency of TP300, we assessed the antitumor activity of TP300 in combination with other anti-cancer drugs. TP300 in combination with capecitabine resulted in synergistic effects in the HCT116 human colon cancer and NCI-N87 human gastric cancer xenograft models and an additive effect in the WiDr human colon cancer xenograft model which is BCRP-positive and CPT-11-insensitive (Fig. 7). Synergistic effects were more profound when TP300 was combined with platinum agents (oxaliplatin or cisplatin) and caused strong tumor remission in the COL-16-JCK human rectal cancer and Calu-6 human lung cancer xenograft models, respectively (Fig. 8). TP300 showed additive effects when combined with monoclonal antibodies such as bevacizumab (anti-VEGF) and cetuximab (anti-EGFR) in the HT-29 and HCT-8 human colon cancer xenograft models, respectively (Fig. 9). The additive and synergistic effects of these combinations were not associated with any additional body weight loss as compared to body weight change caused by each drug alone (not shown). The results clearly demonstrate that TP300 elicits additive or synergistic effects in combination with various anti-cancer drugs and thus is widely eligible for combination therapy.

Discussion

We discovered a novel camptothecin derivative, CH0793076, which is active against cells resistant to topotecan or SN-38. Recently, several oral camptothecin analogs were developed but most have failed to show

efficacy in clinical studies [26]. It is conceivable that an oral formulation has substantially high individual variation in absorption and thereby may cause unpredictable severe toxicity. CH0793076 is hardly soluble in water and, therefore, it cannot be formulated for intravenous injection. We synthesized a water-soluble prodrug of CH0793076, TP300, which is stable in lower pH aqueous solutions, but is rapidly and chemically converted to the lipophilic active drug (CH0793076) under neutral pH conditions and also in blood [25]. The chemical conversion from the parent molecule to the active drug occurs very fast. In rats and dogs, TP300 was detected in plasma at 5 min (the first sampling point) after single intravenous administration, and maximum plasma concentration (C_{max}) of CH0793076 was achieved at 5 min after dosing. It is therefore expected that the administration of TP300 will fully elicit the effect of the active drug, yet minimize individual variation in pharmacokinetics and toxicity as a result of the efficiency of the activation of the prodrug. In addition, TP300 did not affect the AChE activity, at least in the effective dose range, whereas CPT-11 did inhibit the AChE activity [9, 10]. Therefore, the administration of TP300 is unlikely to cause the cholinergic acute diarrhea caused by the inhibition of AChE activity. Moreover, it has been reported that SNPs (single nucleotide polymorphisms) in the UGT1A1, the gene coding for enzymes that form the SN-38 glucuronide, contribute to the inter-patient variability of CPT-11 in toxicity and efficacy [11–16]. Because CH0793076 has no phenolic-OH group for glucuronidation, the variability caused by the SNPs of UGT 1A1 may not be the case with TP300.

Several groups have shown that overexpression of BCRP confers cancer cell resistance to CPT-11 and SN-38 [18–21]. Furthermore, it has recently been demonstrated that the BCRP mRNA content of liver metastatic tumor cells from CRC patients treated with CPT-11 was higher than that of CPT-11-naive patients [27]. Similarly, we found that the antitumor activity of SN-38 was highly influenced by BCRP expression, whilst that of CH0793076 was only slightly affected by BCRP. As a result, TP300 was efficacious against both CPT-11-sensitive and -insensitive tumor xenografts; the antitumor effect of CPT-11 was attenuated in the BCRP-positive xenograft models, whereas the presence or absence of BCRP had much less influence on the antitumor activity of TP300. However, because BCRP is expressed in the hematopoietic stem cells, bone marrow toxicity should be carefully monitored in clinical studies [28].

As determined in the HCT116 xenograft model, the effective dose range for TP300 was approximately 13 times wider than for CPT-11. TP300 showed additive or synergistic effects when combined with other anti-cancer drugs such as capecitabine, oxaliplatin, cisplatin, bevacizumab and cetuximab. Thus, TP300 is expected to have superior efficacy to CPT-11 both as a monotherapy and in combination with other drugs, and TP300 should show less individual variation and thus reduce the unpredictable toxicity to which CPT-11 gives rise in clinical.

Acknowledgment We thank F. Ford for proofreading the manuscript.

Conflict of interest statement None.

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