

# ANTITUMOR ACTIVITY OF A NEW SYNTHETIC CAMPTOTHECIN WITH THE OPEN LACTONE RING

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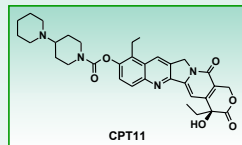
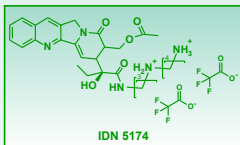
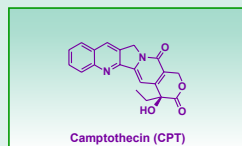
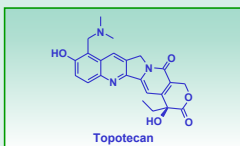
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## ABSTRACT

A number of water-soluble natural camptothecins containing a polyamine linked to the carboxylate function of the opened lactone ring has been identified. To investigate the therapeutic interest of these hydrophilic camptothecins, a series of analogs containing a polyamine moiety has been synthesized, and IDN 5174 was selected for further development. In the NCI-H460 human lung carcinoma cell line the molecule was cytotoxic, even though less potent than topotecan or SN38, in short-term and long-term exposure assay (1 and 72 h). The analysis of topoisomerase-mediated DNA cleavage using purified enzyme indicated that IDN 5174 was an efficient topoisomerase I poison and displayed the same cleavage pattern of SN38. Persistence of the DNA cleavage after NaCl-mediated disruption of the ternary complex was comparable to that of SN38. Stabilization of the cleavable complex was not the result of hydrolysis of the N-C bond between polyamine and the drug, because less than 1% of camptothecin was formed during the enzymatic reaction. Against the NCI-H460 human tumor xenograft, IDN 5174 exhibited an activity comparable to that of topotecan or irinotecan (TW1% ~90%) when given i.v. or p.o. Preliminary evaluation of IDN 5174 pharmacokinetics in CD1 mice, after i.v. administration of 15 mg/kg, showed rapid plasmatic clearance with elimination half-life of 20 minutes. In plasma the camptothecin level were 50-fold lower than that of IDN 5174. In conclusion, such a data was unexpected, since a closed  $\alpha$ -lactone ring has been considered for long time a critical requirement for effective camptothecins. In spite of presenting a polyamine linked through a stable N-C bond to the carboxylate function of the opened lactone ring, the novel camptothecin analog IDN 5174 maintained the biological and antitumor properties of the established camptothecin. The contribution of closed-ring camptothecins in determining the effects of IDN 5174 appeared marginal.

## INTRODUCTION

- Camptothecins (CPTs) act by stabilizing the DNA-topoisomerase complex, and thus inducing an accumulation of a reversible DNA-topoisomerase I-CPT ternary complex (the cleavable complex).
- The six-membered  $\alpha$ -hydroxy lactone E-ring of CPT is important for binding to the DNA-enzyme complex and for the biological activity. However, the E-ring is instable due to its sensitivity to hydrolysis, with formation of the carboxylate form.
- The development of CPT analogs with improved chemical stability while maintaining binding ability to the DNA-enzyme complex is an important goal in CPTs development.
- A series of water-soluble CPT analogs containing a polyamine linked through a stable N-C bond to the carboxylate function of the opened lactone ring has been synthesized, and the analog linked to spermidine, IDN 5174, was selected for preclinical development.
- Aim of the study was to investigate the pharmacological profile of IDN 5174 in *in vitro* and *in vivo* systems, using the NCI-H460 tumor model presenting high expression of topoisomerase I.



## ANTIPROLIFERATIVE ACTIVITY

The non-small cell lung cancer NCI-H460 was used. Tumor cells were seeded into six-well plates. Compounds (dissolved in DMSO, final concentration: 1%) were added after 24 h and left for 1 or 72 h. Cell viability was counted by a counter after 72 h.

Drug	IC <sub>50</sub> (µg/mL)	
	1 h	72 h
Topotecan	0.2, 0.25, 0.53	0.004, 0.0036
SN38	0.083	0.04
IDN 5174	3.1	0.033

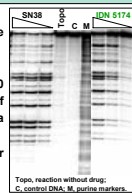
## TOPOISOMERASE I-DEPENDENT DNA CLEAVAGE ASSAY

A gel purified BamHI-EcoRI fragment of SV40 DNA was used for the cleavage assay.

DNA fragments were uniquely 3'-end labeled.

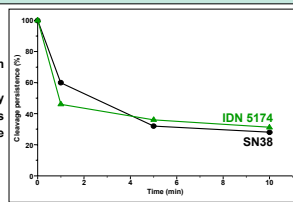
Samples were reacted with 1, 10 and 50 µM drug at 37 °C for 30 min. Reaction was then stopped by adding 1% SDS, 0.3 mg/mL of proteinase K and incubating for 45 min at 42 °C before loading on a denaturing 8% polyacrylamide gel.

Overall DNA cleavage levels were measured with a PhosphorImager 425 model (Molecular Dynamics).



## PERSISTENCE OF TOPOISOMERASE I-MEDIATED DNA CLEAVAGE IN THE PRESENCE OF SN38 AND IDN 5174

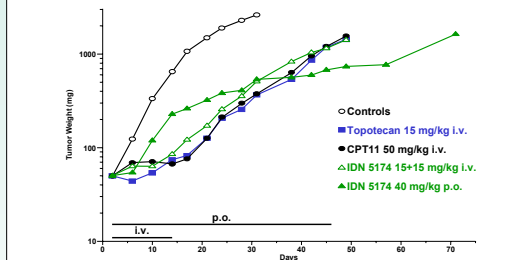
The samples were reacted for 30 min with 10 µM drug. DNA cleavage was then reversed by adding 0.6 M NaCl. The 100% value is referred to the extent of DNA cleavage at 30 min of incubation.



## ANTITUMOR ACTIVITY IN HUMAN TUMOR XENOGRAFTS

- Tumor NCI-H460 implanted s.c. in both flanks of nude athymic mice
- Drugs and treatment schedule: Solvent: distilled water. Topotecan and CPT11: i.v. q4dx4; IDN 5174: i.v. (split in two injections) q4dx4; p.o. q4dx12
- Tolerability: Immediately after i.v. treatment with CPT11 (50 mg/kg) and IDN 5174 (15+15 mg/kg) mice present a rapid shock which was recovered in few minutes

Drug	Dose (mg/kg)	TW1%	LCK	Shock	Tox
Topotecan	15	91	2.0	0/5	0/5
CPT11	50	91	1.8	5/5	0/5
IDN 5174	10+10	73	1.2	0/5	0/5
	15+15	89	1.8	5/5	1/5
	20	n.d.	n.d.	5/5	5/5
	40 p.o.	90	3.3	0/5	0/5

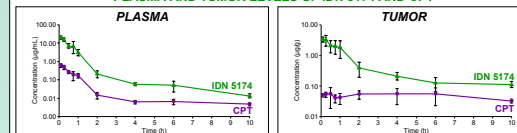


IDN 5174 shows antitumor activity similar to that of Topotecan and CPT11.

## PHARMACOKINETIC STUDIES OF IDN 5174

- CD-1 nude athymic mice s.c. bearing the NCI-H460 tumor were treated i.v. with IDN 5174, 15 mg/kg;
- Time points: four mice were sacrificed at 5', 15', 30', 45', 1 h, 2 h, 4 h, 6 h, 10 h for collection of plasma and tumor;
- Plasma: 50 µL of plasma samples were added with 10 ng CPT11 as internal standard (IS), 10 µL trichloroacetic acid 0.6 N (TCA) and 50 µL CH<sub>2</sub>Cl<sub>2</sub>. A calibration curve was prepared in the range 2.5-500 ng/mL for both IDN 5174 and CPT11;
- Tumor: weighted samples were homogenized in 1 mL CH<sub>2</sub>Cl<sub>2</sub>, added with 50 ng IS and 100 µL TCA and extracted with 4 mL CH<sub>2</sub>Cl<sub>2</sub>;
- Chromatographic conditions: HPLC separation was carried out on Luna 5 µm Phenyl-Hexyl, 150x4.6 mm (Phenomenex). Analytes were revealed by fluorimetric detection with  $\lambda_{em}$  370 nm and  $\lambda_{em}$  510 nm. The LOQs were 2.5 ng/mL and 10 ng/g for plasma and tumor, respectively.

## PLASMA AND TUMOR LEVELS OF IDN 5174 AND CPT



In plasma we noticed the formation of CPT at all the investigated time points. CPT experimental AUC was nearly thirty times lower than that of IDN 5174.

In tumor both CPT and IDN 5174 were quantifiable up to 10 hours. The ratio between IDN 5174 and CPT experimental AUCs was about 10.

## PHARMACOKINETIC PARAMETERS OF IDN 5174 AND CPT

Drug	PLASMA					TUMOR				
	C <sub>max</sub> (µg/mL)	AUC <sub>0-10h</sub> (µg·h/mL)	AUC <sub>0-10h</sub> (µg·h/mL)	% extrap	T <sub>1/2</sub>	Cl (mL/h/kg)	V <sub>d</sub> (mL/kg)	C <sub>max</sub> (µg/g)	AUC <sub>0-10h</sub> (µg·h/g)	Ratio* %
CPT	0.614	0.50	0.53	6.9	5.6	-	-	0.05	0.48	82.8
IDN 5174	21.7	13.52	13.56	0.3	2.2	1106.21	3469.65	3.36	4.96	33.5

\*Ratio between tumor AUC and plasma AUC

IDN 5174 achieves a C<sub>max</sub> of 21.7 µg/mL, it is rapidly distributed and cleared from plasma with a Cl and an elimination half-life of 1106 mL/h/kg and 2.2 h, respectively. The drug is measurable up to 10 h at levels >LOQ.

## ◆ We have shown that IDN 5174 has:

- in vitro* antiproliferative activity with a potency 10 and 40-fold lower than those of Topotecan and SN38, respectively;
- ability to cleave DNA in a topoisomerase I-dependent assay and persistence of the cleavage comparable to those of SN38;
- antitumor activity in a human tumor xenograft, when delivered i.v. or p.o., similar to those of Topotecan and CPT11;
- pharmacokinetic profile indicating that the compound achieves and maintain a favorable distribution in tumor. Formation of CPT was evident at all time points in plasma (3%) and tumor (10%).

## CONCLUSIONS

- IDN 5174, a water soluble CPT containing a spermidine linked to the carboxylate function of the opened lactone ring, maintains the ability to stabilize the topoisomerase I-DNA cleavable complex and exhibits antitumor activity.
- In *in vivo* studies a low amount of CPT molecule is detectable in plasma and tumor, which may contribute to the pharmacological effects of IDN 5174.
- IDN 5174 represents the lead compound of a new series of opened-lactone CPTs.