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7-SILYLCAMPTOTHECINS (SILATECANS): A NEW FAMILY OF CAMPTOTHECIN ANTITUMOR AGENTS

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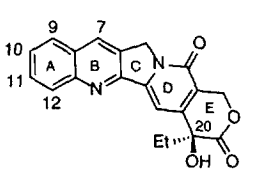
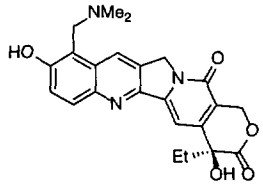
Abstract: The synthesis and biological evaluation of about one dozen 7-silylcamptothecin derivatives are described. Most new compounds show potencies comparable to or better than camptothecin itself. The best compound, 11-fluoro-10-amino-7-trimethylsilylcamptothecin, is more than 20 times more potent than camptothecin in cell assays. © 1997 Elsevier Science Ltd.

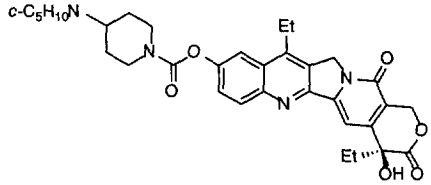
Introduction: (20*S*)-Camptothecin is the parent of a family of antitumor agents that are currently on the front lines of cancer chemotherapy.[1] Topotecan (now called hycamtin) has recently been approved in the US, and irinotecan (formerly called CPT-11 and now called camptosar) is a prodrug that is now approved for sale in the US, France, and Japan. A number of other related compounds show promise and are in various stages of clinical trials.[1]



(20*S*)-camptothecin (CPT)



topotecan (hycamtin, SKB)



irinotecan (campstar, Daiichi/Upjohn)

Medicinal chemistry studies starting in the late 1960's and intensifying over the last decade now provide a good picture of the structure–activity relationship of camptothecin.[1] In general, substitution on the C-E rings is not well tolerated,[2] and the alteration of the core ring system of camptothecin has only been moderately successful.[3] But there is broad latitude with both the number and nature of substituents at C7, C9, C10, and to a lesser extent C11.[4] Early semisynthetic routes to substituted camptothecins often lacked selectivity, and a totally synthetic route based on the Friedlander condensation has been used to make most of the hundreds of known camptothecin analogs.[5] However, limitations associated either with the Friedlander condensation itself or with difficulties in preparing requisite starting materials have left broad gaps in understanding of the effects of certain classes or combinations of substituents.

We have invested several years in development of a cascade radical annulation route to the camptothecin family that we believe is now much more general than the Friedlander route. The discovery of a new radical route to cyclopentaquinolines[6] was followed by first-[7] and second-generation routes to camptothecin and analogs like topotecan, irinotecan, and about a dozen others.[8] We now report the first medicinal chemistry dividends of this work; members of a series of new 7-silylcamptothecins are generally more potent than camptothecin.

Chemistry Results: Related variants of the cascade radical annulation route were used to prepare all the new (20*S*)-7-silylcamptothecins (Scheme 1). Otherwise unsubstituted 7-silylcamptothecins were prepared by the standard route. Reaction of the appropriate silyl chloride with the anion of THP-protected propargyl alcohol followed by direct conversion of the protected alcohol to a bromide provided the needed propargylating agents **1a–c**. The key lactone pyridone **2**[8] was then selectively N-propargylated[9] to provide the radical precursors **3a–c**. These precursors were then reacted with phenyl isonitrile under standard conditions to provide the 7-silyl camptothecins **4a–c**. All the new camptothecin analogs were purified by crystallization or chromatography.

Likewise, 10-substituted-7-silylcamptothecins were prepared by the standard route with the appropriate *para*-substituted arylisonitrile and the propargyl trimethylsilane **3a**. Because the two *ortho*-positions of the isonitrile are equivalent, there is no issue of regioselectivity in the last radical cyclization of the cascade,[8] and single products were produced. Compound **4d** is a silyl analog related to irinotecan. The 10-acetoxy analog **4e** was hydrolyzed to give 10-hydroxy-7-trimethylsilyl camptothecin **4f**. The 10-amino derivative **4h** was prepared via the NH-Boc derivative **4g**. Mixtures of regioisomers are generally produced when *meta*-substituted arylisonitriles are used,[6] but to our surprise the use of the *meta*-NH-Boc phenylisonitrile provided exclusively the 11-NH-Boc-7-trimethylsilyl camptothecin **4i**. Cleavage of the Boc group gave the 11-amino-7-trimethylsilyl-camptothecin **4j**.

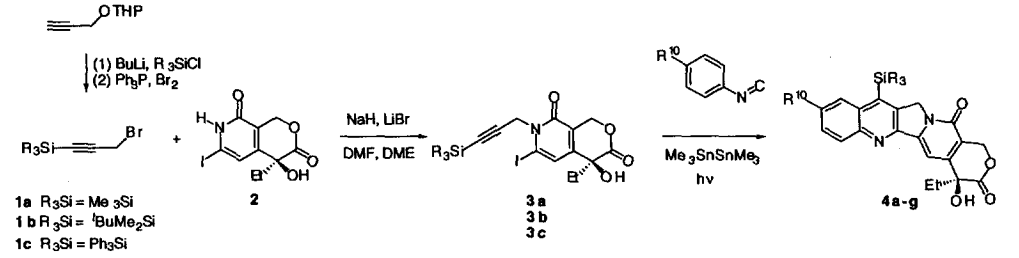
Reactions of the other *meta*-substituted isonitriles according to the standard route were not regioselective, and produced mixtures of isomers. Reaction of **3a** with *m*-fluorophenylisonitrile gave a 9/11-fluorocamptothecin mixture (**4k/4l**) that could not be separated, and this was assayed as a mixture. Reaction with *p*-NH-Boc-*m*-fluorophenylisonitrile also produced an inseparable mixture of regioisomers **4m/4n**. After removal of the Boc group, the resulting amino fluoro camptothecin isomers **4o** and **4p** were separated by flash chromatography. Reaction of *o,m*-difluorophenyl-isonitrile produced a 3/1 mixture of 11,12-difluoro- and 9,10-difluorotrimethylsilyl camptothecin (**4q** and **4r**). The 11,12-isomer **4q** is a normal product that results from 1,6-cyclization to the *ortho* carbon bearing hydrogen. The 9,10-isomer **4r** does not result from 1,6-cyclization to the other ortho position but instead arises from a skeletal rearrangement that is believed to follow 1,5-cyclization.[6,10] Such rearranged products are common in simpler systems, but this is the first time (>50 examples) that one has been observed in the camptothecin series.

The 1/2 mixture of 11-fluoro-7-trimethylsilylcamptothecin and the 9-fluoro isomer (**4k** and **4l**) proved to be quite active, and we suspected that the minor 11-fluoro isomer **4l** was the more active of the two components. To confirm this, we prepared the pure 11-fluoro isomer by the modified route shown in the lower part of Scheme 1. Radical annulation with *m*-fluoro-*o*-trimethylsilylphenyl-isonitrile produced a single regioisomer **4s** with cyclization occurring away from the TMS group. Interestingly, desilylation of 11-fluoro-7,12-bis(trimethylsilyl)camptothecin **4s** (HBr, 50° C) produced exclusively the mono desilylated product 11-fluoro-7-trimethylsilylcamptothecin **4l**. This pure compound indeed proved to be considerably more active than the mixture. The high chemical stability of the 7-silyl groups makes it unlikely that 7-silylcamptothecins simply serve as "prodrugs" for the parent camptothecins. This conclusion is supported by the biological results.

The standard route was also used to make reference samples of camptothecin and irinotecan that were used in the biological assays. In addition, the dimeric camptothecin **4t** was also prepared by the standard route from 1,2-bis(*p*-isocyanophenyl)ethane, albeit in only 20% yield, This dimer is highly insoluble in organic solvents and is not very active. However, the ease with which this unusual compound was made speaks for the generality of the synthesis. Opening of the 7-

trimethylsilyl-10-hydroxycamptothecin **4f** provided a standard prodrug **4u** form of this molecule,[11] which was tested along with the irinotecan analog **4d** to demonstrate the prospects for more water soluble silylcamptothecins.

Scheme 1

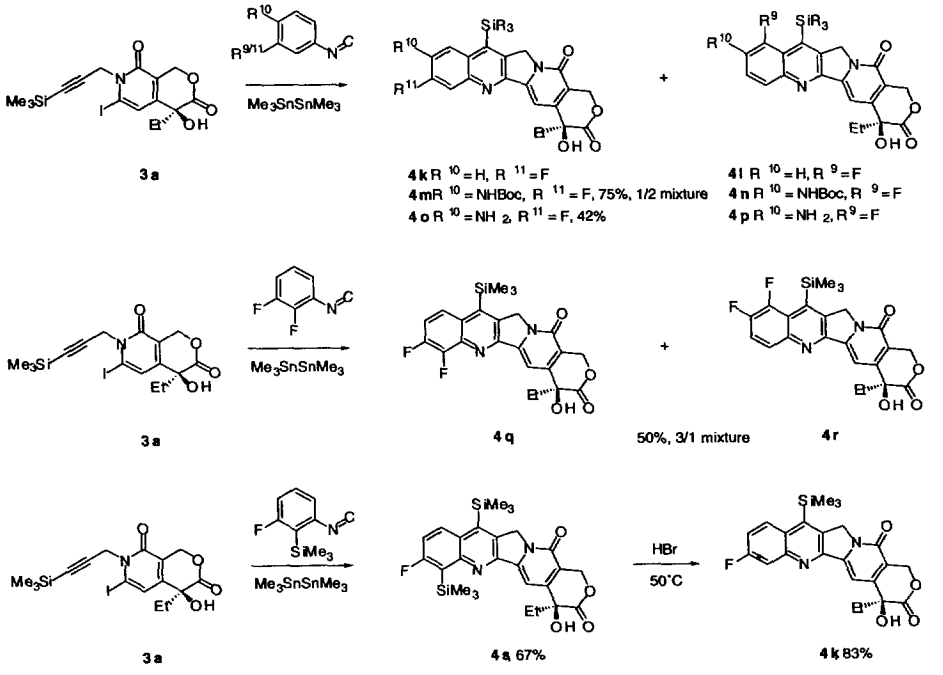


	R^{10}	R^{11}	SiR_3	yield %
4a	H	H	$SiMe_3$	54
4b	H	H	$SiMe_2{}^tBu$	54
4c	H	H	$SiPh_2{}^tBu$	45
4d	OPP[a]	H	$SiMe_3$	19
4e	OAc	H	$SiMe_3$	63

[a]PP = pyrrolidinylpyrrolidine, see irinotecan

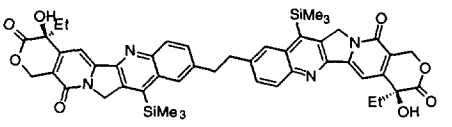
	R^{10}	R^{11}	SiR_3	yield %
4f	OH	H	$SiMe_3$	63[b]
4g	NHBoc	H	$SiMe_3$	60
4h	NH_2	H	$SiMe_3$	54[c]
4i	H	NHBoc	$SiMe_3$	43
4j	H	NH_2	$SiMe_3$	43[d]

[b]from **4e**; [c]from **4g**; [d]from **4i**

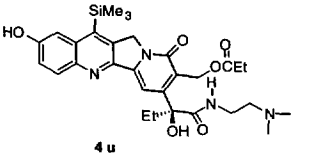


Biology Results: The camptothecin derivatives were evaluated for their inhibition effects on the growth of HL-60 (human promyelocytic leukemic), 833K (human teratorcarcinoma), and DC-3F (hamster lung) cells in vitro. The cells were maintained in a 5% CO_2 humidified atmosphere at 37° C in RPMI-1640 media (GIBCO-BRL, Grand Island, NY) containing penicillin (100 μ/mL)/streptomycin (100 mg/mL) (GIBCO-BRL) and 10% heat inactivated fetal bovine serum. The assay was done in

duplicate in 96-well microplates. The cytotoxicity of the compounds toward HL-60 cells following 72 h incubation was determined by XTT-microculture tertrazolium assay.[12]



4t



4u

The cytotoxicity of the camptothecin compounds toward 833K teratocarcinoma solid tumor cells and DC-3F hamster lung cells was determined in 96-well microplates by a method described by Skehan et al.[13] for measuring the cellular protein content. The experiments were carried out in duplicate each using five to six concentrations of the drugs tested. Data were analyzed by using computer software.[14]

The compounds were also evaluated in a topo I mediated DNA cleavage assay and in an assay for inhibition of topo I mediated relaxation of supercoiled DNA. For the cleavage assay, the reaction mixture consisted of Tris-HCl buffer 10 mM, pH 7.5; PBR_{322} supercoiled double stranded circular DNA 0.125 μg/μmL, drug (camptothecin or its derivatives) concentration at 1, 10, and 100 μM, in the presence of purified DNA topoisomerase I with final volume of 20 μL as described previously.[15] Electrophoresis was carried out on 1% agarose gel plus ethidium bromide (1 mg/mL) and ran at 25V for 18 h. Photographs were taken under UV light using Polaroid film type 55/N and developed as indicated by the manufacturer. To study the inhibiting effect on PBR_{322} DNA topoisomerase I mediated relaxation of DNA, the method described by Liu and Miller[16] was used. The samples were loaded onto 1% agarose in TAE running buffer, electrophoresed overnight at 39 V, stained with EtBr, and photographed under UV light.

Discussion: The biological testing results are summarized in Table 1. With the exceptions of the prodrugs (entries 6 and 9), the dimer (entry 16), and the triphenylsilyl derivative (entry 5), all of the new compounds are as potent as or more potent than camptothecin (entry 1). This is important because even today camptothecin is still regarded as a challenging benchmark whose activity is not easy to surpass. The "irinotecan-like" prodrug (entry 6) is more active than irinotecan (entry 2), and the expected active component of both this prodrug and **4u** (entry 9), 7-trimethylsilyl-10-camptothecin is highly active (entry 8).

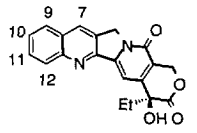
Most of the compounds that showed higher antitumor cytotoxicity also showed higher potency in enhancing the DNA-topoisomerase I-mediated cleavage of PBR_{322}DNA, or in inhibiting the DNA-topoisomerase I-mediated relaxation of PBR_{322}DNA. There is a reasonable correlation between the antitumor cytotoxicity of the camptothecin compounds with their ability to inhibit the function of DNA-topoisomerase I.

Combination substituent effects in this series are especially interesting. The 10-amino and 11-fluoro substituents are both known to increase the potency of camptothecin or its derivatives.[1,4] 7-Trimethylsilyl camptothecin is about two times more potent than camptothecin (entry 3 vs. 4). Combination of the 7-trimethylsilyl group with an 11-fluorine provides a compound (entry 13) that is about five times more potent, while combination with a 10-amino group provides a compound that is about 10 times more potent (entry 10). Adding all three substituents together provides compound **4o** that is more than 20 times more potent than camptothecin (entry 14).

For in vivo chemotherapeutic effects in tumor bearing mice, 7-trimethylsilyl camptothecin **4a** showed superior activity to camptothecin against sarcoma-180 in $B_6D_2F_1$ mice at several equivalent

doses (1.5-3 mg/kg, i.p. twice daily for 5 days) in a dose dependent manner in terms of tumor volume reduction.[17] At the 3 mg/kg dosage, tumor reductions on day 7 were 85% and 66%, respectively. For Lewis lung carcinoma, 7-trimethylsilyl-11-fluoro camptothecin **4k** (i.p. at 0.25 mg/kg, twice daily, 5 days) showed a comparable antitumor effect (56% reduction in tumor volume) to camptothecin (44% reduction in tumor volume) at four-fold lower doses. Thus, there is clear promise that the in vitro results will be indicative of in vivo activity.

Table 1. Biological Activities of (20*S*)-7-Silyl-Camptothecin Derivatives.



Entry	7	9	10	11	12	Inhibition of cancer cell growth IC_{50} (nM) HL-60	833K	DC-3F	Enhancement of Topo I Mediated DNA Cleavage[f]	Inhibition of Topo I mediated DNA relaxation[f]
1 CPT	H	H	H	H	H	5	10	6-9	+++	+++
2 IRT	Et	H	OPP[a]	H	H	270	487	372	nd	nd
3 **4a**[b]	TMS	H	H	H	H	3.8	5.6	4.2	++++	+++
4 **4b**	TBDMS	H	H	H	H	0.12	1.2	2.9	++++	+++
5 **4c**	TBDPS	H	H	H	H	339	243	663	++	+
6 **4d**	TMS	H	OPP[a]	H	H	66	214	256	nd	—
7 **4e**	TMS	H	OAc	H	H	2.7	–	6.7	++++	+++++
8 **4f**	TMS	H	OH	H	H	2.6	7.0	6.9	++++	+++++
9 **4u**	Entry 8 with opened E ring					9.7	15.0	14.2	+++	+
10 **4h**	TMS	H	NH_2	H	H	**0.52**	**5.7**	**0.72**	nd	nd
11 **4j**	TMS	H	H	NH_2	H	2.6	7.4	6.4	nd	nd
12 **4l**[d]	TMS	F	H	H	H	3.0	2.9	8.2	++++	++++
4k[d]	TMS	H	H	F	H (2:1)					
13 **4k**	TMS	H	H	F	H	**0.75**	**0.92**	**2.0**	++++	+++++
14 **4o**	TMS	H	NH_2	F	H	**0.07**	**0.14**	**0.29**	++++	++++
15 **4q**[e]	TMS	H	H	F	F	1.01	2.1	2.5	+++	+++
4r[e]	TMS	F	F	H	H (3/1)					
16 **4t**	TMS		"dimer"[e]			53	84	inactive	nd	±

nd = not determined. [a]OPP = irinotecan's pyrrolidinyl pyrrolidine carbamate. [b]More active than CPT in S-180 in BD_2F_1 mice testing. [c]More active than CPT in Lewis lung Carcinoma in BD_2F1 mice. [d,e]Tested as a mixture. [f]+++++ (<0.1 μM), ++++ (0.1-1 μM), +++(1-2 μM), ++ (2-5 μM), + (5-20 μM), ± (>20 μM)

Essentially all of the active new compounds in the Table can be viewed as suitable preclinical candidates either as is (water insoluble) or in a suitable prodrug form. Beyond that, the results show that the cascade radical route can now step in to provide a wide assortment of new camptothecin analogs that have not been available by the Friedlander condensation. The modularity of the synthesis is an attractive feature for making new derivatives. Especially suitable are derivatives with unusual substituents (like trimethylsilyl) or with multiple substituents that are combined either to boost potency or to provide some other desirable property (such as solubility) without damaging potency.

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