Letters

Discovery of a Fluoroindolo[2,3-*a*]carbazole Clinical Candidate with Broad Spectrum Antitumor Activity in Preclinical Tumor Models Superior to the Marketed Oncology Drug, CPT-11†

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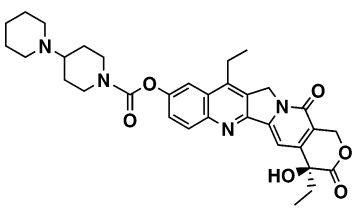
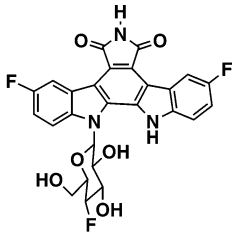
Abstract: A series of fluoroglycosylated fluoroindolocarbazoles was examined with respect to their topoisomerase I activity, cytotoxicity, and selectivity. The lead clinical candidate from this series, BMS-250749, displays broad spectrum antitumor activity superior to CPT-11 against some preclinical xenograft models, including curative antitumor activity against Lewis lung carcinoma.

The glycosylated indolo[2,3-*a*]carbazole pharmacophore is a rich structural motif that is encompassed in many anticancer agents and kinase inhibitors including UCN-01.[1−4] In this context, we and others have discovered that this class of compounds also exhibits topoisomerase I (topo I) inhibition[4−6] and DNA cleaving activity.

Introduction of 3,9-difluoro substitution into the indolocarbazole confers topo I selectivity within this series of analogues.[6−7] From our initial investigations, it became apparent to us that the plasma, tumor levels, physiochemical properties, and overall metabolism and pharmacokinetic (M-PK) properties of the analogues play a significant role in imparting distal site antitumor activity in animal models.[6] In this letter, we disclose the discovery of BMS-250749 (**1**), a fluoroglycosyl-3,9-difluoroindolocarbazole topo I inhibitor which, by virtue of its robust in vitro and in vivo preclinical profile reported herein, has entered human clinical trials.



CPT-11; Camptosar™



BMS-250749; 1

The discovery of **1** stems from a program directed toward the design of topo I selective agents in a novel class of glycosylated indolo[2,3-*a*]carbazoles and benzthiopheno[2,3-*a*]carbazoles, wherein both the core and sugar moieties are modified with fluorine substituents.

The rationale for introduction of fluoroglycosides is based on the hypothesis that substitution of fluorine for sugar hydroxyl may serve to remove potential sites of metabolic liability while concomitantly modulating lipophilicity, one of the parameters influential in imparting in vivo distal site activity. It is also apparent that such fluorine substitution would maintain hydrogen bond accepting potential in the DNA/topo I/analogue ternary complex. Several of these novel analogues reported herein, including **1**, **2**, and **3**, display greatly enhanced topo I and cytotoxic potencies vis-à-vis the parent glucosyl analogues. In addition, it is noteworthy that **3** exhibits highly specific topo I mediated cytotoxicity similar to camptothecin (CPT).[7]

The synthesis of the indolocarbazole core[8] and its glycosylation[9] are well described in the literature.[6,10] The synthetic routes to the analogues described in Table 1 are delineated in Schemes 1 and 2, using the examples pertaining to the 3,9-difluoroindolocarbazole core. Introduction of fluorine into the 2′, 4′, and 6′-positions of the sugar portion of the molecule is accomplished both pre and post glycosylation. To illustrate, 6′-fluoro-2′,3′,4′-tri-(*O*)-benzyl-D-glucopyranose[11] (**4**) is prepared from the known[12] 6′-fluoro-1′,2′,3′,4′-tetra-(*O*)-acetyl-α-D-glucopyranose and then coupled with the *N*-6-(4-*tert*-butyl)-benzyl-3,9-difluoroindolo[2,3-*a*]carbazole core **5**,[13] using the Mitsunobu protocol.[14] Further processing by transfer hydrogenolysis followed by deprotection of the *N*-6-(4-*tert*-butyl)benzyl group[15] provides the desired 6′-fluoro analogue **6** (Scheme 1).

In fashion similar to the method described above, 2′-fluoro-3′,4′,6′-tri-(*O*)-benzyl-D-glucopyranose[16] and 4′-fluoro-2′,3′,6′-tri-(*O*)-benzyl-D-glucopyranose are converted into their respective 2′-fluoro and 4′-fluoro (**1**) analogues.[17]

The optimal procedure for the synthesis of 4′-fluoroglycoside analogues involves post-glycosylative modification of a D-galactofluoroindolocarbazole intermediate **7** as shown in Scheme 2. Thus, conversion of the 4′-axial hydroxyl in **7** to an equatorial 4′-fluorine proceeds in reasonable yield, and with complete inversion of configuration, using 4-DMAP-attenuated DAST. Base-

† Dedicated to the memory of Dr. Monroe Wall, who made significant contributions to the field of natural product-based cytotoxic agents.

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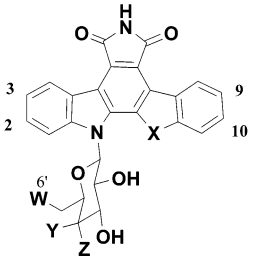
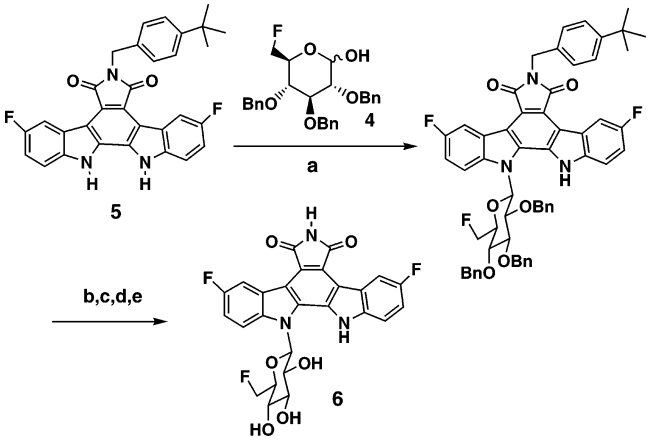
Table 1. Topo I Potency and Cytotoxicity of Fluorosugar Analogues



compd	core	X	W	Y	Z	Topo[a]	P388[b]	*R*/*S*[c]
13	3,9-diF	NH	OH	H	OH	0.24	0.018	183
6	3,9-diF	NH	F	H	OH	0.48	0.050	98
10	3,9-diF	NH	F	OH	H	4.4	0.012	110
11	3,9-diF	S	F	H	OH	0.42	0.016	110
12	Tetra-F[d]	S	F	H	OH	1.50	0.052	119
17	Tetra-F	NH	F	H	OH	3.00	0.034	52
1	3,9-diF	NH	OH	H	F	0.11	0.002	380
18	2,10-diF	NH	OH	H	F	0.20	0.003	59
3	Tetra-F	S	OH	H	F	0.04	0.003[e]	>1658
14	Tetra-F	NH	OH	H	F	0.75	0.004	500
2	3,9-diF	NH	F	H	F	0.41	0.004	450
8	3,9-diF	NH	OH	F	F	0.08	0.003	380
15	Tetra-F	S	OH	F	F	0.50	0.0004	580
16	Tetra-F	NH	NH_2	F	F	0.05	0.060	16
9	3,9-diF	NH	NH_2	H	F	0.07	0.014	37

[a] Ratio of the median effective concentration (EC_{50}, μM)) of drug for inducing single-strand breaks in the DNA substrate divided by that obtained for CPT in the same experiment. CPT has a mean Topo I EC_{50} = 160 nM. [b] Mean cytotoxic concentration (IC_{50}, μM) following 3 days of continuous exposure of P388 murine leukemia cells to drug. CPT has a mean P388 IC_{50} = 36 nM. [c] Ratio of the IC50 value obtained for P388/CPT45 cells (cells that have acquired high levels of CPT resistance) divided by that obtained for parental P388 cells. [d] Tetra-F is 2,3,9,10-tetrafluoro. [e] <0.003.

Scheme 1. Synthesis of 6′-Fluoro Analogue **6**[a]

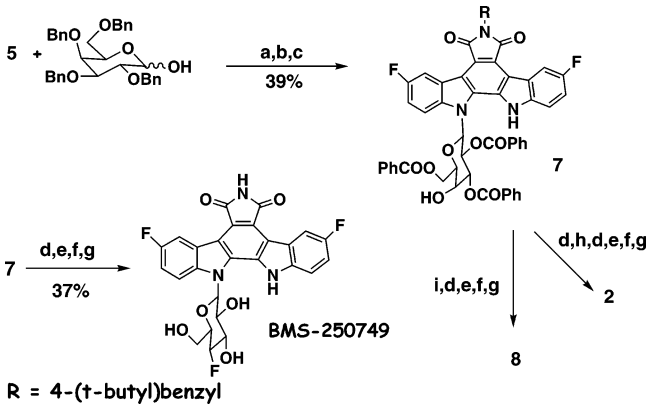


[a] Reagents and conditions: (a) Ph_3P, DIAD, THF. (b) 20% $Pd(OH)_2$/C, cyclohexene, EtOH, reflux. (c) 4.45 M KOH, EtOH, rt, 24 h. (d) concd HCl. (e) NH_4OAc, EtOH reflux 48 h.

induced global deblocking of the resulting 4′-fluoro-2′,3′,6′-tribenzoate provides **1**. Tribenzoate **7** is also a key intermediate for the synthesis of the 4′,6′-difluoro analogue **2** and the 4′,4′-*gem*-difluoro analogue **8**. Both 4′-fluoro and 4′,4′-*gem*-difluoro analogues are suitable substrates for direct conversion of their free 6′-hydroxyl groups to 6′-amino, via reduction of their initially derived 6′-azides.[6] Thus, the 4′-fluoro-6′-amino analogue **9** is easily obtained from **1**.

Our assay for determining the extent of topo I mediated DNA cleavage for the analogues described

Scheme 2. Synthesis of 4′-Fluoro (**1**), 4′,6′-Difluoro (**2**), and 4′,4′-*gem*-Difluoro (**8**) Analogues[a]



R = 4-(t-butyl)benzyl

[a] Reagents and conditions: (a) Ph_3P, DIAD, THF. (b) 20% $Pd(OH)_2$/C, cyclohexene, EtOH, reflux. (c) PhCOCl, Pyr, −5 °C, 20 min. (d) DAST, 4-DMAP, −50 to 45 °C. (e) 4.45 M KOH, EtOH, 5 to 25 °C, 24 h. (f) concd HCl. (g) NH_4OAc, EtOH, reflux 48 h. (h) 4.45 M KOH, EtOH, 5 °C, 20 min. (i) Dess−Martin periodinane ([1,1,1-tris(acetyloxy)-1,1-dihydro-1,2-benziodoxol-3-(1*H*)-one).

herein employs covalently closed, supercoiled phage PM2 DNA as the substrate and was previously described.[6] The topo I selectivity of these analogues is determined as previously reported[6] using P388 cells which express high levels of topo I (and are therefore sensitive to topo I targeting agents such as CPT) and a camptothecin resistant cell line (P388/CPT45) derived from the parental line that expresses little or no topoisomerase I. This cell line overexpresses topoisomerase II, which serves to compensate for the lack of topoisomerase I, and is thus resistant to topo I active agents such as CPT. Cells that do not express detectable levels of topo I are completely resistant to camptothecin and its analogues.[6] Thus, camptothecin treatment results in a very high *R*/*S* ratio and an agent that is cytotoxic by a non-topoisomerase I mechanism would kill both lines equally and result in an *R*/*S* ratio near 1.0. A ratio (*R*/*S*) of the IC_{50} values for P388/CPT45 versus P388 cells for an analogue is an indication of topo I selectivity expressed by the analogue. The cytotoxic effects of topo I active agents are directly proportional to the number of DNA breaks they form. Therefore, cells that express high levels of topo I are sensitive to these agents, and conversely, cells that express low levels of the enzyme are more resistant to these agents. Topo I levels are generally higher in cancer cells than in normal cells, a feature that is most likely a reflection of higher proliferation rates in transformed cells. The topo I activity (for DNA breaks), P388 cell cytotoxic potency, and *R*/*S* ratio of each these novel fluoroglycosylated fluoroindolo[2,3-*a*]carbazoles and fluoroglycosylated fluorobenzthiopheno[2,3-*a*]carbazoles are reported in Table 1.

Inspection of Table 1 reveals some interesting SAR trends regarding the position of fluorine substitution[17] on the glycosyl moiety and its effect on topo I selectivity. In general, substitution of the 6′-OH by 6′-F (as in **6**, **10**, **11**, **12**) results in the loss of topo I selectivity vis-à-vis their corresponding parent glucose analogues[10] (e.g. **13**). In contrast, 4′-fluorine (equatorial) substitution of C-4′-OH (e.g. **1**, **3**, **14**) has the opposite effect: It enhances the topo I selectivity, thus demonstrating the

Table 2. Antitumor Efficacy of Lead Analogues vs CPT-11 in HT29 Xenograft Model

drug	optimal effect in LCK (cures/total)	optimal dose (MTD) (mg/kg/inj)	schedule,[b] route
1	3.1 (2/8)	20 (30)[a]	q2dx5, iv
2	1.6 (0/8)	13[a]	q2dx5, iv
3	>2.3 (2/8)	11[a]	2q2dx5, iv
CPT-11	1.9 (0/8)	50[a]	q2dx5, iv

[a] MTD likely reached. MTD may be distinct from optimal dose, in which case MTD is shown in parentheses. [b] q2dx5; once a day iv dosing administered every other day for five doses: 2q2dx5; twice a day iv dosing administered every other day for five doses.

importance of such fluorine for hydroxyl substitution in the sugar moiety. Substitution of the 2′-hydroxyl for fluorine slightly improves topo I potency, but poor cell permeability of this analogue may account for its weak cytotoxicity.[17] The selectivity ratio for **3** is similar to camptothecin and is the highest we have observed to date for an indolocarbazole analogue. Bis-substitution of C-4′ and C-6′ for fluorine, as in **2**, substantially increases the topo I selectivity vis-à-vis the parent gluco analogue **13**.

Enhancements in P388 cytotoxic potency relative to their corresponding glucose counterparts are also realized with fluorinated glycosyl analogues, e.g. the C-4′ geminal difluoro analogues **8** and **15**. The combination of 4′,4′-*gem*-difluoro substitution with the tetrafluorobenzothiopheno[2,3-*a*]carbazole chromophore (**15**) leads to the most cytotoxic (0.4 nM vs P388), yet still highly topo I specific analogue. The two C-6′-NH_2 analogues **16** and **9** display enhanced fluorine-mediated topo I potency vis-à-vis parent **13**, yet their significantly lower topo I selectivity suggests that additional mechanisms might be involved.

Our initial premise to introduce fluorine into the glycosyl moiety of **13** for imparting distal antitumor activity proved to be correct. As a case in point, it is noteworthy that 6′-fluoro analogue **6** provides significantly increased exposure levels relative to the parent analogue **13**[6] (plasma levels >100 fold above the IC_{50} following iv administration at 24 mg/kg/inj) and also displays in vivo distal site antitumor activity (LCK = 1.2, 21 mg/kg/inj, the MTD in 10:10:80, cremophor: ethanol:water formulation) comparable to CPT-11 against the A2780 xenograft model.

On the basis of the topo I selectivity and cytotoxic potency, as well as the in vivo plasma and tumor concentrations at 6 h after iv administration in tumor bearing mice,[6] a number of analogues were profiled for their antitumor activity in the A2780 human ovarian carcinoma xenograft model as a primary screen. In particular, the mean 6 h plasma concentrations in nude mice (A2780 xenograft model) for the three analogues **3** (12 mg/kg), **1** (24 mg/kg), and **2** (8 mg/kg) are 40, 33, and 27-fold greater than their A2780 cellular IC_{50} values. In fact, all three compounds show in vivo efficacy comparable to CPT-11 in A2780. Thus, the three analogues were identified as leading candidates for advanced in vivo evaluation against a panel of human tumor xenograft animal models.

Table 2 exemplifies the in vivo activity displayed by the three analogues against HT29 human colon carcinoma xenograft model when administered iv, in a 1:1:8 cremophor:ethanol:water vehicle. It is gratifying to see

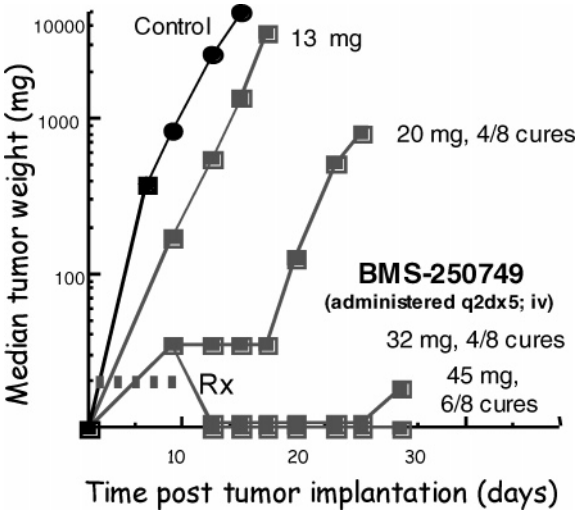


Figure 1. Antitumor activity of **1** vs Lewis lung carcinoma. CPT-11 is about as active as **1** at 13 mg in this model.

that all three fluorosugar analogues show efficacy equal to or better than CPT-11 in this animal model.

Single dose pharmacokinetics of **1** in mice was determined following iv administration using cremophor/ethanol/water (10:10:80) at a MTD efficacious dose of 24 mg/kg. Serial blood samples were collected out to 8 h, and the separated plasma was analyzed for parent drug by UV/HPLC. The clearance is low, 5.2 mL/min^{-1} kg^{-1}, and may be saturated at this dose, with a terminal half-life of 2.3 h and a moderate volume of distribution of 0.44 L/kg. Analogue **1** also exhibits good in vitro metabolic stability when incubated with human liver microsomes.

The superiority of **1** over CPT-11 as an anticancer agent was further demonstrated in the Lewis lung carcinoma model (Figure 1). In this model **1** is active over a range of three doses (20, 32, and 45 mg/kg) and yields 6/8 cures at the MTD. Lewis lung carcinoma is a particularly aggressive tumor model which is only modestly responsive (as active as **1** at 13 mg/kg) to CPT-11 and inactive to adriamycin (doxorubicin). On the basis of these data, and the subsequent broad spectrum antitumor activity of **1**, which is equal or superior to CPT-11 in other models such as murine M5076 sarcoma and human xenografts HCT-116 colon and PC-3 prostate carcinomas, **1** was nominated for clinical development.

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Supporting Information Available: Experimental details and analytical data are available for the preparation of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Sugar **4** was prepared in four steps (26% yield), from 6′-fluoro-1′,2′,3′,4′-tetra-(*O*)-acetyl-α-D-glucopyranose: (a) $PhSH/BF_3{\cdot}Et_2O/ClCH_2CH_2Cl$. (b) K_2CO_3/CH_3OH. (c) $PhCH_2Br/NaOH/BTEAC/CH_2Cl_2$. (d) $Hg(OAc)_2/THF/H_2O$.

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(17) The 2′-fluorogluco isomer of **1** is 5-fold more topo I potent than CPT, yet is poorly cytotoxic vs P388 cells (IC_{50} = 1500 nM). Poor cell permeability of this 2′-fluoro analogue may account for its weak cytotoxicity.

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