Design and Synthesis of a Fluoroindolocarbazole Series as Selective Topoisomerase I Active Agents. Discovery of Water-Soluble 3,9-Difluoro-12,13-dihydro-13-[6-amino-β-D-glucopyranosyl]-5*H*,13*H*-benzo[*b*]-thienyl[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (BMS-251873) with Curative Antitumor Activity against Prostate Carcinoma Xenograft Tumor Model§

Balu N. Balasubramanian,*,# Denis R. St. Laurent,*,# Mark G. Saulnier,# Byron H. Long,† Carol Bachand,‡ Francis Beaulieu,‡ Wendy Clarke,# Milind Deshpande,# Jeffrey Eummer,# Craig R. Fairchild,† David B. Frennesson,# Robert Kramer,† Frank Y. Lee,† Mikael Mahler,‡ Alain Martel,‡ B. Narasimhulu Naidu,# William C. Rose,† John Russell,# Edward Ruediger,‡ Carola Solomon,‡ Karen M. Stoffan,# Henry Wong,# Kurt Zimmermann,# and Dolatrai M. Vyas#

The Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford Connecticut 06492, P.O. Box 4000, Princeton, New Jersey 08543, and Candiac, Montreal, Canada

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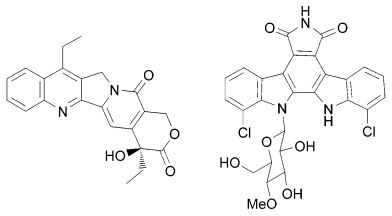
Abstract: A series of fluoroindolocarbazoles were studied with respect to their topoisomerase I activity, cytotoxicity, selectivity, and in vivo antitumor activity. Emerging from this series was BMS-251873, a potential clinical candidate possessing a robust pharmacological profile including curative antitumor activity against prostate carcinoma.

Introduction. Topoisomerase I[1] (topo I) normally functions to form transient single-strand breaks in genomic DNA to relieve the torsional strain that develops during DNA replication or transcription. These transient breaks are formed by a transesterification process involving the hydroxyl group of an active site tyrosine and the 5′-hydroxyl group of deoxyribose involved in the phosphodiester backbone of the DNA molecule.

Camptothecin[2] (CPT, **1**, Figure 1) was identified in 1985 as the first member of a novel class of cytotoxic compounds that acted upon eukaryote topo I to effectively stabilize these transient intermediates. The presence of these persisting DNA breaks triggers the apoptotic cascade in cancer cells expressing wild-type p53. 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. Cells that do not express detectable levels of topo I are completely resistant to camptothecin and its analogues.



1 Camptothecin **2** Rebeccamycin

Figure 1. Camptothecin (**1**) and rebeccamycin (**2**).

To date, campotothecins[2] are the only structural class of antitumor agents that confer the highest degree of topo I selectivity. Our efforts to discover non-camptothecin topo I selective agents stems from an earlier natural products fermentation program that led to the identification of the novel indolocarbazole[1,3] class of compounds represented by rebeccamycin (**2**). These are DNA binding agents that do not share any specific topo I targeting activity. Surprisingly, precursor feeding experiments with fluorotryptophan resulted in the identification of the prototypical fluoroindolocarbazole[4] class of compounds (e.g., **19** and **20**) endowed with selective topo I activity. However, within this fluoroindolocarbazole series, depending on the substitution of the core nucleus, varying degrees of topo I selectivity were observed.

In this communication, we describe the syntheses, in vitro, and in vivo antitumor activity of prototypical fluoroindolocarbazole analogues **19**, **20** (BMS-210287), and their core modified and aminosugar analogues. Several of these possess improved topo I dependent selectivity, aqueous solubility, and in vivo activity against human xenograft antitumor animal models. One such analogue, BMS-251873 (**36**), was identified as a potential clinical candidate.

Results and Discussion. The indolocarbazole nucleus and its corresponding thio and oxo analogues were efficiently assembled via the chemistry described in Schemes 1 and 2. Stepwise addition of an appropriately substituted (e.g., **19**−**26**, **31**−**33**, Table 1) indole-based Grignard reagent to a dihalomaleimide followed by oxidative cyclization furnished the prerequisite fluoroindolocarbazole cores[3a,5] in modest overall yield. In contrast, limited access to the benzofurans and benzothiophenes with the desired substitution pattern warranted the Fischer indole process[6] (Scheme 2) as an attractive alternative (e.g., **27**−**30**, **34**, Table 1). Initially, for the core glycosylation, we explored the epoxide opening protocol described by Danishefsky.[7] The generation of dissacharide in this reaction warranted an alternative approach. Thus, the majority of the examples described herein were synthesized by alkylation[8] or Mitsunobu[9] protocol (Scheme 3).

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

* To whom correspondence should be addressed. For B.N.B.: phone, 203-677-6693; fax, 203-677-7884; e-mail, balu@bms.com. For D.R.S.L. phone, 203-677-6972; fax, 203-677-7702; e-mail, stlaured@bms.com.

The Bristol-Myers Squibb Pharmaceutical Research Institute, CT.

† The Bristol-Myers Squibb Pharmaceutical Research Institute, NJ.

‡ The Bristol-Myers Squibb Pharmaceutical Research Institute, Canada.

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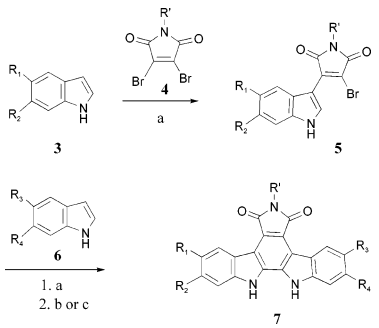
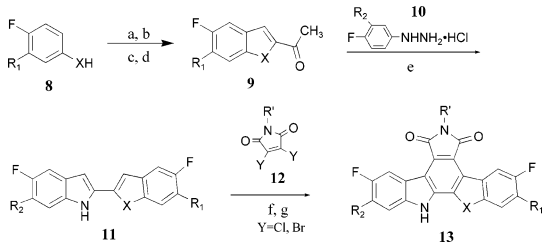
Scheme 1. Grignard Construction of Indolocarbazole Core[a]



[a] Reagents and conditions: (a) 3 M EtMgBr in THF, PhH, 80 °C; (b) DDQ, TsOH, PhH, 80 °C; (c) *hν*, cat. I_2, air, PhH, 80 °C.

Scheme 2. Fischer Indole Construction of Indolocarbazole Core[a]



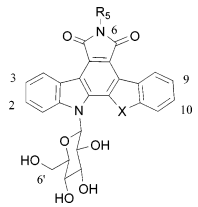
[a] Reagents and conditions: (a) NaOEt, EtOH, $ClCH_2CH(OEt)_2$, room temp; (b) PPA, PhCl, 100 °C; (c) *n*BuLi, CH_3CHO, THF/Et_2O (1:1), −78 to 0 °C; (d) PCC, CH_2Cl_2, Celite, room temp; (e) EtOH, NaOAc, 75 °C; (f) 3 M EtMgBr in THF, THF; (g) *hν*, EtOH, dioxane, 100 °C.

Topo I activity (i.e., DNA strand breaks) of the analogues depicted in Table 1 was measured using a modified Hsiang[10a] protocol, and cytotoxicity was determined by tetrazolium dye conversion assay.[10b] The topo I selectivity for these analogues was determined (as R/S ratio of their IC_{50}'s) by measuring the cytotoxcity against cell lines that are sensitive (S) and resistant (R) to camptothecin.

The resistant cell line expresses little or no functional topo I. Within the fluoroindolocarbazole series, the 3,9-difluoro substitution rendered the best topo I potency and selectivity over the 2,10-difluoro, 2,3,9,10-tetrafluoro, 2,3,9-trifluoro, and 10-monofluoro **24**, **23**, **26**, and **25** analogues, respectively. While N^6-methylation of the imide nitrogen (e.g., **33**) resulted in reduced topo I activity, the N^6-amino and hydroxy substitution maintained the high topo I activity, implying the significance of H-bonding in this structural motif with the topoisomerase I enzyme/DNA ternary complex. In contrast, the conformational distortion[11] imposed by the N^{12}-indole methyl group appears to play a predominant role over N^6-imide H-bonding, which is reflected in the loss of activity observed in example **22**. The thio and oxo analogues (**28** and **30**) were chosen to fully explore the influence of electronic, steric, and H-bonding against the bioactive conformation necessary for topo I activity.

The topo I active, nonselective lead **19**, when evaluated against a subcutaneously (sc) implanted A2780 ovarian carcinoma xenograft model, showed some hints of distal site in vivo activity when administered ip (LCK = 0.9 at 30 mg/kg dose).[12] Interestingly, in the same tumor model, the more selective lead analogue **20** displayed poor distal site activity whether administered intermittently over 9 days or via 24 h infusion at its MTD (LCK = 0.2−0.6). On closer examination of the pharmacokinetic parameters, it became apparent that

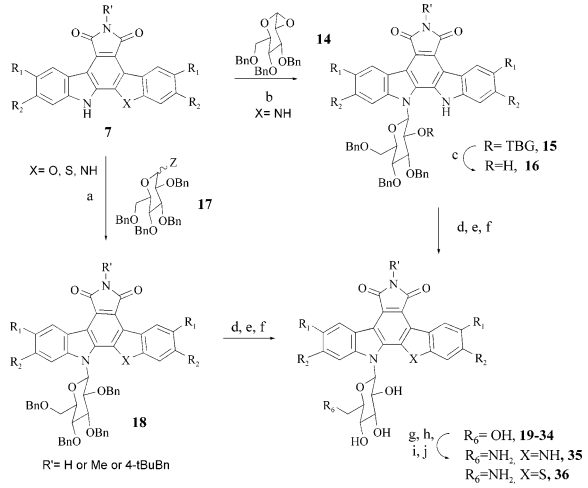
Table 1. Structure−in Vitro Activity Relationships for Some Indolocarbazole Analogues against Human Topoisomerase I and Murine P388 Leukemia Cells



compd	substitution	X	R_5	topo[a]	P388[b]	R/S[c]
2	rebeccamycin	NH	H	>500	0.54	1.26
19	2,10-diF	NH	H	2.0	0.26	8.7
20	3,9-diF	NH	H	0.22	0.018	182.7
21	2,3,9,10-tetraF	NH	H	0.69	0.007	67.1
22	2,3,9,10-tetraF	NMe	H	>600	>8.720	>1.0
23	3-F	NH	H	6.6	1.036	>9.5
24	2-F	NH	H	3.1	0.392	4.6
25	10-F	NH	H	>200	0.098	11.4
26[d]	9-F	NH	H	1.7	0.101	31.7
27	3-F	S	H	2.2	0.155	>51.3
28	3,9-diF	S	H	0.09	0.010	232.5
29	3-F	O	H	1.5	0.529	13.6
30	3,9-diF	O	H	0.27	0.114	63.7
31	3,9-diF	N	NH_2	0.22	0.020	196.9
32	3,9-diF	N	OH	0.08	0.035	19.6
33	3,9-diF	N	CH_3	1.0	0.862	>10.8
34	3,9-diF	S	CH_3	0.48	0.236	14.1
35[e]	3,9-diF	NH	H	0.28	0.326	28
36[e]	3,9-diF	S	H	0.46	0.068	>115

[a] Ratio of the median effective concentration (EC_{50}, μM) of compound for inducing single-strand breaks in the DNA substrate divided by that obtained for CPT in the same experiment. CPT mean topo I EC_{50} = 160 nM. [b] Mean cytotoxic concentration (IC_{50}, μM) following 3 days of continuous exposure of compound to P388 murine leukemia cells. CPT mean P388 IC_{50} = 36 nM. [c] Ratio resulting from the cytotoxicity IC_{50} value obtained for CPT-resistant P388/CPT45 cells divided by that obtained for parental P388 cells. [d] Inseparable mixture with **23**. [e] Both **35** and **36** are 6′-NH_2 analogues.

Scheme 3. Glycosylation of Indolocarbazole Core[a]



[a] Reagents and conditions: (a) Method A, (Z = OH) DIAD, Ph_3P, THF, −78 °C to room temp; Method B, (Z = Cl) $NaN(TMS)_2$, THF or KOH, $NaSO_4$, THF, room temp to 65 °C; (b) $NaN(TMS)_2$, PhH, 80 °C; (c) 4-8 N HCl/MeOH; (d) 20% $Pd(OH)_2$/C, 95% EtOH, cyclohexene, reflux or 20% $Pd(OH)_2$/C, 60 psi of H_2, EtOAc/EtOH, room temp; (e) 5 N KOH or 10% NaOH, EtOH, room temp followed by concentrated HCl, 0 °C; (f) NH_4OAc, AcOH, EtOH, 140 °C or $(TMS)_2NH$−MEOH, DMF, room temp or NH_2OH or NH_2NH_2; (g) MsCl, 4 Å molecular sieves, pyridine; (h) NaN_3, DMF; (i) Ph_3P, wet THF; (j) HCl/MeOH, TBG = 3,4,6-tribenzyl-L-glucopyranose.

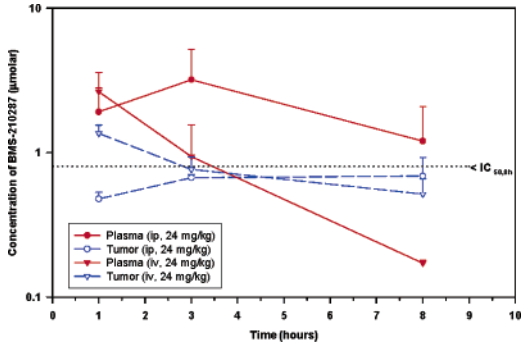


Figure 2. Comparison of **20** in plasma and tumor after iv and ip administration to mice at 24 mg/kg.

Table 2. Pharmacokinetic Profile of a Select Set of Analogues

compd	$T_{1/2}$ [a]	Cl [b]	Vds [c]	IC_{50} [d]	AUC [e]
20 (24 mg/kg)	1.6	3.6	0.10	0.86	0.17
35 (24 mg/kg)	4.0	6.1	0.46	0.17	0.69
28 (5 mg/kg)	7.6	9.33	1.27	0.15	0.068 (6 h)
36 (40 mg/kg)	0.86	4.3	0.12	0.051 (6 h)	>5.0

[a] Half-life (h). [b] Clearance (mL min^{-1} kg^{-1}). [c] Volume of distribution (L/kg). [d] iv IC_{50} (μM) after 8 h. [e] Exposure (μM h mL^{-1}).

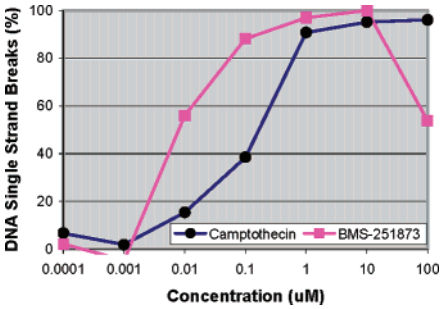


Figure 3. Comparison of topo I mediated DNA cleavage activities between **36** and camptothecin.

the poor plasma and tumor exposure, i.e., tumor levels below cytotoxic IC_{50} levels of **20**, was likely responsible for the lack of distal site activity as reflected in Figure 2. Furthermore, these analogues displayed very low solubility in many of the usual vehicles (such as cremophor/EtOH, PEG) used in the in vivo experiments. This observation prompted us to replace the 6′-hydroxyl group on the sugar moiety with an amino functionality (to render enhanced aqueous solubility), employing a three-step synthetic sequence (OH → OMs → N_3 → NH_2) Consequently, conversion of prototypical lead **20** to its amino analogue **35** did show modest distal site in vivo activity, albeit being much less topo I selective. The increased solubility of **35** seemed to act favorably in the exposure studies, as seen by sustained cytotoxic levels for more than 6 h in mice when administered ip (Table 2). The amino counterpart **36** of the benzthiophene core analogue **28** was also evaluated extensively. Analogue **28** appeared to have maintain the topo I selectivity ratio (see Table 1); however, the plasma levels of **28** were relatively low (Table 2). Remarkably, the corresponding 6′-aminobenzothiophene analogue **36** (BMS-251873) was endowed with higher topo I mediated DNA cleavage activity, cytotoxic potency, and topo I selectivity. Analogue **36** is 2-fold more potent than CPT in vitro, and the mechanism involving topo I is indistinguishable from that of CPT as shown above in Figure 3. Analogue

Table 3. Median Cytotoxic Potencies of **36** against Cell Lines after 72 h of Exposure and Antitumor Activities against Their Corresponding in Vivo Tumor Models

	HCT116	A2780	HCT29	M5076	PC3
IC_{50} [a]	0.010	0.005	0.065	- -	0.049
dose (MTD)[b]	10	60	70	50	60
LCK [c]	1.3	1.7	1.6	1.2	>2.8

[a] IC_{50} (μM). [b] Maximum tolerated dose expressed in mg/kg and administered twice a day iv every other day for 5 days (2q2d × 5) in cremophor/ethanol/water (10:10:80) except for PC3 (2q3d × 5). [c] Log of cell kill.

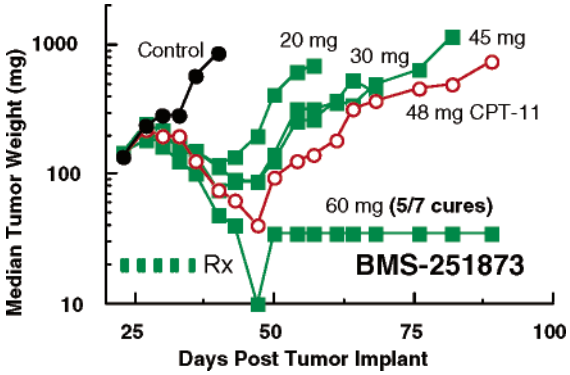


Figure 4. Efficacy of BMS-251873 against CPT-11 at different doses in the PC-3 xenograft model with 2q3d × 5 iv dosing schedule (Rx).

36, however, is about 7-fold less cytotoxic for P388 murine leukemia cells than **28**. The in vitro cytotoxic potencies of **36** against a number of human cell lines are compared in Table 3.

Prostate, colon, and ovarian cell lines are highly sensitive; however, cell lines expressing P-glycoprotein appear to be less sensitive (results not shown). Table 2 illustrates a robust pharmacokinetic profile shown by **36** compared to the prototypical leads. The high levels of exposure in plasma and tumor were sufficiently significant to warrant further exploration of this analogue in a number of in vivo antitumor animal models. Also, analogue **36** did not show any major metabolic and CYP-450 inhibition liabilities. In a panel of in vivo tumor models, this analogue displayed broad spectrum activity against human colon (HCT116 and HCT29), ovarian (A2780), prostate (PC3) carcinomas, and a murine sarcoma (M5076) (see Table 3).[12] In particular, pronounced antitumor efficacy was observed for **36** against the slow-growing human PC3 xenograft model that included cures and superior efficacy over CPT-11 (irinotecan)[2b] (see Figure 4).

The robust in vitro topo I mediated DNA cleavage activity, cytotoxicity, and topo I selective cytotoxic profile of **36** with improved solubility and pharmacokinetic behavior encouraged in vivo efficacy evaluations. The above properties and the broad spectrum of in vivo antitumor activities against a panel of human and murine models, especially efficacy in the prostate tumor xenograft model, mandated the selection of this analogue as a potential clinical candidate.

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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(12) LCK (log of cell kill) is defined as the in vivo antitumor activity obtained against solid tumor implanted sc following iv, ip, or po administration of the agent and is calculated according to the guidelines described in *Anti-Cancer Drugs* **1996**, *7*, 437−460.

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