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Evaluation of antitumor properties of novel saframycin analogs in vitro and in vivo

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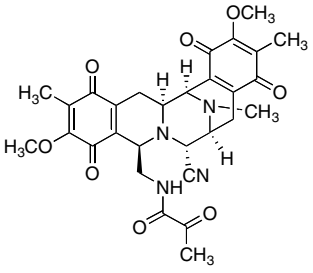
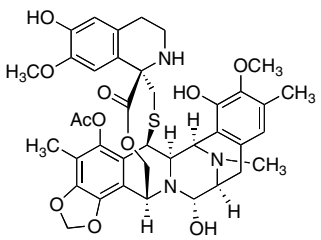
Abstract—Novel analogs of (−)-saframycin A are described. The analogs are shown to be potent inhibitors of the in vitro growth of several tumor cells in a broad panel and promising as leads for further optimization. The first in vivo studies in a solid tumor model (HCT-116) reveal potent antitumor activity with associated toxicity of daily administration.

(−)-Saframycin A (**1**) and (−)-ecteinascidin-743 (ET-743, **2**) are natural products that inhibit the growth of human cancer cells in vitro and in vivo.[1–3] Both molecules have been demonstrated to bind covalently to the exocyclic amino group of guanosine residues of duplex DNA, and this activity is thought to play a role in cancer cell killing.[4,5] In vivo studies of the chemotherapeutic properties of (−)-saframycin A were reported by Arai et al. in 1980 and by Kaneda et al. in 1986. They reported trends toward increased survival in mice following ip administration in P388 leukemia, L1210 leukemia, and B16 melanoma models.[6,7] While promising activities were demonstrated, several of the dose groups suffered from body weight loss. Results of phase I and II clinical studies of ET-743 (**2**) for the treatment of soft tissue sarcomas and breast and ovarian cancers have demonstrated its therapeutic potential, although limitations related to hepatic toxicity were also reported.[8] A number of synthetic analogs of both natural products have been prepared, some with potent antiproliferative effects.[9,10,11a] Here, we explore further the particular class of bis-tetrahydroisoquinoline analogs of saframycin-A (**1**) first described by Myers and co-workers and typified by the quinaldic acid derivative (QAD, **3**).[9,10] We describe novel and potent members of the analog series, extensive evaluations of these compounds in vitro, and the first in vivo studies of members of the class in a murine HCT-116 colon tumor model. In addition, we describe the preparation of a novel analog bearing a fluorescent label and show that this molecule co-localizes with chromatin in resting and dividing cancer cells.



Saframycin A (1)



ET-743 (2)

The saframycin analog QAD (**3**) has been shown to inhibit the growth of A549 lung tumor cells (GI50 value 4.4 nM) and A375 melanoma cells (1.3 nM) in vitro and served as a good comparator compound for pharmacological studies. We synthesized QAD (**3**) by the route previously described and evaluated this synthetic analog

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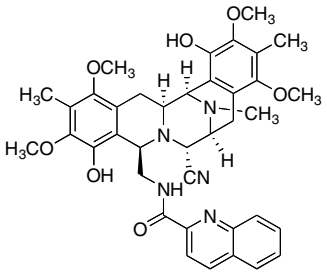
Table 1. GI50 values for the in vitro growth inhibitory effects of reference compound **3** in tumor cells from different tissues



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Cell type	Histology	GI50[a] (nM)
DLD-1	Colon	8.1
HCT-116		5.8
HT-29		7.5
CWR 22Rv1	Prostate	1.1
DU145		6.9
PC-3		6.2
A549	Lung	10.7
H1299		6.2
MDA-MB-231	Breast	4.5
HeLa-S3	Cervical	6.6
HT-1080	Fibrosarcoma	5.0
BxPC-3	Pancreatic	5.8
Nalm-6	B-cell leukemia[b]	0.6
P12	T-cell leukemia[b]	1.9

[a] Cells were grown in culture to a specific cell density and treated with compound for a period equal to two cell doubling times.
[b] Ref. 13.

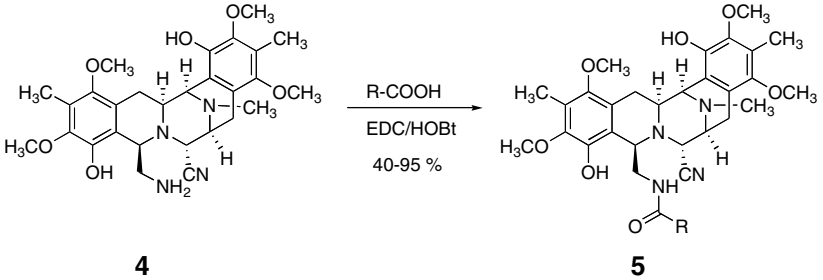
against a broad panel of cultured human cancer cells to establish benchmark activities (Table 1).[9,10] In all cases potent growth inhibition was observed, with a range of GI50 values from a low of 0.6 nM (Nalm-6 B-cell leukemia) to a high of 10.7 nM (A549 lung tumor cells), confirming that **3** exhibits significant potency relative to other known cytotoxic agents.[11b] We then prepared a series of analogs by the established synthetic route, using as a final step amide bond formation with a water-soluble carbodiimide reagent (Scheme 1). Novel, potent analogs were identified. Those containing a benzofuran-2-carboxamide in place of the quinoline-2-carboxamide of analog **3** were particularly active (**5a**–**e**, **6**), and are illustrated in Table 2 along with yields for the final step of synthesis and their measured GI50 values in four different cancer cell lines.

Analog **5d** was prepared as a structural homolog of the saframycin S class,[2] characterized by the replacement of the cyano group with an hydroxyl group. It is believed that both groups undergo elimination, albeit at different rates, forming a common electrophilic iminium species that is proposed to lead to DNA alkylation.[12]

Analogs **5b** (X = CN) and **5d** (X = OH) both produced potent growth inhibition in the four cell lines assayed. In a DNA alkylation study, both molecules efficiently alkylated a GC-rich dsDNA oligonucleotide 21-mer sequence [5′-GGAACCGGGCTCGGGCCAAGG-3′] as detected by a gel-shift assay (data not shown). Analog **5e** lacks the ability to form an electrophilic iminium species through elimination of hydroxide or cyanide and, not surprisingly, did not show evidence of DNA alkylation in the gel-shift assay. It was also substantially weaker at inhibiting the growth of HCT-116 cells (GI50 > 300 nM), which supports the idea that the ability to alkylate DNA in this series of analogs correlates with cytotoxicity. Analog **6** is a bis-quinone derivative of the analog **5b**, and while active in growth inhibition, was a somewhat poorer inhibitor relative to **5b** (Table 2) (note: analogs **5a**–**e** contain *O*-methyl hydroquinones on the distal aryl rings).

Fluorescently labeled analog **7** was prepared to assist in visualizing intracellular localization of the drug. While less potent compared to **5a**–**e**, analog **7** (GI50 value 201 nM) accumulated within HCT-116 cells relative to control cells (not shown). The compartmentalization is illustrated in Figure 1, where immunofluorescence was used to enhance the image. Association with organized nuclear DNA was most apparent in cells in metaphase, where highly structured chromatin appeared as brighter bodies. The apparent weaker staining of other cellular compartments suggests that DNA is a preferred target, independent of cell cycle. Compounds that are capable of alkylating DNA in cells may in turn activate DNA damage response pathways that eventually lead to cell death. Others have reported that cellular proteins may be involved in this process by associating at sites of DNA that have been alkylated with cytotoxic agents such as mercaptopurine,[13] ET-743 (**2**),[14] and QAD (**3**).[15] Further studies of these protein complexes and their roles in cell death may be facilitated by fluorescent molecules such as **7**.

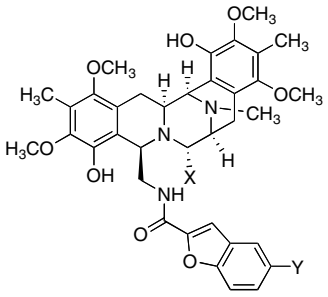
Using evidence from in vitro DNA footprinting assays, Rao et al. proposed that saframycin A covalently and reversibly binds in the presence of reducing agents to



4 **5**

Scheme 1. Coupling reactions used to prepare saframycin analogs **5**.

Table 2. GI50 values for the growth inhibitory effects of saframycin analogs in tumor cells



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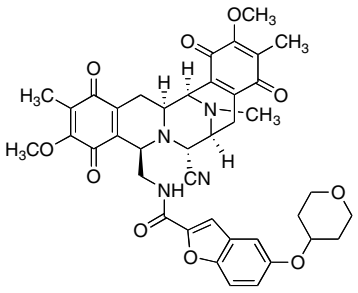

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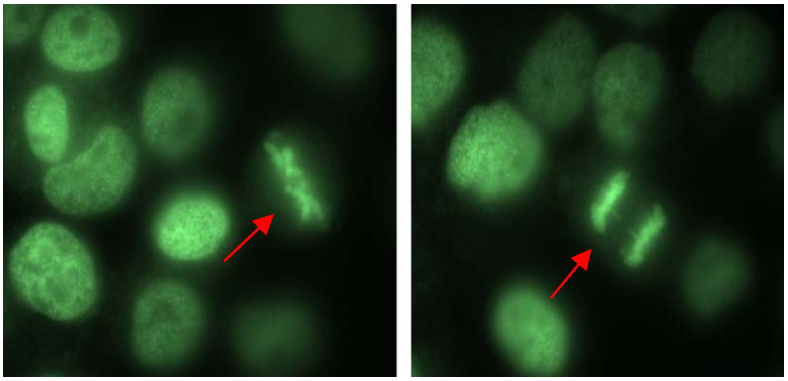
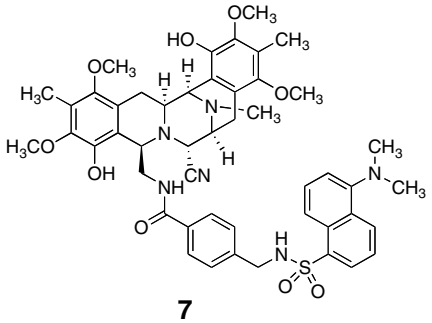
	X	Y	Yield (%)	GI50 (nM)			
				HCT-116	DLD-1	PC-3	A549
5a	CN	H	72	5.5	ND[b]	ND[b]	ND[b]
5b	CN	Tetrahydropyran-4-yloxy	60	3.2	8.3	2.7	18.9
5c	CN	2-(*N*-Morpholino)-ethoxy	ND[a]	8.1	22.4	11.7	57.5
5d	OH	Tetrahydropyran-4-yloxy	58	5.5	10.4	5.0	20.6
5e	H	Tetrahydropyran-4-yloxy	45	>300	ND[b]	ND[b]	ND[b]
6			44	17.3	26.5	16.5	ND[b]

[a]Yield of last step not determined; [b]GI50 value not determined.





7

Figure 1. Analog **7** was incubated in vitro with HCT-116 cells for 24 h, after which, whole cells were fixed and imaged by standard immunofluorescence using an anti-dansyl antibody. Extracted DNA could be readily visualized under UV light. The arrows point to metaphase cells where DNA association of **7** is more easily detected. The in vitro GI50 value of **7** was 201 nM (HCT-116).

the minor groove of dsDNA in a sequence-selective manner, preferring G-C base pairs.[16] Later, a two-dimensional ^{1}H NMR study of the related tetrahydroisoquinoline antiproliferative agent ET-736 bound to DNA provided proton distance constraints that led to a model suggesting the structural basis for sequence-selective DNA binding of this drug.[17] These distance constraints were employed by García-Nieto and co-workers in computer simulations of DNA binding by ET-743 and phthalascidin.[18] We used the coordinates of García-Nieto et al. as a starting point to model computationally the binding of novel analog **5b** in the minor groove of DNA, with particular focus on the positioning of the benzofuran side chain. Our computational results suggested that the bis-hydroquinone scaffold of **5b** is oriented similarly to that of E-743 and phthalascidin,

Table 3. Pharmacokinetic parameters for analogs **3** and **5b** following iv (0.5 mg/kg) and ip (2.0 mg/kg) administration to female Balb/C mice

	3	**5b**
C_{max} (μM)	1.1	3.7
Clearance (mL/min/kg)	72	45
AUC (μM min)	10	29
α-$T_{1/2}$ (min)	3.5	3.0
β-$T_{1/2}$ (min)	17	25
ip bioavailability (%)	46	38

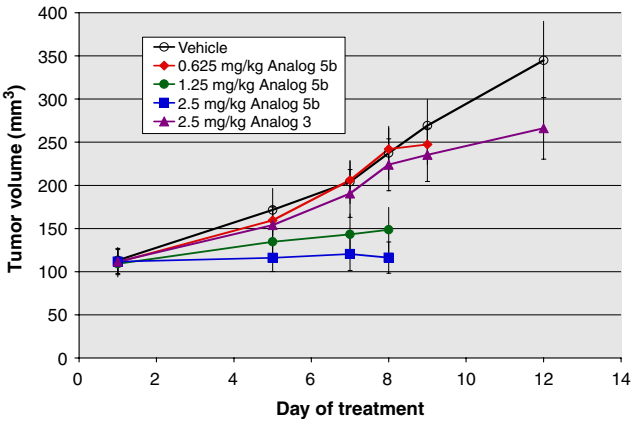


Figure 2. HCT-116 tumor growth curves for mice receiving either compound **3** or **5b** by once daily ip administration. Treatment was ceased on day 8 for 1.25 and 2.5 mg/kg of compound **5b**, day 9 for 0.625 mg/kg of compound **5b**, and day 12 for 2.5 mg/kg of compound **3**. Statistical analysis was performed using one-way ANOVA (Dunnett's) on comparison between treatment groups and the vehicle control.

and that the benzofuran group occupies an extended conformation, oriented away from the DNA and toward solvent. Figure S1 in supplementary material shows two perspectives of this model.[19]

We also studied the activity of analogs **3** and **5b** in vivo in a mouse model of colon cancer (HCT-116). The pharmacokinetics of these analogs were studied following intravenous (iv) and intraperitoneal (ip) administration in female Balb/C mice and showed a similar profile of disposition for both compounds (Table 3).[20] Mice with palpable tumors (100–150 mm^3 in size) received once-daily ip injections of the analogs followed by measurement of tumor size and body weight. The growth curves are shown in Figure 2. Statistically significant antitumor activity was seen in the groups receiving daily injections of compound **5b** at 1.25 mg/kg (68% tumor growth inhibition, $p = 0.01$) and 2.5 mg/kg (96% tumor growth inhibition, $p < 0.001$). However, these doses were accompanied by significant body weight loss due to toxic effects of the daily administration, requiring cessation of dosing after the eighth day. A single dose group receiving 0.625 mg/kg per day of compound **5b** did not show nearly as much antitumor activity or body weight loss. For comparison, reference compound **3** was administered for 12 days at 2.5 mg/kg. Antitumor activity was observed, but was weaker in comparison to the higher dose groups of compound **5b**.

In summary, we have described the preparation of novel bis-tetrahydroisoquinoline analogs of saframycin-A (**1**) and evaluations of these compounds in vitro. We have also presented the first in vivo studies of members of the class in a murine HCT-116 colon tumor model. In addition, we have presented evidence to support the idea that active compounds in the series are DNA associated. The potent antitumor activity of analog **5b** at relatively low doses is promising and sets the stage for further effort to explore therapeutic potential with this and other bis-tetrahydroisoquinoline analogs of saframycin. Future studies with **5b** and other analogs to incorporate dose scheduling would be helpful to establish a therapeutic index.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.06.085.

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19. Figure S1 is contained in supplementary material which may be accessed online alongside the article.
20. Plasma concentrations of analogs **3** and **5b** were determined by LC/MS/MS. The plasma sample was processed using acetonitrile precipitation, and then the supernatant was injected onto the LC column. The limit of quantitation of the assay was 0.002 and 0.0002 micromolar, respectively. Pharmacokinetic data were analyzed by WinNonlin-Pro (Pharsight Corp.), using compartmental and non-compartmental analysis for iv and ip data, respectively. ip bioavailability was evaluated from dose-normalized AUC values as follows: bioavailability = AUC_{ip}/AUC_{iv}.