Synthesis and Evaluation of Bishydroquinone Derivatives of (−)-Saframycin A: Identification of a Versatile Molecular Template Imparting Potent Antiproliferative Activity

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Received February 5, 2001

The saframycins are a family of microbial fermentation products with significant antiproliferative activity.[1] The most potent member of the series is saframycin A (**1**),[2] a bisquinone alkaloid containing an α-amino nitrile function. The molecular basis of the antiproliferative activity of this compound series is not known. In vitro experiments suggest that guanine residues of double-stranded DNA are alkylated by an iminium ion generated from the amino nitrile function of saframycin A in the presence of reducing cofactors, such as thiols, leading to the speculation that covalent modification of DNA is integral to the mechanism of action of this natural product.[3] Recently, the identification of the structurally related, non-quinoid natural products, the ecteinascidins,[4] has renewed interest in the saframycins, from the standpoints of synthesis, analogue preparation, and the possibility that a relationship may exist in the mechanisms of action of the two natural product series. Et-743 (**2**) (Figure 1) is currently undergoing phase II clinical trials for cancer therapy. Although its potency in antiproliferative assays is profound (sub-nM, ∼50-fold more potent than Taxol), it is now apparent that this potency can be maintained in the face of substantial structural modification. For example, Corey and Martinez have reported the discovery of the equipotent, structurally simpler molecule phthalascidin,[5] exploiting the power of their synthetic route to the ecteinascidins.[6] It has been suggested that DNA alkylation alone is insufficient to account for the biological activity of ecteinascidin; evidence continues to accrue that the mechanism of action of the compound class may involve protein−drug as well as drug−nucleic acid interactions.[5,7]

As a product of our own synthetic studies in the saframycin area, we had developed a short and enantioselective synthesis of saframycin A by the simple condensation of *N*- and *C*-protected α-amino aldehyde components.[8,9] The advanced intermediate **3**, a bishydroquinone dimethyl ether with a protected primary amino group, can be easily prepared by this route in hundred-milligram to gram quantities.[8] *N*-Deprotection of **3** and pyruvoylation of the resultant amine (**4**) afforded the intermediate **5**; simultaneous oxidation of both hydroquinone rings of **5** then provided totally synthetic saframycin A.[8] In considering the bioreductive activation mechanism proposed for saframycin activity, and the putative bishydroquinone intermediate **6** in particular,[3d,f] we wondered whether the final oxidation step in our initial route could be avoided entirely. That is, it seemed reasonable to propose that the bishydroquinone dimethyl ether **5** that served as the immediate precursor to saframycin A would be a viable mimic of the proposed bioactive intermediate **6** and, thus, was potentially a compound of significant bioactivity in its own right. Although **5** had been earlier described in racemic form by Fukuyama and co-workers (in the first laboratory synthetic route to saframycin A),[9c] we are unaware of any investigations of the antiproliferative activity of this compound. In the present context, the implications of a positive finding in bioassays of intermediate **5** were enormous with regard to an analogue synthesis program, given the efficiency of the synthetic route to optically pure **4** and the potential adaptability of this route for analogue synthesis by modification of the α-amino aldehyde and aldehyde condensation components (see, e.g., compound **16**,[10] Table 1). It has emerged from this investigation, as disclosed herein, that the bishydroquinone dimethyl ether scaffold does indeed provide a platform with high innate antiproliferative activity, activity that greatly exceeds that of the natural product in many cases. To exploit this finding fully we have developed simple protocols to transform the readily available, optically pure precursor **4** into a wide variety of bioactive analogues, in a single step and with high chemical purity.

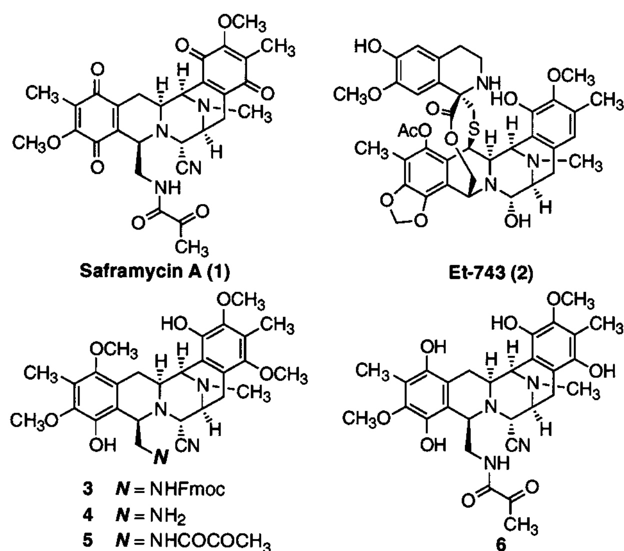

Figure 1.

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(10) See Supporting Information for the preparation of compound **16**, a detailed experimental protocol for amide coupling, and a complete table of compounds prepared and their biological activities.

10.1021/ja0103086 CCC: $20.00 © 2001 American Chemical Society
Published on Web 05/08/2001

Table 1. Some of the Most Potent Bishydroquinone Derivatives of Saframycin A and Their Antiproliferative Activities

N =	IC_{50}, nM A375	IC_{50}, nM A549	*N* =	IC_{50}, nM A375	IC_{50}, nM A549
Saframycin A (**1**)	5.3	133		2.7	31
(**5**)	4.5	160	(**10**)	1.7	9.2
	13	290		3.3	40
	2.4	39		2.5	32
	2.5	37	(**11**)	1.3	4.4
(**7**)	1.4	14	(**12**)	1.4	4.6
(**8**)	1.2	11	(**13**)	2.0	3.5
(**9**)	1.2	6.5	(**14**)	1.5	4.1
	1.7	25	(**15**)	1.2	4.7
	1.9	37	(**16**)[10]	3.6	78

To assess the biological activity of the intermediate **5** (synthesized as previously described),[8] antiproliferative assays using two human cancer cell lines, A375 melanoma and A549 lung carcinoma, were run in parallel against a range of drug concentrations. Cell proliferation in the presence of synthetic **5** was quantitated after 72 h of continuous drug exposure using a CellTiter 96 AQ_{ueous} Assay (Promega Corporation, Table 1).[11] The data showed that saframycin A and **5** were virtually indistinguishable in both cell lines. Although these data may be taken to support the idea that the bioactive form of saframycin A is the bishydroquinone **6**, the greater significance of the finding was considered to be the breadth of opportunity for analogue synthesis made possible by simple modification of the *N*-Fmoc appendage within this now-established bioactive structural platform.

Toward this end, the primary amino group of **4** was modified in a series of reliable, high-yielding coupling reactions, namely, amide bond-forming and reductive amination reactions. Results from the bioassay data suggested that the presence of a basic nitrogen atom in the side-chain residue was disadvantageous. Biological data from acylamine derivatives were much more promising. Acyl derivatives were readily prepared by a variety of solution-phase methods, including reactions with chloroformates, isocyanates, acid chlorides, and active esters formed in situ using carbodiimide reagents. Sulfonamides were also efficiently prepared. When bioassay data showed that amide derivatives were particularly potent antiproliferative agents (Table 1), attention was turned to develop a protocol for the rapid synthesis of these derivatives in high purity. For this purpose, solid-phase activation proved ideal. A simple mix-and-filter protocol was used to prepare the majority of the amide derivatives (Scheme 1), typically in milligram quantities and in >95% purity (high-resolution ^{1}H NMR analysis with additional confirmation of chemical composition by HRMS). The potential for transformation of this technology to automated systems is evident.

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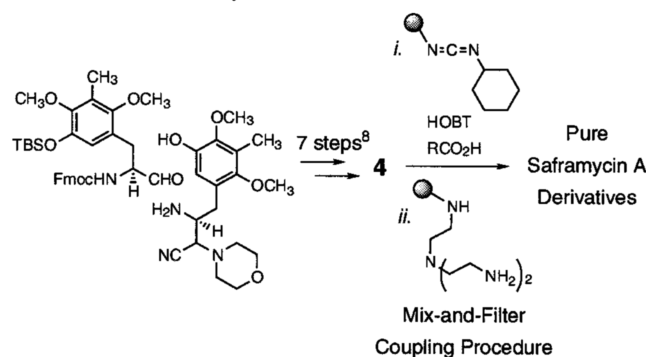
Scheme 1. Rapid Synthesis of Large Numbers of Potent Derivatives of Saframycin A



We have prepared and evaluated more than 70 derivatives of saframycin A to illustrate the scope of the procedure for analogue synthesis by modification at this single site; selected examples are shown in Table 1. Of these derivatives, approximately half show greater activity in antiproliferative assays than the natural product, in some cases, more than 20-fold greater activity. Although a detailed structure−activity analysis is not the focus of this report, it is noteworthy that salicylamide (**7**−**9**) and quinoline-2-carboxylic acid amide derivatives (**11**−**15**) are especially potent. In keeping with the observations of Corey and Martinez, the phthalimide side-chain is also found to be a potent inhibitor (**10**).[5] It is worth emphasizing that an analogue synthesis program of the magnitude described would not have been possible with a less efficient synthetic route to the key optically active precursor **3**, or with a more laborious final-step sequence, that is, one requiring product purification (Scheme 1).

The studies described have identified a versatile and readily available molecular template with innate, potent antiproliferative activity. In addition to their greater chemical stability and ease of synthesis, hydroquinone derivatives of the saframycins may be inherently less toxic, as suggested by biological studies of the natural monohydroquinone derivative, saframycin R.[12] Finally, the series of compounds described may be considered as a further structural link between the ecteinascidin and saframycin classes, particularly when viewed alongside ecteinascidin−saframycin hybrid structures previously prepared.[9d] The sensitivity to structural modification we observe would seem to support the notion that we are probing a rather precise binding interaction, perhaps consistent with the involvement of a protein or proteins.

Note Added in Proof: In further biological evaluation of a subset of the synthetic compounds described herein, compound **11** was shown to possess single-digit picomolar potency against three human sarcoma cell lines (100× more potent than Et-743). We gratefully acknowledge Drs. Joseph Bertino and Wei Wei Li, Memorial Sloan-Kettering Cancer Center, for these experimental data.

Acknowledgment. We gratefully acknowledge helpful conversations with Professor E. J. Corey and are indebted to Dr. Eduardo Martinez for kindly guiding us through the procedures for the biological evaluation of our compounds, and to Professor Stuart Schreiber for generous access to his laboratories. Financial support from the National Institutes of Health is gratefully acknowledged.

Supporting Information Available: General experimental procedures, listings of selected spectral data, reproductions of ^{1}H NMR spectra for selected, more potent analogues, and a complete table of all saframycin A derivatives prepared and bioassay data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA0103086