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Analogs of the marine alkaloid makaluvamines: Synthesis, topoisomerase II inhibition, and anticancer activity

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Abstract—Twelve analogs of makaluvamines have been synthesized. These compounds were evaluated for their ability to inhibit the enzyme topoisomerase II. Five compounds were shown to inhibit topoisomerase catalytic activity comparable to two known topoisomerase II targeting control drugs, etoposide and *m*-AMSA. Their cytotoxicity against human colon cancer cell line HCT-116 and human breast cancer cell lines MCF-7 and MDA-MB-468 has been evaluated. Four makaluvamine analogs exhibited better IC_{50} values against HCT-116 as compared to control drug etoposide. One analog exhibited better IC_{50} value against HCT-116 as compared to *m*-AMSA. All 12 of the makaluvamine analogs exhibited better IC_{50} values against MCF-7 and MDA-MB-468 as compared to etoposide as well as *m*-AMSA.

For the past quarter of a century, global marine sources have proven to be a rich source of a vast array of new medicinally valuable compounds.[1] These natural products exist as secondary metabolites in marine invertebrates such as sponges, bryazoa, tunicates, and ascidians. As a result of the potential for new drug discovery, marine natural products have attracted scientists from different disciplines, such as organic chemistry, bioorganic chemistry, pharmacology, and biology. About a dozen of marine alkaloids are currently in various phases of human clinical trials for treatment of different cancers.[2] The largest number of bioactive marine alkaloids with novel structures has been isolated from marine sponges.[3,4] Sponges produce a plethora of chemical compounds with widely varying carbon skeletons. Most bioactive compounds from sponges have exhibited a variety of activities such as anti-inflammatory, antitumor, immunosuppressive, neurosuppressive, antiviral, antimalarial, and antibiotic activities.[4] While a number of these alkaloids have been isolated in quantities sufficient to ascertain their biological profile, many with unique structures are available only in minute quantities, precluding their thorough biological evaluations. Laboratory synthesis of these alkaloids is the only practical solution to this problem.

Marine sponges of the genera *Latrunculia*, *Batzella*, *Prianos*, and *Zyzzya* are a rich source of alkaloids bearing a pyrrolo[4,3,2-*de*]quinoline skeleton.[5,6] This series of alkaloids comprises of about 60 metabolites including discorhabdins,[7] epinardins,[8] batzellines,[9] isobatzellines,[9] makaluvamines,[10–15] and veiutamine.[16] Pyrrolo[4,3,2-*de*]quinoline alkaloids have shown a variety of biological activities such as inhibition of topoisomerase I[14] and II,[10] cytotoxicity against different tumor cell lines,[10,17] and antifungal[14] and antimicrobial activities.[18] Pyrrolo[4,3,2-*de*]quinoline alkaloids have recently received increasing attention as a source of new anticancer drugs.[19–24] Their unique fused ring skeletons carrying interesting biological properties have made them targets for several synthetic and biological studies. There has been a rapid growth of interest in the synthesis and biological evaluation of this class of compounds and their analogs. Several reviews have been published on the chemistry and bioactivity of this class of compounds.[6,25,26] Our interest is focused on makaluvamines which belong to this family of alkaloids. Makaluvamines A–P are a group of 16 marine alkaloids isolated mainly from four species of marine sponges, namely the Fijian sponge *Zyzzya*cf. *marsailis*,[10] Indonesian sponge *Histodermella* sp.,[14] Pohnpeian sponge *Zyzzya fuliginosa*[11], and Jamaican sponge *Smenospongia aurea*[15] (Fig. 1).

Keywords: Makaluvamine; Analog; HCT-116; MCF-7; MDA-MB-468; Topoisomerase II.
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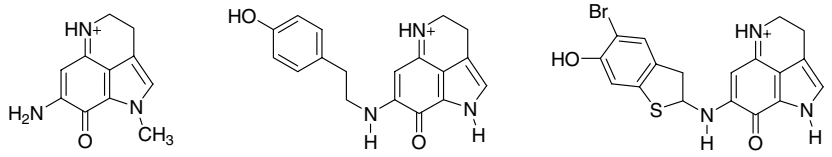


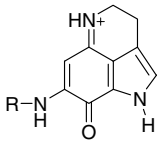
1, Makaluvamine A **2**, Makaluvamine D **3**, Makaluvamine F

Figure 1.

Makaluvamines have exhibited in vitro cytotoxicity against human colon tumor cell line, HCT-116. The cytotoxicities exhibited by makaluvamines against a Chinese hamster ovary (CHO) cell line Xrs-6 have paralleled the data obtained with HCT-116.[10] Makaluvamines produce their anticancer activity by targeting the enzyme topoisomerase II as shown by a decatenation inhibition assay.[10] According to a recent publication it is possible that makaluvamines produce their anticancer activity by other mechanisms as well.[27]

As a part of our research on marine natural product analogs with potential pharmacological value, we have been interested in studying the cytotoxicity of makaluvamine analogs. We propose to make these analogs by introducing different substituents at the 7-position of the pyrroloiminoquinone ring present in makaluvamines. Many pyrroloiminoquinone alkaloids with proven cytotoxicities were found to have substitutions at the 7-position of the ring. We decided to explore some simple substitutions with increased steric bulk, hydrophobicity, and hydrophilicity at the 7-position of the pyrroloiminoquinone ring and study their effects on topoisomerase II inhibition and cytotoxicity. Proposed target structures are given in Figure 2.

Makaluvamine analogs were synthesized from the pyrroloiminoquinone derivative **5**. Synthesis of compound **5** has been reported in the literature by several groups.[28] We prepared this compound following the 4,6,7-trimethoxyindole approach described previously.[29] This compound was converted to the makaluvamine analogs in two steps as outlined in Scheme 1.



4, Makaluvamine analogs

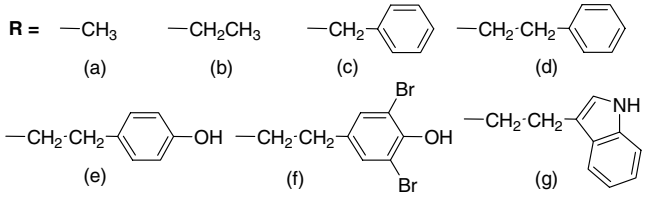
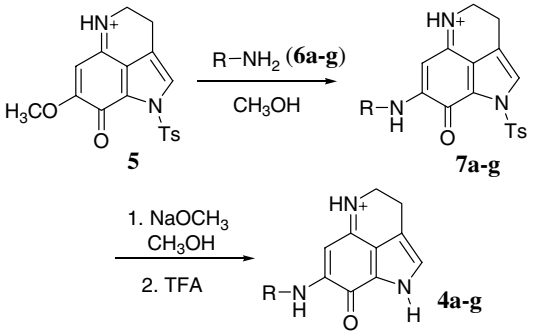


Figure 2.

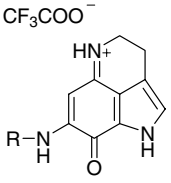
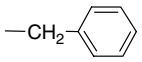
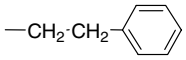
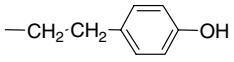
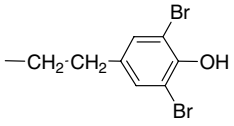
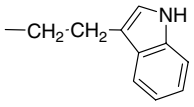
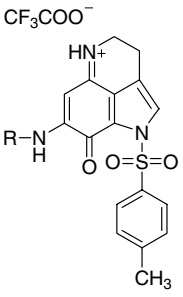
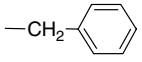
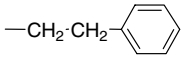
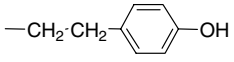
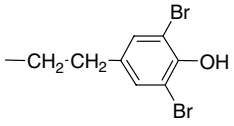
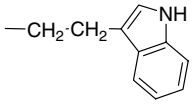


Scheme 1.

Treatment of the pyrroloiminoquinone derivative **5** with different amine derivatives (**6a**–**g**) in anhydrous methanol at room temperature provided the aminated compounds **7a**–**g** in 51–68% yield. Removal of tosyl protecting group from the compounds **7a**–**g** was accomplished by treatment with NaOMe in MeOH to obtain the final products **4a**–**g** in 48–62% yield. In the synthesis of compounds **4a**–**b**, intermediate products **7a**–**b** were not isolated as the amination and detosylation took place in a single step leading to the formation of final products. This type of detosylation in the presence of amines was not unusual and has been previously reported in the literature.[29] All these compounds were individually synthesized and fully characterized.

Compounds **4a**–**g** and **7c**–**g** were evaluated for their cytotoxicities against human breast cancer cell lines MCF-7 and MDA-MB-468, and human colon cancer cell line HCT-116. The dose of the compound that inhibits 50% cell proliferation (IC_{50}) was calculated using the data generated from 2 to 4 independent tetrazolium-based (XTT) cytotoxicity assays (R&D Systems Inc., Minneapolis, MN). Two known topoisomerase II targeting drugs, *m*-AMSA and etoposide, were included for comparisons. Results of cytotoxic assays are summarized in Table 1. HCT-116 cells were shown to be the most sensitive to etoposide and *m*-AMSA with IC_{50} doses of 1.7 and 0.7 μM, respectively. MDA-MB-468 cells showed IC_{50} doses of 13.6 and 8.5 μM for etoposide and *m*-AMSA, respectively. MCF-7 cells were shown to be the least sensitive with IC_{50} values of 35.6 and 21.7 μM for etoposide and *m*-AMSA, respectively. Several of makaluvamine analogs have shown significantly better inhibition than the control drugs in these assays. Four makaluvamine analogs (**4c**, **7d**, **7f**, and **7g**) exhibited better IC_{50} values against HCT-116 as compared to control drug etoposide. One analog (**7d**) exhibited

Table 1. In vitro cytotoxicity and inhibition of topoisomerase II catalytic activities of makaluvamine analogs

Structure	Compound	R =	Inhibition of cancer cell proliferation (IC_{50}[a], μM)			Topoisomerase II inhibition[b]
			HCT-116	MCF-7	MDA-MB-468	
	**4a**	–CH_3	2.5 ± 1.3	1.3 ± 0.1	0.4 ± 0.05	–
	4b	–CH_2CH_3	3.6 ± 0.7	1.3 ± 0.3	0.4 ± 0.03	–
	4c		1.3 ± 0.2	1.0 ± 0.4	0.3 ± 0.18	++
	4d		3.9 ± 1.2	1.7 ± 0.5	0.3 ± 0.27	+
	2		13.2 ± 0.9	3.2 ± 0.6	0.6 ± 0.05	–
	4f		14.4 ± 1.2	13.2 ± 3.1	4.5 ± 0.8	+++
	4g		5.2 ± 0.8	1.6 ± 0.2	0.4 ± 0.01	–
	**7c**		2.7 ± 1.5	1.8 ± 0.2	1.0 ± 0.01	+++
	7d		0.5 ± 0.03	1.5 ± 0.1	0.8 ± 0.1	—[d]
	7e		5.3 ± 0.6	5.1 ± 0.4	1.5 ± 0.14	+++
	7f		1.0 ± 0.2	2.3 ± 1.1	1.5 ± 0.9	—[d]
	7g		0.8 ± 0.3	1.2 ± 0.03	1.4 ± 0.9	—[d]
Etoposide	—[c]		1.7 ± 0.2	35.6 ± 3.4	13.6 ± 0.6	+
m-AMSA	—[c]		0.7 ± 0.3	21.7 ± 2.5	8.5 ± 1.2	++

[a] The dose that inhibits 50% cell proliferation (IC_{50}) was determined in human colon cancer cell line, HCT-116, and the human breast cancer cell lines, MCF-7 and MDA-MB-468 (ATCC, Manassas, VA). The IC_{50} doses from 2 to 4 independent XTT assays performed in triplicate were combined for an average ± standard deviation.

[b] Topoisomerase II (4U) was incubated with 500 ng plasmid DNA containing vehicle (DMSO) or 100 μM drug compound as described in the Topo II Drug Screening Kit protocol (TopoGEN, Inc). *m*-AMSA and etoposide were used as positive controls. Relaxed DNA was separated using non-ethidium bromide (EtBr) agarose gels, then stained with EtBr and quantified using Kodak Gel Logic Imaging System and Molecular Imaging software (Eastman Kodak Co., Rochester, NY). Inhibition of relaxation of plasmid DNA or catalytic activity is reported as: – none, + low, ++ moderate, or +++ strong.

[c] Not applicable.

[d] Not determined.

better IC_{50} value against HCT-116 as compared to *m*-AMSA. All twelve of the makaluvamine analogs exhibited better IC_{50} values against MCF-7 and MDA-MB-468 as compared to etoposide as well as *m*-AMSA. Compound that showed best activity against HCT-116 is the *N*-tosyl-6-phenethylamino derivative (**7d**) with an IC_{50} value of 0.5 μM. The compound that showed best activity against MCF-7 is the benzyl amino derivative (**4c**) with an IC_{50} value of 1.0 μM. Benzyl amino derivative, **4c**, and phenethyl amino derivative, **4d**, showed the best activity against MDA-MB-468 with IC_{50} value of 0.3 μM for each.

The final products (**4a**–**g**) and the intermediate products (**7c**–**g**) were also screened for their ability to inhibit topoisomerase II enzymatic activity. Topoisomerase II functions by generating a double-stranded DNA break followed by resealing of the break. Topoisomerase II inhibitors will interfere with the breakage-rejoining reaction thereby trapping the enzyme in a cleavage complex. In order to find out the ability of our compounds to inhibit topoisomerase II enzymatic activity, we used a topoisomerase-II drug screening kit (TopoGEN, Inc., Port Orange, FL) that uses a supercoiled plasmid DNA substrate (pRYG) which contains one topoiso-

merase II cleavage/recognition site. Two topoisomerase II inhibiting cancer drugs *m*-AMSA and etoposide were used as controls in these assays. The results of our assays are summarized in Table 1. Five out of nine tested makaluvamine analogs exhibited inhibition of topoisomerase II catalytic activity comparable to that of etoposide and *m*-AMSA. Three of these compounds (**4f**, **7c**, and **7e**) showed the strongest inhibition of catalytic activity of topoisomerase II.

In conclusion, several analogs of makaluvamines have been synthesized and characterized. Anticancer activity of these analogs against human colon tumor cell line, HCT-116, and human breast cancer cell lines, MCF-7 and MDA-MB-468, was evaluated. Four makaluvamine analogs exhibited better IC_{50} values against HCT-116 as compared to control drug etoposide. One analog exhibited better IC_{50} value against HCT-116 as compared to *m*-AMSA. All 12 of the makaluvamine analogs exhibited better IC_{50} values against MCF-7 and MDA-MB-468 as compared to etoposide as well as *m*-AMSA. Makaluvamine analogs were also evaluated for their ability to inhibit topoisomerase II enzymatic activity and found that five makaluvamine analogs exhibited inhibition of topoisomerase II comparable to etoposide and *m*-AMSA.

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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.2007.02.065.

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