Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 823–825

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Cytotoxic, Antifouling Bromotyramines: A Synthetic Study on Simple Marine Natural Products and Their Analogues

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Received 15 November 2001; accepted 3 January 2002

Abstract—Synthesis and biological evaluation of two naturally-occurring bromotyramines, moloka'iamine **1** and 3,5-dibromo-4-methoxy-β-phenethylamine **2**, together with several analogues, have been completed. Bromotyramine **2** is cytotoxic, and was found to be a potent antifoulant. Analogues **15** and **16** also displayed significant cytotoxic and antifouling activities.

Marine organisms are a rich source of chemically diverse bioactive substances. Over the past three decades, bromotyrosine-derived natural products have frequently been isolated from sponges of the order Verongida,[1] as well as from a few other marine sources such as ascidians.[2] Those compounds exhibit antifouling, antiviral, cytotoxicity, antifungal, and antibacterial activities.[3] Moloka'iamine (**1**), a compound first isolated in 1993 from a Verongid sponge of unidentified species,[4] is based on an *O*-alkylated dibromotyramine core, and displays cytotoxic and antifouling activity.[5] The *O*-alkylated dibromotyramine motif can be found in nature in both large, structurally complex natural products such as ceratinamide B (**4**),[6] as well as in its simplest form, that is 3,5-dibromo-4-methoxy-β-phenethylamine (**2**) (Fig.1)[7]

The antifouling activity of **1** against barnacle cyprids of *Balanus amphitrite* was particularly noteworthy.[5] Natural product antifoulants have been extensively investigated recently in search of environmentally benign replacements for tributyltin in maritime coatings applications.[8] We felt that the relatively simple structure of **1** invited the synthesis of analogues in an effort to probe antifouling structure–activity relationships. Since **1** was also reported to be active against P388 murine leukemia cells ($IC_{50} = 2.1$ μg/mL),[5] further antitumor and cytotoxic screening also seemed warranted.

We previously reported the synthesis of **1** in conjunction with the total synthesis of ceratinamine **3**,[9] a functionalized bromotyramine exhibiting potent antifoulant and cytotoxic activities.[5] For the present study, a new synthetic plan was devised that would allow for the convenient preparation of **1** and its analogues on a suitably

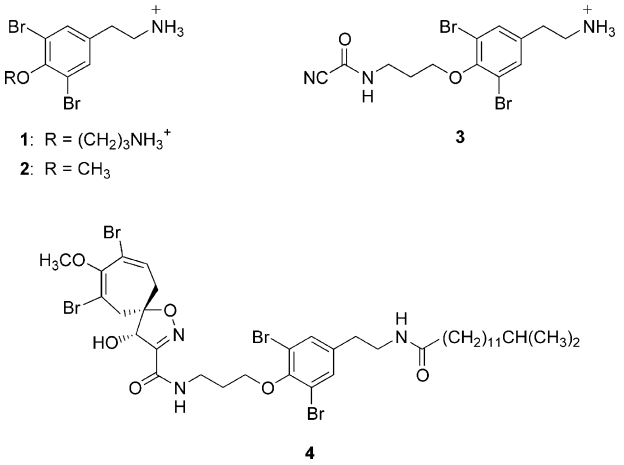

Figure 1. Bromotyramine marine natural products moloka'iamine (**1**), 3,5-dibromo-4-methoxy-β-phenethylamine (**2**), ceratinamine (**3**), and ceratinamide B (**4**).

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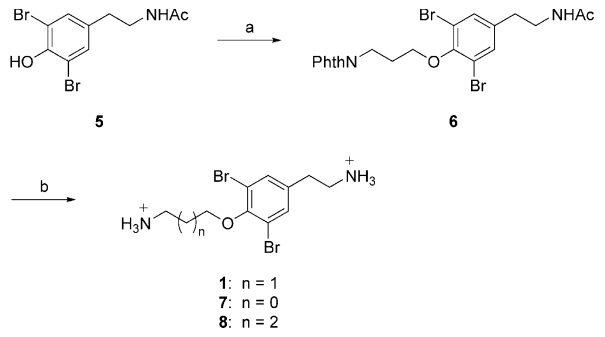
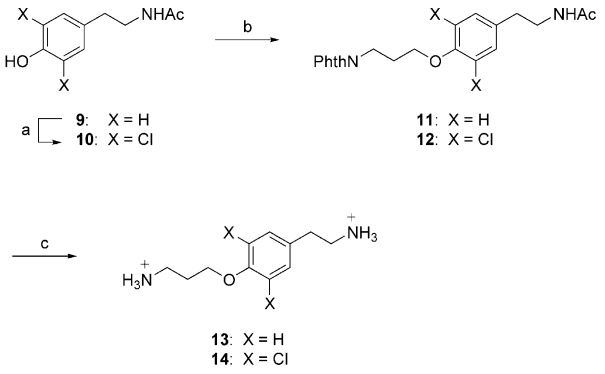
0960-894X/02/$ - see front matter
PII: S0960-894X(02)00022-7

large scale (Scheme 1). Reaction of *N*-acetyl-3,5-dibromo-4-hydroxy-β-phenethylamine **5**[10] with 3-bromopropylphthalimide, potassium carbonate, and catalytic potassium iodide in acetonitrile at reflux smoothly afforded protected diamine **6** in 98% yield. Exhaustive hydrolysis with concentrated HCl at reflux furnished moloka'iamine **1** as its dihydrochloride salt. The ethyl and *n*-butyl analogues **7** and **8** were prepared in similar fashion.

N-Acetyltyramine **9**[11] was used as starting material for the synthesis of additional analogues (Scheme 2). Chlorination of **9** with sulfuryl chloride in ether afforded **10** in modest yield. Phenols **9** and **10** were then aminopropylated and deprotected as described above in the synthesis of **1** to give the unhalogenated and chlorinated analogues **13** and **14**, respectively.



Scheme 1. Reagents and conditions: (a) $PhthN(CH_2)_3Br$, K_2CO_3, KI, acetonitrile, reflux, 98%; (b) concd HCl, reflux, 84%.

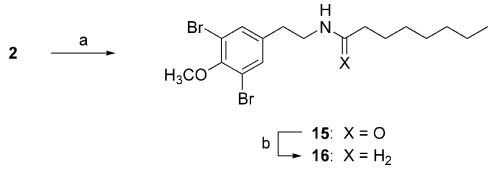


Scheme 2. Reagents and conditions: (a) SO_2Cl_2, diethyl ether, 38%; (b) $PhthN(CH_2)_3Br$, K_2CO_3, KI, acetonitrile, reflux, 87–98%; (c) concd HCl, reflux, 21–86%.

Initially, **1** and its analogues **7**, **8**, **13**, and **14** were assayed against *B. amphitrite* using a previously described cyprid settlement assay (Table 1).[12] The weak activity observed with **13** and **14** indicated that bromines were strictly required for antifouling performance. The most active compound tested was the ethyl analogue **7**.

Noting the increased activity associated with a shorter alkyl chain, we decided to investigate the naturally-occurring bromotyramine **2**, in which a methyl group replaces the aminopropyl chain found in **1**. Compound **2**, first synthesized in 1958 as part of a veterinary clinical study[10] and more recently isolated from an *Eudistoma* sp. Ascidian,[7] was found to be the most potent antifouling bromotyramine reported to date, a full two orders of magnitude more active than **1**.

Bromotyramines **15** and **16** were designed to hybridize the aromatic portion of **2** with the aliphatic portion of an antifouling natural product such as ceratinamide B (**4**, $IC_{50} = 2.4$ μg/mL vs *B. amphitrite*),[6] having a long-chain alkyl group appended to the phenethylamine nitrogen. We reasoned that a more lipophilic version of **2** would be more soluble in a coating, and therefore more practical as an antifouling paint additive. Acetylation of **2** with octanoyl chloride afforded amide **15** in near-quantitative yield (Scheme 3). Reduction of **15** with borane-THF, followed by an acidic workup, furnished octylamine **16** as its HCl salt. Compounds **15** and **16** strongly inhibited the settlement of barnacle cyprids, with IC_{50} values of 0.2 and 0.008 μg/mL, respectively. It



Scheme 3. Reagents and conditions: (a) $C_7H_{15}COCl$, triethylamine, methylene chloride, 99%; (b) BH_3-THF, 98%.

Table 1. Antifouling activity of bromotyramine natural products (**1** and **2**) and their analogues (**7**–**8** and **13**–**16**) against barnacle *Balanus amphitrite*

Compd	Settlement inhibition EC_{50} (μg/mL)	Lethality LD_{50} (μg/mL)[a]
1	5.0	nt
2	0.07	0.2
7	0.8	nt
8	6.0	nt
13	>50	nt
14	33	nt
15	0.2	1.0
16	0.008	0.03

nt, not tested.
[a]Concentrations at which the compounds tested were lethal to 50% of the barnacle cyprids.

Table 2. Growth inhibition of human cancer cell lines by selected bromotyramine compounds (**1**, **2**, **3**, **5**, **7**, **8**, **13**, **14**, **15**, and **16**) at 100 μM

	% Growth		
Compd	NCI-H460 (lung)	MCF-7 (breast)	SF-268 (CNS)
1	68	38	62
2	53	8	16
3[a]	82	7	39
5	105	88	84
7	70	39	70
8	25	19	50
13	120	104	120
14	93	−53	85
15	1	−74	−68
16	−83	−82	−84

[a]Compound **3** (ceratinamine) was synthesized as described previously.[9]

should be noted that bromotyramines **2**, **15**, and **16** exhibited varying levels of cyprid toxicity (Table 1), to which the observed antisettlement activity may well be ascribed. Investigation into the use of **16** as an antifouling paint additive is being pursued.

Some of the compounds described herein were submitted to the US National Cancer Institute for screening against several human tumor cell lines.[13] Prescreening results (Table 2) indicated that compounds **2**, **15**, and **16** demonstrated sufficient activity to pass to the 60-cell line screen. All three compounds displayed cytotoxicity at a mean panel GI_{50} concentration of 10 μM. Bromotyramine **2** was especially active in the CCRF-CEM leukemia, NCI-H226 lung cancer, and SW-620 colon cancer cell lines ($GI_{50} < 0.01$ μM for each), as well as in the Hs578T breast cancer cell line ($GI_{50} = 0.2$ μM). Compound **15** was most active in the CCRF-CEM leukemia cell line ($GI_{50} = 0.4$ μM), while **16** displayed the greatest activity in the NCI-H460 lung cancer cell line ($GI_{50} = 0.05$ μM).

Acknowledgements

We thank the National Science Foundation (#OCE-9216629, to D.R.) for generous financial support. R.C.S. was the recipient of a Sokol Summer Fellowship at Cornell. Support of the Cornell NMR Facility by the National Science Foundation (CHE 7904825, PGM 8018643) and the National Institutes of Health (RR02002) is gratefully acknowledged.

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