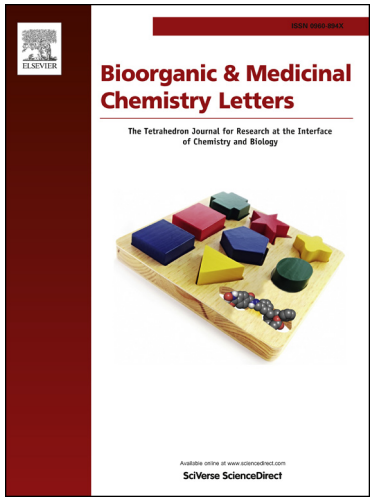

Synthesis and SAR studies of marine natural products ma'edamines A, B and their analogues

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PII: S0960-894X(13)00862-7
DOI: http://dx.doi.org/10.1016/j.bmcl.2013.07.017
Reference: BMCL 20682

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 19 June 2013
Revised Date: 10 July 2013
Accepted Date: 12 July 2013

Please cite this article as: Saha, S., Venkata Ramana Reddy, Ch., Xu, S., Sankar, S., Neamati, N., Patro, B., Synthesis and SAR studies of marine natural products ma'edamines A, B and their analogues, *Bioorganic & Medicinal Chemistry Letters* (2013), doi: http://dx.doi.org/10.1016/j.bmcl.2013.07.017

Graphical Abstract

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Synthesis and SAR studies of marine natural products ma'edamines A, B and their analogues

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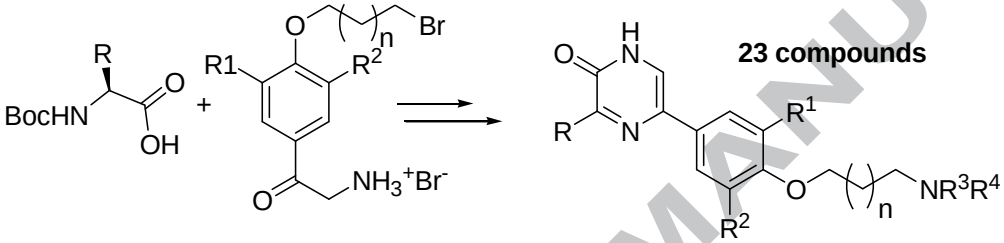
Sanjay Saha[a,b], Ch. Venkata Ramana Reddy[b],
Shili Xu[c], Saranya Sankar[c], Nouri Neamati[c], Balaram Patro[a*]

[a]GVK Biosciences Pvt. Ltd. Medicinal Chemistry Division, Nacharam, Hyderabad, India; [b] Department of Chemistry, JNT University, Kukatpally, Hyderabad, India; [c]Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California.



The synthesis of several analogues of ma'edamines A and B are reported. The synthesized compounds were tested on hormone receptor positive and HER2 positive breast cancer cell lines by MTT assay. MED-114, 115, 117, 119, 120, 124, 128 and 131 were found to be equally active as Lapatinib on HER2 +ve cell line SKBR3.

Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

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ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Keyword_1
Keyword_2
Keyword_3
Keyword_4
Keyword_5

ABSTRACT

The synthesis of several analogues of ma'edamines A and B are reported. The synthesized compounds were tested on hormone receptor positive and HER2 positive breast cancer cell lines, by MTT assay. MED-114, 115, 117, 119, 120, 124, 128 and 131 were found to be equally active as Lapatinib on HER2 +ve cell line SKBR3.

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The marine world is a source of excellent biodiversity which translates into great chemical diversity as well. Eight compounds derived from marine source have been approved till date by the FDA or EMEA for various disorders. They are Cephalosporin C, Cytarabine (Ara-C), Vidrabine (Ara-A), Ziconotide (Prialt), omega-3-acid ethyl esters (Lovaza), ET-743 (Yondelis), E7389 (Halaven) and Brentuximab vedotin (SGN-35).[1] A recent review gives an account of on-going efforts in marine pharmacology and anti-cancer drug discovery from marine natural products which are in various phases of clinical development.[2] This includes: bryostatin 1, dolastatin 10, aplidine, kahalalide F, squalamine, discodermolide, hemiasterlin analogue, bengamide analogue, cemadotin and eulotherabin. Excellent reviews featuring the discovery of antiviral and anti-malarial compounds from marine source have also appeared in literature.[3, 4]

Ma'edamines A (Med-111) and B (Med-114) (Table 1) which have a unique pyrazine-2-(1H)-one core structure was isolated by Kobayashi and co-workers from a marine sponge *Suberea sp*.[5] Kobayashi et. al. have reported cytotoxic activity of Med-111 and Med-114 in human and murine tumor cell lines and c-erbB-2 kinase inhibition (IC_{50}: 3.9-6.7 µg/mL for ma'edamine A) in enzymatic assays.[5] We have recently reported the total synthesis of ma'edamines A and B by two different approaches.[6] Ma'edamines A and B were found to be cytotoxic on human colon cancer line COLO 205 (IC_{50} 7.9 and 10.3 µM, respectively), breast cancer cell line MCF-7 (IC_{50}: 6.9 and 10.5 µM, respectively) and human lung adenocarcinoma cell line A549 (IC_{50}:12.2 and 15.4 µM, respectively). We report herein the synthesis of several analogues of ma'edamines and their biological evaluation for antiproliferative activity on breast cancer cell lines SKBR3 and T47D. Compounds Med-114, 115, 117, 119, 120, 124, 128 and 131 were found to have cyctotoxicity with IC_{50} values in the range of 3.5-8.15 µM which is comparable to HER2 inhibitor lapatinib.[7]

The synthesis of ma'edamines A and B was achieved *via* two different approaches.[6] The first approach involved coupling an α-keto acid with an α-amino ketone in a key step, followed by cyclization of the product with ammonium acetate which resulted in formation of the 2-[1*H*]-pyrazinone core of ma'edamines. In the second approach an α-amino acid was coupled with an α-amino ketone and the resulting product was de-protected, cyclized and oxidized to give the ma'eadamine core (Scheme 2). The potent *in-vitro* cyctotoxicity of ma'edamines on breast cancer, lung cancer and colon cancer cell lines prompted us to synthesise several analogues. In the present study, the second approach was followed to synthesize analogues of ma'edamines. The design of analogues was based on intuitive modification of the ma'edamines template. The structure of ma'edamine A was divided into two hydrophobic regions A and B around the pyrazin-2[1*H*]-one ring as shown in Figure 1. The following modifications were done: substituents on the ring in A region (designated as E) was modified; the ring in A region was replaced with different aliphatic and aromatic residues; the substituents on the ring in region B was modified; the number of carbon atoms (designated as n) connecting the oxygen and nitrogen (designated as D) was varied between 2 and 4; and the substituent on nitrogen (region D) was modified.

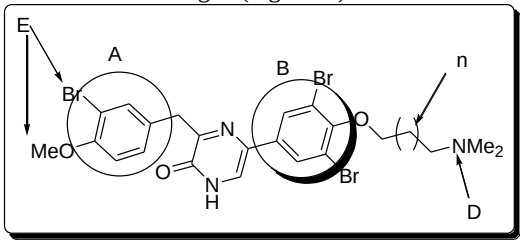


Figure 1: Design considerations and parameters: **a**: Replacement of the 3-bromo-4-methoxyphenol group; b: replacement of the 3,5-dibromophenyl group; **c**: variation of the number of c-atoms connecting the oxygen and the N atoms; **d**: replacing the substitution on nitrogen or **e**: changing the bromo or the methoxy substituents.

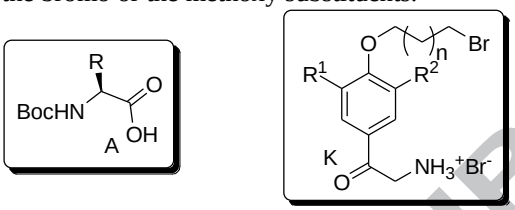


Figure 2: Key Building blocks required for synthesis

Following amino acid building blocks were used for synthesis of ma'edamines analogues (Figure 3). The compounds **a2** and **a3** were prepared by bromination and chlorination respectively, of L-4-methoxyphenyl alanine, by reported methods followed by N-protection with di-tert-butyldicarbonate.[8, 9] All other amino acid derivatives were obtained from commercial sources and used as such.

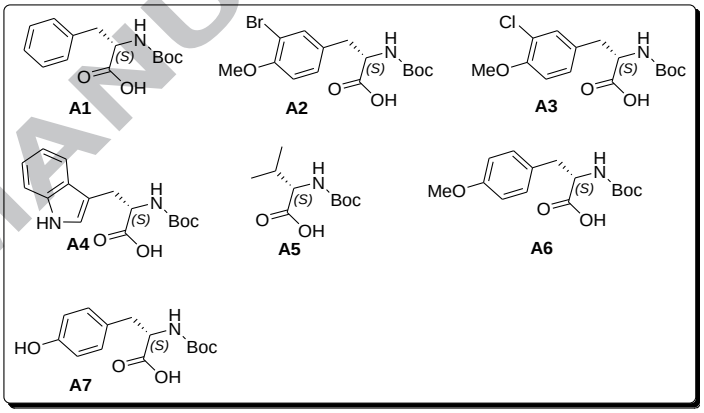
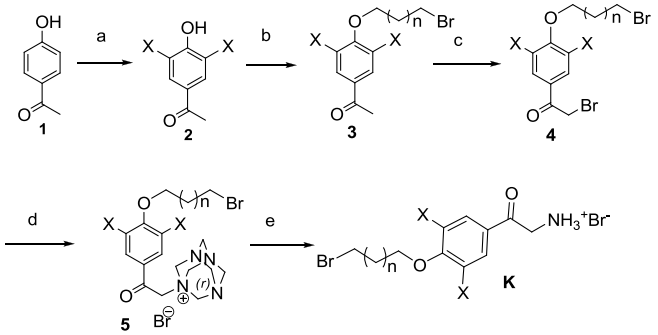


Figure 3: α-amino acid building blocks

A key reaction step in the synthesis of the α-Amino ketones building blocks (Figure 4) was a Delepine reaction of an α-bromoketone compound **4** (Scheme 1). [10] The synthesis was started with bromination of 4-hydroxyacetophenone with pyridinium bromochromate which afforded 3,5-dibromo-4-hydroxyacetophenone in 93 % yield.[11] Chlorination of 4-hydroxyacetophenone was done by bubbling chlorine gas through its solution in acetic acid and water to afford 3,5-dichloro-4-hydroxyacetophenone in 30 % yield.[12] Alkylation of the resulting di-halo products **2** with 1,2-diboroethane / 1,3-dibromopropane / 1,4-dibromobutane in DMF solvent using potassium carbonate as a base afforded the O-alkylated product **3**. The acetyl group of the resulting product **3** was brominated using bromine in chloroform at room temperature. A Delépine reaction on the intermediate **4** which involved reaction with hexamethylenetetramine followed by hydrolysis of the resulting salt with HBr, afforded the desired α-amino ketone K.



Scheme 1: Reagents and Conditions: a) pyridinium bromochromate, AcOH, 40 °C, 2h, ~70%; or Cl_2, AcOH, H_2O, ~75%; b) $BrCH_2(CH_2)_nCH_2Br$, (n=0-2), K_2CO_3, DMF, rt, 36h, 60%; c) Br_2, $CHCl_3$, rt, 2h, 95%; d) Hexamethylenetetramine, $CHCl_3$, rt, 18h; e) MeOH, HBr (aq.), 48h, 66% for two steps.

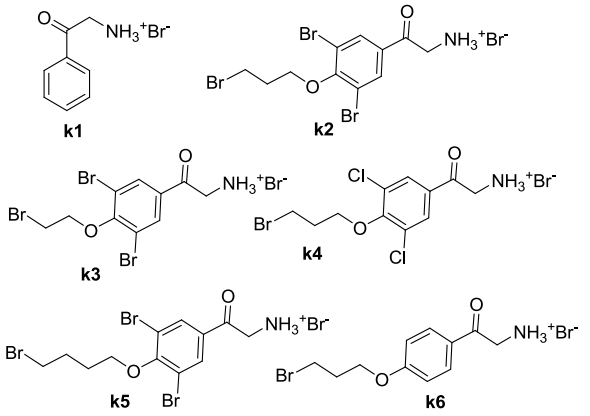
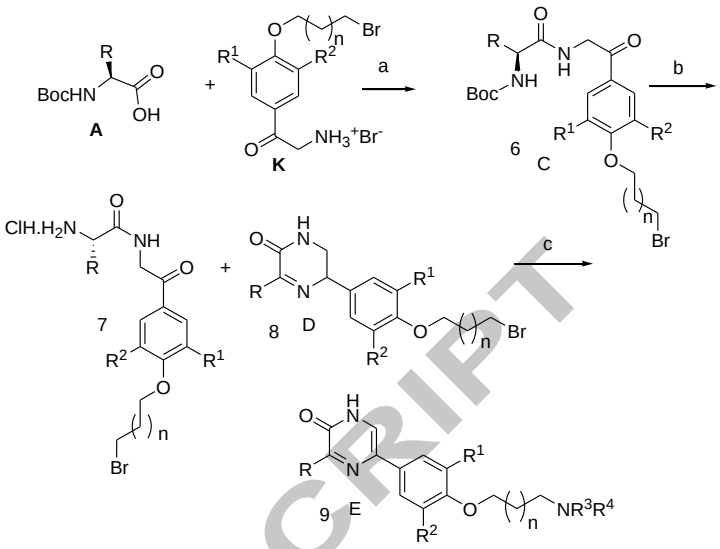


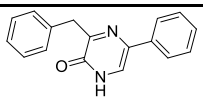
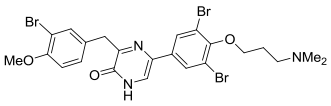
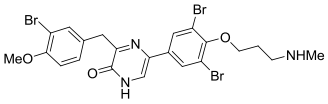
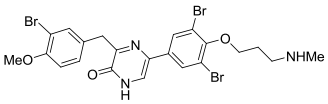
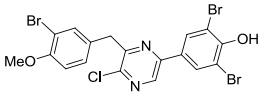
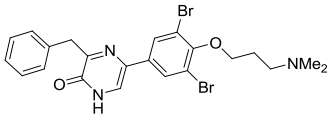
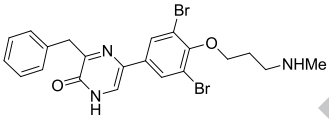
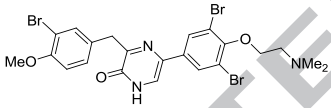
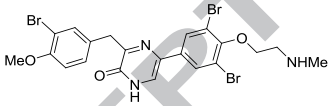
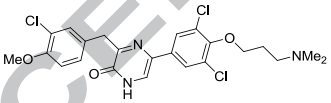
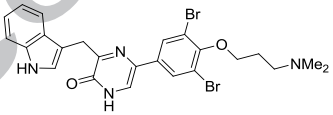
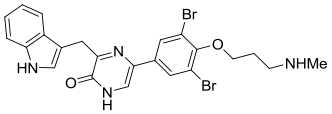
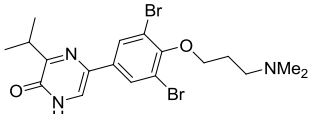
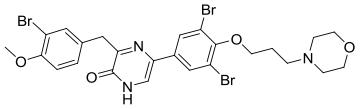
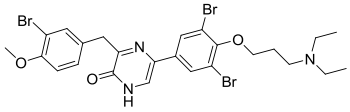
Figure 4: Synthesized α-Amino ketone building blocks.

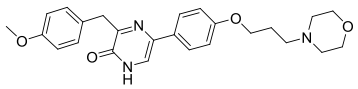
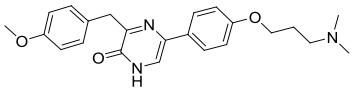
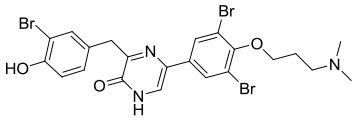
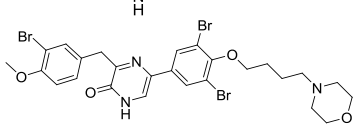
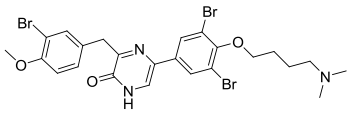
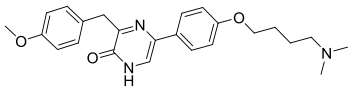
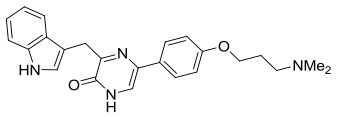
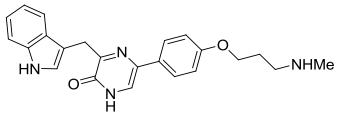
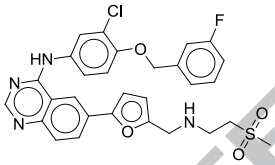


Scheme 2 : Reagents and Conditions: a) HATU, TEA, DMF, 4h, 40-60%; b) 4M HCl in 1,4 - dioxane, rt, 4h; c) Amine in THF, rt, 16- 24h, ~30-40 % for two steps.

The analogues of ma'edamines A and B were synthesized by a route shown in Scheme 2. The first step in this route is based on the coupling of an α-amino acid **A** with an α-amino ketone derivative **K** mediated by HATU which afforded us the amide **6**. De-protection of the amide with 4M HCl in 1,4-dioxane at room temperature afforded a mixture of de-protected derivative **7** and cyclized compound **8**. Treatment of this mixture with the appropriate amine offered us the aromatized compound **9** suitably substituted by an alkyl amino group. All the synthesized compounds were purified by flash column chromatography over silica gel followed by crystallization. The prepared analogues were characterized by ^{1}H NMR and HPLC-MS and obtained in sufficiently pure form (>95 %). They were screened for anti-proliferative activity by MTT (3,4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay[13] on SKBR3 [estrogen receptor (ER) -ve, progesterone receptor (PR) –ve and human epidermal growth factor receptor (HER2) +ve], T47D (ER +ve, PR +ve and HER2 -ve) and CAMA 1 (ER +ve, PR –ve and HER 2 -ve) cell lines. [14] The IC_{50} values are reported in Table 1.

Table 1: Synthesized compounds and their anti-proliferative activity against breast cancer cell lines

Compound	**Structure**	IC_{50} (μM)		
MED-		**SKBR3**	**T47D**	**CAMA1**
110		>10	>10	>10
111		>10	>10	>10
114		6.17±0.27	6.18±1.45	5.77±0.37
115		4±0.80	6±0.0	6±0.0
116[6]		>10	>10	>10
117		5.5±0.0	6.33±0.53	7.10±0.96
118		> 10	>10	>10
119		5.73±1.24	5.67±1.87	>10
120		8.10±0.21	6.18±1.45	>10
121		> 10	>10	>10
123		7±0.0	6.33±1.07	>10
124		4.33±0.53	4±2.4	5.77±0.37
125		> 10	>10	>10
127		> 10	>10	>10
128		6±0.0	>10	5.23±0.05

129		> 10	>10	>10
130		> 10	>10	>10
131		5.67±0.53	4.77±0.03	6.67±1.07
132		> 10	>10	>10
133		> 10	>10	>10
134		> 10	>10	>10
137		>20	-	>20
138		>20	-	>20
Lapatinib		5.05±0.28	>10	>10

The cytotoxicity of compounds MED-114 (ma'edamine B), 115, 117, 119, 120, 124, 128 and 131 on SKBR3 cell lines were comparable to Lapatinib (IC_{50} 5.05±0.28 µM), a compound approved by FDA for HER2 targeted therapy of breast cancer. MED-115 and MED-124 were found to be the most potent compounds in this series with a mean IC_{50} 4 µM and 4.33 µM respectively against SKBR3 cell line. MED-117, a compound without a bromo and a methoxy group on the phenyl ring (in A region) was also quite potent (IC_{50} 5.5 µM, SKBR3) suggesting that these groups on the phenyl ring weren't absolutely essential for activity.

When the hydrophobic phenyl group of MED-111 (ma'edamine A) was replaced by an indole in compound MED-123 (IC_{50} ~7 µM, SKBR3) and MED-124 (IC_{50}4.33±0.53 µM, SKBR3) the activity was retained. However when the aromatic group was replaced with an aliphatic group in MED-125 the resulting compound was inactive. Replacement of the bromo groups of MED-111 with chloro groups (MED-121) didn't result in any improvement in activity. De-methylation of MED-111 resulted in restoration of antiproliferative activity in compound MED-131 (IC_{50} 5.67±0.53 µM, SKBR3). The replacement of methyl groups with ethyl (MED-128) also resulted in restoration of activity (IC_{50}6µM, SKBR3).

Completely de-brominated compounds MED-129, MED-130 (IC_{50}>10 µM) and MED-134 (IC_{50}>10 µM) didn't show any improvement in activity over their brominated counterparts MED 111 (IC_{50}> 10 µM) and MED 133 (IC_{50}>10 µM). De-bromination resulted in loss of activity in MED-137 (IC_{50}>20 µM) and MED-138 (IC_{50}>20 µM) compared to their halogenated couterparts MED-123 (IC_{50} ~7 µM, SKBR3) and MED-124 (IC_{50}4.33±0.53 µM, SKBR3).

The amino-alkyl side chain is required for activity since removal of side chain of MED-115 (IC_{50} 4±0.80 µM, SKBR3) resulted in complete loss of activity in MED-116 (IC_{50} >10 µM, SKBR3). Reducing the length of the amino-alkyl side chain of MED-111 to two carbons resulted in improvement of activity in MED 119 (IC_{50}5.73±1.24 µM, SKBR3), whereas increasing the chain length to four carbons did not improve the activity in MED-133 (IC_{50} >10 µM, SKBR3). Reduction of chain length of active compound Med-114 (IC_{50} 6.17±0.27 µM, SKBR3) did not changed its activity significantly in MED-120 (IC_{50} 8.10±0.21 µM, SKBR3).

MED-114, 115, 117, 119, 120, 123, 124 and 131 were also had good cyctotoxicity on hormone receptor positive cell line T47D with IC_{50} values in the range of 4-6.33 µM (Table 1).

In conclusion, we have prepared 23 analogues of ma'edamines A and B and tested them on three breast cancer cell lines representing hormone receptor positive and HER2 positive breast cancer. It is evident from the cytotoxicity data that MED-114, 115, 117, 119, 120, 123, 124, 128 and 131 were equally active as Lapatinib on HER2 +ve cell line SKBR3. MED-114, 115, 117, 124 and 131 were active on all three types of breast cancer cell lines.

Acknowledgments

We sincerely thank GVK Biosciences Private Limited for financial support and encouragement. Support from analytical department is also acknowledged.

References and notes

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Supplementary Material

1H NMR scans and mass spectral scans of all newly synthesized compounds are available.