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The first total synthesis and biological evaluation of marine natural products ma'edamines A and B

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ABSTRACT

We have developed the first total syntheses of marine natural products ma'edamines A (**18**) and B (**20**). Structurally, they contain a pyrazine-2-(1*H*)-one core and were screened for antiproliferative activity on several cancer cell lines. Out of the six cell lines tested, ma'edamines A and B showed significant cytotoxicity against human colon cancer line COLO 205 (IC₅₀ 7.9 and 10.3 μM, respectively), breast cancer cell line MCF-7 (IC₅₀: 6.9 and 10.5 μM, respectively) and human lung adenocarcinoma cell line A549 (IC₅₀: 12.2 and 15.4 μM, respectively). The apoptotic effect of ma'edamines was confirmed by comet assay. Hence ma'edamines are likely to be useful as leads for development of a new class of anti-cancer agents.

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The marine ecosystem is a treasure of diverse compounds and a rich source of biological diversity which translates into excellent chemical diversity.^{1–3} Many potent compounds of marine origin or their analogues have entered clinical trials or have been approved as drugs.⁴ Selected examples include: bryostatin 1, dolastatin 10, discodermolide, bengamides and Ecteinascidine 743.^{5–9} Ma'edamines A (**18**) and B (**20**) are cytotoxic compounds, isolated from the extract of the Okinawan marine sponge *Suberea* sp., exhibiting ErbB2 kinase inhibitory activity (IC₅₀: 6.7 μg/mL).¹⁰ They belong to a family of marine bromotyrosine alkaloids,¹¹ containing a unique pyrazine-2-(1*H*)-one core structure. Compounds containing this scaffold are not known in literature for exhibiting anti-cancer activity, whereas many selective ErbB2 (human epidermal growth factor receptor or HER2) kinase inhibitors, or HER2/EGFR dual inhibitors bearing a 4-amino-quinazoline, 4-aminoquinoline are approved as drugs (Fig. 1).¹² Due to the involvement of the EGFR family of tyrosine kinases in the development of several types of solid tumors like non small cell lung cancer, breast cancer, head and neck cancer, etc., they continue to be the subject of further development through rational design.¹³ Recent examples include development of pyrrolo[3,2-*d*]pyrimidine and pyrimidine scaffolds as dual EGFR/HER2 inhibitors.¹⁴

We report herein the syntheses of ma'edamines A and B, and results of their biological evaluation; which includes assessment of anti-proliferative activity on several cancer cell lines. The ma'edamines were found to be moderately potent in vitro (low micromolar IC₅₀) and induced apoptosis, which was confirmed by comet assay.¹⁵

We envisioned the synthesis of ma'edamines by two routes shown below in the retro synthesis (Fig. 2).

Both routes revealed an α-amino aryl ketone **F3** as the common building block, the second component being an α-keto acid derivative **F2** in the former and an α-amino acid **F4** in the latter. The α-keto acid derivative **F2** (**10**) was synthesized from *p*-anisaldehyde in three steps by a reported procedure.¹⁶ The second building block **8** was readily synthesized from 4-hydroxyacetophenone via brominations, followed by a Delépine reaction of the resulting bromoaryl ketone **4**¹⁷ (Scheme 1). Compound **9** was also prepared by the same route.

The acid–amine coupling of the key intermediates **8** and **10** by a standard protocol using TBTU/*n*-methylmorpholine/DMF afforded intermediate **11** (Scheme 2). Ring closure using ammonium acetate in ethanol yielded pyrazinone derivative **12**. Alkylation of **12** with 1,3-dibromopropane,¹⁸ furnished a mixture of mono and bis-alkylated products, which was difficult to purify. Alkylation of **12** with (3-chloropropyl)-dimethyl-amine **14**, was not regiospecific and also resulted in formation of a complex mixture. Therefore, chloropyrazine derivative **13** was prepared by reaction of

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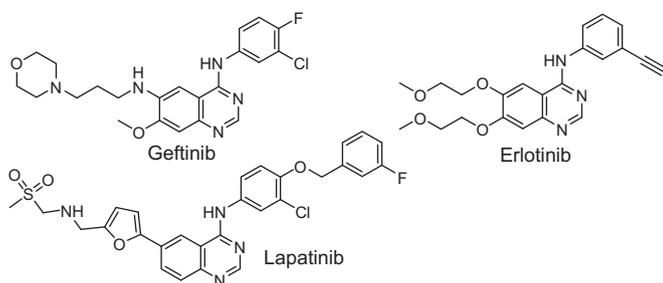


Figure 1. FDA approved tyrosine kinase inhibitors.

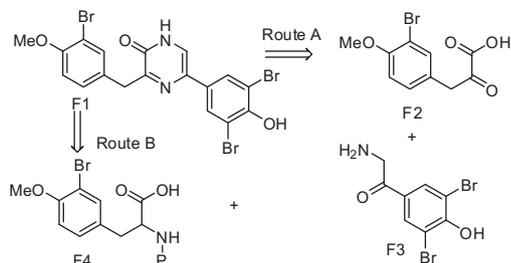
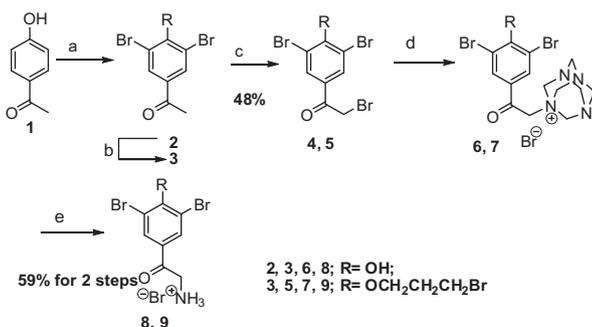


Figure 2. Retro synthetic approach for synthesis of pyrazin-2(1H)-ones showing key fragments (F1–F4).

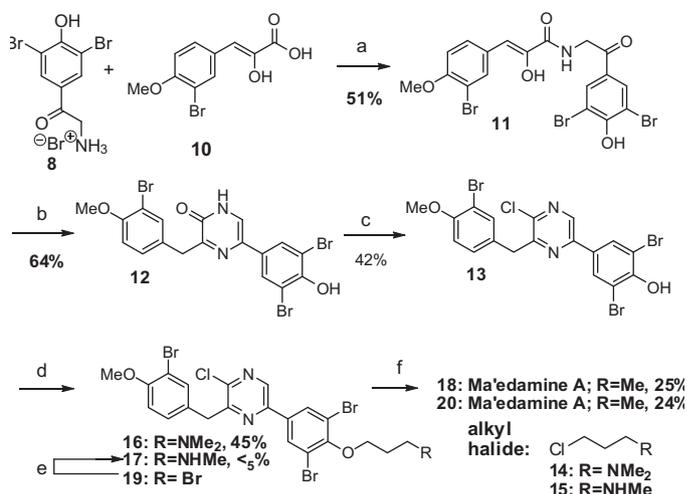
pyrazinone **12** with phosphoryl chloride. O-Alkylation of **13** with (3-chloropropyl) dimethyl-amine **14** afforded **16**. The best yields were obtained when the alkylation was done in butanone, in presence of cesium carbonate as base and one equivalent of sodium iodide added as an additive. Treating the chloropyrazine derivative **16** with 90% acetic acid and water at 160 °C for 12–16 h in a sealed tube afforded ma'edamine A **18**.

The alkylation of chloropyrazine derivative **13** with (3-chloropropyl)-methyl-amine **15** was very low yielding (<5%). Therefore **13** was alkylated with 1,3-dibromopropane and the resulting product was treated with dimethylamine to afford **17**. Similar de-protection of **17** yielded ma'edamine B in a modest yield. Both **18** and **20** were characterized by ¹H NMR, ¹³C NMR and HR-MS analyses and the analytical data was consistent with the literature.¹⁰ The structure of ma'edamine A was further confirmed by NOESY, HMBS and HSQC NMR analyses.

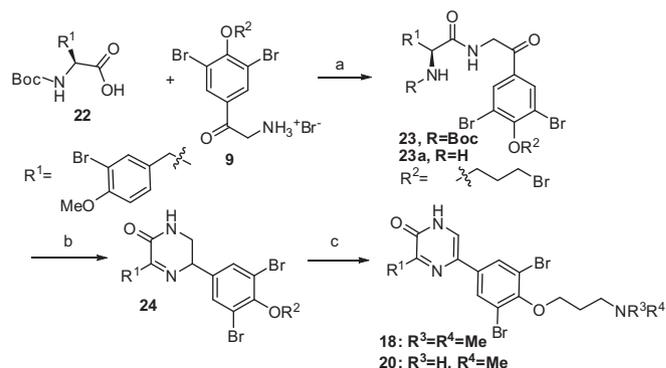
In order to expand the scope of our synthetic method for creating small focused library of analogues we have also developed a second route using different building blocks. The second approach of synthesis of ma'edamines is depicted in Scheme 3. *N*-Boc α-amino acid **22** and α-amino aryl ketone **9** were coupled by a standard



Scheme 1. Reagents and conditions: (a) Br₂, AcOH, rt, 2 h; (b) BrCH₂CH₂CH₂Br, K₂CO₃, DMF, rt, 18 h; (c) Br₂, CHCl₃, rt, 2 h; (d) Hexamethylene tetramine, CHCl₃, rt, 18 h; (e) MeOH, HBr (aq), 48 h, 59% for two steps.



Scheme 2. Reagents and conditions: (a) TBTU/NMM/DMF, rt, 2 h; (b) NH₄OAc, EtOH; (c) POCl₃/DMF; (d) alkyl halide, base; (e) MeNH₂ (MeOH), rt; (f) dil AcOH, 160 °C, 16 h.



Scheme 3. Reagents and conditions: (a) HATU, TEA, DMF, 4 h; (b) 4(M) HCl in 1,4-dioxane, rt, 4 h; (c) amine in THF, rt, 16–24 h.

coupling protocol. Treatment of the amide **23**, with hydrochloric acid (4 M in dioxane) afforded a mixture of **23a** and **24**. We found that treatment of this mixture with anhydrous dimethylamine in methanol resulted in cyclization of **23a** to **24** followed by air oxidation to afford ma'edamine A **18**. Ma'edamine B was obtained similarly by treating the mixture of **23a** and **24** with methylamine in THF. These compounds were characterized by ¹H NMR and mass spectra which were identical to the ones prepared by Scheme 2. Ma'edamines A and B were studied for their antiproliferative activity by MTT assay,¹⁹ trypan blue staining²⁰ and apoptotic potential by comet assay^{15,21} as well as DNA laddering.²²

Cells in the exponential phase of growth were exposed to the test substance. The duration of exposure was usually set as the time required for minimal damage to occur. After addition of the drug, the cells were allowed to proliferate for two to three populations doubling times (PDT's) in order to distinguish between cells that remain viable and are capable of proliferation and those that cannot proliferate. The number of surviving cells were then determined indirectly by reduction of MTT ((3,4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) by the mitochondrial dehydrogenase of live cells to a purple formazan product.¹⁹ The amount of MTT–formazan produced was determined spectrophotometrically at 570 nm after dissolving it in DMSO.

The cytotoxicity data is expressed as IC₅₀ values in μM (Table 1). Etoposide, a well-known anti-cancer drug was used as the positive control for comparison.²³ Ma'edamines and intermediates were

Table 1
Antiproliferative activity of ma'edamines and its intermediates

Compound	Structure	IC ₅₀ (μM)					
		COLO-25	MCF-7	A-549	THP-1	B16-F10	HepG2
18		7.9	6.9	12.2	46.2	16.7	29.6
16		nt	nt	14.4	87.3	63.2	nt
12		nt	nt	52	124.7	146	nt
20		10.3	10.9	15.4	90.7	53.3	42.8
17		nt	nt	25.3	133	75.8	nt
13		nt	nt	41.2	108.2	25.6	nt

nt: not tested.

tested for their antiproliferative activity on human colon cancer line COLO 205, breast cancer cell line MCF-7, human lung adenocarcinoma cell line A549, human acute monocytic leukemia cell line THP-1, liver hepatocellular carcinoma cell line HepG2 and mouse melanoma cell line B16-F10. Both ma'edamines A and B were found to show significant in vitro cytotoxicity against COLO 205 (IC₅₀ 7.9 and 10.3 μM, respectively), MCF-7 (IC₅₀: 6.9 and 10.9 μM, respectively) and A549 (IC₅₀: 12.2 and 15.4 μM, respectively). Ma'edamine A was found to be about equally cytotoxic on MCF-7 and A-549 cell line compared to etoposide (IC₅₀: 16.3 μM and 11 μM, respectively). Ma'edamines A and B showed low activity on THP-1, B16-F10 and HEPG2 cell lines. The intermediates **12**, **13**, **16** and **17** were less potent compared to the ma'edamines.

The ability of ma'edamines to cause programmed cell death was assessed by comet assay (Figs. 3a–3c),^{15,21} which is a very sensitive and most commonly used experiment to find out double strand breaks in the DNA of eukaryotic cells. The cells treated with the

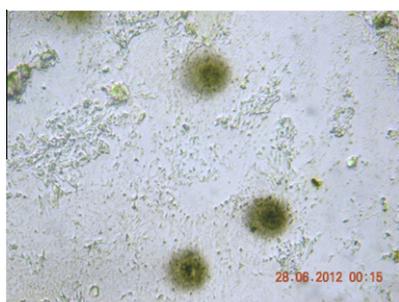


Figure 3a. The untreated control cells are damage free. The entire DNA is intact without any double stranded DNA breaks. No tail is observed after comet assay.

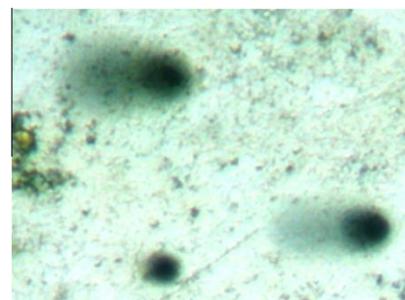


Figure 3b. Cells treated with the ma'edamine A (IC₅₀ = 6.93 μMol). The comets with large tail indicate DNA damage.

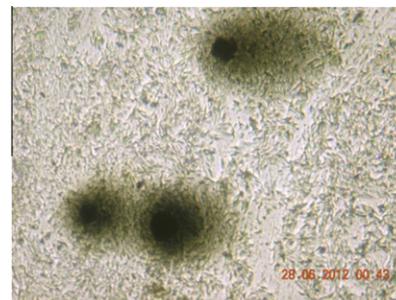


Figure 3c. Cells treated with the ma'edamine B (IC₅₀ = 10.95 μMol). The comets with large tail indicate DNA damage.

drug were embedded in low melting agarose immobilized on a microscopic slide and subjected to cell lysis at alkaline pH followed by electrophoresis at high pH. The slides were observed under

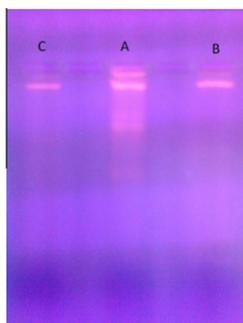


Figure 4. The gel picture of DNA (C: untreated cells, A – ma'edamine A treated cells, B – ma'edamine B treated cells) indicate that sample A has fragmented DNA which is a major event in case of apoptosis. Whereas in case of sample B there is not much fragmentation of DNA. This proves that ma'edamine A is very effective in inducing cell death by apoptosis.

microscope and scanned for comets. As DNA is a highly supercoiled structure and is negatively charged, any double stranded breaks (DSB) in its structure results in the formation of a tail towards the anode. Higher the DNA damage longer is the tail length. DNA damage can also be used to conclude whether the cells have undergone apoptosis or necrosis. If the tail length is more and head length is less, it symbolizes apoptosis whereas, a large head and small tail lengths represents necrosis. Further confirmation of the ability of ma'edamines to cause apoptosis was confirmed by DNA laddering assay (Fig. 4).²²

In conclusion, we have reported two methods for the preparation of ma'edamines A and B. The cytotoxicity (IC₅₀) data of ma'edamines A and B for COLO-205, MCF-7 and A-549 cell lines was found to be of the same order as that of Etoposide. The comet assay results show that both the compounds cause DNA damage (double stranded breaks). The extent of damage is higher in case of ma'edamine A as the tail is longer when compared to ma'edamine B comet tails. This is further supported by lower IC₅₀ value of ma'edamine A. Hence, these two compounds present scaffolds which can be used as template for development of a novel class of anti-cancer agents. Synthesis of analogues of ma'edamines and study of their biological activity is in progress and will be reported in future.

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Supplementary data

Supplementary data (1H and ¹³C NMR scans of compound **12**, **13**, **16**, **17**, **18**, **19**, **20** and **23** are available. Data related to MTT

assay, trypan blue staining, comet assay and DNA laddering) associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmcl.2012.12.033>.

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