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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 (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). 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.

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 (IC_{50}: 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 pyrollo[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 IC_{50}) 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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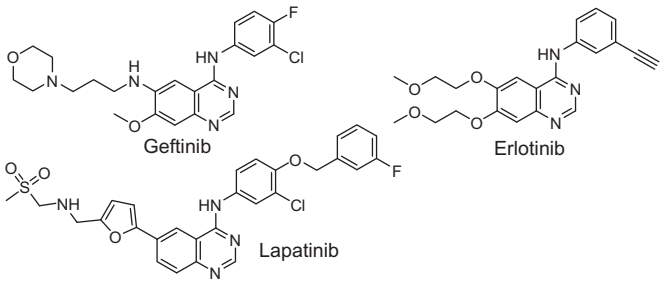


Figure 1. FDA approved tyrosine kinase inhibitors.

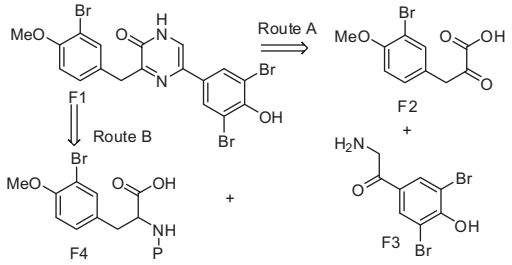
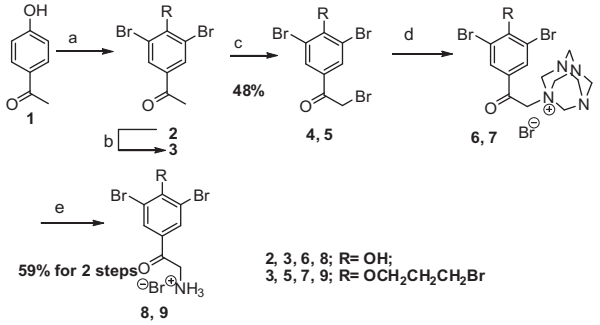


Figure 2. Retro synthetic approach for synthesis of pyrazin-2(1*H*)-ones showing key fragments (**F1**–**F4**).

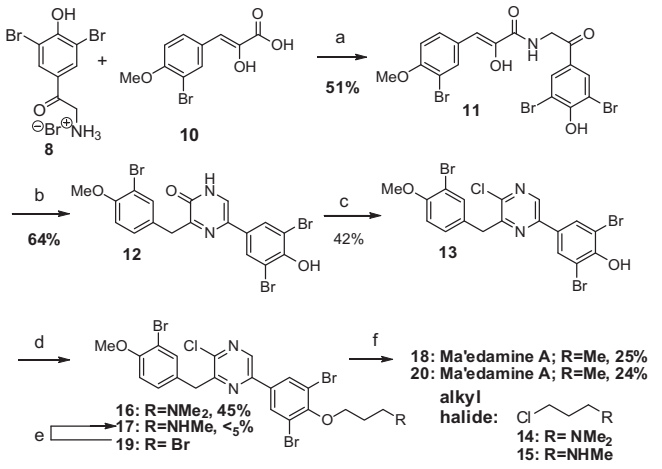
pyrazinone **12** with phosphoryl chloride. O-Alkylation of **13** with (3-chloropropyl) dimethyl-amine **14** afforded **16**. The best yields were obtained when the alkylation was done in butanone, in presence of cesium carbonate as base and one equivalent of sodium iodide added as an additive. Treating 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 ^{1}H NMR, ^{13}C 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.

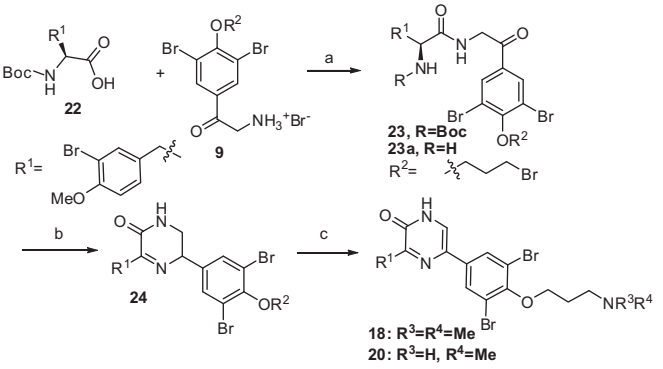
In order to expand the scope of our synthetic method for creating small focused library of analogues we have also developed a second route using different building blocks. The second approach of synthesis of ma'edamines is depicted in Scheme 3. *N*-Boc α-amino acid **22** and α-amino aryl ketone **9**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



Scheme 1. Reagents and conditions: (a) Br_2, AcOH, rt, 2 h; (b) $BrCH_2CH_2CH_2Br$, K_2CO_3, DMF, rt, 18 h; (c) Br_2, $CHCl_3$, rt, 2 h; (d) Hexamethylene tetramine, $CHCl_3$, 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) NH_4OAc, EtOH; (c) $POCl_3$/DMF; (d) alkyl halide, base; (e) $MeNH_2$ (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 ^{1}H 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 staining[20] 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 IC_{50} 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