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# Synthesis and cytotoxicity of novel imidazo[4,5-*d*]azepine compounds derived from marine natural product ceratamine A

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ABSTRACT

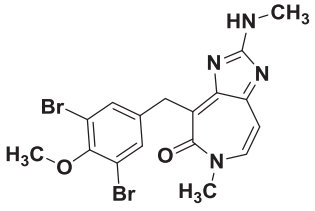
A series of novel imidazo[4,5-*d*]azepine compounds derived from marine natural product ceratamine A were designed and synthesized in 7 steps. Most compounds exhibited comparable cytotoxicity against five human cancer cell lines (HCT-116, HepG2, BGC-823, A549 and A2780) to natural product ceratamine A. Compound **1k**, bearing methoxy group at C-14, C-15 and C-16, showed the best *in vitro* cytotoxicity, which was better than ceratamine A. The structure and activity relationships study showed that the benzyloxymethyl group on *N*-3 played an important role on the cytotoxicity.



Over the past 30 years, marine natural products have gained a lot of attention as an important source of drug candidates.[1] Several anticancer drugs derived from marine natural products have achieved great successes in clinic.[2] This trend indicates that marine natural products would continue to play a key role in anticancer drug discovery.[3]

Ceratamine A (Fig. 1) is a heterocyclic alkaloid isolated from marine sponge *Pseudoceratina* sp., which displayed potent antimitotic activity through promoting tubulin polymerization. The fact that ceratamine A has a totally different binding site on microtubule from paclitaxel makes it a promising lead compound in anticancer drug discovery.[4] However, the lack of availability from natural source brings huge difficulty in its further biological evaluation.

In our previous work, we have reported an efficient approach to synthesize ceratamine A[5] and its analogues.[6] Some analogues exhibited slight increases in cytotoxicity compared with ceratamine A, and a preliminary structure and activity relationship was obtained. It was found that the introduction of bulky groups at C-14 and C-16 could increase the cytotoxity, and that the substituents on *N*-7 played a significant role on high potency. During the evaluation of cytotoxicity, we were pleased to find that the intermediate **1a**[6] with the imidazo[4,5-*d*]azepine skeleton exhibited comparable cytotoxicity against four human cancer cell lines




ceratamine A

**Fig. 1.** Marine natural product ceratamine A.

(HCT-116, HepG2, BGC-823 and A2780) to ceratamine A and better cytotoxicity against A549 than ceratamine A (Fig. 2). Because the synthesis of intermediate **1a** was apparently easier than ceratamine A and its analogues, we attempted to synthesize some derivatives with the imidazo[4,5-*d*]azepine skeleton in order to discover potential anticancer candidate with simpler structure.

In this paper, a series of analogues of compound **1a** as ceratamine A simplified derivatives were synthesized in 7 steps as depicted in Scheme 1. The synthetic route generally follows that reported by us previously.[6] The key step was to employ Heck reaction[7] to construct the imidazo[4,5-*d*]azepine core. Firstly, amine **5** was easily prepared from commercially available histamine dihydrochloride over 4 steps. Treatment of amine **5** with substituted cinnamic acids **6** generated by Knoevenagel condensation[8] afforded compounds **7a-d** in satisfying yields. Benzylation of **7a-d** with NaH afforded amides **8a-n**. The target compounds **1a-n**

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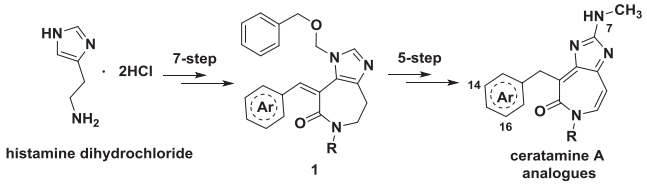
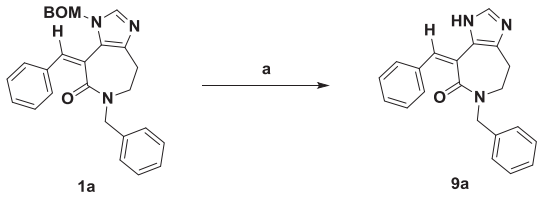



| Compound | $IC_{50}$ (μmol/L) | | | | |
|---|---|---|---|---|---|
| | HCT-116 | HepG2 | BGC-823 | A549 | A2780 |
| ceratamine A | 12.4 | 7.8 | 20.0 | 26.8 | 11.2 |
| 1a | 20.0 | 13.9 | 20.4 | 9.9 | 20.4 |

**Fig. 2.** Synthesis and cytotoxicity of ceratamine A analogues and the intermediate **1a**.

were then successfully prepared through Heck reaction. We also synthesized compound **9a** to check the effect of the benzyloxymethyl (BOM) group on the cytotoxicity (Scheme 2). The structures of the target compounds were confirmed by $^{1}$HNMR, $^{13}$CNMR and HRMS data. It should be noted that the methylene protons signals of 7-membered ring broadened in the $^{1}$H NMR spectra due to the flipping of 7-membered lactam ring[9] (see the Supplementary data). This phenomenon was well studied by variable-temperature $^{1}$H NMR in our previous work.[6]

The cytotoxicity of these compounds against five human cancer cell lines (HCT-116, HepG2, BGC-823, A549 and A2780) was evaluated by the standard MTT assay. The results are shown in Table 1.

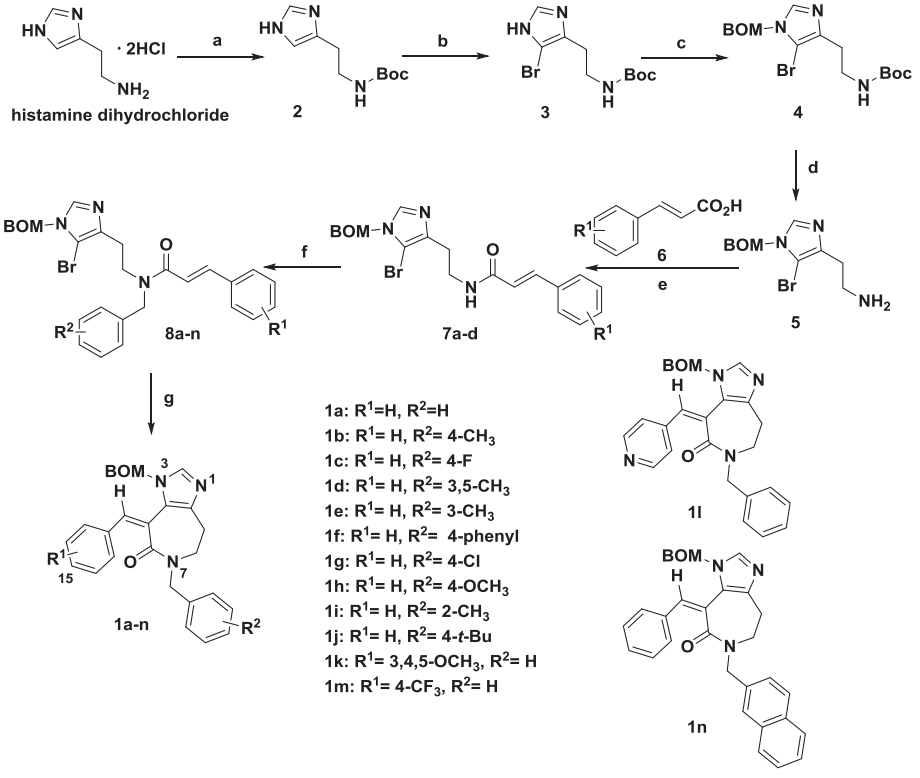



**Scheme 2.** Reagents and conditions: (a) $AlCl_3$, $CH_2Cl_2$, r.t., 1 h.

**Table 1**
Cytotoxicity of compounds **1a-n** and **9a**.

| Compound | $IC_{50}$ (μmol/L)[a] | | | | |
|---|---|---|---|---|---|
| | HCT-116 | HepG2 | BGC-823 | A549 | A2780 |
| **Ceratamine A** | 12.4 | 7.8 | 20.0 | 26.8 | 11.2 |
| **1a**[b] | 20.0 | 13.9 | 20.4 | 9.9 | 20.4 |
| **1b** | 7.94 | 44.65 | 39.08 | 11.38 | 21.19 |
| **1c** | 11.19 | 39.40 | 72.94 | 19.56 | 24.77 |
| **1d** | 6.23 | 40.87 | 52.60 | 22.47 | 31.29 |
| **1e** | 3.80 | 34.30 | 14.57 | 16.05 | 26.28 |
| **1f** | 13.95 | 72.54 | 80.99 | 18.34 | 48.29 |
| **1g** | 10.50 | 47.94 | 75.70 | 11.79 | 22.20 |
| **1h** | 31.05 | 44.40 | 71.59 | 46.80 | 43.11 |
| **1i** | 24.69 | 53.32 | 31.28 | 25.55 | 25.25 |
| **1j** | 8.46 | 18.03 | 27.84 | 20.58 | 23.85 |
| **1k** | 8.90 | 10.04 | 12.92 | 8.56 | 8.97 |
| **1l** | 67.06 | >100 | >100 | 36.02 | >100 |
| **1m** | 36.00 | 51.41 | 42.62 | 52.49 | 28.24 |
| **1n** | 19.36 | 23.71 | 93.26 | 26.43 | 72.83 |
| **9a** | >100 | >100 | >100 | 51.14 | >100 |

[a] The $IC_{50}$ values represent the inhibitory concentration of 50% of cell growth.
[b] Compound **1a** was synthesized previously.[6]




**Scheme 1.** Reagents and conditions: (a) $Boc_2O$, 4N NaOH, dioxane/$H_2O$ (2:1), r.t., 2 h, 95%; (b) NBS, THF, r.t. 5 h, 92%; (c) BOMCl, $Et_3N$, THF, r.t., overnight, 82%; (d) $CF_3COOH$, $CH_2Cl_2$, r.t., 4 h, 93%; (e) EDCI, DMAP, $CH_2Cl_2$, r.t., 30 h; (f) NaH, DMF, r.t., overnight, 76–92% (over 2-step); (g) $Pd(PPh_3)_4$, Methyl dicyclohexylamine, DMF, 120 °C, 20 h, 50–65%.

As illustrated in Table 1, most compounds exhibited cytotoxicity against these five cell lines with $IC_{50}$ values of micromolar level. It is worth noting that compound **1a** exhibited better cytotoxicity than the analogues **1b-j** and **1n**, indicating that substituents at $R^2$ (whether bulky groups, electron-donating groups or electron-withdrawing groups) may be not preferable. It is evident that $R^1$ had an appreciable influence on the cytotoxicity. In particular, compound **1k** ($R^1$ = 3, 4, 5-$OCH_3$) had the most potent inhibitory activities, even better than the natural product ceratamine A. However, introduction of $CF_3$ group at C-15 (compound **1m**) or pyridine ring (compound **1l**) led to a decrease in cytotoxicity. Compound **9a**, with the benzyloxymethyl (BOM) group being cleaved, exhibited obviously weaker cytotoxicity than compound **1a**, which indicated that the benzyloxymethyl (BOM) group on *N*-3 was critical for high potency.

In conclusion, a series of ceratamine A simplified derivatives possessing imidazo[4,5-*d*]azepine core was designed and synthesized from histamine dihydrochloride in 7 steps. Compared with natural product ceratamine A, these newly synthesized compounds exhibited comparable cytotoxicity. Especially, compound **1k**, bearing methoxy group at C-14, C-15 and C-16, showed even better cytotoxicity than ceratamine A. In addition, the synthesis of these compounds was more concise and the total yield was higher (7-step, 26.4–38.7%) than that of ceratamine A and its analogues (12-step, 7.8–13.8%). These results demonstrated that this type of ceratamine A simplified derivatives were more appropriate for further exploration on developing efficient anticancer candidates. Further structural optimization and biological studies of compound **1k** are in progress and will be reported in due course.

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## A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2018.02.004.

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