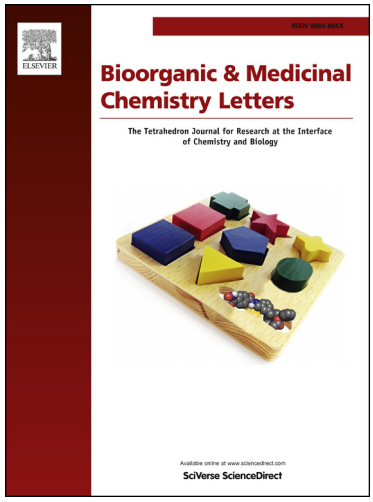


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# Synthesis and anticancer evaluation of spermatinamine analogues

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## Abstract

Spermatinamine was isolated from an Australian marine sponge, *Pseudoceratina* sp. as an inhibitor of isoprenylcystiene carboxyl methyltransferase (Icmt), an attractive and novel anticancer target. Herein, we report the synthesis of spermatinamine analogues and their cytotoxic evaluation against three human cancer cell lines i.e. cervix adenocarcinoma (HeLa), breast adenocarcinoma (MCF-7), and prostate carcinoma (DU145). Analogues **12**, **14** and **15** were found to be the most potent against one or more cell lines with the $IC_{50}$ values in the range of 5 - 10 µM. The obtained results suggested that longer polyamine linker along with aromatic oxime substitution provided the most potent analogue compounds against cancer cell lines.

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Bromotyrosine secondary metabolites are marine invertebrates derived natural products and have been described for their variety of biological activities including: anticancer, antimicrobial, antifouling, antiviral, ATPase regulator, calcium channel modulator, etc [1]. More than 300 bromotyrosine-derived alkaloids are currently known and divided into six categories according to their chemical structures: simple bromotyrosine derivatives (suberedamines A) [2], oximes (spermatinamine) [3], bastadins ((*E,E)-Bastadin* 19) [4], spirocyclohexadienylisoxazolines (11-Hydroxyaerothionin) [5], and other more complex structural classes. The anticancer activity of bromotyrosine-derived natural products has also been investigated and a significant number of compounds have been found to elicit anticancer activity, both in vitro and in vivo [6-8].

Spermatinamine (**1**), a polyamine alkaloid, containing a bromotyrosyl-spermine-bromotyrosyl sequence was isolated from an Australian marine sponge, *Pseudoceratina* sp. as an inhibitor of isoprenylcystiene carboxyl methyltransferase (Icmt) [3]. Icmt is an attractive and novel anticancer target and the various studies have provided strong evidence that tumorigenesis can be markedly impaired in cells by blocking Icmt activity [9]. Polyamines have been described earlier for their variety of cellular functions and cancer associations [10-12]. Polyamines analogues are being developed as anticancer drugs to target polyamines metabolic enzymes [10, 12-16]. The ability of different types of polyamines to recognize different receptor systems provided the

rationale for developing polyamines as drugs selective for different biological targets [17]. Polyamines could be considered as a skeleton key in the drug-receptor recognition process because it can assume different conformations in order to enable interaction between the drug and the receptor [18]. Recently, Hillgren et al [19] reported the isolation and characterization of four novel bromotyrosine polyamine alkaloids, pseudoceramines A-D (**2-5**) from marine sponge, *Pseudoceratina* sp. as inhibitors of the type III secretion (T3S) system of Gram-negative bacterium *Yersinia pseudotuberculosis*.

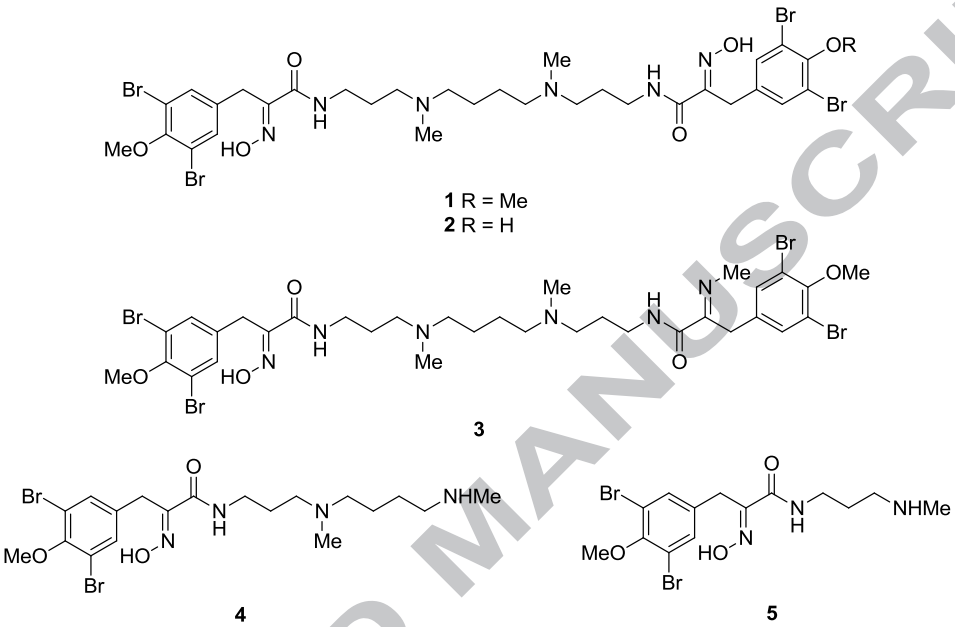
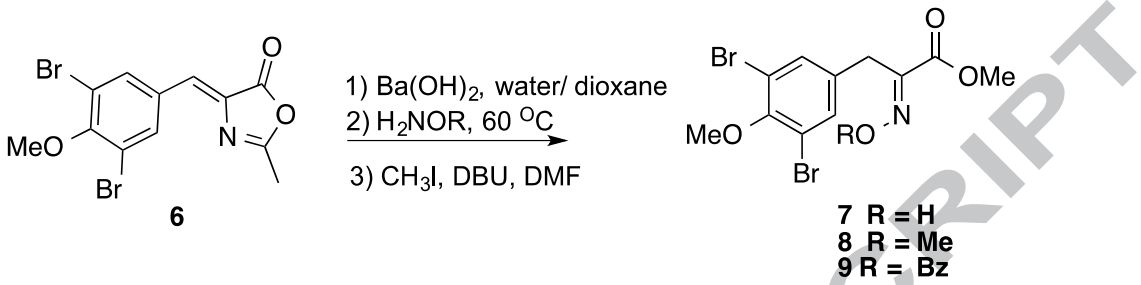



**Fig. 1** Structures of spermatinamine (**1**) and pseudoceramines A-D (**2-5**)

As minor structural variations can have considerable effect on the biological activity of these natural compounds, we synthesized a number of spermatinamine analogues by modifying the polyamine linker between the two-bromotyrosine rings and the substitution on oxime group. The compounds were then evaluated against three human cancer cell lines i.e. cervix adenocarcinoma (HeLa), breast adenocarcinoma (MCF-7), and prostate carcinoma (DU145) by using MTT assay. To the best of our knowledge no such study on the anticancer activities of spermatinamine analogues has been performed to date, which is necessary for an eventual lead optimization within this class of modified natural products.

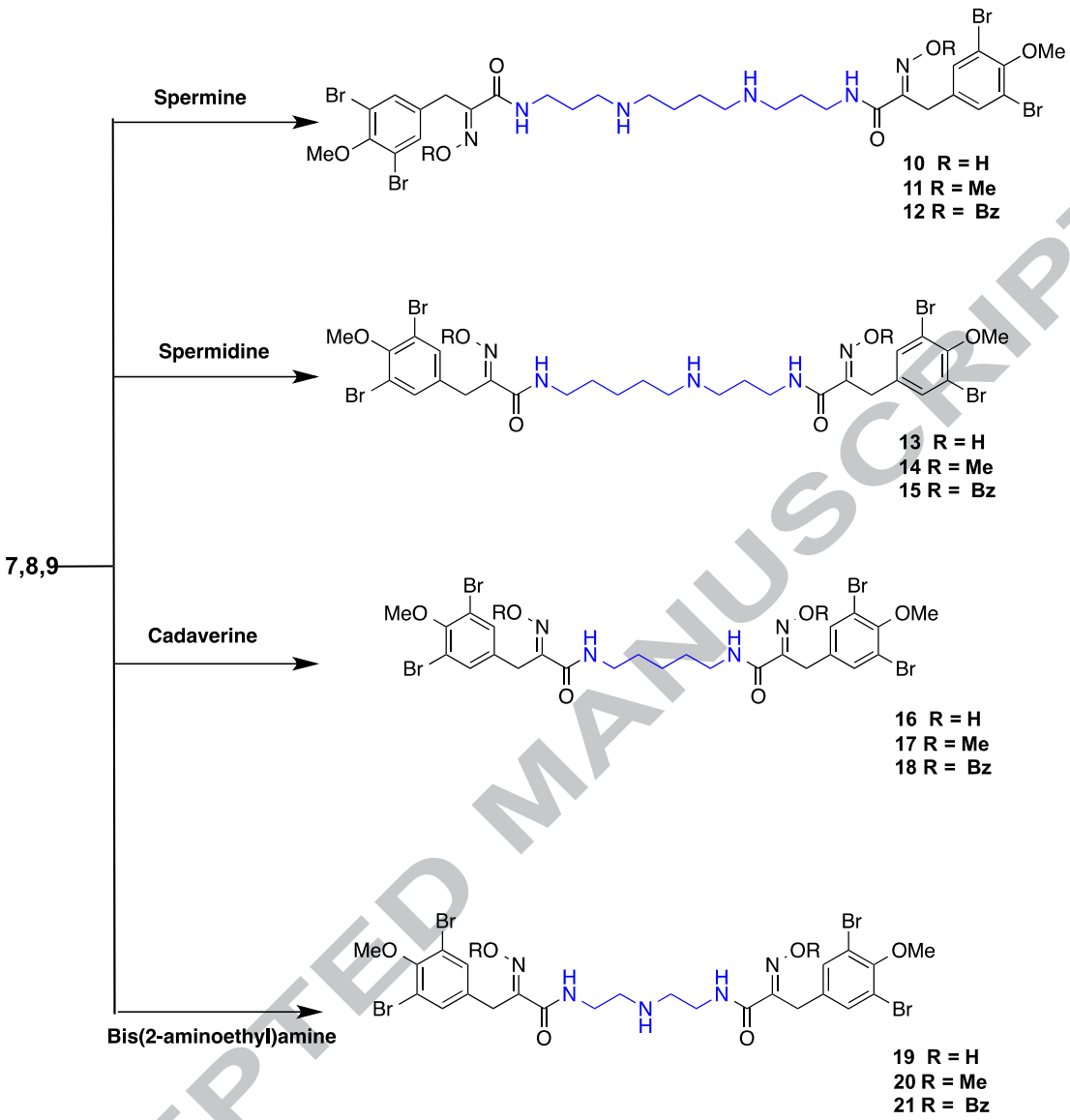
Spermatinamine was synthesized by following the recently reported synthetic route [19] with an overall yield of ~ 55% (over 4 steps). In our synthetic strategy, 3,5-dibromo-5-methoxybenzaldehyde was used as starting material to prepare *dibromo-O-methyltyrosine* which was then converted to azalactone (**6**) by reacting it with *N-acetylglycine* [20]. The synthesized azalactone (1) was then hydrolyzed by using barium hydroxide followed by addition of different types of hydroxylamine hydrochloride (R = H, Me, Bz) which yielded three types of substituted hydroxamic acid. The hydroxamic acid analogues were converted to methyl esters (**7-9**) by reacting it with methyl iodide in presence of DBU as a catalyst as shown in Scheme 1 [21]. It is noteworthy that most of reaction products were reasonably pure and column chromatography

purification was needed only in the final step. The oxime geometry was determined as (E) based on NMR and as reported earlier [22, 23].




**Scheme 1** Synthesis of substituted oxime methyl esters (2-4)

Methyl esters (**7-9**) were then coupled with various types of polyamines [20, 24] as shown in scheme 2. This efficient synthesis afforded the products (**10-21**) with approx. 50-55% yield. The polyamines chosen for the coupling were biogenic polyamines i.e. naturally available such as spermine, spermidine and cadaverine. its well-known that biogenic polyamines such as putrescine, spermidine, and spermine, are ubiquitous cellular cations and exert multiple biological functions [25]. Nayvelt et.al. [26] described that several polyamine analogues down-regulate polyamine levels, inhibit cellular uptake, and activate catabolism. Casero and Woster [27] also indicated that polyamine chains have been used to make hybrid drug molecules in order to improve cellular import, to increase affinity for chromatin, or to serve as carriers. The $^{1}$H NMR analyses of the purified compounds in scheme 2 revealed the symmetric character of the final products (**10-12** and **16-18**). While compounds (**13-15**) showed the unsymmetrical characteristics (see supporting information). The configuration of the oxime was confirmed for the final products to be (E) based on NMR and as reported in the literature for spermatinamine and other similar natural product like psammapline A [23].




**Scheme 2** Synthesis of spermatinamine analogues (**10-21**)

The synthesized spermatinamine analogues were evaluated for their cytotoxicity against three human cancer cell lines representing breast, cervical and prostate. MTT (3-(4,5-Dimethylthiazol-z-yl)-2,5-diphenyltetrazolium bromide) assay was used to assess the $IC_{50}$ (compound concentration required to inhibit 50% cell viability) and growth inhibition of the cells after treatment with test compounds. This assay targets the activity of succinate dehydrogenase enzyme in mitochondria, which reduces the tetrazolium salt into formazan crystals. The intensity of the color of formazan dye correlates to the number of viable cells [28]. The $IC_{50}$ values were calculated and are summarized in Table 1. Out of the 12 test compounds, five compounds (**11, 12, 14, 15** and **20**) were found to be active at lower concentrations while other compounds showed $IC_{50}$ values greater than 100 µM. Comparison of cytotoxicities of active analogues based on the type of linker suggested that spermine, spermidine and Bis(2-aminoehtyl)amine linkers

were able to induce significant cytotoxicity in all the cell lines, however analogues with cadaverine linker were found to be inactive. It is also noteworthy that the only difference between spermatinamine and spermine tethered compound (R = H) is the presence of methyl group, but their cytotoxic effects differ widely. Activity profiles of these five compounds were also suggestive of thefact that methyl and aromatic substitution of oxime group is useful for their potent activity.

**Table 1**: Cytotoxicity ($IC_{50}$ in µM) of test compounds against cancer cell lines (HeLa, MCF-7 and DU145).

| | | **HeLa** | | **DU145** | | **MCF-7** | |
|---|---|---|---|---|---|---|---|
| **Analogue No.** | **cLog P[a]** | **24 h** | **48 h** | **24 h** | **48 h** | **24 h** | **48 h** |
| **10** | 6.29 | >100 | >100 | >100 | >100 | >100 | >100 |
| **11** | 8.50 | 26.3 | 25.0 | 26.2 | 27.7 | 24.8 | 19.1 |
| **12** | 11.25 | **4.0** | **2.9** | **4.0** | **4.6** | **4.5** | **3.4** |
| **13** | 6.73 | >100 | >100 | >100 | >100 | >100 | >100 |
| **14** | 9.01 | **8.9** | **8.4** | **8.9** | **8.5** | **11.1** | **7.5** |
| **15** | 11.74 | **7.3** | **3.3** | **7.3** | **5.8** | **5.4** | **5.7** |
| **16** | 6.64 | >100 | >100 | >100 | >100 | >100 | >100 |
| **17** | 9.01 | >100 | >100 | >100 | >100 | >100 | >100 |
| **18** | 11.70 | >100 | >100 | >100 | >100 | >100 | >100 |
| **19** | 5.84 | >100 | >100 | >100 | >100 | >100 | >100 |
| **20** | 8.00 | 25.8 | 10.8 | 25.8 | 27.9 | 27.1 | 30.9 |
| **21** | 10.80 | >100 | >100 | >100 | >100 | >100 | >100 |
| Spermitinamine [29] | 7.45 | - | - | 1.60 | - | 1.60 | - |

cLogP was calculated using Chem draw software.

The effect of test compounds on cell growth/proliferation was also analyzed. Figure 2 represents the growth inhibition of cancer cells after treatment with five (**11, 12, 14, 15** and **20**) active compounds selected based on $IC_{50}$ values (Table 1). Based on cell growth inhiition, compounds **12**, **14** and **15** were found to be most active compounds (below 10 µM). Compound **12** was found to be the most potent as it inhibited growth of approximately 70% of DU145 cells (Fig 2 (a)), >90 % of HeLa cells (Fig 2 (b)) and approximately 60% of MCF-7 cells (Fig 2 (c)) at <5 µM concentration. None of the other test compound was found to be active below 5 µM within 24 h of treatment. The anticancer activity of the compound **10** was found to be time dependent as the growth inhibition was observed to be significantly increased from approximately 60% at 24 h to 90% at 48 h in HeLa cells at 5 µM concentration (data not shown). Both **12** and **15** were most active against HeLa cell line as compared to DU145 and MCF-7 and this shows that these compounds have selective activities against cervical cancer. Comparing the structure activity relationship of spermatinamine analogues, it has been understood that the longer chains linkers with the aromatic substitution of oxime group are essential for their cytotoxicity profiles.

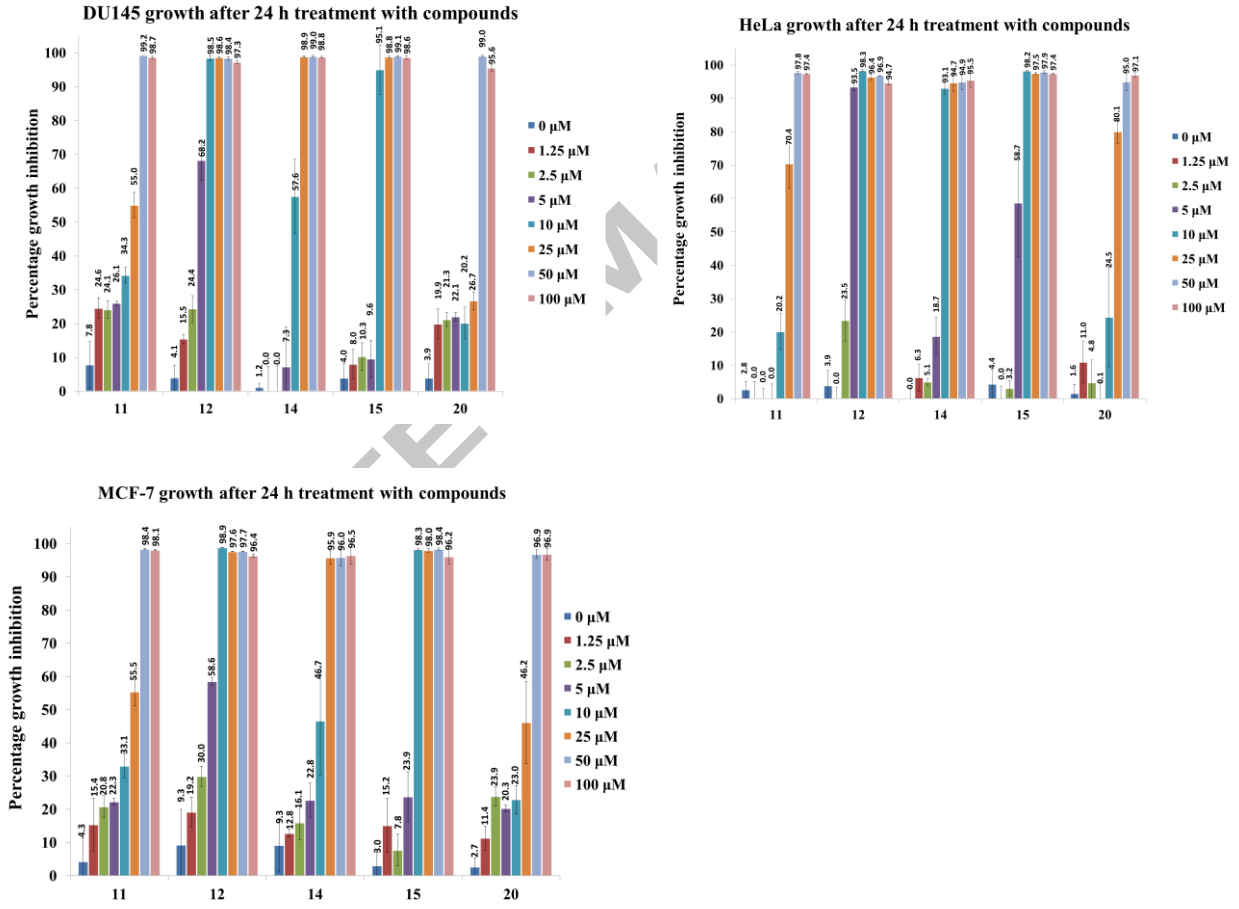



**Fig. 2** Percentage Growth inhibition of (a) DU145, (b) HeLa and (c) MCF-7 cancer cells after treatment with five most potent test compounds for 24 h. Data were normalized to untreated controls, and error bars represent the standard deviation for triplicate experiments.

In conclusion, present manuscript describes the synthesis of spermatinamine analogues by using different biogeneic polyamine linkers and oxime substitutions and their cytotoxicity/growth inhibition against three human cancer cell lines namely HeLa, DU145 and MCF-7. Syntheses of spermatinamine analogues have been achieved in an overall yield of ~34%. Based on $IC_{50}$ and growth inhibition results, **12**, **14** and **15** were found to be the most potent compounds. Structure activity relationship of these compounds suggested that the longer chains linkers with the aromatic substitution of oxime group are essential for their cytotoxicity profiles. The study provided significantly important results for the lead optimization process and needs further studies in order to improve the activity profiles of scaffolds.

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**Supplementary data**

Supplementary data (1H and 13C NMR, IR and HRMS data, theoretical methods, cell culture method and MTT assay procedure) is associated with this article.

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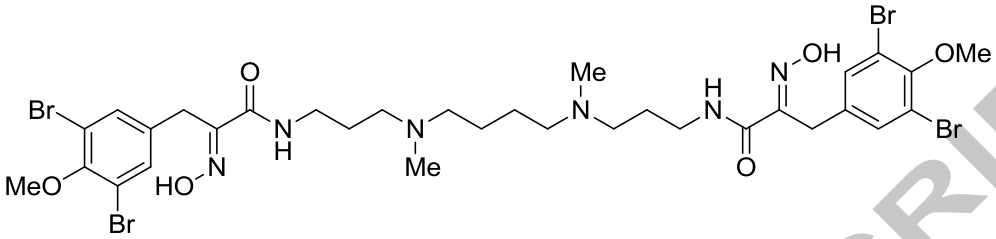
**Graphical abstract**

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