The discovery, synthesis and antimalarial evaluation of natural product-based polyamine alkaloids

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Abstract

Bioassay-guided fractionation of an antimalarial extract derived from the fungus *Ramaria subaurantiaca* afforded the known polyamine alkaloid, pistillarin. Nine pistillarin analogues were synthesised via EDC-mediated chemistry and these compounds along with the previously reported natural product polyamines, ianthelliformisamines A–C and spermatinamine, were evaluated against *Plasmodium falciparum* (3D7) parasites and a normal human cell line to determine parasite-specific activity. Spermatinamine (IC_{50} 0.23 μM) and pistillarin (IC_{50} 1.9 μM) were the two most potent antimalarials identified during these studies.

Polyamines are aliphatic alkaloids that have been reported from various natural sources including terrestrial and marine animals, plants, fungi, and bacteria.[1] Two of the simplest naturally occurring polyamines include spermidine and spermine. Substructure searching of the Dictionary of Natural Products database[1] against these two molecules identified >400 natural products that contain these aliphatic alkaloid moieties. Knowledge associated with polyamine chemistry and metabolism in human cells (both normal and tumour lines), bacteria, and parasites is expanding rapidly.[2,3] The polyamine chemotype has been shown to effect multiple biological processes including: the regulation of gene expression, cell proliferation and translation, the modulation of cell signalling, membrane stability, inhibition of carbonic anhydrase function, inhibition of certain ion channels, and free radical-scavenging.[2,4-7] It has been reported that, at physiological pH, polyamines can bind with various anionic macromolecules in the cell including DNA, RNA, ATP, proteins, and phospholipids.[8] Consequently, polyamine-based drug discovery is an evolving field with various compounds currently under development as potential therapeutics.[2,3] Furthermore, clinical success has been achieved for the polyamine-derivative eflornithine [(*DL*)-2,5-diamino-2-(difluoromethyl)pentanoic acid; syn. difluoromethylornithine (DFMO)], which was approved by the FDA for both the systemic treatment of African trypanosomiasis[9,10] [Ornidyl®, Sanofi-Aventis] and the topical treatment of hirsuitism [Vaniqa®, Gillette]. Of significance, this compound has also progressed to phase III clinical trials as an anticancer agent.[3,11] Mechanism of action studies for DFMO have shown that this compound is a potent suicide inhibitor of the polyamine biosynthetic enzyme, ornithine decarboxylase.[12,13]

As part of our continuing research into the discovery of new antimalarial leads from nature[14-18] we screened a unique fungi-derived fraction library (2,035 fractions) against a chloroquine-sensitive *Plasmodium falciparum* line (3D7), using a radiometric growth inhibition assay.[19,20] A total of 20 fractions (0.98% hit rate) derived from 18 fungal species (10 genera, 8 families) showed promising antimalarial activity. One fraction derived from *Ramaria subaurantiaca* Corner was prioritised for further investigation since it demonstrated potent malarial

growth inhibition along with no cytotoxicity towards neonatal foreskin fibroblast (NFF) cells at the highest concentration tested.[21] Bioassay-guided fractionation of the large-scale CH_2Cl_2/MeOH extract from *R. subaurantiaca* using reversed phase semi-preparative C_{18} HPLC [MeOH/H_2O/0.1% CF_3CO_2H] yielded the trifluoroacetate salt of the known fungal metabolite pistillarin (**1**).[22-24] This molecule was identified on the basis of spectroscopic and spectrometric data, and by comparison with literature values.[22-24] Pharmacological evaluation of **1** (Table 1) showed that it inhibited the growth of a drug-sensitive *P. falciparum* line (3D7) with an IC_{50} value of 1.9 μM, and displayed no cytotoxicity against NFF cells at 100 μM.

Pistillarin (**1**) has been isolated from a variety of fungal species including *Penicillium bilaii*,[22] *Gomphus floccosus*,[23] *Clavariadelphus pistillaris* and several *Ramaria* species[24] and is a known siderophore (i.e., a low molecular weight ferric-ion specific ligand produced by microorganisms to sequester iron under aerobic conditions and at low iron concentrations).[25-27] Of note, many reported siderophores possess a hydroxamic acid or a catechol moiety, such as that present in **1**.[26] Since iron is essential for parasitic growth and multiplication, it was reasoned that chelation within infected erythrocytes may be a selective mechanism to control *P. falciparum*.[25-29] Importantly, the antimalarial activity of iron chelating agents has been established both *in vitro* and *in vivo*.[28-31] In addition, compound **1** has been shown to exhibit significant protective effects against DNA damage by hydroxyl radicals generated from the Fenton reaction *via* iron chelation, as well as free radical-scavenging activity.[23]

Encouraged by the antimalarial data of **1**, we subsequently performed substructure searching on an in-house natural product library using the spermidine fragment in an attempt to identify additional polyamine alkaloids for antiplasmodial evaluation. This process identified four previously reported polyamines, ianthelliformisamines A–C (**2**–**4**), and spermatinamine (**5**; Fig.1). Ianthelliformisamines A–C (**2**–**4**) were isolated initially from the marine sponge *Suberea ianthelliformis*[7] and displayed varying levels of inhibitory activity against the Gram-negative bacteria *Pseudomonas aeruginosa*.[7] Specifically, ianthelliformisamine A was the most potent

antibacterial agent with an IC_{50} value of 6.8 μM (MIC 35 μM).[7] Spermatinamine (**5**) was originally isolated from the sponge *Pseudoceratina* sp. and was the first natural product inhibitor of isoprenylcysteine carboxyl methyltransferase (ICMT), which catalyses the carboxyl methylation of oncogenic proteins in the final step of a series of post-translational modifications.[4] ICMT has been proposed as an attractive and novel anticancer target.[4] More recently spermatinamine (**5**) and a series of related natural products have been shown to inhibit Gram-negative bacteria.[32]

In order to evaluate the pharmacological activity of additional polyamine alkaloids, we embarked on a synthetic program that aimed to generate several modified pistillarin analogues. It was hoped that these compounds, in conjunction with the previously isolated natural products **2**–**5**, would enable us to elucidate preliminary structure activity relationships.

The approach undertaken for the target analogues **6**–**12, 16** and **17** (Fig.2) relied on an EDC-mediated amide formation between benzoic acid or 3,4-dimethoxybenzoic acid with either spermidine or N^1,N^5-bis-Boc-spermidine; yields ranged from 2–30%.[33,34] Treatment of analogue **8** with boron tribromide-dimethylsulfide complex conveniently effected global de-protection and afforded the desired catechol **10**.[35,36] Analogues **11** and **12** were synthesised in a similar manner through a two-step coupling/deprotection protocol from 3,4-dimethoxybenzoic acid with either *N*-Boc-1,4-diaminobutane or *N*-Boc-1,3-diaminopropane. During the preparation of this manuscript, we identified literature that reported the antimalarial activity of the marine natural product orthidine F (**13**), and several synthetic analogues, two of which (**14** and **15**) displayed potent antimalarial activity (with IC_{50} values of 19.0 and 8.6 nM respectively, against the *P. falciparum* K1 line).[37] Due to these data, we elected to synthesise and screen the related compounds **16** and **17**. These molecules were generated by coupling 2,5-dimethoxybenzoic acid or acetylsalicylic acid with spermidine by using EDC as the coupling reagent, which afforded **16** and **17** in yields of 6% and 2%, respectively.[33,34] The acetylated derivative of **17** was not identified during these studies and we propose that ester hydrolysis occurred during the purification step. In order to obtain larger quantities of **17**, a different coupling methodology was employed. Accordingly, spermidine was

exposed to salicyloyl chloride at –20 °C in CH_2Cl_2, and after work-up and purification, **17** was afforded in an improved yield of 8%.

All synthetic analogues generated during these studies were characterized using 1D (^{1}H, ^{13}C) and 2D (gCOSY, gHSQC, gHMBC) NMR, UV and MS data. The majority of these compounds have been previously reported as either natural products (**6**,[38] **7**,[39] and **9**[40]), or they have been synthesised (**10**[41,42] and **11**[43]), but only partially characterized. We report the full spectroscopic characterisation of **6**, **8**, **10**–**12**, **16** and **17** (see Supplementary Data for details). Prior to pharmacological evaluation all compounds were assessed for purity by ^{1}H NMR spectroscopic analysis and confirmed to be ≥95%.

Compounds **1**–**12**, **16** and **17** were all tested for *in vitro* growth inhibitory activity against *P. falciparum* 3D7 parasites and for mammalian cell toxicity using the NFF cell line (Table 1). Pistillarin (**1**) showed the best selectivity index (SI >53), while spermatinamine (**5**) displayed the most potent antimalarial and cytotoxic activity. The poor selectivity of **5** (SI = 9) prevented further evaluation of this compound as a potential antimalarial lead. Compounds **6** and **7** were both significantly less active than pistillarin indicating that the catechol group(s) present in **1** are important for antimalarial activity. However, the moderate antimalarial data for **16** and **17** suggests substitution variability about the aryl units is tolerated, and this is supported by other literature reports.[37]

When comparing the significance of the number of aryl substituents it was observed that the series of monoaryl amides **2**, **3** and **8**–**12** were the least active against *P. falciparum* 3D7 parasites and NFF cells, and modifying the length of the amide side chain did not affect the activity in this study. Whilst the monoaryl brominated natural products **2** and **3** showed slight antimalarial activity, they displayed no cytotoxicity against NFF cells at 100 μM. In contrast, the diaryl brominated natural products **4** and **5** were significantly more active against 3D7 parasites and more cytotoxic than their monoaryl congeners **2** and **3**. Lastly, Boc protection of the two amine groups (monoaryl synthetics **8** and **9**) maintained some antimalarial activity; a similar pattern of activity for Boc-

substituted compounds has been reported previously.[44] The improved activity associated with Boc protection was proposed by others[44] to be due to enhancement of the membrane permeability of the cationic amine analogues, which subsequently increased accumulation of the compound(s) within the digestive vacuole of the malaria parasite.[44]

The micromolar antimalarial activity for compounds **16** and **17** (IC_{50} 16.7–17.2 μM, 3D7) compared to the nanomolar activity for the previously reported analogues **14** and **15** (IC_{50} <20 nM, K1),[37] indicated that the extended polyamine chain length [spermine (**14** and **15**) vs spermidine (**16** and **17**)] and/or the amide functionality [phenylacetamide (**14** and **15**) vs benzamide (**16** and **17**)] is critical for parasite growth inhibition in the 2,5-dimethoxy- and 2-hydroxy-aryl series. A caveat to this observation is that the screening for the previously reported **14** and **15** (IC_{50} <20 nM), was conducted against *P. falciparum* K1 parasites,[37] which is a drug-resistant line, whereas in our studies, chloroquine-sensitive *P. falciparum* 3D7 parasites were used.

In summary, bioassay-guided fractionation of the CH_2Cl_2/MeOH extract from the Australian macrofungus *R. subaurantiaca* resulted in the identification of the known secondary metabolite, pistillarin (**1**). Four additional polyamine alkaloid natural products and nine synthetic pistillarin analogues were tested against *P. falciparum* (3D7) and NFF cells. Although a variety of small molecules belonging to the polyamine chemotype have been previously identified as antimalarial agents[41,45-47] this is the first report of antimalarial activity for the polyamine alkaloids pistillarin, ianthelliformisamines A–C, and spermatinamine.

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Supplementary data

Supplementary data (general experimental procedures, fungus collection and identification details, isolation and extraction protocols, synthetic reaction details, spectroscopic/spectrometric data for compounds **6**, **8**, **10**–**12**, **16** and **17**, malaria and cytotoxicity assay protocols and ^{1}H and ^{13}C NMR spectra for compounds **6**, **8**, **10**–**12**, **16** and **17**) associated with this article can be found in the online version at doi:

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Table 1. Biological activities for compounds **1**–**12**, **16** and **17**

	IC_{50} ± SD (μM)		
Compound	3D7[a]	NFF[b]	SI[c]
1	1.9 ± 0.6	>100	>53
2	14.5 ± 0.4	>100	>7
3	12.1 ± 1.0	>100	>12
4	4.4 ± 0.5	17.5 ± 0.4	4
5	0.23 ± 0.04	2.1 ± 0.4	9
6	>25	78.8 ± 15.5	<3
7	>25	89.8 ± 6.2	<4
8	20.3 ± 0.4	>100	>5
9	20.5 ± 0.5	>100	>5
10	>25	>100	>4
11	>25	>100	>4
12	>25	>100	>4
16	16.7 ± 2.7	>100	>6
17	17.2 ± 13.8	>100	>6
chloroquine	0.04 ± 0.004	40.2 ± 3.4	1005

[a] 3D7 = *Plasmodium falciparum* chloroquine-sensitive line
[b] NFF = neonatal foreskin fibroblast cells
[c] SI = selectivity index = NFF cell line IC_{50} / *P. falciparum* IC_{50}

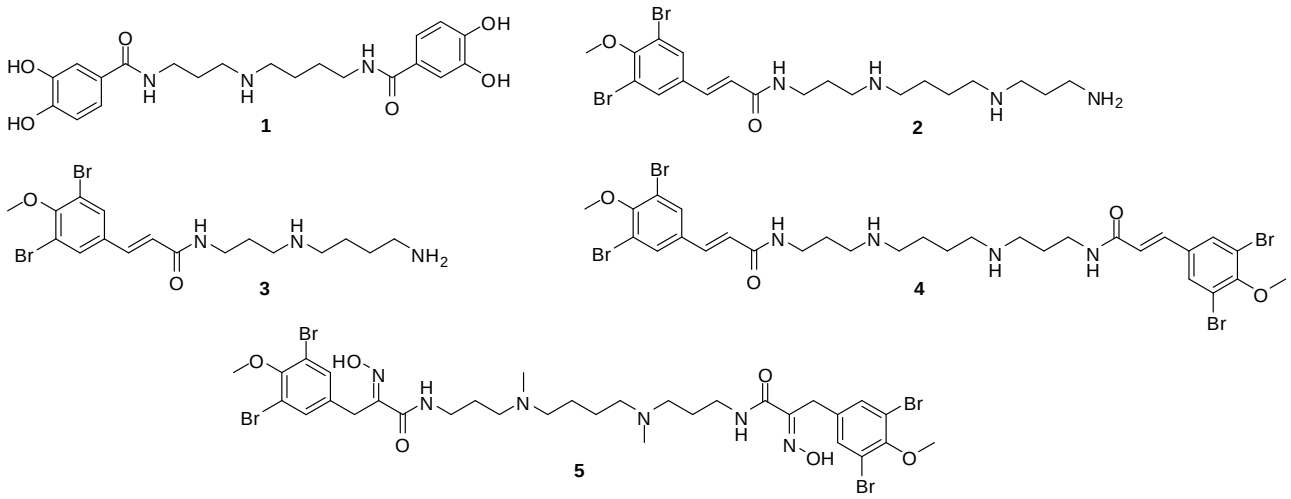

Figure 1. Chemical structures of the natural product polyamines pistillarin (**1**), ianthelliformisamines A–C (**2**–**4**) and spermatinamine (**5**)

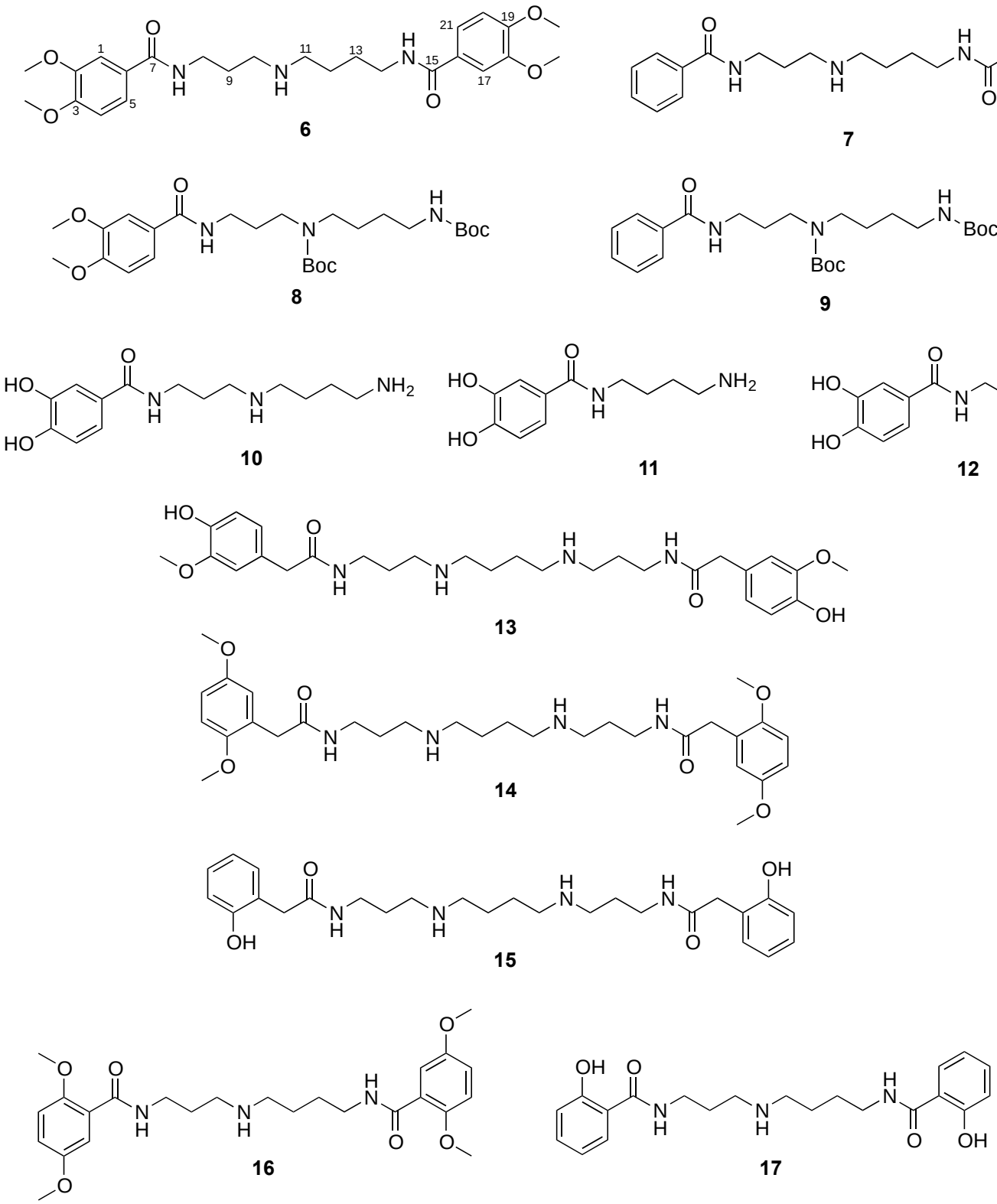

Figure 2. Chemical structures of synthetic polyamine analogues (**6**–**12**, **14**–**17**) and the ascidian natural product orthidine F (**13**)