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## Valorisation of the diterpene podocarpic acid – antibiotic and antibiotic enhancing activities of polyamine conjugates.

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### Abstract

As part of our search for new antimicrobials and antibiotic adjuvants, a series of podocarpic acid-polyamine conjugates have been synthesized. The library of compounds made use of the phenolic and carboxylic acid moieties of the diterpene allowing attachment of polyamines (PA) of different lengths to afford a structurally-diverse set of analogues. Evaluation of the conjugates for intrinsic antimicrobial properties identified two derivatives of interest: a PA3-4-3 (spermine) amide-bonded variant **7a** that was a non-cytotoxic, non-hemolytic potent growth inhibitor of Gram-positive *Staphylococcus aureus* (MRSA) and **9d**, a PA3-8-3 carbamate derivative that was a non-toxic selective antifungal towards *Cryptococcus neoformans*. Of the compound set, only one example exhibited activity towards Gram-negative bacteria. However, in the presence of sub-therapeutic amounts of either doxycycline (4.5  $\mu$ M) or erythromycin (2.7  $\mu$ M) several analogues were observed to exhibit weak to modest antibiotic adjuvant properties against *Pseudomonas aeruginosa* and/or *Escherichia coli*. The observation of strong cytotoxicity and/or hemolytic properties for subsets of the library, in particular those analogues bearing methyl ester or *n*-pentylamide functionality, highlighted the fine balance of structural requirements and lipophilicity for antimicrobial activity as opposed to mammalian cell toxicity.

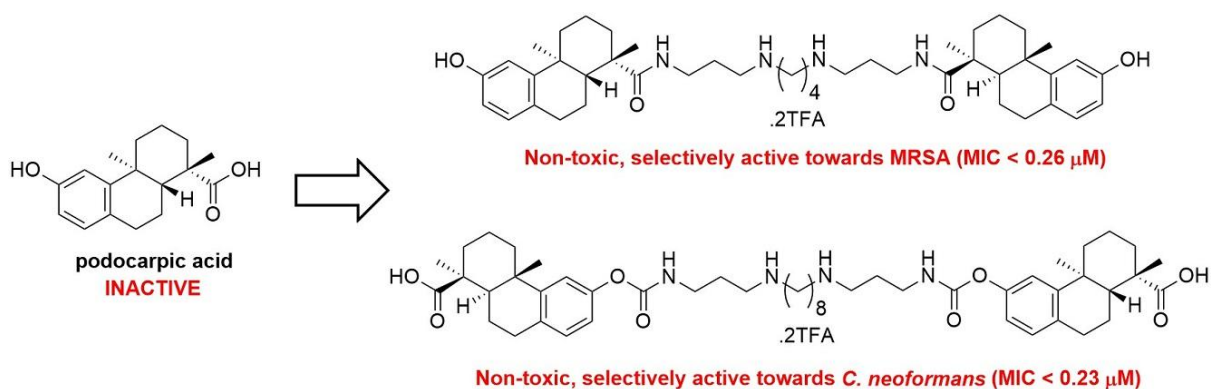
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## Highlights:

- Library of amide and/or carbamate linked podocarpate – polyamine conjugates prepared
- Amide derivative exhibited potent and selective anti-MRSA activity
- Carbamate derivative exhibited potent and selective antifungal activity
- Weak to modest ability to enhance the action of antibiotics towards G- bacteria

## Graphical abstract:

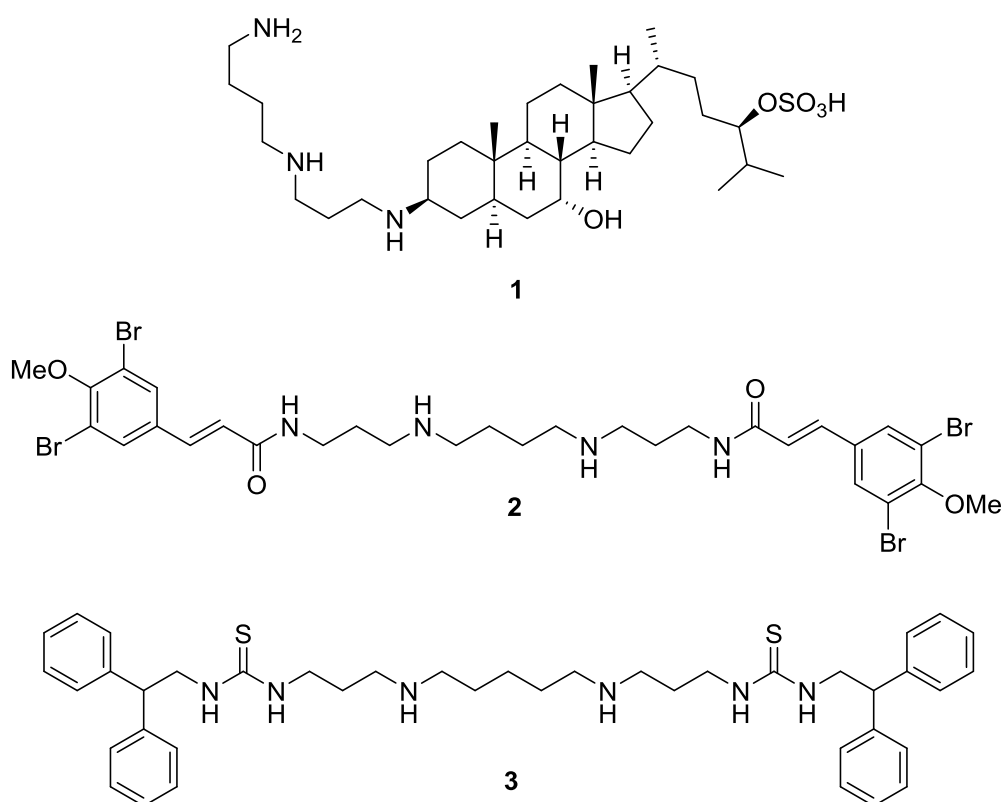


## Keywords

Antimicrobial activities; diterpene polyamine conjugates; podocarpic acid; antibiotic adjuvant

## 1. Introduction

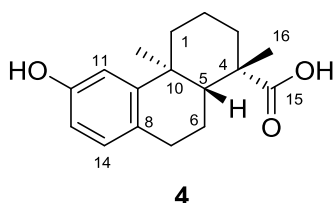
Polyamines (PAs) are ubiquitous naturally-occurring amines that play multiple critical roles in biological functions including the regulation of cell growth and differentiation and modulation of cell signalling pathways.<sup>1-5</sup> Natural product and synthetic analogues of polyamines have been reported to exhibit epigenetic modulation,<sup>6-8</sup> antiproliferative,<sup>9,10</sup> antiparasitic<sup>11-18</sup> and antibacterial properties. Examples of the latter include the marine natural products squalamine (**1**)<sup>19,20</sup> and ianthelliformisamine A (**2**)<sup>21,22</sup> and non-natural 1, $\omega$ -(bis)thiourea analogues (e.g. **3**)<sup>(Figure 1)</sup><sup>23</sup> all of which exhibit antibacterial activities and can also sensitize bacteria to antibiotics.<sup>24,25</sup> Previously reported structure-activity relationship studies have highlighted the requirement of central charged nitrogens and terminal lipophilic aryl for antibacterial activity.<sup>23,26</sup> Presumably these features aid electrostatic interaction between the positively charged central nitrogens and the negatively charged bacterial membrane, while the lipophilic end-group(s) facilitate membrane penetration. Examples of end-groups incorporated into antibacterial mono-substituted or  $\alpha,\omega$ -disubstituted polyamines include lipids,<sup>27,28</sup> naphthyl,<sup>29</sup> cinnamic acids,<sup>21,22</sup> indoles,<sup>30,31</sup> sterols<sup>32-34</sup> and triterpenes.<sup>35-37</sup>



**Figure 1.** Structures of antimicrobial polyamines **1–3**.

We have sought to expand the scope of lipophilic end groups by preparing polyamine conjugates of the diterpene natural product podocarpic acid (**4**) (**Figure 2**). Podocarpic acid is a readily available diterpene isolated from the heartshakes of the New Zealand conifer *Dacrydium cupressinum* (rimu). While extensive

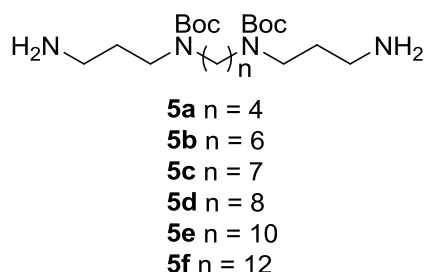
studies have explored the use of podocarpic acid as a chiral intermediate for synthesis,<sup>38</sup> there are limited accounts of biological activity associated with the acid and/or its derivatives, including inhibiting IL-1 $\beta$  cytokine release,<sup>39</sup> acting as agonists of liver X receptor (cholesterol metabolism),<sup>40,41</sup> inhibition of influenza A viruses *in vitro*,<sup>42</sup> opening large-conductance calcium-activated potassium channels<sup>43</sup> and activation of the transient receptor potential TRPA1-Nrf pathway.<sup>44</sup> Herein, we present details of our exploration of the potential of podocarpic acid diamino and polyamino-derivatives to act as antimicrobials and antibiotic adjuvants.



**Figure 2.** Structure of podocarpic acid (**4**).

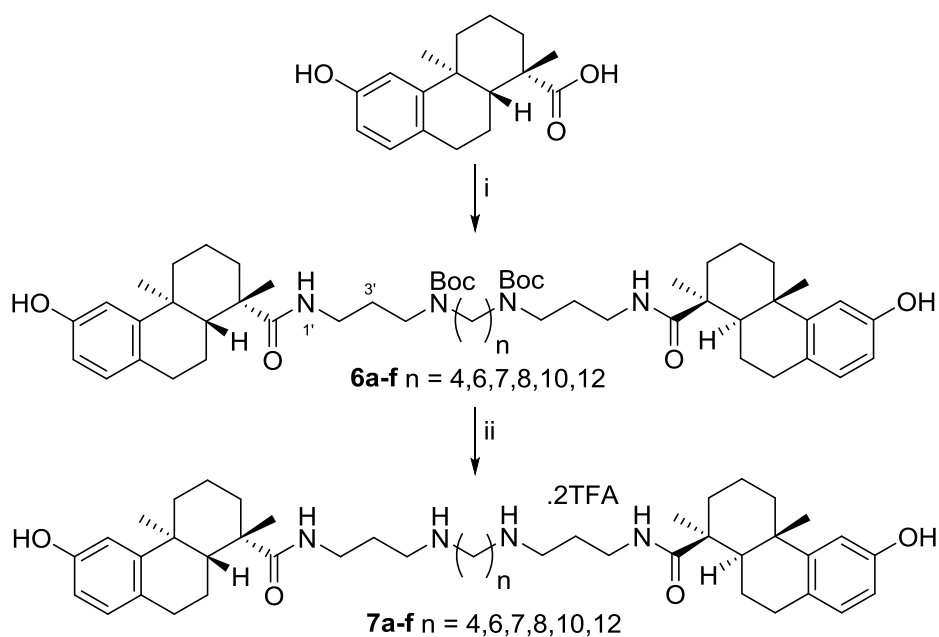
## 2. Chemistry

The first requirement was preparation of the Boc-protected polyamine scaffolds **5a-f** (**Figure 3**) which have been previously described.<sup>45-48</sup> These six polyamines (PA3-4-3, PA3-6-3, PA3-7-3, PA3-8-3, PA3-10-3, PA3-12-3) were targeted as they collectively allow exploration of the influence of chain length, lipophilicity and spatial requirements of dialkylammonium ion positive charges on bioactivity.



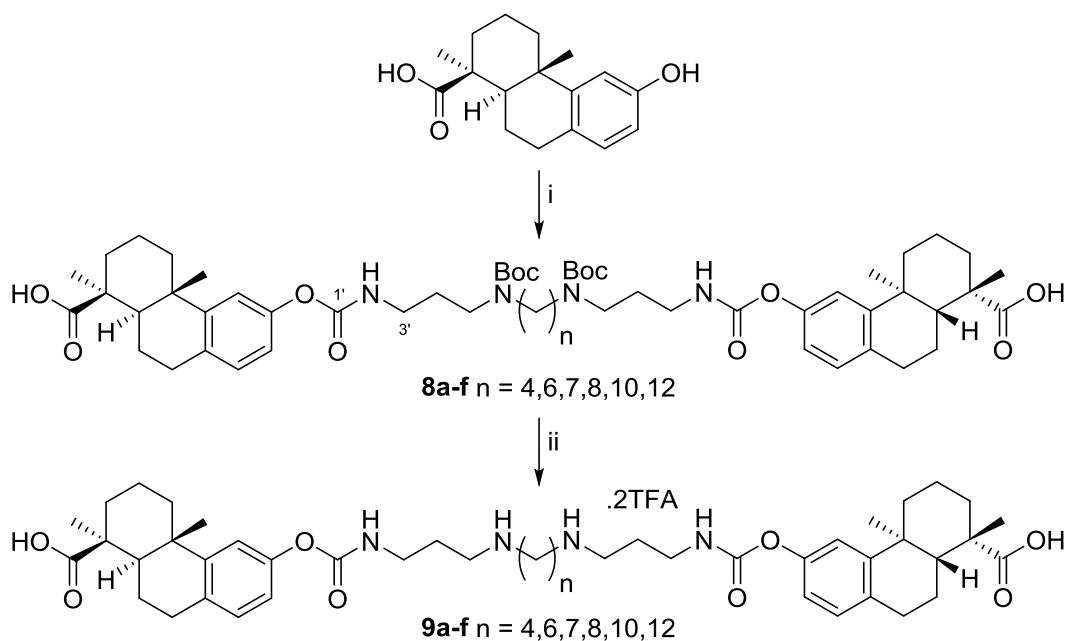
**Figure 3.** Polyamine scaffolds **5a-f**.

Reaction of podocarpic acid with the Boc-protected polyamine scaffolds **5a-f** mediated by the coupling agent PyBOP/DIPEA in DMF afforded the Boc-protected diamides **6a-f** in modest yields of 27–58%. Deprotection of the Boc-protected diamides was carried out using TFA in CH<sub>2</sub>Cl<sub>2</sub> to afford the target analogues **7a-f** as their di-TFA salts in 80–100% yield (**Scheme 1**).



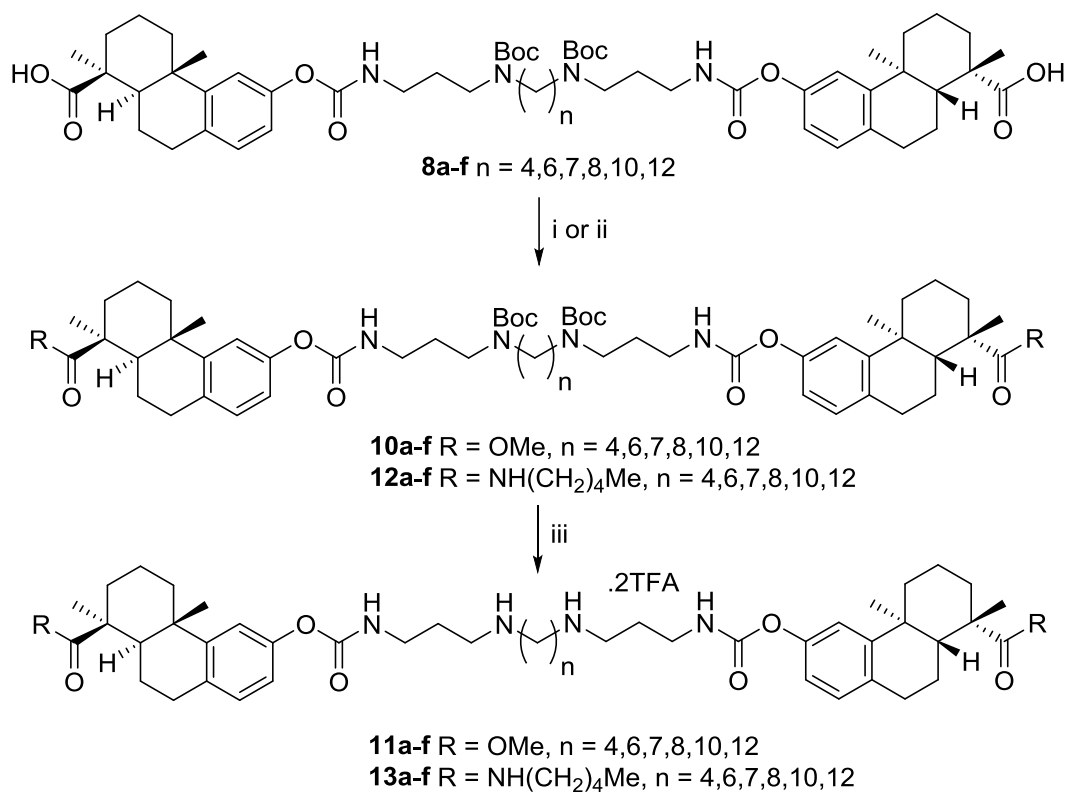
**Scheme 1.** General method for the preparation of polyamine-diamides **7a-f**. Reagents and conditions: (i) pre-incubate podocarpic acid: PyBOP, DIPEA, DMF, 5 h, then polyamine **5a-f**, 72 h, 27–58% yield; (ii) TFA/CH<sub>2</sub>Cl<sub>2</sub> (10% v/v), 3 h (80–100% yield).

Changing the coupling agent to 1,1'-carbonyldimidazole (CDI) for the reaction of podocarpic acid and polyamines **5a-f** afforded the Boc-protected dicarbamates **8a-f** that were subsequently deprotected using TFA in CH<sub>2</sub>Cl<sub>2</sub> to afford dicarbamates **9a-f** as their di-TFA salts (**Scheme 2**).



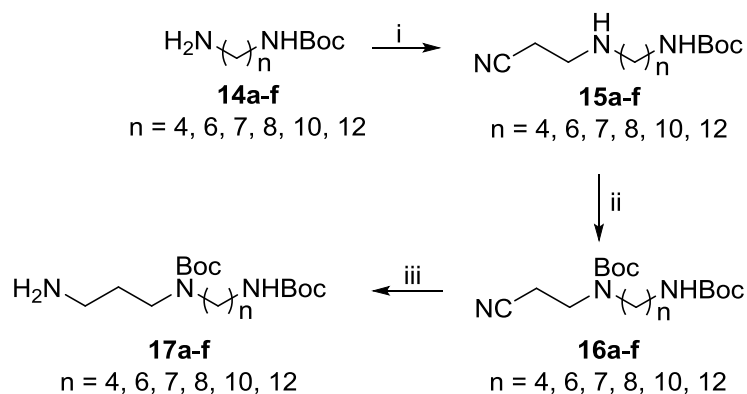
**Scheme 2.** General method for the preparation of polyamine-dicarbamates **9a-f**. Reagents and conditions: (i) pre-incubate podocarpic acid: CDI, CH<sub>2</sub>Cl<sub>2</sub>, 5 h, then polyamine **5a-f**, 72 h, 12–26% yield; (ii) TFA/CH<sub>2</sub>Cl<sub>2</sub> (10% v/v), 3 h (55–100% yield).

Further elaboration of the carboxylic acid moiety present in carbamates **9a-f** to less polar ester and amide derivatives were then explored. Conversion of Boc-protected dicarbamates **8a-f** to their respective methyl esters **10a-f** using trimethylsilyldiazomethane (TMSCHN<sub>2</sub>) followed by Boc deprotection afforded analogues **11a-f** (Scheme 3). The corresponding set of *n*-pentylamide analogues were prepared by PyBOP-mediated coupling of protected carbamates **8a-f** with *n*-pentylamine to give **12a-f**, deprotection of which afforded amides **13a-f**.



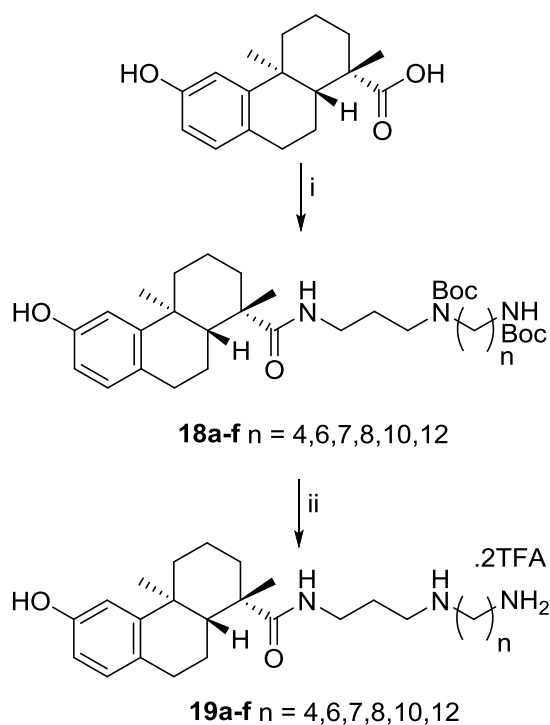
**Scheme 3.** General method for the preparation of methyl ester polyamine-carbamates **11a-f** and *n*-pentylamide polyamine-carbamates **13a-f**. Reagents and conditions: (i) for **10a-f**: trimethylsilyldiazomethane, CH<sub>2</sub>Cl<sub>2</sub>/MeOH (3:2), 2 h, 60–100% yield; for **12a-f**: (ii) pre-incubate **8a-f** with PyBOP, DIPEA, DMF, 5 h, then *n*-pentylamine, 72 h, 50–100% yield; (iii) TFA/CH<sub>2</sub>Cl<sub>2</sub> (10% v/v), 3 h (44–100% yield).

The preceding sets of analogues (**7a-f**, **9a-f**, **11a-f**, **13a-f**) all contained a core polyamine that was 1, $\omega$ -disubstituted via an amide or carbamate linker to podocarpic acid. To explore monosubstituted variants, di-Boc-protected triamines were prepared using the general methodology summarized in Scheme 4. Briefly, mono-Boc protected diamines **14a-f**<sup>49</sup> were reacted with acrylonitrile (to give **15a-f**), followed by Boc-protection (to give **16a-f**) and subsequent catalytic hydrogenation to give **17a-f**.<sup>50</sup>



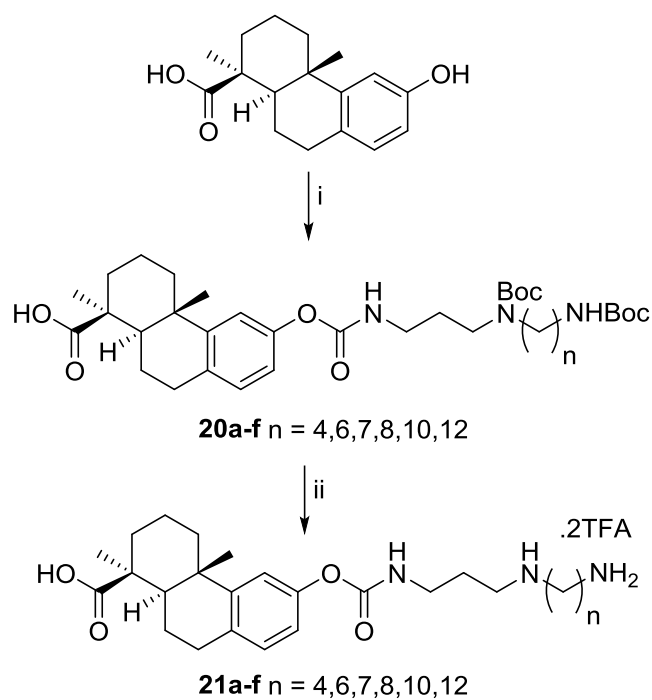
**Scheme 4.** General method for the preparation of di-Boc-triamines **17a-f**. Reagents and conditions: (i) acrylonitrile (2 equiv.), Et<sub>3</sub>N, EtOH, 20 h (52–91% yield); (ii) Boc<sub>2</sub>O, Et<sub>3</sub>N, MeOH, 20 h (77–93% yield); (iii) Raney-Nickel, LiOH, Pd/C, dioxane/water (2:1), 50 °C, 24 h, H<sub>2</sub> (66–85% yield).

Applying our established workflow, reaction of podocarpic acid with protected polyamines **17a-f** using PyBOP as the coupling agent gave **18a-f** which were then deprotected using TFA/CH<sub>2</sub>Cl<sub>2</sub> to give podocarpic amides **19a-f** (Scheme 5). Using CDI as the coupling agent afforded Boc-protected carbamates **20a-f**, deprotection of which gave carbamates **21a-f** (Scheme 6).



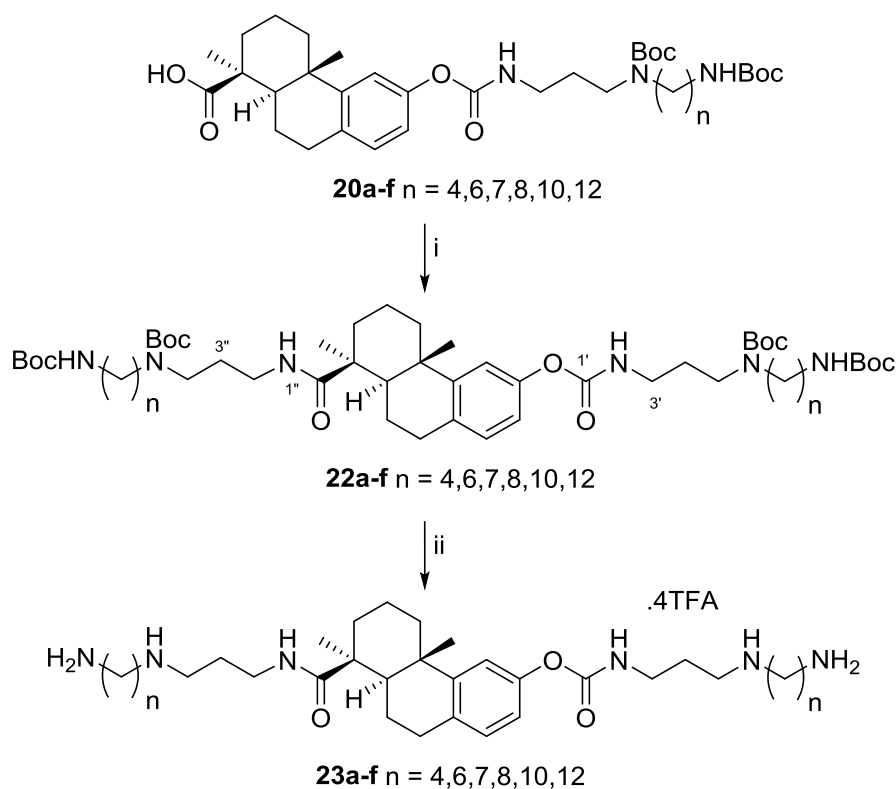
**Scheme 5.** General method for the preparation of polyamine-podocarpic acid amides **19a-f**. Reagents and conditions: (i) pre-incubate podocarpic acid: PyBOP, DIPEA, DMF, 5 h, then polyamine **17a-f**, 72 h (28–84% yield); (ii) TFA/CH<sub>2</sub>Cl<sub>2</sub> (10% v/v), 3 h (79–100% yield).





**Scheme 6.** General method for the preparation of polyamine-podocarpic acid carbamates **21a-f**. Reagents and conditions: (i) pre-incubate podocarpic acid: CDI, CH<sub>2</sub>Cl<sub>2</sub>, 5 h, then polyamine **17a-f**, 72 h, 11–61% yield; (ii) TFA/CH<sub>2</sub>Cl<sub>2</sub> (10% v/v), 3 h (50–100% yield).

A final set of analogues were prepared that had the unusual structural motif of a diterpenoid bearing two polyamine substituents. Protected carbamates **20a-f** were reacted with polyamines **17a-f** to give disubstituted intermediates **22a-f**, with subsequent deprotection using TFA/CH<sub>2</sub>Cl<sub>2</sub> affording products **23a-f** (Scheme 7).



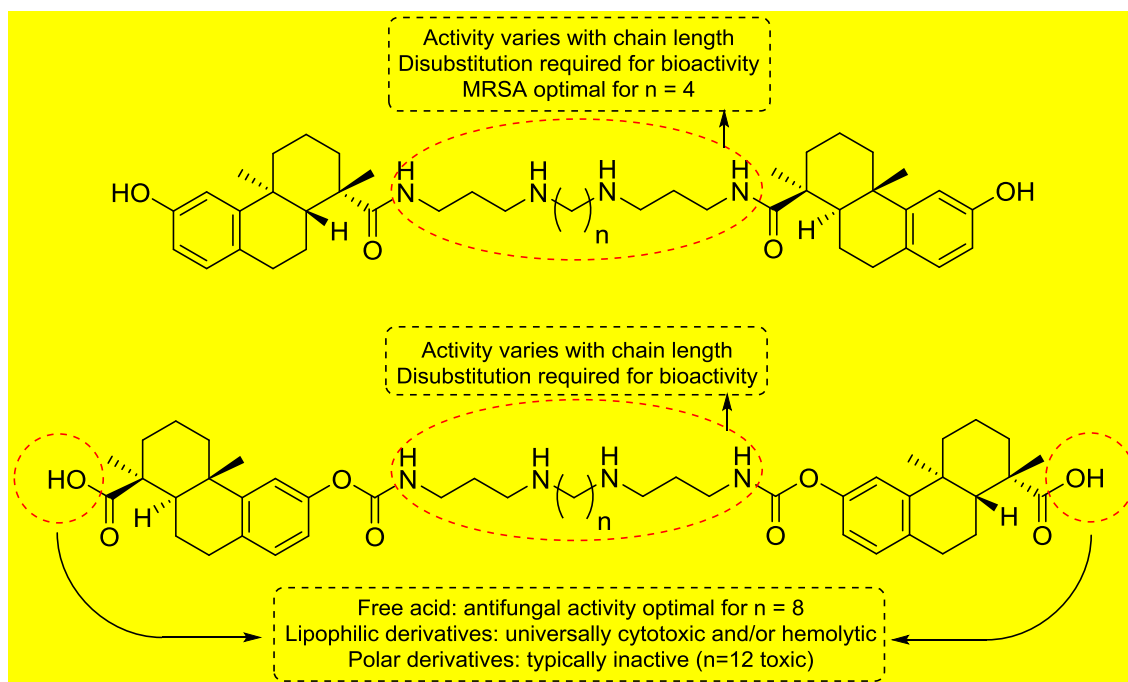
**Scheme 7.** General method for the preparation of dipolyamine substituted podocarpic acid analogues **23a-f**. Reagents and conditions: (i) pre-incubate podocarpic acid carbamate: PyBOP, DIPEA, DMF, 5 h, then polyamine **17a-f**, 72 h (38–81% yield); (ii) TFA/CH<sub>2</sub>Cl<sub>2</sub> (10% v/v), 3 h (85–100% yield).

### 3. Biological Results and Discussion

The intrinsic antimicrobial efficacy of podocarpic acid (**4**) and the set of polyamine analogues **7a-f**, **9a-f**, **11a-f**, **13a-f**, **19a-f**, **21a-f** and **23a-f** were evaluated against a range of Gram-positive (*Methicillin-resistant Staphylococcus aureus* (MRSA)) and Gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*) bacteria and two fungal strains (*Candida albicans* and *Cryptococcus neoformans*) (Table 1).<sup>51</sup> Podocarpic acid was found to be devoid of activity against any of the microbes in the test panel however some polyamine analogues did exhibit growth inhibition properties. While all but one of the analogues (**23f**) failed to inhibit the growth of Gram-negative bacteria, some analogues exhibited moderate to potent activities towards the Gram-positive bacteria *S. aureus* (MRSA) and the fungal strains *C. albicans* and *C. neoformans*.

For the amide series of analogues **7a-f**, potent anti-*S. aureus* activity (MIC  $\leq 0.24$ – $0.26$   $\mu$ M) was observed for **7a** (polyamine 3-4-3), **7e** (3-10-3) and **7f** (3-12-3), while only the longer polyamine chain examples in this set exhibited antifungal activity. We and others have previously observed a general trend indicating that progressive lengthening of the polyamine chain leads to antibiotic activity,<sup>52–54</sup> but in the current set, it was interesting to note the potent anti-*S. aureus* activity of the shortest chain variant (**7a**) but that the PA3-6-3 and PA3-7-3 examples (**7b** and **7c**) were devoid of activity. Interest in the potent anti-*S. aureus* and moderate to potent antifungal activities of **7e** and **7f** (*C. neoformans*, MIC 3.9 and  $\leq 0.24$   $\mu$ M, respectively)

were tempered somewhat however with the observation of cytotoxicity towards the human embryonic kidney (HEK-293) cell line for the former ( $IC_{50}$  0.87  $\mu$ M) and human erythrocyte hemolytic properties for the latter ( $HC_{10} \leq 0.24$   $\mu$ M) (Table 2). A lack of *in vitro* toxicity properties for the PA3-4-3 variant **7a** identified it to be a non-toxic potent selective growth inhibitor of *S. aureus* (MRSA) with a selectivity index (SI, defined as cytotoxicity  $IC_{50}$  / MIC) of >130. Of the carbamates **9a-f**, the only activity observed was towards one or both of the fungal strains, with *C. neoformans* being the most susceptible and **9d** being the most potent (MIC  $\leq 0.23$   $\mu$ M). Additional evaluation of **9a-f** against the yeast *Saccharomyces cerevisiae* strains BY4743 (laboratory isolate) and YJM789 (clinical isolate) identified modest activity ( $IC_{50}$  3.59 and 14.3  $\mu$ M, respectively) for the PA3-10-3 variant **9e**, with all of other analogues being deemed inactive ( $IC_{50} > 25$   $\mu$ M) (data not shown). This result will prompt future mechanism of action studies of **9e** using gene-deleted mutants of *S. cerevisiae*.<sup>55</sup> No cytotoxic or hemolytic properties were observed for **9d**, identifying it as a non-toxic selective antifungal with an SI >128. Conversion of carbamates **9a-f** to their methyl ester (**11a-f**) or *n*-pentylamide (**13a-f**) derivatives led to marked increases in activity towards *S. aureus* and the fungal strains *C. albicans* and *C. neoformans* (Table 1) but, unfortunately, with concomitant increases in cytotoxicity and/or hemolytic properties (Table 2). It would appear that the increase in lipophilicity of the pendant podocarpic acid units in **11a-f** and **13a-f** has exceeded a hydrophobicity threshold leading to increased penetration/perturbation of mammalian cell membranes.<sup>56</sup> Truncated polyamines bearing a single podocarpic acid attached via an amide bond (**19a-f**) or a carbamate (**21a-f**) were almost completely devoid of antimicrobial activity, with the single exception of the amide derivative **19f** (*S. aureus*, MIC 5.4  $\mu$ M). Modest cytotoxicity ( $IC_{50}$  26.2  $\mu$ M, SI 4.9) was observed for the compound. These results highlighted the requirement for 1, $\omega$ -disubstitution for the biological activities observed for analogues **7a-f** and **9a-f**. The final set of dipolyamino-podocarpate analogues, **23a-f**, were almost universally inactive, except for the variant bearing the longest polyamine chains (**23f**) which exhibited strong *S. aureus* growth inhibition in addition to activity towards some of the Gram-negative strains (*E. coli* MIC  $\leq 0.2$   $\mu$ M, *Klebsiella pneumoniae* 12.8  $\mu$ M and *Acinetobacter baumannii* MIC 6.4  $\mu$ M). Unfortunately, poor selectivity was observed for this compound, with it being found to be cytotoxic to the HEK293 cell line with  $IC_{50}$  0.33  $\mu$ M. These structure-activity relationships are summarized in Figure 4.



**Figure 4.** Summary of structural analogues prepared and the resultant intrinsic antimicrobial, cytotoxic and hemolytic properties.

Functionalized polyamines are capable of acting to synergize the action of antibiotics towards otherwise drug-resistant bacterial species.<sup>24,25</sup> Compound sets **7a-f**, **9a-f**, **19a-f**, **21a-f**, **23a-f** were evaluated for the ability to enhance the antibiotic activity of the tetracycline doxycycline towards *P. aeruginosa* ATCC27853 and for erythromycin against *E. coli* ATCC28922.<sup>21,28,37</sup> The assay uses a low fixed dose of 2  $\mu\text{g/mL}$  of either of the antibiotics doxycycline (4.5  $\mu\text{M}$ ) or erythromycin (2.7  $\mu\text{M}$ ), both concentrations of which are substantially less than their respective MIC's (doxycycline, *P. aeruginosa*, 40  $\mu\text{g/mL}$ , 90  $\mu\text{M}$ ; erythromycin, *E. coli*, 128  $\mu\text{g/mL}$ , 174  $\mu\text{M}$ ). Of the compounds tested, only three exhibited any degree of antibiotic enhancement of doxycycline against *P. aeruginosa*, with **23a** being classified as a moderate enhancer (32-fold increase in potency from MIC 800 to 25  $\mu\text{M}$ ) and **23e** and **23f** being more modestly active (potency increase MIC 200 to 50  $\mu\text{M}$ ). Four- to eight-fold increases of potency of erythromycin towards *E. coli* were observed for five analogues (**7a**, MIC 212 to 26.5  $\mu\text{M}$ ; **23a**, MIC 200 to 25  $\mu\text{M}$ ; **23b**, MIC 400 to 50  $\mu\text{M}$ ; **23c**, MIC 200 to 50  $\mu\text{M}$ ; **23e**, MIC 12.5 to 3.125  $\mu\text{M}$ ).

**Table 1:** Antibacterial and antifungal activities of podocarpic acid (**4**) and analogues **7a-f**, **9a-f**, **11a-f**, **13a-f**, **19a-f**, **21a-f** and **23a-f**.

Compound	MIC ( $\mu\text{M}$ )						
	<i>S. a</i> <sup>a</sup>	<i>P. a</i> <sup>b</sup>	<i>E. c</i> <sup>c</sup>	<i>K. p</i> <sup>d</sup>	<i>A. b</i> <sup>e</sup>	<i>C. a</i> <sup>f</sup>	<i>C. n</i> <sup>g</sup>
<b>4</b>	>117	>117	>117	>117	>117	>117	>117
<b>Diamide</b>							
<b>7a</b>	<b><math>\leq 0.26</math></b>	>33.9	>33.9	>33.9	>33.9	>33.9	17.0
<b>7b</b>	32.9	>32.9	32.9	>32.9	>32.9	32.9	32.9
<b>7c</b>	32.5	>32.5	32.5	>32.5	>32.5	32.5	32.5
<b>7d</b>	8.0	>32.0	32.0	>32.0	32.0	32.0	32.0
<b>7e</b>	<b><math>\leq 0.24</math></b>	>31.1	>31.1	>31.1	>32.1	>32.1	<b>3.89</b>
<b>7f</b>	<b><math>\leq 0.24</math></b>	>30.3	>30.3	>30.3	>30.3	7.58	<b><math>\leq 0.24</math></b>
<b>Dicarbamate</b>							
<b>9a</b>	>31.0	>31.0	>31.0	>31.0	>31.0	7.76	7.76
<b>9b</b>	30.2	>30.2	>30.2	>30.2	>30.2	30.2	30.2
<b>9c</b>	>29.8	>29.8	>29.8	>29.8	>29.8	29.8	7.45
<b>9d</b>	>29.4	>29.4	>29.4	>29.4	>29.4	14.7	<b><math>\leq 0.23</math></b>
<b>9e</b>	>28.7	>28.7	>28.7	>28.7	>28.7	>28.7	7.17
<b>9f</b>	14.0	>28.0	>28.0	>28.0	>28.0	>28.0	3.50
<b>Methyl ester</b>							
<b>11a</b>	<b>1.89</b>	>30.2	30.2	>30.2	30.2	15.1	<b>1.89</b>
<b>11b</b>	<b><math>\leq 0.23</math></b>	>29.4	14.7	>29.4	>29.4	<b><math>\leq 0.23</math></b>	<b>0.46</b>
<b>11c</b>	<b><math>\leq 0.23</math></b>	>29.1	>29.1	>29.1	>29.1	<b><math>\leq 0.23</math></b>	<b>7.26</b>
<b>11d</b>	<b><math>\leq 0.22</math></b>	>28.7	>28.7	>28.7	>28.7	<b><math>\leq 0.22</math></b>	<b>0.45</b>
<b>11e</b>	<b>3.50</b>	>28.0	>28.0	>28.0	>28.0	<b><math>\leq 0.22</math></b>	<b><math>\leq 0.22</math></b>
<b>11f</b>	<b><math>\leq 0.21</math></b>	>27.3	>27.3	>27.3	>27.3	<b><math>\leq 0.21</math></b>	<b>0.43</b>
<b><i>n</i>-Pentyl amide</b>							
<b>13a</b>	<b><math>\leq 0.21</math></b>	>27.4	>27.4	>27.4	>27.4	13.7	>27.4
<b>13b</b>	<b><math>\leq 0.21</math></b>	>26.7	>26.7	>26.7	>26.7	<b>1.67</b>	<b>6.68</b>
<b>13c</b>	<b><math>\leq 0.21</math></b>	>26.4	>26.4	>26.4	>26.4	<b><math>\leq 0.21</math></b>	<b>3.30</b>
<b>13d</b>	<b>1.63</b>	>26.1	>26.1	>26.1	>26.1	<b><math>\leq 0.20</math></b>	<b><math>\leq 0.20</math></b>
<b>13e</b>	<b><math>\leq 0.20</math></b>	>25.5	>25.5	>25.5	>25.5	<b><math>\leq 0.20</math></b>	<b>3.19</b>
<b>13f</b>	<b>1.56</b>	>25.0	>25.0	>25.0	>25.0	<b><math>\leq 0.19</math></b>	<b><math>\leq 0.19</math></b>
<b>Mono-amide</b>							
<b>19a</b>	>50.8	>50.8	>50.8	>50.8	>50.8	>50.8	>50.8
<b>19b</b>	>48.6	>48.6	>48.6	>48.6	>48.6	>48.6	>48.6
<b>19c</b>	>47.6	>47.6	>47.6	>47.6	>47.6	>47.6	>47.6
<b>19d</b>	46.7	>46.7	>46.7	>46.7	>46.7	>46.7	>46.7

<b>19e</b>	>44.8	>44.8	>44.8	>44.8	>44.8	>44.8	>44.8
<b>19f</b>	<b>5.4</b>	>43.1	43.1	>43.1	43.1	43.1	43.1
<b>Mono-carbamate</b>							
<b>21a</b>	>47.5	>47.5	>47.5	>47.5	>47.5	>47.5	>47.5
<b>21b</b>	>45.6	>45.6	>45.6	>45.6	>45.6	>45.6	>45.6
<b>21c</b>	>44.7	>44.7	>44.7	>44.7	>44.7	>44.7	>44.7
<b>21d</b>	>43.8	>43.8	>43.8	>43.8	>43.8	>43.8	>43.8
<b>21e</b>	>42.2	>42.2	>42.2	>42.2	>42.2	>42.2	>42.2
<b>21f</b>	>32.8	>32.8	>32.8	>32.8	>32.8	32.8	>32.8
<b>Dipolyamine</b>							
<b>23a</b>	>31.1	>31.1	>31.1	>31.1	>31.1	>31.1	>31.1
<b>23b</b>	>29.5	>29.5	>29.5	>29.5	>29.5	>29.5	>29.5
<b>23c</b>	>28.7	>29.7	>29.7	>29.7	>29.7	>29.7	>29.7
<b>23d</b>	>28.0	>28.0	>28.0	>28.0	>28.0	>28.0	>28.0
<b>23e</b>	26.7	>26.7	>26.7	>26.7	>26.7	>26.7	>26.7
<b>23f</b>	<b>≤0.20</b>	>25.5	<b>≤0.20</b>	12.8	<b>6.38</b>	25.5	>25.5

<sup>a</sup> *Staphylococcus aureus* ATCC 43300 (MRSA) with vancomycin (MIC 0.7 µM) used as positive controls and values presented as the mean (n = 2).

<sup>b</sup> *Pseudomonas aeruginosa* ATCC 27853 with colistin (MIC 0.2 µM) used as positive controls and values presented as the mean (n = 2).

<sup>c</sup> *Escherichia coli* ATCC 25922 with colistin (MIC 0.1 µM) used as positive controls and values presented as the mean (n = 2).

<sup>d</sup> *Klebsiella pneumoniae* ATCC 700603 with colistin (MIC 0.2 µM) as a positive control and values presented as the mean (n = 2).

<sup>e</sup> *Acinetobacter baumannii* ATCC 19606 with colistin (MIC 0.2 µM) as a positive control and values presented as the mean (n = 2).

<sup>f</sup> *Candida albicans* ATCC 90028 with fluconazole (MIC 0.4 µM) as a positive control and values presented as the mean (n = 2).

<sup>g</sup> *Cryptococcus neoformans* ATCC 208821 with fluconazole (MIC 26 µM) as a positive control and values presented as the mean (n = 2).

**Table 2:** Cytotoxicity and hemolytic activity of analogues **7a-f**, **9a-f**, **11a-f**, **13a-f**, **19a-f**, **21a-f** and **23a-f**.

Compound	Cyto <sup>a</sup>	Hem <sup>b</sup>	Compound	Cyto <sup>a</sup>	Hem <sup>b</sup>
<b>Diamide</b>			<b>Mono-amide</b>		
<b>7a</b>	>33.9	>33.9	<b>19a</b>	n.d. <sup>c</sup>	n.d. <sup>c</sup>
<b>7b</b>	28.6	>32.9	<b>19b</b>	>48.6	>48.6
<b>7c</b>	19.1	>32.5	<b>19c</b>	>47.6	>47.6
<b>7d</b>	18.1	>32.0	<b>19d</b>	>46.7	>46.7
<b>7e</b>	<b>0.87</b>	>31.1	<b>19e</b>	>44.8	>44.8
<b>7f</b>	>30.3	≤ <b>0.24</b>	<b>19f</b>	26.2	>43.1
<b>Dicarbamate</b>			<b>Mono-carbamate</b>		
<b>9a</b>	>31.0	>31.0	<b>21a</b>	n.d. <sup>c</sup>	n.d. <sup>c</sup>
<b>9b</b>	>30.2	26.3	<b>21b</b>	>45.6	>45.6
<b>9c</b>	>29.8	>29.8	<b>21c</b>	>44.7	>44.7
<b>9d</b>	>29.4	>29.4	<b>21d</b>	>43.8	>43.8
<b>9e</b>	>28.7	20.2	<b>21e</b>	n.d. <sup>c</sup>	n.d. <sup>c</sup>
<b>9f</b>	>28.0	>28.0	<b>21f</b>	>32.8	>32.8
<b>Methyl ester</b>			<b>Dipolyamine</b>		
<b>11a</b>	16.4	14.6	<b>23a</b>	>31.1	>31.1
<b>11b</b>	<b>4.68</b>	>29.4	<b>23b</b>	>29.5	>29.5
<b>11c</b>	>29.1	≤ <b>0.23</b>	<b>23c</b>	>28.7	>28.7
<b>11d</b>	>28.7	≤ <b>0.22</b>	<b>23d</b>	>28.0	>28.0
<b>11e</b>	<b>1.30</b>	≤ <b>0.22</b>	<b>23e</b>	20.6	>26.7
<b>11f</b>	>27.3	<b>0.77</b>	<b>23f</b>	<b>0.33</b>	>25.5
<b>n-Pentyl amide</b>					
<b>13a</b>	>27.4	<b>4.10</b>			
<b>13b</b>	>26.7	<b>2.77</b>			
<b>13c</b>	>26.4	≤ <b>0.21</b>			
<b>13d</b>	>26.1	≤ <b>0.20</b>			
<b>13e</b>	>25.5	≤ <b>0.20</b>			
<b>13f</b>	>25.0	≤ <b>0.19</b>			

<sup>a</sup> Concentration of compound at 50% cytotoxicity on HEK293 human embryonic kidney cells and values presented as the mean (n = 2). Tamoxifen was the positive control (IC<sub>50</sub> 9 µg/mL, 24 µM).

<sup>b</sup> Concentration of compound at 10% hemolytic activity on human red blood cells and values presented as the mean (n = 2). Melittin was the positive control (HC<sub>10</sub> 2.7 µg/mL).

<sup>c</sup> not determined.

## 4. Conclusions

We have synthesized a series of 42 podocarpic acid polyamine conjugates functionalized at the phenolic and/or carboxylic acid positions of the natural product diterpene. Wide variation in intrinsic antimicrobial properties were observed for the compound set, with the Gram-positive bacterium *S. aureus* (MRSA) and the fungus *C. neoformans* being the most susceptible. Of note was the discovery of two analogues, **7a** and **9d** that exhibited selective, non-toxic activity towards *S. aureus* and *C. neoformans*, respectively. The complete set of results indicate variation of biological activity was dependent upon polyamine structure and attachment point (phenolic vs carboxylic acid) of the diterpene and that lipophilic substitution of the carboxylic acid leads to sharp increases in undesirable cytotoxicity and/or hemolytic properties. When compared to previously reported 1, $\omega$ -disubstituted polyamines e.g. **2** and **3**, the present compound set were overall substantially less active as antimicrobial agents and as antibiotic adjuvants. This highlights the delicate balance of structural features and properties of the end-group(s) attached to polyamines for the conjugates to exhibit desirable antimicrobial/adjuvant activities. The discovery of novel end-groups that exhibit these properties are the focus of our ongoing studies.

## 5. Experimental

Full experimental details are given in the Supplementary Material (Appendix A).

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary material (experimental details)



Experimental details and compound characterization data can be found online at:

## Appendix B. Supplementary material (NMR spectra)

NMR spectra ( $^1\text{H}$  and  $^{13}\text{C}$ ) of compounds **7a-f**, **9a-f**, **11a-f**, **13a-f**, **19a-f**, **21a-f**, **23a-f** can be found online at:

## Appendix C. Supplementary material (HRESIMS)

HRESIMS spectra for compounds **7a-f**, **9a-f**, **11a-f**, **13a-f**, **19a-f**, **21a-f**, **23a-f** can be found online at:

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