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Parallel synthesis of a bis-triazoles library as psammaplin A analogues: A new wave of antibiofilm compounds?

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ABSTRACT

Synthesis of psammaplin A analogues is described. Screening for antibiofilm activity of the targeted library afford some interesting elements in terms of structure-activity relationships. Some compounds exhibited EC50 in the range of ampicillin against three strains of gramnegative bacteria without toxic effect.

Nowadays, eradication of bacterial biofilms still remains a challenge for chemists and microbiologists in many economical sectors. These include medical health care since they colonize implants such as artificial joints or catheters.[1] While in marine environment, the formation of biofilms on immersed substrata, leads to major economic problems which conducted to the use of toxic biocides to fight against these communities.[2–4] In this context, development of original compounds that specifically target the biofilm formation is of great need in view of rational use of antibiotics and/or biocides. Such biofilm inhibitors should have the potential to be used in a preventive treatment of a wide diversity of industrial and/or medical surfaces. Some of the anti-biofilm techniques, that are tested today, are based on the observation of sessile marine macroorganisms (sponges, corals) which are constantly exposed to undesirable bacterial colonization (e.g. biofouling).[5] To cope with biofouling and maintain unfouled exterior surfaces, several of them have developed various defense systems.[5–9] This observation has motivated investment in the research of potential "non-toxic" antibiofilm compounds from their arsenal of secondary metabolites.[10–14] Therefore, use of such secondary metabolites on a large scale appears to be difficult to achieve since they are obtained in small quantities. These factors led to the synthesis of analogues, by maintaining the natural framework in order to retain biological activity. For this purpose, we have developed a new synthetic plan, based on click chemistry, allowing a rapid and efficient synthesis of libraries of bromotyramine/triazole analogues at a suitably large scale.[15,16] Click chemistry is a highly efficient process for the generation of compound libraries.[17] In addition, the 1,2,3-triazole ring has been explored as bioisosteres in medicinal chemistry of several chemical functions.[18] Number of compounds containing this ring have shown a broad spectrum of biological activities.[19–22] In continuation of our investigations, the present study consists in the preparation of a series of psammaplin A analogues possessing a two triazolic core. Psammaplin A, was extracted from the marine sponge *Pseudoceratina* sp. and was shown to exhibit interesting biological activities, such as antibacterial or anticancer properties.[23,24]

The targeted library (Fig. 1) was constructed by considering three points of chemical diversity: 1) a biosoteric replacement of the oxime/amide fonctions by 1,2,3-triazole core, since we have demonstrated that the bioisoteric replacement of an oxime by a 1,2,3-triazole was successful to afford somesinteresting elements of structure–activity relationships (SAR) in the field of anti-biofilm compounds derived from the group of bromotyrosine alkaloids.[15,16] In addition, recent reports described the diversity-oriented synthesis of pyrrolidinyl triazoles as biosisosteres of some pyrrolidinyl oxime (an mPTP blocker as a viable therapeutic target for the treatment of Alzheimer's disease).[25] 2) The second point concerns the replacement of the disulfide linker by different chemical classes of linker.3) Finally, substituents on aromatic rings were considered in agreement with those found in the bromotyrosine alkaloids.[26,27]

Ability of the resulting analogues to inhibit biofilm formation of three bacterial strains was investigated in order to establish SAR.

Access to the desired psammaplin A analogues was achieved in a one-step by means of double Copper-catalyzed 1,3-dipolar cycloaddition between starting azide derivatives**3a-d** and different dialkynes. Azides **3a-d**, giving the first level of chemical diversity, were easily accessible in three steps from 4-(2-azidoethyl)-2-bromo-1-methoxybenzene[15] in excellent yields (Scheme 1).

The second level of chemical diversity was introduced by three kinds of linkers chosen from commercial sources: alkyl chain containing

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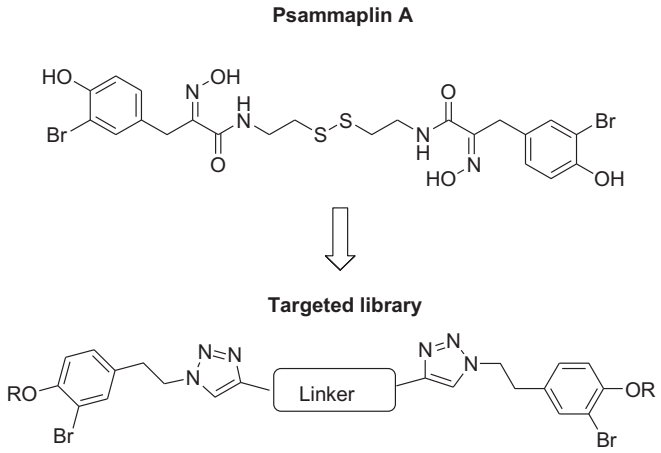


Figure 1. Structure of psammaplin A and of targeted library.

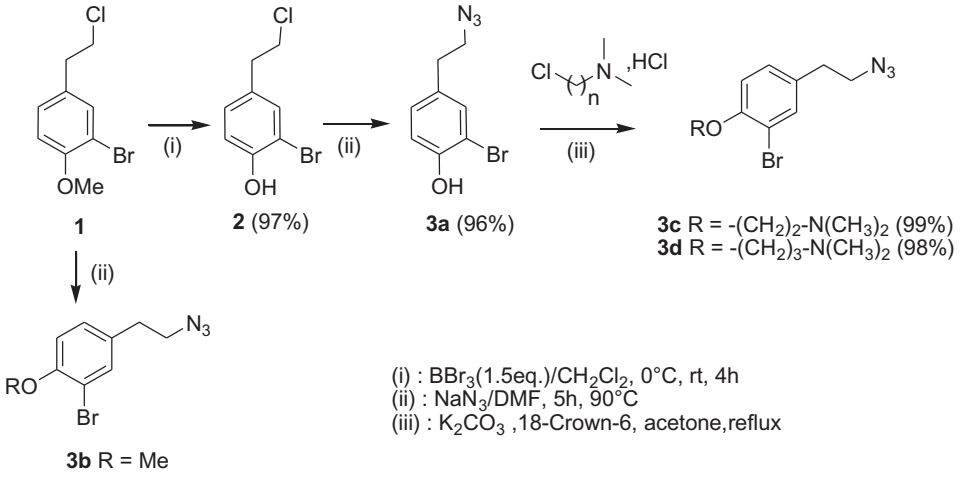
3,4 and 6 carbons, two heteroatomoic dialkynes (oxygen and nitrogen), and finally two aromatic systems. The formation of the bis-triazole analogues was then achieved by performing the copper(I)-catalyzed 1,3-dipolar cycloaddition of the organic azides with appropriate dialkynes resulting in the formation of two 1,2,3-triazoles. In general, these reactions usually proceed to completion in 6–36 h at room temperature in water with a variety of organic co-solvents, such as *tert*-butanol, ethanol, DMF, DMSO, THF or CH_3CN.[28,29] Ethanol was usually chosen rather than DMF to allow an easier workup and a better purity of products as described in our previous work but in this case DMF was used because of the poor solubility of the resulting bis-triazoles in ethanol. Practically, 1 equivalent of dialkyne was added to a solution of appropriate azide (**3a-d,** 2.6 equivalents), $CuSO_4$/sodium ascorbate in a water/DMF mixture (50/50) and the reaction time was optimized at 24 h at room temperature. Results reported in Table 1 show that all compounds were obtained in excellent yields (> 77%), but it is notable that compound **5g** bearing an 1,4-linked aromatic ring could not be isolated and purified for further biological tests.

In order to assess anti-biofilm activity of these compounds against representative Gram-negative bacterial biofilms, three strains were chosen for their capacity to form biofilms: *Pseudoalteromonas lipolytica* (TC8), *Pseudoalteromonas ulvae* (TC14) and a *Paracoccus* sp. strain (4 M6).[30] In an initial screening process, all compounds were tested for their ability to modulate biofilm formation at concentration of 200 µM by using our previous method adapted from Leroy et al. using the specific fluorophore Syto®61[31,32]. Partial information about structure–activity relationship (SAR) could be highlighted at this stage: all compounds possessing an alkyl-type linker (series **a, b, c**) were inactive (less than 50% of inhibition of the adhesion). Replacement of the carbon in the series **a** by an oxygen (series **d**) or nitrogen (series **e**) enhanced the activity. Considering aromatic linkers (series **f** and **g**), only the compounds connected in the 1,4-positions were founded to be active (series **g**). In order to precise structure-activity relationships, effective concentrations to inhibit 50% of the bacterial adhesion (expressed as EC_{50}) were determined for compounds **4d-g, 5d-e, 6d-g, 7d-g** which inhibited > 50% of adhesion at 200 µM. Results of this screen are outlined in Table 2. In this way, first observation was to note that globally the TC14 strain was more sensitive to this class of bis-triazoles than TC8 and 4 M6 strains. Among the three classes tested, series **g** possessing an 1,4-linked aromatic ring as central part (**4g, 6g, 7g**) were the more potent compounds especially **6g** an **7g** with EC_{50} closed to ampicillin and tributyltin oxide (TBTO). In term of SAR, it is interesting to note that the dimethylaminoethyl chain (**6g**) as well as the dimethylaminopropyl chain (**7g**) are common natural framework found in bromotyrosine alkaloïds possessing antifouling properties, and that this class of substituents afforded a beneficial aspect when compared to simple hydroxyl or methyl groups found in alkaloids extracted from sponges such as aplysamines or hemibastadins or simple 2-(3′-Bromo-4′-hydroxyphenol)ethanamine.[33]

In order to determine if these compounds exhibited a specific anti-biofilm activity or if this observation was simply related to a general toxic effect on the bacteria, a growth inhibition and viability assay was performed. Active compounds **6g** and **7g** were tested for their capacity to inhibit the growth of the three strains TC14, TC8 and 4M6. Experiments were performed at the high concentration of 100 µM (Figs. 2a, 2b, 2c) and using ampicillin at a concentration of 5 µM.

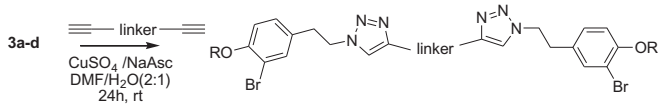
At these concentrations, the two compounds presented bacteriostatic effects on the three bacterial strains. A slight effect was observed for compound **7g** while compound **6g** exhibited effects much more closed to ampicillin especially on the TC8 and 4M6 strains which seems to be more sensitive than TC14.

For viability, the same methodology used for antiadhesion assay with Syto®61 was applied using resazurin test at the concentrations of 5, 10, 20, 50, 100, 200 µM. Results at 5 and 100 µM are reported in Fig. 3 (see supplementary materials for detailed results). At a low concentration of 5 µM, concentration closed to their EC_{50} as antibiofilm compounds, compounds **6g** and **7g** were not lethal to bacteria. This suggested that their anti-biofilm activities were not directly connected to antibacterial effect in contrast to ampicillin which is toxic especially to TC14 and TC8. Furthermore, at the high concentrations (100 µM), compounds **6g** and **7g** presented a slight bactericidal effect on the three



Scheme 1. Preparation of starting azides **3a-d.**

Table 1
Synthesis of psammaplin A analogues.



28 analogues

BT1-28

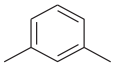
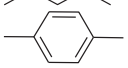
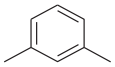
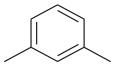
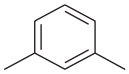
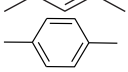
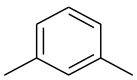
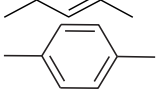
Compound	R	Linker	Yield
4a	H	-$(CH_2)_3$-	92%
4b	H	-$(CH_2)_4$-	87%
4c	H	-$(CH_2)_6$-	93%
4d	H	-CH_2-O-CH_2-	87%
4e	H	-CH_2-NH-CH_2-	63%
4f	H		77%
4g	H		80%
5a	CH_3	-$(CH_2)_3$-	91%
5b	CH_3	-$(CH_2)_4$-	96%
5c	CH_3	-$(CH_2)_6$-	97%
5d	CH_3	-CH_2-O-CH_2-	93%
5e	CH_3	-CH_2-NH-CH_2-	88%
5f	CH_3		90%
5g	CH_3		Not purified
6a	$(CH3)_2N$-$(CH_2)_2$-	-$(CH_2)_3$-	93%
6b	$(CH3)_2N$-$(CH_2)_2$-	-$(CH_2)_4$-	94%
6c	$(CH3)_2N$-$(CH_2)_2$-	-$(CH_2)_6$-	89%
6d	$(CH3)_2N$-$(CH_2)_2$-	-CH_2-O-CH_2-	98%
6e	$(CH3)_2N$-$(CH_2)_2$-	-CH_2-NH-CH_2-	84%
6f	$(CH3)_2N$-$(CH_2)_2$-		84%
6g	$(CH3)_2N$-$(CH_2)_2$-		88%
7a	$(CH3)_2N$-$(CH_2)_3$-	-$(CH_2)_3$-	89%
7b	$(CH3)_2N$-$(CH_2)_3$-	-$(CH_2)_4$-	93%
7c	$(CH3)_2N$-$(CH_2)_3$-	-$(CH_2)_6$-	87%
7d	$(CH3)_2N$-$(CH_2)_3$-	-CH_2-O-CH_2-	85%
7e	$(CH3)_2N$-$(CH_2)_3$-	-CH_2-NH-CH_2-	79%
7f	$(CH3)_2N$-$(CH_2)_3$-		92%
7g	$(CH3)_2N$-$(CH_2)_3$-		88%

Table 2
Antibiofilm activity of psammaplin A bioactives analogues.

cpd	TC14[a]		TC8[a]		4M6[a]	
	% of adhesion[b]	EC_{50}	% of adhesion[b]	EC_{50}	% of adhesion[b]	EC_{50}
Series a						
4a	53.1 ± 4.1	> 200 µM	63.5 ± 8.1	> 200 µM	83.4 ± 3.9	> 200 µM
5a	50.4 ± 2.2	> 200 µM	50.0 ± 4.9	> 200 µM	49.2 ± 0.8	> 200 µM
6a	55.8 ± 3.8	> 200 µM	57.0 ± 2.2	> 200 µM	51.5 ± 3.0	> 200 µM
7a	53.8 ± 5.9	> 200 µM	59.9 ± 6.4	> 200 µM	77.2 ± 2.5	> 200 µM
Series b						
4b	54.4 ± 1.8	> 200 µM	85.6 ± 2.1	> 200 µM	52.4 ± 2.3	> 200 µM
5b	53.3 ± 6.3	> 200 µM	56.9 ± 1.7	> 200 µM	50.9 ± 0.3	> 200 µM
6b	61.5 ± 1.0	> 200 µM	58.5 ± 4.3	> 200 µM	57.4 ± 1.6	> 200 µM
7b	66.5 ± 5.6	> 200 µM	89.2 ± 4.8	> 200 µM	80.7 ± 8.8	> 200 µM
Series c						
4c	61.7 ± 6.7	> 200 µM	75.0 ± 5.2	> 200 µM	78.1 ± 1.2	> 200 µM

(*continued on next page*)

Table 2 (*continued*)

cpd	TC14[a]		TC8[a]		4M6[a]	
	% of adhesion[b]	EC_{50}	% of adhesion[b]	EC_{50}	% of adhesion[b]	EC_{50}
5c	62.7 ± 3.1	> 200 μM	78.3 ± 0.6	> 200 μM	69.2 ± 0.5	> 200 μM
6c	67.4 ± 4.0	> 200 μM	54.4 ± 10.8	> 200 μM	59.5 ± 2.2	> 200 μM
7c	71.0 ± 4.1	> 200 μM	72.3 ± 1.4	> 200 μM	84.7 ± 1.4	> 200 μM
Series d						
4d	40.3 ± 3.3	127.1 ± 27.6	50.3 ± 5.8	194.9 ± 10.7	47.8 ± 0.1	198.3 ± 4.1
5d	38.5 ± 7.4	159.8 ± 33.5	42.8 ± 6.6	180.8 ± 21.3	44.9 ± 3.2	164.1 ± 6.3
6d	34.8 ± 2.8	91.8 ± 17.9	34.6 ± 5.1	101.6 ± 46.2	40.4 ± 7.8	146.2 ± 81.9
7d	41.7 ± 1.7	121.7 ± 59.1	51.6 ± 1.5	> 200	53.7 ± 4.0	> 200
Series e						
4e	29.3 ± 7.5	59.9 ± 27.2	28.9 ± 17.0	80.3 ± 18.9	27.9 ± 1 6.0	88.3 ± 46.3
5e	37.4 ± 0.1	138.4 ± 8.7	48.2 ± 2.2	187.9 ± 3.0	46.3 ± 2.7	194.5 ± 8.0
6e	40.9 ± 5.0	126.2 ± 21.6	47.1 ± 1.0	176.5 ± 3	51.8 ± 4.4	> 200
7e	45.0 ± 0.8	> 200	55.4 ± 0.4	185.2 ± 16.4	56.3 ± 2.4	> 200
Series f						
4f	56.8 ± 1.3	> 200 μM	66.5 ± 11.6	> 200 μM	61.5 ± 4.2	> 200 μM
5f	74.1 ± 1.1	> 200 μM	101.1 ± 4.8	> 200 μM	76.2 ± 1.7	> 200 μM
6f	62.2 ± 0.4	> 200 μM	65.1 ± 8.8	> 200 μM	66.9 ± 1.8	> 200 μM
7f	65.3 ± 6.9	> 200 μM	71.6 ± 2.8	> 200 μM	78.1 ± 2.2	> 200 μM
Series g						
4g	31.1 ± 6.2	40.9 ± 11.41	58.3 ± 8.7	> 200	31.8 ± 3.5	59.9 ± 7.7
6g	0 ± 5.3	0.9 ± 0.83	14.6 ± 11.8	5.1 ± 1	10.1 ± 8.1	5.1 ± 1.0
7g	0.6 ± 0.8	0.4 ± 0.28	5.3 ± 2.5	15.0 ± 3.38	5.1 ± 1.43	3.5 ± 3.0
TBTO	ND	0.7 ± 0.3	ND	7.0 ± 3.0	ND	4.0 ± 3.0
ampicillin	ND	9.3 ± 0.2	ND	17.9 ± 0.9	ND	144.1 ± 12.3

[c]ND: not determined, EC_{50} > 200 μM.

[a] **TC14**: *Pseudoalteromonas ulvae,* **TC8** : *Pseudoalteromonas lipolytica,* **4M6** : *Paracoccus* sp.

[b] % of adhesion at 200 μM.

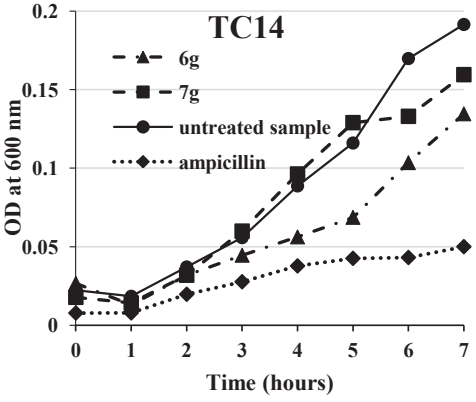


Figure 2a. Effect on TC14 growth of compounds **6g, 7g** at concentrations of 100 μM.

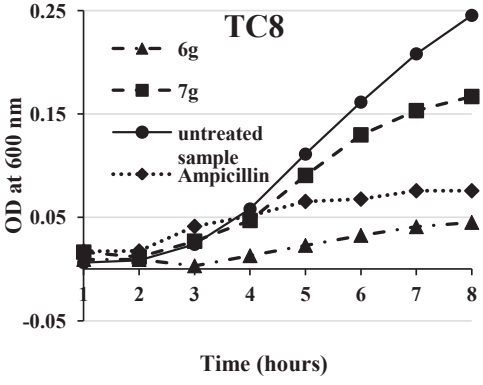


Figure 2b. Effect on TC8 growth of compounds **6g, 7g** at concentrations of 100 μM.

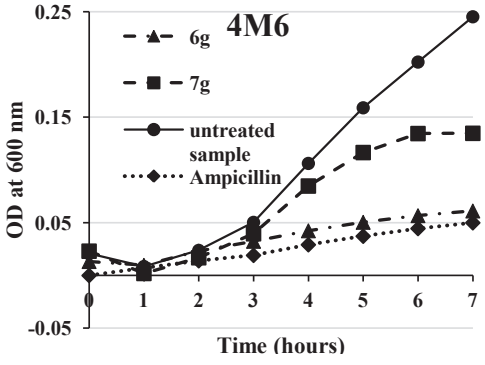


Figure 2c. Effect on 4M6 growth of compounds **6g, 7g** at concentrations of 100 μM.

strains. In addition, it is important to note that the antibiofilm effect of ampicillin seems to be directly related to its antibacterial effect. In fact, the good antibiofilm activities observed on TC14 and TC8 (EC_{50} in the range of 5 μM) were related to high toxicity, while no antibiofilm effect (EC_{50} = 146 μM) was associated with a low toxicity on 4M6 strains.

In conclusion, we have used click chemistry to generate a library of psammaplin A analogues based on a bis-triazole framework. In the present paper, we have generated a preliminary screening for analogues that can be designed to be selective against gram negative bacteria. Potent inhibitors of biofilm formation have been identified. Finally, the low toxicity of the more potent anti-biofilm leads allows us to focuse on future interest in the development of these molecules as non-toxic anti-biofilm compounds for their potential use as non-toxic co-biocides or co-antibiotic in view of rational eradication of persistent biofilms.

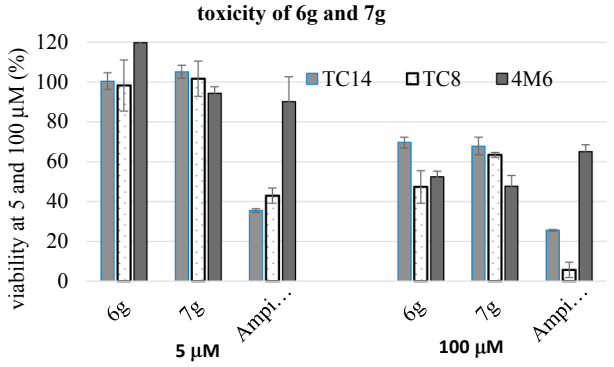


Figure 3. Effect on TC14, TC8, and 4M6 viability of compounds **6g** and **7g** at concentrations of 5 and 100 µM.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2018.12.047.

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