GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Potentiation of the antibiofilm effect of natural compound analogs in a marine bacterium by the cell permeabilization mechanism

Emmanuel Gozoua [1, 2, 3, *], Rose Koffi-Nevry [2] and Yves Blache [1]

[1] *University of Toulon, MAPIEM, Toulon, France.*
[2] *Nangui-Abrogoua University, Microbiology and Biotechnology Laboratory,Abidjan, Ivory Coast, Cote d'Ivoire.*
[3] *University of San Pedro, Faculty of Marine Sciences, Ivory Coast, Cote d'Ivoire.*

GSC Biological and Pharmaceutical Sciences, 2023, 25(01), 154–163

Publication history: Received on 03 September 2023; revised on 10 October 2023; accepted on 13 October 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.25.1.0417

Abstract

The potentiation of the anti-adhesion and anti-biofilm effects of the three compounds (OB1, AS194, AS162) was evaluated in this study. This potentiation is demonstrated by the combination of each of the three compounds with molecules of permeabilizing types which are Polymyxin B nanopeptide hydrochloride (PMBN), Phenylalanine-Arginine Beta-Naphthylamide (PAßN) and Carbonyl cyanide chlorophenylhydrazone (CCCP). The association of this type of molecule with antibiofilm compounds showed a synergistic effect in TC14. This effect was more pronounced with CCCP (5µM). Thus, the AS162 having the highest effect between the three compounds previously induced a reduction in adhesion of up to 30.80% at only 10µM. In combination with the CCCP and at the same concentration, AS162 reduces TC14's adherence by up to 11.23%, a reduction of more than 63.53%. The AS162 also reduces the biofilm mass of the same bacterium to 10.15% in the presence of CCCP, i.e. a reduction of 89.75%. When taking the case of compounds with relatively low anti-biofilm effect such as OB1 and AS194, the reduction rate under the effect of the combinations is found to be higher than that of AS162. Thus, OB1 with an EC_{50} of 75.39 µM when used alone, in the presence of the CCCP, experiences a reduction of this EC_{50} up to 3.6 µM, i.e. 95.22% reduction. In AS194 (with a relatively stronger effect than OB1), the EC_{50} is reduced by 74.02% and 52.79% for AS162. This method has been shown to be effective in enhancing the anti-biofilm effect of the compounds. It is therefore important to note that the less active the compound, the greater the synergistic effect with the permeabilizers.

Keywords: Potentiation; Antibiofilm; Adhesion; Permeabilizers

1. Introduction

Increasingly, bacterial biofilm control uses alternative technologies including naturally occurring active compounds. Numerous substances with antifouling properties have been extracted from various marine organisms, whether microscopic (bacteria, fungi) or macroscopic (algae, sponges, corals, gorgons). Many of these compounds have been developed by the MAPIEM laboratory. These are derived from marine organisms or molecules designed on the basis of known models. Some of these molecules have been screened during previous research, notably those of Linares et *al*., in 2011 and those of Chambers et *al.,* 2011. The natural compounds used in this study come from marine organisms or from synthetic molecules designed on the basis of known models. The origin of these molecules does not guarantee their non-toxicity, even if they have the advantage of having been selected during evolution (Sall et *al*., 2018). At 200µM, one of these compounds, AS162 (very active on the biofilm), has a significant effect on the viability of bacterial cells in TC14 and two other bacteria. The potentiation of the antifouling effect of these analogs is more than necessary and requires a better understanding of the biofilm formation steps and a knowledge of the parameters associated with them. The potentiation consists in optimizing the antiadhesion and/or antibiofilm effect of the compounds by its combination with an adjuvant. The aim of this method is to obtain a combined molecule which contains a relatively low dose of

* Corresponding author: Emmanuel Gozoua

antifouling compound having a lower risk of contamination for the bacterial cell but with a very high antibiofilm effect. The control of the various parameters involved in the formation of the biofilm makes it possible to determine the targets to be targeted for a better optimization of the effect of an analog. It appeared necessary to use organic molecules with targets related to biofilm formation. One of the main parameters related to biofilm formation in gram negative bacteria is the bacterial communication system known as Quorum detection (QS) (Decho et *al*., 2011). A previous study by Gozoua et *al*., 2019 showed that QS modulation in TC14 and two other Gram-negative bacteria does not necessarily induce a synergistic effect on biofilm inhibition in these microorganisms. Indeed, the combination of the antibiofilm compounds with QS modulators showed a restitution of the biofilm and therefore a reduction in the antibiofilm effect of the analogs to more than 80%. This reduction occurred with both a QS inhibitor (3-oxo-C6) and a QS activator (3-oxo-C8). In order to investigate other pathways of inhibition of the biofilm, molecules with targets other than QS were used. These include adjuvants directed to the plasma membrane in the bacteria tested. Indeed, a group of molecules had been described as adjuvants in the work of Aurelien Stutzmann, 2017. According to this author, the adjuvants are presented as compounds which generally have no intrinsic antibacterial activity. However, they allow another compound to have a better effect on its target. The adjuvants used in this study are characterized by their action properties. These are capable of permeabilizing the bacterial membrane. These are polymyxin B nanopeptide hydrochloride (PMBN), phenylalanine arginine beta-naphthylamide (PAßN) and carbonyl cyanide, chlorophenylhydrazone (CCCP). These methods have yielded conclusive results in combination with several antibiotics (Hancock and Wong, 1984). In contrast, the combination of synthetic analogs with membrane permeabilizers provided a synergistic effect on biofilm adhesion and formation in marine strains such as *Pseudoalteromonas ulvae* TC14, *Pseudoalteromonas lipolytica* TC8, and *Paracoccus* sp. 4M6 (Gozoua et *al.,* 2019). This bacterial biofilm inhibition pathway was evaluated with the goal of developing alternative biofilm control pathways other than QS.

1.1. Mechanism of cell permeabilization

Membrane permeabilization is a method that promotes increased antimicrobial activity. Permeabilizers are compounds that are designed on the basis of cationic or peptide forms as found in lipids (Erin et *al*., 2015). They are also in the form of polymers such as, for example, antimicrobial peptides (Hancock, 1997; Scott et *al*., 1999) and cholic acid (Savage, 2001; Schmidt et *al*., 2001). These physico-chemical properties are quite general and therefore some agents are much more effective permeabilizers than others (Vaara, 1991). A study examined the ability of various compounds to permeabilize the outer membrane of *P. aeruginosa* strains and demonstrated the efficacy of citric acid, polyL-lysine, EDTA and polymyxin B nonapeptide (PMBN), a deacylated version of polymyxin without antibiotic activity but retaining the membrane permeabilizing activity of polymyxin B) (Hancock and Wong, 1984).

2. Material and methods

2.1. Analogs of natural compounds

The MAPIEM laboratory has a large repertoire of natural compounds derived from marine organisms (such has sea sponges) or molecules designed on the basis of known models. Many of these molecules have been screened in previous research including those of Linares et *al*. in 2011 and those of Chambers et *al*., 2011. The first authors showed that the molecule called OB1 (Olivier Bottzeck 1) had a significant inhibitory effect on the adhesion and biofilm formation in marine bacteria such as *Paracoccus* sp. 4M6 and *Pseudoalteromonas* sp. D41. As regards the second authors, they have succeeded in producing compounds having a significant antifouling effect on *Cobetia marina*. These compounds include AS194 (Andjouh Sofyane 194) and AS162 (Andjouh Sofyane 162). It has been described in a previous study (Gozoua et *al*., 2019), that although the mode of action of this molecule is not yet elucidated, AS162 is a potent and non-biocidal antibiofilm composed against various strains of marine bacteria at low concentrations (EC_{50} of the order of 5 µM), whereas at high (>100 µM), a biocidal effect was observed decrease in viability (more than 50% after 7 h) Biggerstaff et *al*., 2006). In this study, a combination of permeabilizers and antibiofilm compounds was used. The experiment was carried out in transparent 92-well microplates. A concentration range between 10 µM and 200 µM was developed with the antifouling compounds. The permeabilizers were added in the following concentrations: Polymyxin B nanopeptide hydrochloride, PMBN (10 µM); Phenylalanine-Arginine Beta-Naphthylamide, PAßN (10 µM) and Carbonyl cyanide mchlorophenylhydrazone, CCCP (5 µM). In this study, the estimation of EC_{50} was performed using a comparative approach based on various regression models implemented in the specialized computer program: GraphPad Prism® version 5.01. This method corresponds to that adopted by Zheng et *al*., 2013. The bacteria tested in this study were selected in the harbor of Toulon, France.

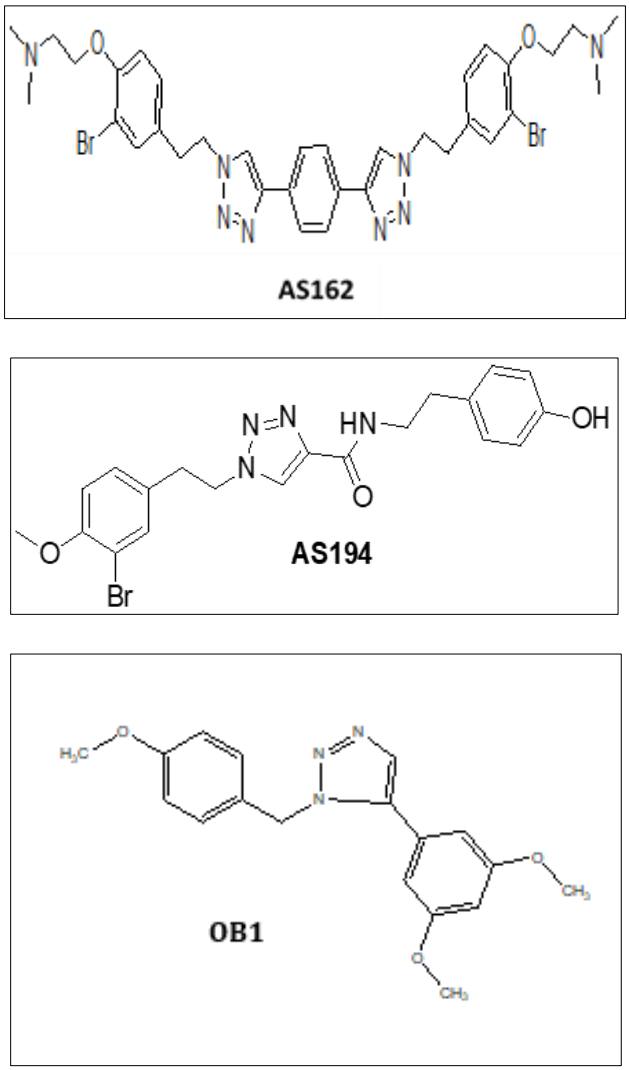
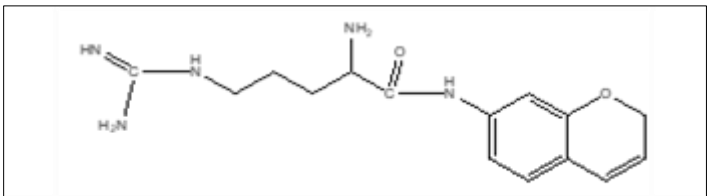


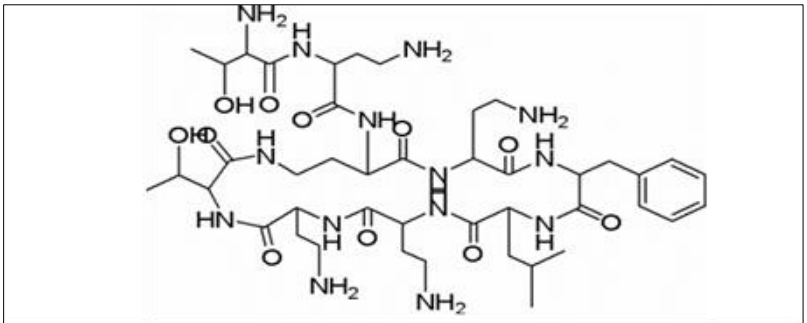
Figure 1 Structures of Analogs of Marine Natural Compounds

2.2. Functions and schematics of permeabilizers

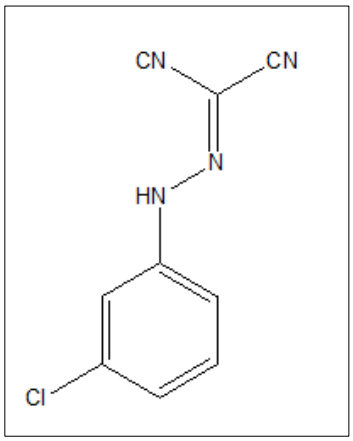
Three molecules, namely: Polymyxin B nanopeptide hydrochloride (PMBN), Phenylalanine-Arginine Beta-Naphthylamide (PAßN) and Carbonyl cyanide mchlorophenylhydrazone (CCCP), made it possible to carry out the combination test. These molecules, which may increase the level of permeability of bacterial cells, are each expected to produce effects necessary to reinforce the anti-biofilm effect of natural compounds. The schematic representation of the three molecules reveals structural variations, but a functional approximation. Thus, Polymyxin B nonapeptide hydrochloride (PMBN) (**FIG. 2a**) is a cationic cyclic peptide derived by enzymatic transformation of the natural polymyxin B peptide. The latter is capable of increasing the permeability of the outer membrane of Gram-negative bacteria to hydrophobic antibiotics, probably by binding to the bacterial lipopolysaccharide (LPS) (Tsubery et *al*., 2000). Phenylalanine-Arginine Beta-Naphthylamide (PAßN) (**FIG. 2b**) has sometimes been classified as an inhibitor of efflux pumps in Gram-negative bacteria. However, in this study it was considered a permeabilizer. In fact, studies conducted by Lamers et al. in 2013 showed that PAßN is capable of considerably reducing the MIC of the antibiotic, the "Lactamine", in *P. aeruginosa*. Finally, the m-chlorophenylhydrazone carbonylcyanide (CCCP) shown in **FIG. 2c** is a hydrazone bound to two nitriles and a chlorobenzene ring. In combination with reserpine, it showed membrane permeabilization activity in Acinetobacter baumannii, although it also showed inhibitory activity on efflux pumps in a large number of Gram-negative bacteria (Shi et *al*., 2005).



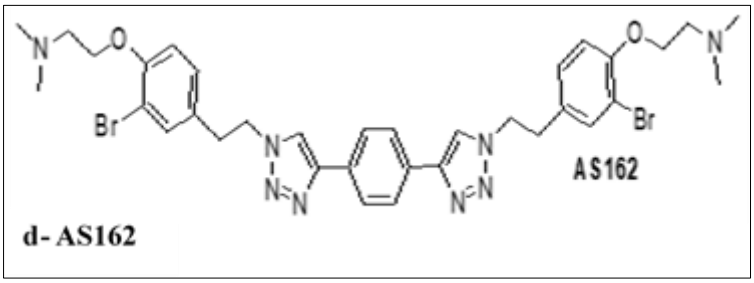
a- Polymyxin B nonapeptide hydrochloride (PMBN)



b- Phenylalanine-Arginine Beta-Naphthylamide (PAßN)



c- Carbonyl cyanide mchlorophenylhydrazone (CCCP)



d- AS162

Figure 2 Schemas of the three membranes permeabilizers and the AS162

3. Results and discussion

3.1. Study of the toxicity of membrane permeabilizers

The toxicity of the three adjuvants was determined in *P. ulvae* TC14 and two other marine strains, namely P. lipolytica TC8 and Paracoccus sp. 4M6 in transparent microplates. Resazurine was used to label living cells (blue) and dead cells (red). These molecules showed no toxic effect in the different strains at 24 h. After 48 h, the PMBN and the acetate sho

wed toxic effects from 20 µM and CCCP from 7 µM. Thus, the concentrations of permeabilizers chosen for the tests for combination with the synthetic analogs are 10 µM for PMBN and 1000 and 5 µM for CCCP. These did not show signific ant toxicity in *P. ulvae* TC14 or in other bacteria after 48 h. These results are summarized in **FIG 3** below:

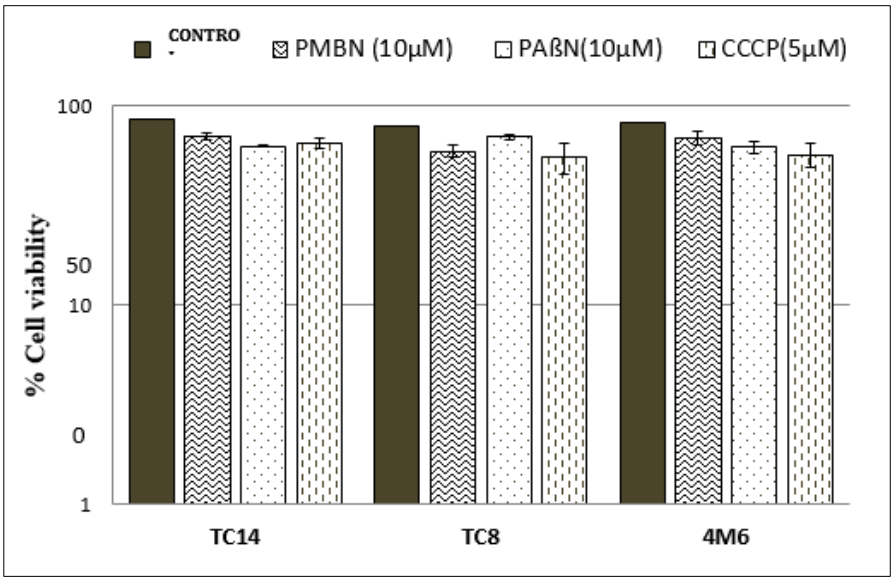


Figure 3 Evaluation of the toxicity of the permeabilizers in the three strains after 48 h. Three tests were carried out in 96-well transparent microplates. 10% of Resazurine was used for the labeling of the cells and the reading was made at TECAN at 570 nm. The results were reduced to a percentage. Statistical tests at $p<0.05$ showed no significant difference

3.2. Effect of the combination of analogs and permeabilizers on adhesion in *P. ulvae* TC14

The different concentrations of permeabilizers previously tested and not having a significant toxic effect on the strains were chosen for the combination tests. The results presented in FIGS. 4 below showed that the anti-adhesion effect of the compounds increased significantly. This increase follows a decreasing order from lower concentrations (10µM) to higher concentrations (up to 200µM) these results are similars to those of Salta, 2012. It should be noted that AS162, having a very marked individual release effect from the outset on *P. ulvae* TC14 (FIG. 4A), the synergistic effect is not marked with the permeabilizers in *P. ulvae* TC14 except with CCCP. Indeed, the combination of AS162 (10µM) and CCCP (5µM) reduces adhesion by almost 89% compared to 70% when AS162 is used alone at this same concentration. In addition, the combination of 10 µM with 5 µM CCCP induces a rate of reduction of bacterial adhesion close to that of AS162 (alone) at high concentrations, namely: 100 µM (88%), 150 µM (89%), 200 µM (90%). This method resulted in a very high anti-adhesion effect of AS162 at a relatively low concentration. In establishing a dose-toxicity ratio of the molecules, it should be said that this method is very advantageous, especially since there is less risk of toxicity at a low dose. At high concentrations (100 µM to 200 µM) the effect becomes stable (FIG. 4A). However, the combination of the permeabilizers with the other two analogs (AS194 and OB1) produces a well-marked synergistic effect on the adhesion of TC14 (FIGS. 4B and 4C). This shows that the less active the molecule is, the more its association with the permeabilizer increases its effect up to a zone of stability. Thus, OB1, which has a relatively low individual effect, has a very marked synergy in association with the permeabilizing agents. The association between 5 µM of CCCP and 100 µM of OB1, for example, induced a reduction rate of 92% (FIG. 4C) higher than that of AS162 (89%) employed alone at 10 µM. These results show that the cell permeabilization method allows optimization of the anti-adhesion effect while increasing the activity of the analogs. These results are presented in FIGS 4 below.

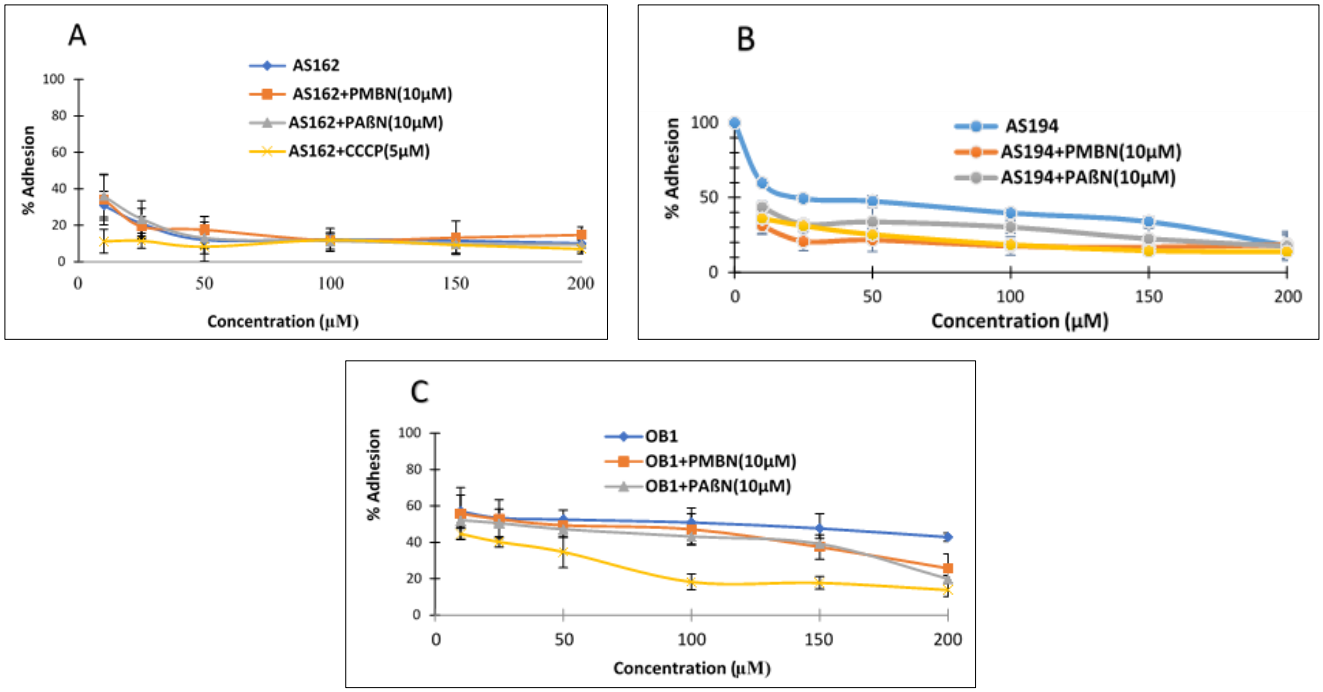


Figure 4 Effect of the combination of synthesized analogs with permeabilizers on adhesion in TC14. The tests were carried out three times in replicates in 96-well black microplates. Tests marked with letters were significantly different (P<0.05) from the control (cultures of bacteria without permeabilizers)

3.3. Study of EC_{50} of combinations on adhesion in TC14 and evaluation of potentiation of the effect of analogs.

The effective concentration to remove 50% of the adhesion of *P. ulvae* TC14 (EC_{50}) was calculated for each compound. The higher the anti-adhesion effect of the compound, the lower the EC_{50}. This method was used to evaluate the increase in the anti-biofilm effect of the compounds in the presence of the permeabilizers. It should be noted that the highest rates of reduction of bacterial adhesion are found in compounds with relatively low individual effect. Thus, OB1, the least active compound, has a strong increase in the anti-adhesion effect in combination with the permeabilizers. Indeed, the rate of reduction of the OB1 EC_{50} on accession is between 90,3 and 95,2 µM. In the increasing order of anti-adhesion effects, AS194 is second only to OB1. Under the same experimental conditions, the AS194 allowed a reduction rate of EC_{50} in the order of 74 to 81.5 µM, which remained below those of OB1. As for AS162, the most active compound, this rate of reduction is relatively low. Better still, the anti-adhesion effect of AS162 in combination with PMBN (10µM) and PAßN (10µM) has a fairly significant decrease. On the other hand, the EC_{50} is reduced by 52.6% with CCCP. This value remains below the EC_{50} values determined for OB1 and AS194. All these results described are presented in Table 1. Through these results, the permeabilization method shows a very high efficiency with molecules with a relatively low effect. This could be explained by the fact that the higher the individual effect of the compound, the more it competes with the permeabilizer. Thus, AS162, being the most active of the three molecules, could compete with the adjuvants so that the effect of the combined molecule remains quite small. Moreover, this difference in effect may be related to the different densities of the molecules. AS162 is a relatively dense molecule. It has a molar mass of 752.54 g/mol compared to 462.00 g/mol for AS194 and 325.00 g/mol for OB1. This density of AS162 could prevent it from entering the intracellular medium in large numbers. On the other hand, the other two less dense compounds are found at a high level inside the cell, hence the increase in their effect. In contrast, AS162 may have an extracellular effect. As a result, the presence of permeabilizer has little effect on its effect. The potentiation of the effect of these compounds having been demonstrated on the adhesion of TC14, in the next stage of our study, it is appropriate to carry out these same tests on the biofilm of this same bacterium at 48 h and 72 h. This step will evaluate the potentiation of the anti-biofilm effect of these compounds.

Table 1 Evaluation of EC_{50}s of the combination of natural compounds and permeabilizers on adhesion in P. ulvae TC14. The EC50 was determined using GraphPadPrism 5 software. The reduction rate was calculated by the formula: [100-(EC_{50} combination/EC_{50} natural compound alone)] X 100]. Tests marked with letters were significantly different ($P<0.05$) from the control. The values given by the sign (-) correspond to a reduction in the effect of AS162

Bacterial strains	Analogs + combinations with permeabilizers	EC50 (µM) on the adhesion of bacterial strains	EC50 Reduction Rate (%)
TC14	OB1	75,39±5,4	
	OB1+PMBN (10µM)	5,68±1,5c	92,5
	OB1+PAßN (10µM)	7,32±0,9c	90,3
	OB1+CCCP (5µM)	3,6±1,0c	95,2
	AS194	38,27±4,7	
	AS194+PMBN (10µM)	7,077±1,5c	81,5
	AS194+PAßN (10µM)	15,63±2,8b	59,2
	AS194+CCCP (5µM)	9,94±1,8a	74,0
	AS162	4,3±1,1	
	AS162+PMBN (10µM)	6,646±1,5b	-54,6
	AS162+PAßN (10µM)	6,887±1,0a	-60,2
	AS162+CCCP (5µM)	2,038±0,8b	52,6

3.4. Evaluation of the effect of combinations on biofilm in TC14 (48h/72h)

Since the anti-adhesion effect of the combinations between analogs and permeabilizers is thus evaluated, the effect of these same combinations on the biofilm should be tested in TC14 at 48 and 72 h. This step was used to assess the potentiation of the effect of the compounds over the long term. The results show that the dose effect observed during the anti-adhesion test reproduces during the biofilm test. It is therefore important to note that the anti-biofilm effect of the compounds is classified in the same order as the anti-adhesion effect. OB1 has the lowest anti-biofilm effect compared to AS194 and AS162. The latter compound has the highest antibiofilm effect with a reduction of almost 60% in the mass of biofilm at a concentration of only 10 µM. The combination of OB1 and the permeabilizers produces a very strong synergistic effect on the biofilm after 48 h. This synergistic effect is more visible with the CCCP (5 µM) (FIG. 5D and FIG. 5D′). The effect of the combination is more reinforced after 72 hours of culture (FIG. 5D′). The results obtained with OB1 are similar to those of AS194, that is to say a very marked synergism with CCCP at 48 h and which increases after 72 h (FIGS. 5E and 5E′). Combinations with PMBN and Paenzoya give a less pronounced synergistic effect which improves after 72 h of culture. Indeed, after 48 h, the combination of 50 µM of AS194 with these two permeabilizers reduces the mass of biofilm up to 41.16% and 43.18% respectively. At 72 hours, these same combinations give rise to a biofilm reduction of up to 30.61% for PMBN and 31.26% for Paositol (FIGS. 5E and 5E′). These results show that the anti-biofilm effect of the combinations persists and increases with time. Moreover, as in the adhesion tests, biofilm tests have shown that AS162 admits a stronger individual effect than the other two analogs. By adopting this combinatorial method, the effectiveness of these compounds almost reaches that of the molecules used by Xiurong et *al*., 2014. As a result, its combination with the permeabilizers gives rise to a less pronounced or non-existent synergistic effect in certain cases. This could be explained by a stability of effect. It is therefore important to note that the combination of AS162 and CCCP gives a very interesting long-term result (72 h). This analog, when used at a high concentration (200µM) in the presence of CCCP, tends to inhibit almost the entire mass of biofilm. Thus, the rate of biofilm which had decreased to 2.51%, i.e. a reduction of 97.49% in the presence of the AS162 alone, reached 1.54%, i.e. a reduction of 98.46% (i.e. a little less than 99%) in the presence of the combined molecule. This shows that by continuing this potentiation method with concentration adjustments, these values could reach 100% reduction of the biofilm (FIG. 5F′)

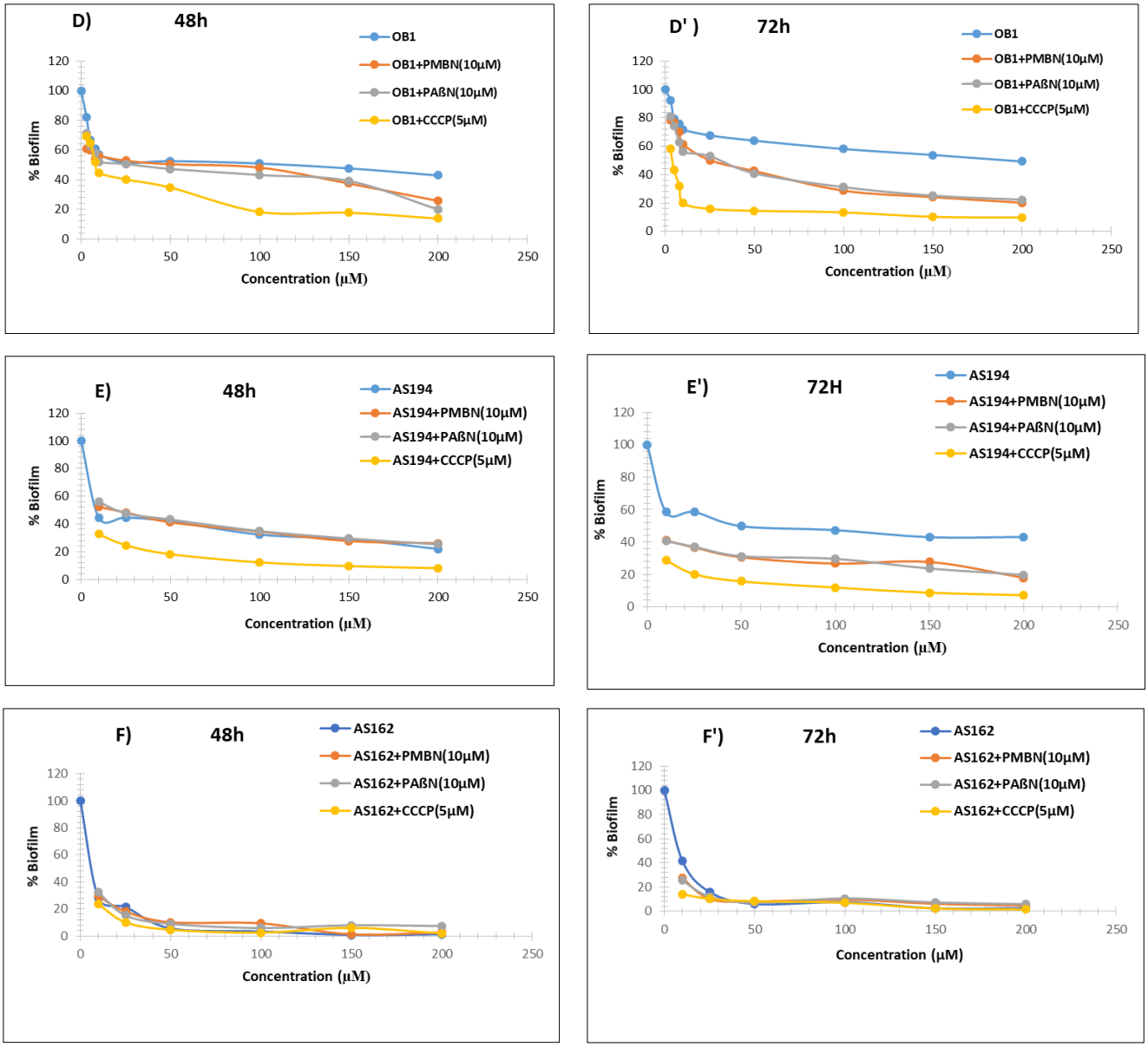


Figure 5 Effect of the combination of the analogs synthesized with the permeabilizers on the Biofilm in TC14. The tests were carried out three times in remnants in transparent microplates 96 wells. Tests marked with letters were significantly different (P<0.05) from the control (cultures of bacteria without permeabilizers)

3.5. Evaluation of the EC_{50}s of the different combinations on the biofilm in TC14

The effective concentrations to remove 50% of the biofilm (EC_{50}) were calculated for each combination. The EC_{50}s vary in the same direction as the anti-biofilm effects of the compounds. The lower they are, the stronger the effect of the compound. The EC_{50}s of the biofilm combinations after 48 h showed that the combination of the two types of compounds (synthetic analogs and permeabilizers) enhances the anti-biofilm effect of the natural compounds. However, in P. ulvae TC14, the synergistic effect appears to be less pronounced. This is expressed by cases of increases in the EC_{50} of combinations comprising AS194 on the one hand and AS162 on the other hand with PMBN and PAßN. On the other hand, with OB1, the synergistic effect is more pronounced under these same conditions. These results are similar to those of accession. OB1, having a lower individual effect, admits a more pronounced synergistic effect when combined with the permeabilizers. For example, the combination of OB1 and CCCP allows an EC_{50} in the order of 3.6 µM after 72 hours (Table 1, row 5, column 4). This concentration is lower than the EC_{50} of AS162 alone, the most active molecule, which is 6.006 µM. It is therefore important to note that potentiation of the effect of OB1 (the least active compound) resulted in a higher effect than that of AS162 (the most active compound).

4. Conclusion

This work evaluated the potentiation of the anti-adhesive and antibiofilm effect of synthetic analogues by permeabilizers. The results showed that the combination with these adjuvants induced an increase in the antifouling effect of these analogues without altering their level of toxicity. This work allows us to consider that membrane permeabilization can be considered an effective method of enhancing the effect of antibiofilm compounds.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Andjouh S, Perrin FX, Blache Y. In "bis-triazoles compounds with anti-bioflm and anti-corrosion properties". WO 2017/102883 A1, International Application Number. 2017. PCT/EP2016/081068. US 2018 / 0370953 A1. 80370953A1/en.

[2] Biggerstaff J., Le Puil M., Weidow B., Prater J., Glass K., Radosevich M., White D. New methodology for viability testing in environmental samples. Molecular and Cellular Probes. 2006. 20: 141-146.

[3] Chambers L D, Hellio C, Stokes K, Dennington S, Goodes L, Wood R, Walsh F Investigation of Chondrus crispus as a potential source of new antifouling agents. International Biodeterioration & Biodegradation. 2011. 65: 939-946.

[4] Decho W., Frey R. , Ferry J., Chemical Challenges to Bacterial AHL Signaling in the Environment. Chem. Rev. 2011, 111, 1, 86–99. Publication Date:December 10, 2010https://doi.org/10.1021/cr100311q.

[5] Erin E. G., Octavio L. F., Hancock R. E. W. Antibiotic Adjuvants: Diverse Strategies for ControllingDrug-Resistant Pathogens. Chem Biol Drug Des 2015; 85:56–78.

[6] Gozoua E., Koffi-Nevry R., Blache Y., Bioflm formation in marine bacteria and biocidal sensitivity: interplay between a potent antibioflm compound (AS162) and quorum-sensing autoinducers. 3 Biotech 2019. volume 9, Article number: 338.

[7] Hancock R. E. W. Peptide antibiotics. Lancet; 1997. Vol 349:418–422. February 8, 1997.

[8] Hancock R. E. W.et Wong P. G. W. Compounds which increase the permeability of the Pseudomonas aeruginosa outer membrane. Antimicrob Agents Chemother. 1984; 26:48–52.

[9] Linares D, Bottzeck O, Pereira O, Praud-Tabariès A, Blache Y Designing 2-aminoimidazole alkaloids analogs with anti-bioflm activities: structure–activities relationships of polysubstituted triazoles. Bioorg Med Chem Lett 21:6751.

[10] Linares D., Bottzeck O., Pereira O., Praud-Tabariès A. et Blache Y. Designing 2- aminoimidazole alkaloids analogs with anti-biofilm activities: structure–activities relationships of polysubstituted triazoles. Bioorg. Med. Chem. Lett. 2011. 21(22): 6751-6755.

[11] Sall C., Ayé M., Bottzeck O., Praud A., Blache Y. Towards smart biocide-free anti-biofilm strategies: Click-based synthesis of cinnamide analogues as anti-biofilm compounds against marine bacteria. Bioorganic & Medicinal Chemistry Letters. 2018. Volume 28, Issue 2. Pages 53-230.

[12] Salta M., Method development for enhanced antifouling testing using novel natural products against marine biofilms. Thesis submitted for the degree of doctor of biology. Faculty of engineering and the environment engineering sciences. 2012, Southampton, England.

[13] Savage P. B. Multidrug-resistant bacteria: overcoming antibiotic permeability barriers of gram-negative bacteria. Ann. Med. 2001. 33:167–171.

[14] Schmidt E. J., Boswell J. S., Walsh J. P., Schellenberg M. M., Winter T. W., Li C., Allman G. W. et Savage P. B. Activities of cholic acid-derived antimicrobial agents against multidrug-resistant bacteria. J. Antimicrob. Chemother 2001; 47:671–674.

[15] Scott M. G., Yan H. et Hancock R. E. W. Biological properties of structurally related a-helical cationic antimicrobial peptides. Infect. Immun. 1999. 67:2005–2009.

[16] Shi W., Jiang J., Xu Ning L., Huang Z., Wang Y., Inhibitory effects of reserpine and carbonyl cyanide m-chloro-phenylhydrazone on fluoroquinolone resistance of Acinetobacter baumannii In Chinese Medical Journal. 2005. 118(4). p.340-343.

[17] Stutzmann A. Synthesis of phenothiazine derivatives and study of their role as inhibitors of antibiotic resistance in Burkholderiaceae. Thesis defended on 12/2017. University of Aix-Marseille.

[18] Tsubery H., Ofek I., Cohen S., et Fridkin M., Structure-function studies of polymyxin B nonapeptide: implications to sensitization of gram-negative bacteria. J. Med. Chem. 2000 Aug 10; 43(16):3085-92.

[19] Vaara M. Agents that increase the permeability of the outer membrane. Microbiol Rev. 1991 56:395–411.

[20] Xiurong D., Zhizhong L., Jianrong S., Donghui Y. Human serum inhibits adhesion and biofilm formation in Candida albicans. BMC Microbiology. 2014. volume 14, Article number: 80 (2014).

[21] Zheng C., Riccardo B., Guglielmina F., EC50 estimation of antioxidant activity in DPPHradical dot assay using several statistical programs. Food Chemistry. 2013. Volume 138, Issue 1, 1 May 2013, Pages 414-420.