



PEGylated triarylmethanes: Synthesis, antimicrobial activity, anti-proliferative behavior and in silico studies

Christophe Ricco, Fatma Abdmouleh, Charlotte Riccobono, Léna Guenineche, Frédérique Martin, Elizabeth Goya-Jorge, Nathalie Lagarde, Bertrand Liagre, Mamdouh Ben Ali, Clotilde Ferroud, et al.

► To cite this version:

Christophe Ricco, Fatma Abdmouleh, Charlotte Riccobono, Léna Guenineche, Frédérique Martin, et al.. PEGylated triarylmethanes: Synthesis, antimicrobial activity, anti-proliferative behavior and in silico studies. *Bioorganic Chemistry*, 2020, pp.103591. <10.1016/j.bioorg.2020.103591>. <hal-02453626>

HAL Id: hal-02453626

<https://hal.science/hal-02453626v1>

Submitted on 7 Mar 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Copyright - All rights reserved

Graphical abstract

PEGylated triarylmethanes: Synthesis, antimicrobial activity, anti-proliferative behavior and *in silico* studies

Christophe Ricco^{a,§}, Fatma Abdmouleh^{a,b,§}, Charlotte Riccobono^a, Léna Guenineche^a, Frédérique Martin^c, Elizabeth Goya-Jorge^d, Nathalie Lagarde^a, Bertrand Liagre^c, Mamdouh Ben Ali^b, Clotilde Ferroud^a, Mehdi El Arbi^b, Maité Sylla-Iyarreta Veitía^{a*}

^a *Equipe de Chimie Moléculaire du Laboratoire Génomique, Bioinformatique et Chimie Moléculaire (EA 7528), Conservatoire national des arts et métiers (Cnam), 2 rue Conté, 75003, HESAM Université, Paris, France.*

^b *Laboratoire de Biotechnologie Microbienne et d'Ingénierie des Enzymes (LBMIE). Centre de Biotechnologie de Sfax, Université de Sfax, Route de Sidi Mansour Km 6, BP 1177, 3018, Sfax, Tunisie.*

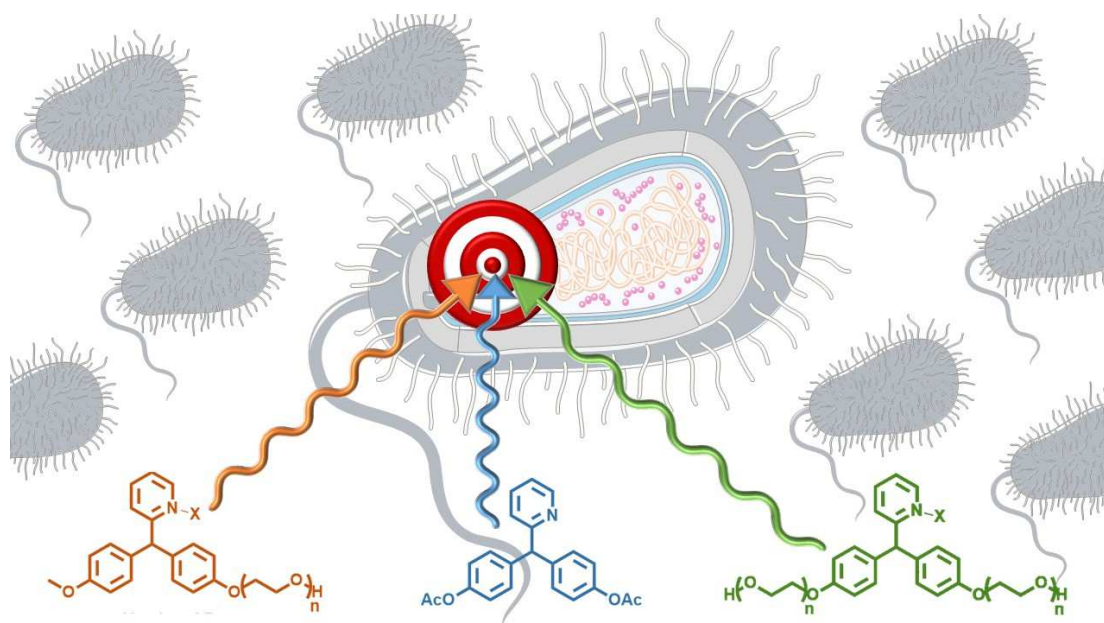
^c *Laboratoire PEIRENE, EA 7500, Département de Biochimie et de Biologie Moléculaire. Faculté de Pharmacie, Université de Limoges, 2, rue du Dr Marcland, 87025 Limoges, CEDEX*

^d *ProtoQSAR SL. CEEI (Centro Europeo de Empresas Innovadoras), Parque Tecnológico de Valencia, Av. Benjamin Franklin 12, 46980 Paterna, Valencia, Spain.*

§ authors contributed equally to this work

*Corresponding author

E-mail address: maite.sylla@lecnam.net (M. Sylla-Iyarreta Veitía)



PEGylated triarylmethanes: Synthesis, antimicrobial activity, anti-proliferative behavior and *in silico* studies

Christophe Ricco^{a, §}, Fatma Abdmouleh^{a,b,§}, Charlotte Riccobono^a, Léna Guenineche^a, Frédérique Martin^c, Elizabeth Goya-Jorge^d, Nathalie Lagarde^a, Bertrand Liagre^c, Mamdouh Ben Ali^b, Clotilde Ferroud^a, Mehdi El Arbi^b, Maité Sylla-Iyarreta Veitía^{a*}

^a *Equipe de Chimie Moléculaire du Laboratoire Génomique, Bioinformatique et Chimie Moléculaire (EA 7528), Conservatoire national des arts et métiers (Cnam), 2 rue Conté, 75003, HESAM Université, Paris, France.*

^b *Laboratoire de Biotechnologie Microbienne et d'Ingénierie des Enzymes (LBMIE). Centre de Biotechnologie de Sfax, Université de Sfax, Route de Sidi Mansour Km 6, BP 1177, 3018, Sfax, Tunisie.*

^c *Laboratoire PEIRENE, EA 7500, Département de Biochimie et de Biologie Moléculaire. Faculté de Pharmacie, Université de Limoges, 2, rue du Dr Marcland, 87025 Limoges, CEDEX*

^d *ProtoQSAR SL. CEEI (Centro Europeo de Empresas Innovadoras), Parque Tecnológico de Valencia, Av. Benjamin Franklin 12, 46980 Paterna, Valencia, Spain.*

We describe herein the synthesis, characterization and biological studies of novel PEGylated triarylmethanes. Non-symmetrical and symmetrical triarylmethanes series have been synthesized by Friedel-Crafts hydroxyalkylation or directly from bisacodyl respectively followed by a functionalization with PEG fragments in order to increase bioavailability and biological effectiveness. The antimicrobial activity was investigated against Gram-positive and Gram-negative foodborne pathogens and against *Candida albicans*, an opportunistic pathogenic yeast. The anti-biocidal activity was also studied using *Staphylococcus aureus* as a reference bacterium. Almost all PEGylated molecules displayed an antifungal activity comparable with fusidic acid with MIC values ranging from 6.25 to 50 µg/mL. Compounds also revealed a promising antibiofilm activity with biofilm eradication percentages values above 80% for the best molecules (compounds **4d** and **7**). Compounds **7** and **8b** showed a modest antiproliferative activity against human colorectal cancer cell lines HT-29. Finally, *in silico* molecular docking studies revealed DHFR and DNA gyrase B as potential anti-bacterial targets and *in silico* predictions of ADME suggested adequate drug-likeness profiles for the synthesized triarylmethanes.

Keywords: triarylmethane, PEGylation, antimicrobial and anti-proliferative activity, antibiofilm activity, drug-likeness, Molecular modeling, DHFR and DNA gyrase B inhibitors

§ authors contributed equally to this work

*Corresponding author: maite.sylla@lecnam.net (M. Sylla-Iyarreta Veitía)

1. Introduction

Triarylmethanes (TAMs) are molecules with three aryl groups (phenyls or heterocycles) bonded to a central carbon atom and they have numerous applications. They are essentially known as compounds derived from the dye industry and used for the dyeing of cotton, wool and silk [1, 2].

The various and very interesting properties of TAMs have attracted the attention of many scientists for several years [3]. They are used in the food and textile industry, in the field of health, and as antifungal agents and antiparasitic agents in the fish farming industry or else as cell division inhibitors for plants [4, 5]. Recent studies show that TAMs and derivatives are interesting from the therapeutic point of view and in particular in the development of anti-cancer[6, 7], anti-tuberculosis drugs [8, 9], antivirals [10, 11] and anti-inflammatory drugs [12]. TAMs made up of heterocycles such as indoles, pyridines, pyrroles and aromatic rings such as naphthalenes, are often used in the pharmaceutical and cosmetic industries [13-17].

Concerned by the high interest in infectious disease research, we previously reported the antibacterial activity of bisacodyl (BSL), drug triarylmethane type, used in therapeutics as laxative and a series of analogues bearing the ferrocenyl moiety. Our study revealed the potentialities of bisacodyl and its derivatives as antibacterial agents against gram-positive pathogens showing an antimicrobial activity comparable to fusidic acid, a steroid antibiotic of narrow spectrum known as a protein synthesis inhibitor [18]. However, some solubility problems were highlighted during the biological studies.

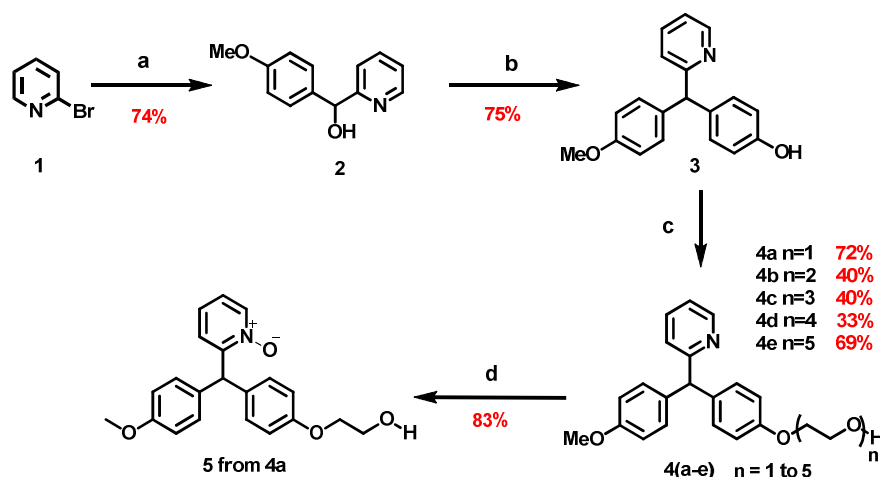
Small molecular drugs often suffer some drawbacks, such as low solubility, high toxicity, rapid excretion or untargeted biodistribution. To overcome these obstacles, one promising approach is to use a PEGylation strategy. Thanks to their favorable properties (nontoxic, non-immunogenic, non-antigenic and amphiphilic), PEG as modifying polymer plays an important role in drug discovery. PEG-drug conjugates have several advantages: prolonged residence in body, decreased degradation by metabolic enzymes, reduction or elimination of protein immunogenicity, and so on [19-22].

As part of our ongoing research and considering the importance of triarylmethane scaffold as a privileged structure in drug discovery, the present work deals with the synthesis and the biological evaluation of novel PEGylated triarylmethanes **4a-e**, **5**, **7**, **8a-f**, **9**, from commercial and simple raw materials. All the PEGylated compounds were evaluated against Gram-positive and Gram-negative pathogens, *Candida albicans* and by their antibiofilm activity. Furthermore, the antiproliferative activity of these derivatives was evaluated against two human colorectal cancer cell lines (HT-29 and HCT-116). Finally, docking studies were carried out to try to understand the mechanism of antimicrobial activity. *In silico* predictions were also used to analyze and comment ADME properties and drug-likeness of the novel PEGylated compounds.

2. Results and discussions

2.1 Chemistry

The synthesis described here involves the preparation of a novel series of PEGylated triarylmethanes to evaluate their biological potential. General synthetic methods to obtain the target compounds are outlined in Scheme 1 and 2. The non-symmetrical PEGylated derivatives were prepared following the synthetic pathways represented in Scheme 1. First the synthesis of the (4-methoxyphenyl)(pyridin-2-yl)methanol **2** has been accomplished by a magnesium-bromine exchange following the procedure described by Park *et al.* 2013[23], from 2-bromopyridine and *p*-anisaldehyde in anhydrous THF.

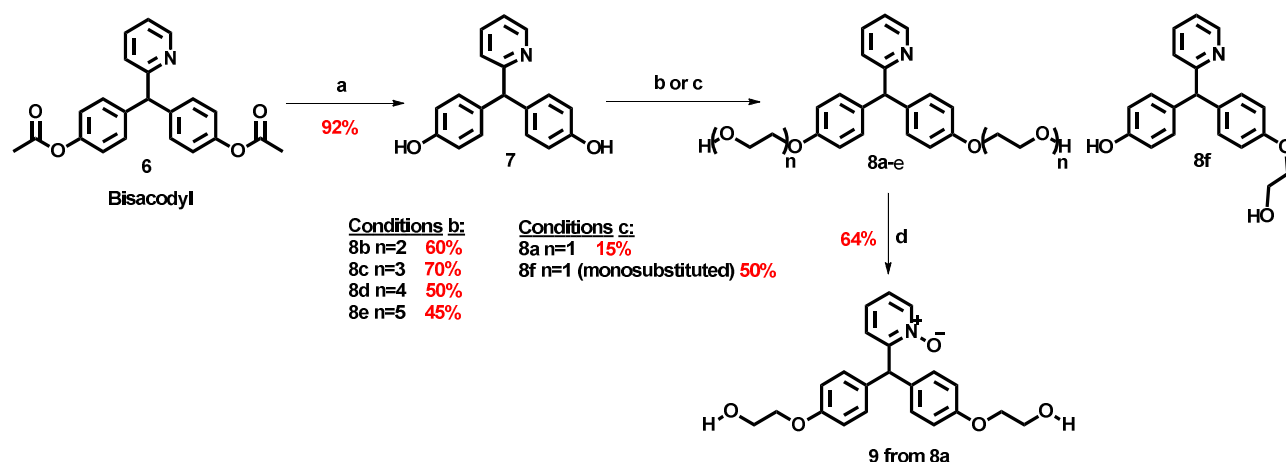


Scheme 1. Synthesis of non symmetrical PEGylated derivatives (a): (i) *i*-PrMgCl, THF, r.t., 2 h, Ar (ii) *p*-anisaldehyde, r.t., 2 h, Ar (b): phenol, H₂SO₄, PhNO₂, 0 °C, 5 min (c): corresponding glycol mono-tosylate, K₂CO₃, DMF anh, 80 °C, 4-7 days, Ar; (d): *m*-CPBA, dichloromethane, r.t., 2 h.

The synthesis of the 4-((4-methoxyphenyl)(pyridin-2-yl)methyl)phenol **3** was carried out by Friedel-Crafts hydroxylalkylation of compound **2** with phenol using concentrated sulfuric acid as brønsted acid. The reaction was very simple, avoiding the use of costly catalyst systems. The PEGylated non-symmetrical triarylmethanes **4(a-e)** were prepared from compound **3** and obtained in a range of 33% to 72% in high purity (¹H NMR > 96%).

For the preparation of symmetrical PEGylated triarylmethanes **8(a-e)**, bisacodyl was previously saponified using standard conditions. After 72 h at room temperature compound **7** was obtained in 92% yield and was pure enough to be used in the next step without any purification as suggested by the ¹H NMR analysis. The preparation of symmetrical PEGylated triarylmethanes **8(a-e)**, was performed by reaction of deacetylated bisacodyl **7** with the corresponding glycol mono-tosylates in DMF. The reaction was conveniently carried out by mixing the reagents at 80 °C in an inert solvent and then allowing the mixture to stirring overnight. Under these conditions the PEGylated compounds **8(b-e)** were obtained with yields ranging between 45% and 70% while compound **8a** could not be obtained. Consequently, a different procedure has been employed to prepare

compound **8a** using 2-bromoethanol in DMF in the presence of K₂CO₃ and KI. After 96 hours the desired compound **8a** was afforded in 15% yield mostly accompanied by the monosubstituted derivative **8f** (50% yield) (Scheme 2).



Scheme 2. Synthesis of symmetrical PEGylated derivatives (a): KOH, H₂O/EtOH, r.t., 72 h (b): corresponding glycol mono-tosylate, K₂CO₃, DMF anh, 80 °C, 24 h (c): 2-bromoethanol, KI, K₂CO₃, DMF anh, 80 °C, Ar, 96 h, (d): *m*-CPBA, dichloromethane, r.t., 3 h

N-oxide PEGylated derivatives **5** and **9** were prepared from the corresponding PEGylated triarylmethanes **4a** and **8a** respectively by oxidation with *m*-chloroperbenzoic acid in dichloromethane at room temperature (Scheme 1 and 2). 2-((4-(2-hydroxyethoxy) phenyl)(4-methoxyphenyl)methyl)pyridine *N*-oxide **5** and 2-(bis(4-(2-hydroxyethoxy) phenyl)methyl)pyridine *N*-oxide **9** were isolated after purification by flash chromatography on silica gel with non-optimized yields (83% and 64% respectively). These results have not been optimized and could probably be improved by modifying the treatment conditions. All synthesized compounds were biologically evaluated.

2.2. Biological evaluation

2.2.1 Antimicrobial activity

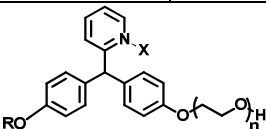
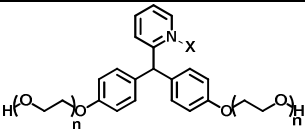
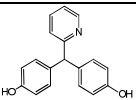
All synthesized final molecules were screened for their antibacterial activity against Gram-positive and Gram-negative pathogens by the 96-well ELISA microdilution method. Gram-positive strains *Micrococcus luteus* (LB14110), *Staphylococcus aureus* (ATCC 9144), Gram-negative strains *Escherichia coli* (ATCC 8739), *Salmonella enterica* (NCTC 6017) and *Pseudomonas aeruginosa* (ATCC 9027), were used for inhibitory tests, using levofloxacin, a broad spectrum antibiotic and fusidic acid, a narrow antibiotic as controls.

The previous results obtained with bisacodyl concerning its potential as antimicrobial agent, encouraged us to develop a new series of PEGylated triarylmethanes with improved antimicrobial potency and good pharmacological profile. The Table 1 summarizes the minimum inhibitory

concentration (MIC) values expressed in micrograms per milliliter ($\mu\text{g/mL}$) obtained for PEGylated derivatives (compounds **4a-e**, **5**, **7**, **8a-f**, **9**), bisacodyl and controls. The MIC and minimum bactericidal concentration (MBC) values for levofloxacin were found to be $< 1.52 \mu\text{g/mL}$ and 12.5-25 $\mu\text{g/mL}$ for fusidic acid for all pathogens. The results revealed that the MBC of all derivatives was superior than 100 $\mu\text{g/mL}$ which could mean that all evaluated compounds seem to be more bactericidal than bacteriostatic (MBC/MIC ratio is less than or equal to four) [24]. All the synthesized compounds **4a-e**, **5**, **7**, **8a-e**, **9** had a moderate *in vitro* antimicrobial activity against all microorganisms tested.

Table 1

Antimicrobial activities of non-symmetrical and symmetrical PEGylated triarylmethanes. Minimum inhibitory concentration (MIC) in $\mu\text{g/mL}$.

Cpd. Num	R, n, X	Minimum inhibitory concentration (MIC)) in $\mu\text{g/mL}$					
		Gram (+)		Gram (-)			Yeast
		<i>M. luteus</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. enterica</i>	<i>C. albicans</i>
							
4a	R= Me; n= 1	[6.25-12.5]	[50-100]	[25-50]	[25-50]	[25-50]	[25-50]
4b	R= Me; n= 2	[3.125-6.25]	[50-100]	[25-50]	[25-50]	[25-50]	[12.5-25]
4c	R= Me; n= 3	[3.125-6.25]	[25-50]	[25-50]	[25-50]	[25-50]	[25-50]
4d	R= Me; n= 4	[6.25-12.5]	[50-100]	[25-50]	[50-100]	[12.5-25]	[25-50]
4e	R= Me; n= 5	[6.25-12.5]	[12.5-25]	[25-50]	[12.5-25]	[25-50]	[12.5-25]
8f	R= H; n= 1	[3.125-6.25]	[50-100]	[25-50]	[25-50]	[25-50]	[12.5-25]
5	R= CH ₃ ; n= 1, X= O ⁻	[6.25-12.5]	[50-100]	[25-50]	[12.5-25]	[25-50]	[12.5-25]
							
7		[6.25-12.5]	[25-50]	[25-50]	[25-50]	[25-50]	[12.5-25]
8a	n= 1	[6.25-12.5]	[12.5-25]	[12.5-25]	[25-50]	[12.5-25]	[6.25-12.5]
8b	n= 2	[6.25-12.5]	[25-50]	[12.5-25]	[25-50]	[25-50]	[6.25-12.5]
8c	n= 3	[12.5-25]	[25-50]	[12.5-25]	[25-50]	[50-100]	[6.25-12.5]
8d	n= 4	[6.25-12.5]	[50-100]	[12.5-25]	[12.5-25]	[25-50]	[12.5-25]
8e	n= 5	[12.5-25]	[25-50]	[12.5-25]	[25-50]	[12.5-25]	[12.5-25]
9	n= 1, X= O ⁻	[6.25-12.5]	[50-100]	[12.5-25]	[12.5-25]	[12.5-25]	[12.5-25]
Lvf		<1.52	<1.52	<1.52	<1.52	<1.52	<1.52
FA		[12.5-25]	[12.5-25]	[12.5-25]	[12.5-25]	[12.5-25]	[12.5-25]

*MCB of levofloxacin $<1.52 \mu\text{g/mL}$, MCB of fusidic acid >100 except for *S. aureus* 50 $\mu\text{g/mL}$

BSL: bisacodyl; TAM OH, deacetylated metabolite of bisacodyl, Lvf: Levofloxacin, FA fusidic acid

In the non-symmetrical PEGylated series, compounds **4a-e**, **5**, **8f** seem to be more active against Gram (+) strains than Gram (-) ones. The greatest antimicrobial activity was obtained against *Micrococcus luteus* Gram (+) strains. Compounds **4a-e**, **5**, **8f** exhibited better activities (MIC $<$

3.125-6.25 or 6.25-12.5 $\mu\text{g/mL}$) as compared to fusidic acid MIC (12.5-25 $\mu\text{g/mL}$). Compounds **4b**, **4c** and **8f** showed the best antibacterial activity against *Micrococcus luteus* with MIC values between 3.125-6.25 $\mu\text{g/mL}$. The influence of the size of the PEGylated chain on the antibacterial activity is not easily discernable. Compounds **4b** and **4c** bearing methoxy group and a PEGylated chain with n equal 2 or 3 carbon atoms showed a higher activity against *Micrococcus luteus* (MIC < 3.125-6.25 $\mu\text{g/mL}$). Nevertheless, the activity slightly declines when the length of the PEGylated chain increases ($n=4$ or 5) (MIC < 6.25-12.5 $\mu\text{g/mL}$). Furthermore, when comparing the activities of compounds **4a** (MIC < 6.25-12.5 $\mu\text{g/mL}$) and **8f** (MIC < 3.125-6.25 $\mu\text{g/mL}$) against *Micrococcus luteus*, it could be assumed that methoxy group would be not critical for the activity. Compound **4e** showed the best activity against *Staphylococcus aureus*, with MIC values between 12.5-25 $\mu\text{g/mL}$. No difference for antimicrobial activity against both gram-negative pathogens was observed between non-symmetrical PEGylated triarylmethanes.

The symmetrical PEGylated derivatives (**8a-e**, **9**) like their non-symmetrical analogues also exhibited the greatest antimicrobial activity against *Micrococcus luteus* (MIC values between 6.25-25 $\mu\text{g/mL}$). However, no significant improvement of the activity was observed compared with the non-symmetrical ones. A similar trend was noticed for the antibacterial activity against *Staphylococcus aureus*. These results could suggest that hindered compounds would have difficulties in crossing the thick peptidoglycan layer of Gram (+) pathogens.

In addition, compared with their non-symmetrical analogues, the symmetrical PEGylated compounds **8a-e** showed better activity against Gram (-) pathogens. The best results were obtained against *Escherichia coli* with MIC values between 12.5-25 $\mu\text{g/mL}$ for **8a-e** versus 25-50 $\mu\text{g/mL}$ for **4a-e**.

Even if no significant difference in activity against *Candida albicans* was observed for all evaluated compounds, symmetrical PEGylated derivatives **8a-e** seem to be more active than the corresponding non-symmetrical ones. Compounds **8a-c** displayed the greatest antimicrobial activity against *Candida albicans* (MIC values between 6.25-12.5 $\mu\text{g/mL}$). Almost all compounds displayed an antifungal activity comparable with that of fusidic acid with MIC values ranging from 6.25-12.5 or 12.5-25 $\mu\text{g/mL}$ except for **4a**.

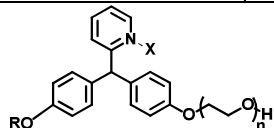
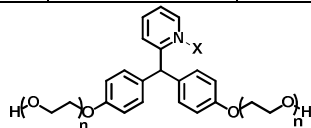
Furthermore, *N*-oxide moiety was introduced (compound **5** and **9**) in order to improve the antimicrobial activity. It has been noticed that the introduction of a pyridine *N*-oxide moiety could play a crucial role in the biological activity. This group could increase oxidation resistance improving the pharmacokinetic parameters [25-28]. By matching the compounds **4a** and **8a** with their corresponding pyridine *N*-oxides **5** and **9** no significant differences in antimicrobial activity were observed for the different microorganisms tested.

Considering the antimicrobial activity of synthesized analogues, we estimate the antimicrobial effect of PEGylated compounds on *Micrococcus luteus* and *Candida albicans* strains by

determining EC₅₀ (effective concentration 50%), using levofloxacin and fusidic acid as controls. The antibiotic potential is indicated by the fraction of bacteria killed by the drug and compounds were evaluated at concentrations between 200 and 1.152 μM. The EC₅₀ was recorded as the concentration of drug required to produce 50% of the maximum biological response. The results obtained are outlined in Table 2.

Table 2

EC₅₀ in μM of non-symmetrical and symmetrical PEGylated triarylmethanes for *Micrococcus luteus*.

Cpd. Num	R, n, X	EC ₅₀ (μM)	Cpd. Num	R, n, X	EC ₅₀ (μM)
		<i>M. luteus</i>			<i>M. luteus</i>
					
4a	R= Me ; n= 1	128	7	TAMOH	144
4b	R= Me; n= 2	88	8a	n= 1	105
4c	R= Me; n= 3	62	8b	n= 2	113
4d	R= Me; n= 4	120	8c	n= 3	110
4e	R= Me; n= 5	86	8d	n= 4	100
8f	R= H; n= 1	101	8e	n= 5	81
5	R= CH ₃ ; n= 1, X= O ⁻	312	9	n= 1, X= O ⁻	63
BSL		79			
LvF		4.10 x 10 ⁻⁶			
FA	.	3.7			

BSL: bisacodyl; TAM OH deacetylated metabolite of bisacodyl, LvF: Levofloxacin, FA fusidic acid

It may be noticed that compounds **4c** and **9** are the more active derivatives against *Micrococcus luteus* with EC₅₀ values of 62 and 63 μM respectively and comparable to that of bisacodyl (79 μM).

2.2.2. Biocidal activity

All synthesized compounds and bisacodyl were evaluated for their biocidal activity. The results are illustrated in Figure 1 and Table 3. *Staphylococcus aureus* was selected as a reference bacterium for biofilm production using Congo red agar (CRA) and 96 wells plate methods. The details of these procedures are given in the experimental section and supplementary data.

The evaluation of the ability of the synthesized compounds to inhibit the early biofilm formation was performed according to the minimum inhibitory concentration of each compound. The obtained results revealed that three symmetrical PEGylated compounds (**8b**, **8c** and **8d**), the bisacodyl and its desacetylated derivative **7**, inhibited the biofilm formation with percentages superior to 45%. Compounds from non-symmetrical series seemed to be less active with a decrease of anti-

adherence activity when the PEGylated chain increase. Results showed that the highest anti-adhesive effect was observed for compound **8d** with an inhibition percentage of 58% at 100 µg/mL. The results displayed that all compounds induce efficient eradication of the biofilm formed with eradication activity percentages between 45% and 83% (Figure 1, Table 3). Compounds from non-symmetrical series seemed to be better biofilm eradication agents than their symmetrical analogues. These results could suggest that the introduction of a second PEGylated chain it is not necessary for the eradication of biofilm. Compounds **4d** and deacetylated bisacodyl (compound **7**) showed the best antibiofilm activities with eradication percentage superior to 80%.

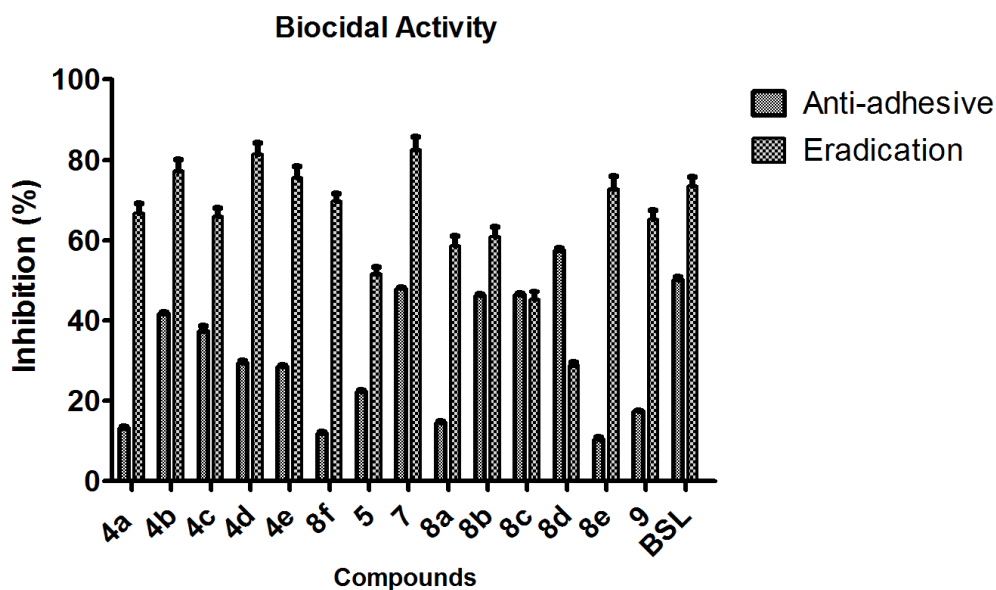


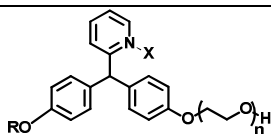
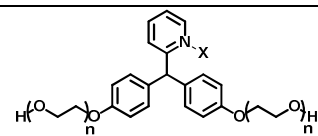
Fig.1 Evaluation of anti-adhesive activity (pre-treatment) of PEGylated compounds, using *Staphylococcus aureus* as reference bacterium. The final concentrations into the wells were ranged between 100 µg/mL and 25 µg/mL according with MIC values against *Staphylococcus aureus*. The percentage of the adhesion inhibition was determined by the following formula: % adhesion inhibition (at 570 nm) = $[(\% \text{ OD (control)} - \text{OD (treated bacterium)}) / \text{OD (control)}] \times 100$

Evaluation of antibiofilm activity (eradication) of PEGylated compounds at concentration of 200 µg/mL after 24 h of incubation at 37 °C. The percentage inhibition of the biofilm was determined at 570 nm by the following formula: % inhibition of biofilm = $[(\% \text{ OD (control)} - \text{OD (treated bacterium)}) / \text{OD (control)}] \times 100$. Data are shown as mean \pm SEM of antibiofilm activity in three independent experiments (n=3).

Table 3

Evaluation of adhesion inhibition* and eradication of biofilm**

Cpd. Num	R, n, X	% of adhesion inhibition	% of eradication	Cpd. Num	R, n, X	% of adhesion inhibition	% of eradication
----------	---------	--------------------------	------------------	----------	---------	--------------------------	------------------

							
4a	R= Me; n= 1	13	67	7	TAMOH	48	83
4b	R= Me; n= 2	42	77	8a	n= 1	15	59
4c	R= Me; n= 3	37	66	8b	n= 2	46	61
4d	R= Me; n= 4	29	81	8c	n= 3	46	45
4e	R= Me; n= 5	29	76	8d	n= 4	58	28
8f	R= H; n= 1	12	70	8e	n= 5	11	73
5	R= CH ₃ ; n= 1, X= O ⁻	22	52	9	n= 1, X= O ⁻	17	65
				BSL		50	74

* Each compound was evaluated at a concentrations according to MIC values against *S. aureus*: At 100 µg/mL for compounds **4a**, **4b**, **4d**, **8f**, **5**, **8d**, **9**; at 50 µg/mL for compounds **4c**, **7**, **8b**, **8c**, **8e**; at 25 µg/mL for compounds **4e** and **8a**.

** Compounds were evaluated at concentration of 200 µg/mL

The antibiofilm activity of the compound with the best eradication percentage (compound **7**, 83%) was confirmed by fluorescence microscopy. The images of the acridine orange staining treated slides with compound **7** displayed the reduction in the biofilm-coated surface compared to the control (Figure 2). The obtained results demonstrated the ability of compound **7** to modify the surface properties of bacterial cells and to reduce their adhesive properties. These results also confirm that compound **7** is more effective in eliminating the biofilm by eradication than by anti-adhesion (83% and 48% respectively).

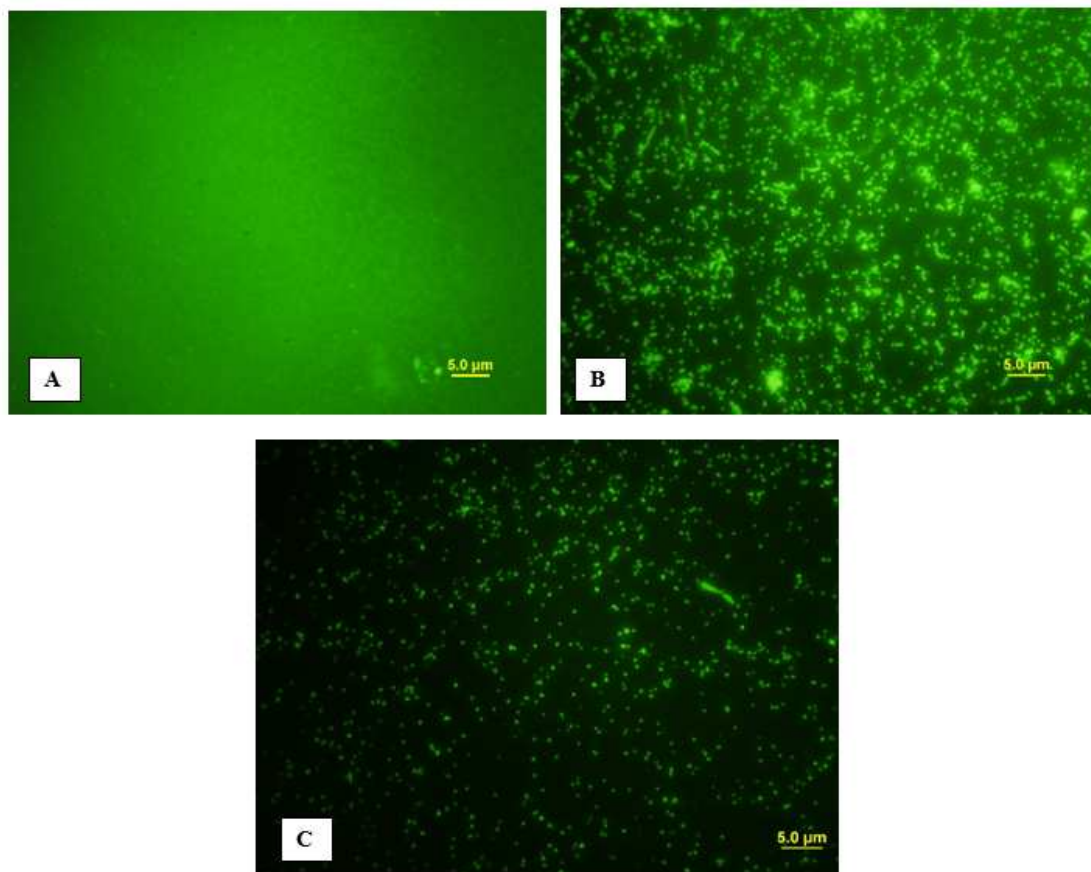


Fig.2 Fluorescence microscopy images (9×40) of anti-adherence activity of non-treated biofilm (A) compared with biofilm treated by the compound **7** against *Staphylococcus aureus* by anti-adhesion (treatment at 50 $\mu\text{g/mL}$) (B) and eradication (treatment at 200 $\mu\text{g/mL}$) (C) The formation of biofilm (untreated biofilm) is represented by the green areas and the necrosis zones of the biofilm are represented by the black ones.

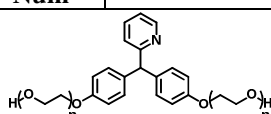
The biofilm metabolic activity of compound **7** was measured using the MTT assay. The compound **7** reduced significantly the metabolic activity in the *Staphylococcus aureus* biofilm by anti-adhesion (78%) and by eradication (75%). These results suggest that the inhibition of the biofilm metabolism may be one of the mechanisms of action of antibiofilm activity.

2.2.3. Antiproliferative activity

Antiproliferative activity of derivatives **7**, **8a-e**, **9** was evaluated by MTT assay using human colorectal cancer cell lines (HT-29 and HCT-116). MTT assay were performed 24, 48 and 72 h after treatment and cell viability was expressed in percentage of each condition of treatment by normalizing to untreated cells. The results obtained for symmetrical triarylmethane series are outlined in Figure 3 and Table 4. Antiproliferative effects of evaluated PEGylated triarylmethanes were moderate. Comparing the cell viability percentages, HT-29 cells line seemed to be more sensitive than HCT-116. However, for HT-29 cells, some significant differences were observed for bisacodyl (BSL) and their PEGylated analogues at 24 h or 48 h after 100 μM treatment. At 72 h, BSL, its deacetylated derivative **7** and compound **8b** displayed the best results with inhibition percentages of cell viability of 34.06%, 35.76% and 54.42% respectively. At this high *in vitro* concentration (100 μM) and due to a relatively long treatment time (72 h), it would therefore appear that these molecules are not only no-cytotoxic but also have no antiproliferative effect on HT-29 cancer cells.

Table 4

Cell viability of bisacodyl (BSL) TAM OH and their PEGylated analogues **8a-e** and **9**.

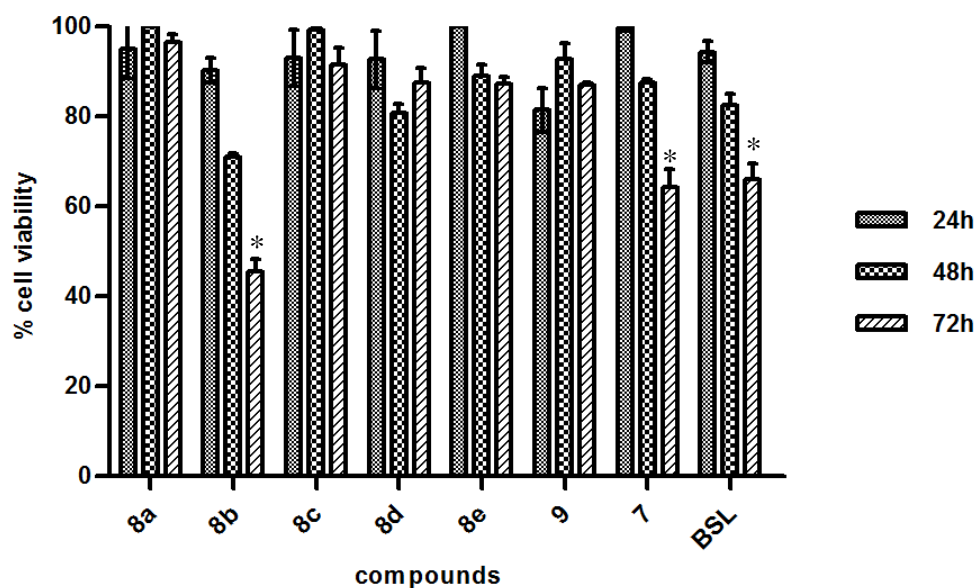
Cpd. Num		% cell viability					
		HT-29			HCT-116		
							
		24 h	48 h	72 h	24 h	48 h	72 h
7	TAMOH	99.72	87.65	64.24	98.84	77.79	81.51
8a	n= 1	95.04	100	96.54	99.06	73.76	75.27
8b	n= 2	90.33	71	45.58	100	91.28	93.23
8c	n= 3	92.97	99.25	91.60	89.65	77.58	87.36

8d	n= 4	92.68	80.83	87.47	87.35	69.57	72.95
8e	n= 5	100	88.94	87.31	84.04	67.10	79.84
9	n= 1, X= O ⁻	81.50	92.70	86.96	92.90	74.02	83.60
BSL		94.35	82.44	65.94	99.97	77.01	73.28
Control		100	100	100	100	100	100

TAMOH: deacetylated metabolite of bisacodyl

A

Antiproliferative activity (HT-29 cell line)



B

Antiproliferative activity (HCT-116 cell line)

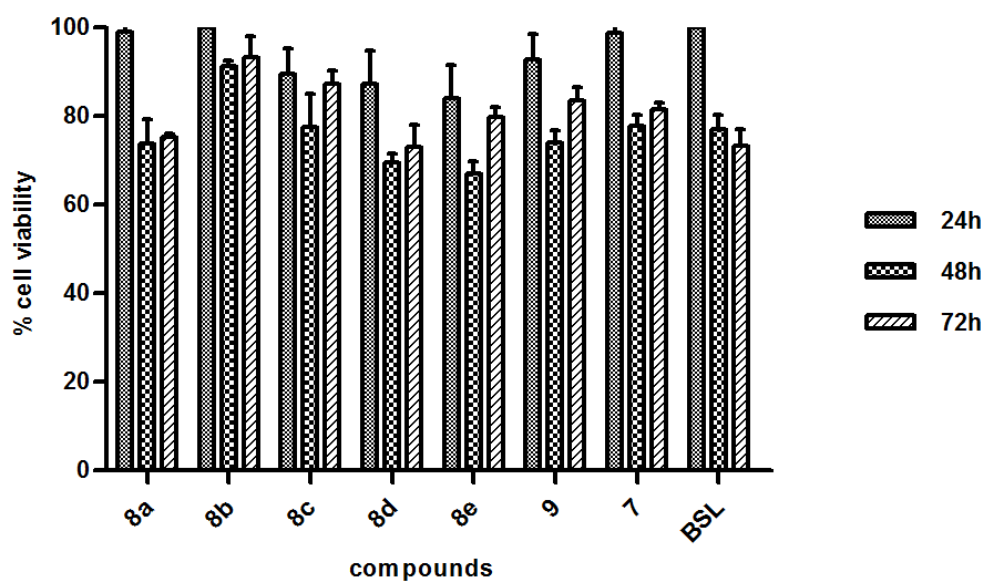


Fig. 3 Effect of compounds **7**, **8a-e**, **9** and bisacodyl (BSL) on cell proliferation. HT-29 and HCT116 cells were treated at 100 μ M and relative cell viability was assessed by MTT dye exclusion method for 24, 48 and 72 h. Data are shown as mean \pm SEM of cell viability compared to untreated cells as control (100%) in three independent experiments (n=3). *p < 0.05.

2.2.4. *In silico* studies

2.2.4.1 Docking studies

Following the biological evaluation, docking studies were conducted in order to predict potential anti-bacterial targets for the synthesized molecules. Three well-known antibacterial compounds targets were selected according to the scientific literature: dihydrofolate reductase (DHFR)[29], DNA gyrase B [30] and heptaprenyl diphosphate synthase (HepPPS)[31]. For each of these targets, molecules BSL, **4a**, **4d**, **7**, **8a** and **8d** were docked into the defined binding sites. For each target, the pose associated with the best docking score for each conformation of each molecule was conserved and submitted to a re-scoring procedure using the scoring function HYDE. For each pose, we thus obtained a range of estimated affinity with a lower limit and an upper limit (Figure 4).

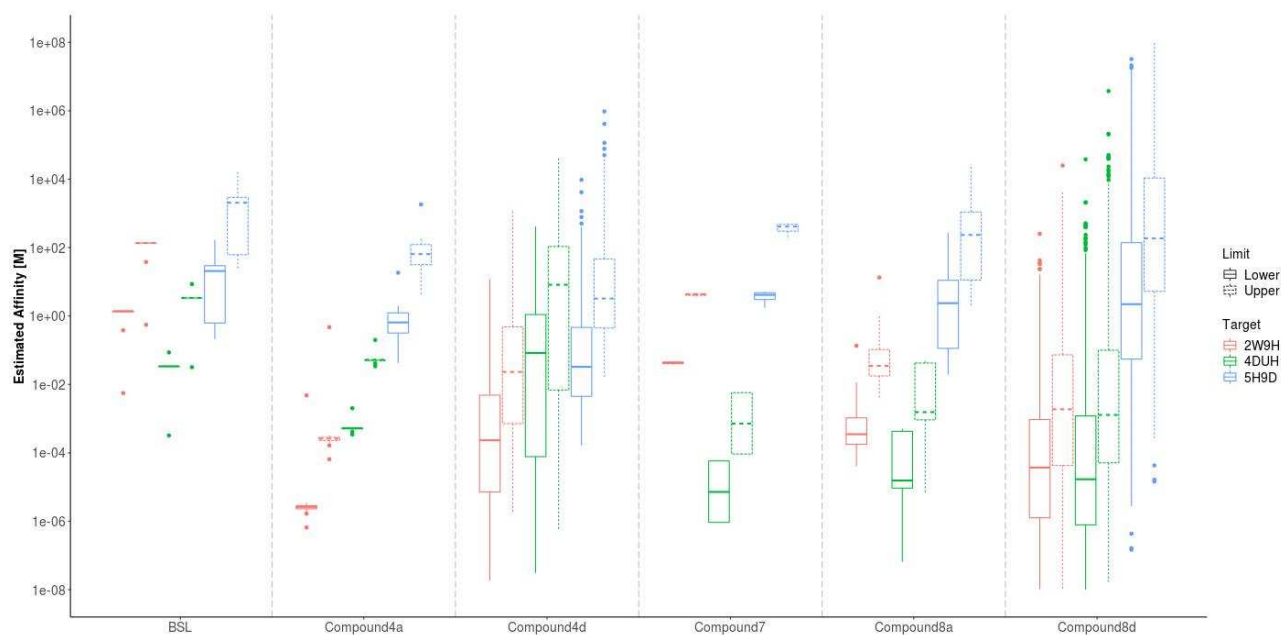


Fig. 4 Boxplots representing the estimated affinity values (in M) as predicted by HYDE for the docking poses obtained with the DHFR structure (2W9H, salmon), the DNA gyrase B structure (4DUH, green) and the HepPPS structure (5H9D, blue). The plain lines and dashed lines represent respectively the lower and the upper limits of estimated affinity

Focusing on the outcomes associated with HepPPS, the median estimated affinity is very low for all compounds, around 1 M for the lower limit (see Figure 4, blue boxes). Results obtained with DHFR (Figure 4, salmon boxes) show that compounds BSL and **7** present very low estimated affinities

(around 1 M and 10^{-2} M respectively for the lower limits). For compounds **4d** and **8a**, the estimated affinity median values are in the 0,1mM to 10 mM range and the best results are associated with compounds **4a** and **8d** with estimated affinity median values in the 1/10 μ M to mM range. Finally, compounds docked into the DNA gyrase B binding site (Figure 4, green boxplots) present estimated affinity median values in the 10 μ M to mM range for compounds **7**, **8a** and **8d**. Lower estimated affinity median values were obtained for BSL and compound **4d** (10 $^{-1}$ to 10 M range) and compounds **4a** (mM to 0,1M range). Comparison between these estimated affinity values and the experimental results obtained for the synthesized compounds tend to exclude HepPPS from the potential targets. Indeed, very low estimated affinity values were associated with this target whereas the synthesized compounds demonstrated *in vitro* anti-bacterial activity. On the opposite, the estimated affinity values obtained for the docking poses into DHFR and DNA gyrase B are compatible with the experimental results. Visual inspection of the docking poses of the synthesized compounds into the DHFR and DNA gyrase B binding sites also revealed that the predicted binding mode are quite similar to the binding mode of the co-crystallized ligands (Figure 5 A and B).

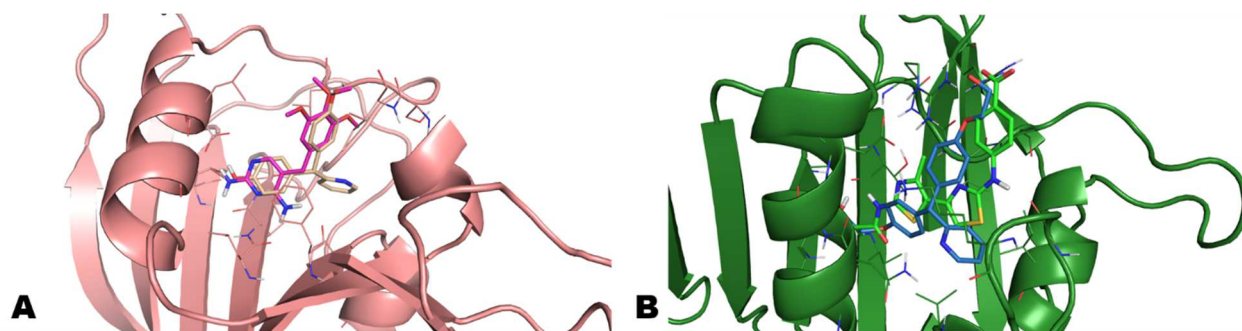


Fig. 5 Binding mode prediction of (A) compound **7** (represented in stick and colored in wheat) into the DHFR binding site and (B) compound **8a** (represented in stick and colored in blue) into the DNA gyrase B binding site. The co-crystallized ligands are also represented in stick in their respective binding site (in pink for DHFR and in green for DNA gyrase B) and used as the reference.

Two anti-bacterial targets, DHFR, a key enzyme controlling purine and thymidine nucleotide biosynthesis [32] and DNA gyrase B that is able to modulate DNA topology [33] constitute potential targets for the synthesized compounds herein presented.

2.2.4.2 ADME and drug-likeness profiles

In view of describing the potential for good bioavailability and to evaluate the drug-likeness of the synthesized PEGylated compounds, *in silico* predictions of ADME (absorption, distribution, metabolism and excretion)-related pharmacokinetic and physicochemical properties were

performed. The results obtained are shown in Table 5 and the recommended values for each parameter are listed below[34, 35].

Table 5

Predictions of ADME-related parameters and drug-likeness of compounds **4a-e**, **5**, **7**, **8a-e**, **9**.

Compound	MW ^a	#stars ^b	#rotor ^c	PSA ^d	HBD ^e	QLogPo/W ^f	QLogS ^g	QPPCaco ^h	QPPMDCK ⁱ	QPPKp ^j	Lipinski's Rule of five ^k
4a	335.402	0	8	48.623	1	4.676	-5.343	2543.778	1357.141	-0.5	0
4b	379.455	0	11	59.757	1	4.682	-5.245	2156.846	1135.453	-0.407	0
4c	423.508	0	14	68.373	1	4.931	-5.749	2156.855	1135.458	-0.12	0
4d	467.561	3	17	76.83	1	5.159	-6.192	2149.919	1131.511	0.166	1
4e	511.614	4	20	85.409	1	5.215	-6.05	2151.926	1132.653	0.446	2
8f	321.375	0	8	62.896	2	3.785	-4.67	774.734	375.437	-1.475	0
5	351.401	0	8	57.487	1	4.627	-5.456	1784.746	925.283	-0.836	0
7	277.322	0	5	54.363	2	3.536	-4.163	828.409	403.629	-1.65	0
8a	365.428	0	11	73.898	2	3.779	-5.215	581.905	275.539	-1.507	0
8b	453.534	3	17	91.238	2	4.285	-5.763	582.486	275.836	-0.932	0
8c	541.64	5	23	106.507	2	4.625	-6.197	584.107	276.666	-0.353	1
8d	629.746	8	29	121.093	2	5.147	-7.016	721.751	347.763	0.441	3
8e	717.852	11	35	138.463	2	5.398	-7.359	734.68	354.502	1.004	3
9	381.427	0	11	80.292	2	3.394	-5.338	508.191	238.013	-1.611	0

a Molecular weight of the molecule (recommended value: 130-725).

b Number of property or descriptor values that fall outside the 95% range of similar values for known drugs (recommended value: 0-5).

c Number of non-trivial (not CX3), non-hindered (not alkene, amide, small ring) rotatable bonds (recommended value: 0-15).

d Van der Waals surface area of polar nitrogen and oxygen atoms (recommended value: 7-200).

e Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution (recommended value: 0-6).

f Predicted octanol/water partition coefficient (recommended value: -2.0 to 6.5).

g Predicted aqueous solubility, log S. S in mol dm⁻³ is the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid (recommended value: -6.5 to 1.5).

h Predicted apparent Caco-2 cell permeability in nm/sec. Caco-2 cells are a model for the gut-blood barrier (< 25 is poor and > 500 is great).

i Predicted apparent MDCK cell permeability in nm/sec. MDCK cells are considered to be a good mimic for the blood-brain barrier (< 25 is poor and > 500 is great).

j Predicted skin permeability, log Kp (recommended value: -8.0 to -1.0).

k Number of violations of Lipinski's rule of five (max is 4). Coloured cells correspond to excellent (green), medium (yellow) or poor (grey) compliance of the rule.

The molecular weight (MW), van der Waals surface area of polar nitrogen and oxygen atoms (PSA) as well as the estimated number of hydrogen bonds donors (HBD) available to establish hydrogen bonds in an aqueous solution are consistent with the recommended values for drug-like compounds. Meanwhile, the number of rotatable bonds exceeded the recommended maximum of 15 in the cases of **4d**, **4e** and from **8b-8e**.

The 95% range of similarity respect to known drugs (#stars) reached the recommended values for all except for compounds **8d** and **8e**. The aqueous solubility (QPlogS) of these last two triarylmethanes was predicted as insufficient as well. These results could suggest that the introduction of bulky PEGylated chains on the triarylmethane skeleton is not favourable to develop drug-like compounds. It should be noticed that three of the predicted ADME properties satisfied the recommended values for all the compounds analysed: the octanol/water partition coefficient, the apparent Caco-2 cell permeability, and the MDCK cell permeability. Such predictions suggested a good bioavailability of the triarylmethanes synthesized. However, the skin permeability reached the recommended values only for the compounds **7**, **8a**, **8f** and **9**.

Overall, all the synthesized triarylmethanes are compliant with the Lipinski's rule of five, which demonstrates an adequate drug-likeness profile of the set. In fact, more than 60% of the compounds comply with the five rules, as recommended.

3. Conclusions

In summary two series of PEGylated triarylmethanes symmetrical and non-symmetrical were prepared, characterized and biologically evaluated. Twelve new compounds were straightforwardly synthesized by Friedel-Crafts hydroxylalkylation reaction or by nucleophilic substitution from deacetylated bisacodyl and then functionalized with PEG moieties. All compounds were tested on Gram-positive and Gram-negative foodborne pathogens and against *Candida albicans*. The biological results revealed that these compounds are potentially more effective against Gram-negative bacteria in particular against *Micrococcus luteus*. Almost all PEGylated molecules displayed an antifungal activity comparable to that of fusidic acid with MIC values ranging from 6.25 to 50 µg/mL. They also showed a promising antibiofilm activity with inhibition percentages superior to 80% for the best molecules (compounds **4d** and **7**). The MTT assay suggested that the inhibition of the biofilm metabolism could be one of the mechanisms of action of antibiofilm activity. Nevertheless, those compounds have shown a modest anti-proliferative activity against colorectal cancer cell lines HT-29. Finally, *in silico* molecular docking studies proposed that DHFR and DNA gyrase B could be a potential antibacterial targets and *in silico* ADME predictions suggested that synthesized compounds have good drug-likeness and ADME profiles, except for **8d** and **8e**. Thus, the introduction of long PEGylated chains in triarylmethane skeleton seems to disfavor the drug-likeness properties of the studied compounds.

The importance of anti-infective research field encourages us to continue the study of based triarylmethane skeleton compounds in order to find new potential antimicrobial molecules. The development of new series of triarylmethane bearing various aminoalkyl chains is the subject of current investigations. Moreover, additional studies focus on the biochemical mechanism of action

of antibiofilm activity are now underway in our laboratory and results will be published in due course.

4. Experimental section

4.1. Chemicals

All reagents were obtained from commercial sources unless otherwise noted, and used as received. Heated experiments were conducted using thermostatically controlled oil baths and were performed under an atmosphere oxygen-free in oven-dried glassware. All reactions were monitored by analytical thin layer chromatography (TLC). TLC was performed on aluminium sheets precoated silica gel plates (60 F₂₅₄, Merck). TLC plates were visualized using irradiation with light at 254 nm or in an iodine chamber as appropriate. Flash column chromatography was carried out when necessary using silica gel 60 (particle size 0.040-0.063 mm, Merck).

4.2. Physical measurements

The structure of the products prepared by different methods was checked by comparison of their NMR, IR and MS data and by the TLC behavior. ¹H and ¹³C-NMR spectra were acquired on a Bruker BioSpin GmbH spectrometer 400 MHz, at room temperature. Chemical shifts are reported in δ units, parts per million (ppm). Coupling constants (*J*) are measured in hertz (Hz). Splitting patterns are designed as follows: s, singlet; d, doublet; dd, doublet of doublets; dm, doublet of multiplets, ddd, doublet of doublets of doublets; m, multiplet; br, broad. Various 2D techniques and DEPT experiments were used to establish the structures and to assign the signals. For the assignments of the NMR signals, we use the convention presented in Figure 3. Low resolution mass spectra (ESI-LRMS) were performed from ionization by electrospray on a Waters Micromass ZQ2000. Infrared spectra were recorded over the 400-4000 cm⁻¹ range with an Agilent Technologies Cary 630 FTIR/ ATR/ ZnSe spectrometer. High-resolution mass spectra (HRMS) analyses were acquired on a Thermo Scientific LTQ Orbitrap mass spectrometer.

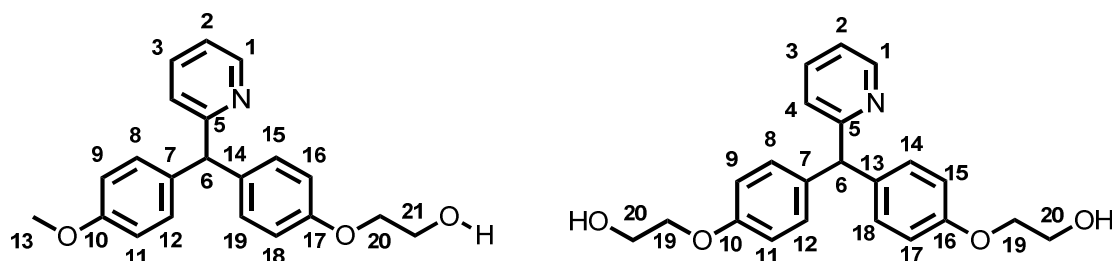


Fig. 3 Convention adopted to assign signals of ¹H and ¹³C-NMR spectra

4.2.3. General Procedure for the preparation of PEGylated non-symmetrical triarylmethanes

To a solution of compound **3** (1 equiv) in anhydrous DMF (8 mL) was added K₂CO₃ (4 equiv). The mixture was stirred at 80 °C for 1 h, under argon. Then a solution of the corresponding glycol

mono-tosylate (1 equiv, except for **4a**, 3 equiv) in dry DMF (2 mL) was added dropwise and the mixture was stirred at 80 °C (monitoring by TLC). After reaction completion, the reaction mixture was diluted with water (80 mL) and extracted with CH₂Cl₂ (5 x 80 mL). The organic layer was washed with a saturated solution of NaHCO₃ (3 x 80 mL), brine (3 x 80 mL) and water (3 x 80 mL), and then dried over MgSO₄. The solvent was removed *in vacuo* and then the product was purified by flash column chromatography on silica gel to afford the corresponding PEGylated non-symmetrical triarylmethanes **4a-e**. Yields: **4a**, 72% ; **4b**, 40% ; **4c**, 40% ; **4d**, 33% ; **4e**, 69%.

4.2.3.1. 2-(4-((4-methoxyphenyl)(pyridin-2-yl)methyl)phenoxy)ethanol **4a**

From compound **3** (0.58 mmol; 169 mg) and ethylene glycol mono-tosylate (1.74 mmol ; 376 mg) Reaction time: 7 days. FCC: (EtOAc/CH₂Cl₂, 25:75) to afford the desired compound **4a** as an oil in 72% yield (141 mg). **TLC** (EtOAc/CH₂Cl₂ 40:60, R_f : 0.22); **IR (ATR)** (cm⁻¹): ν 751 ; 808 ; 1031 ; 1088 ; 1175 ; 1242 (ν_{C-O-C}) ; 1464 (ν_{C-OH}) ; 1507 ; 1608 (ν_{C=C}) ; 2931 (ν_{O-Me}) ; 3394 (ν_{O-H}) ; **LRMS(ESI- CV=30, 35)**: 336.17 (M-H⁺) ; 358.17 (M-Na⁺). **¹H NMR (DMSO-d₆, 400 MHz)** δ (ppm) = 3.69 (m, 5H, H₁₃ + H₂₁) ; 3.93 (t, 2H, H₂₀, J = 5.0 Hz) ; 4.85 (br, 1H, H_{OH}) ; 5.54 (s, 1H, H₆) ; 6.85 (m, 4H, H₉ + H₁₁ + H₁₆ + H₁₈) ; 7.09 (m, 4H, H₈ + H₁₂ + H₁₅ + H₁₉) ; 7.21 (m, 2H, H₂ + H₄) ; 7.71 (ddd, 1H, H₃, J = 1.9, 7.7 and 9.6 Hz) ; 8.54 (ddd, 1H, H₁, J = 0.9, 1.8 and 4.6 Hz); **¹³C NMR (DMSO-d₆, 100 MHz)** δ (ppm) = 55.0 (C₁₃) ; 56.5 (C₆) ; 59.6 (C₂₀) ; 69.4 (C₂₁) ; 113.6 (C₉ + C₁₁) ; 114.2 (C₁₆ + C₁₈) ; 121.5 (C₂ or C₄) ; 123.4 (C₂ or C₄) ; 129.9 (C₈ + C₁₂ + C₁₅ + C₁₉) ; 135.3 (C₇ or C₁₄) ; 135.4 (C₇ or C₁₄) ; 136.7 (C₃) ; 149.1 (C₁) ; 157.1 (C₁₇) ; 157.6 (C₁₀) ; 163.1 (C₅). **HRMS**: calcd. for C₂₁H₂₁NO₃H (336.1594); found (336.1596).

4.2.3.2. 2-(2-(4-((4-methoxyphenyl)(pyridin-2-yl)methyl)phenoxy)ethoxy)ethanol **4b**

From compound **3** (0.89 mmol ; 259 mg) and diethylene glycol mono-tosylate (0.89 mmol ; 231 mg) Reaction time: 5 days. FCC: (EtOAc/CH₂Cl₂ 20:80 then EtOAc/CH₂Cl₂ 40:60 then EtOAc/CH₂Cl₂ 70:30) to afford the desired compound **4b** as an oil in 40% yield (137 mg). **TLC** (EtOAc/CH₂Cl₂ 20:80, R_f : 0.26. **IR (ATR)** (cm⁻¹): ν 752 ; 807 ; 929 ; 1031 ; 1128 ; 1176 ; 1240 (ν_{C-O-C}) ; 1464 (ν_{C-OH}) ; 1507 ; 1587 (ν_{C=C}) ; 2873 (ν_{O-Me}) ; 2931 (ν_{Csp3-H}) ; 3391 (ν_{O-H}). **LRMS(ESI- CV=30, 35)** : 380.18 (M-H⁺) ; 402.20 (M-Na⁺). **¹H NMR (DMSO-d₆, 400 MHz)** δ (ppm) = 3.50 (m, 4H, H₂₂ + H₂₃) ; 3.71 (m, 5H, H₁₃ + H₂₁) ; 4.04 (m, 2H, H₂₀) ; 4.62 (t, 1H, H_{OH}, J = 5.5 Hz) ; 5.54 (s, 1H, H₆) ; 6.85 (m, 4H, H₉ + H₁₁ + H₁₆ + H₁₈) ; 7.10 (m, 4H, H₈ + H₁₂ + H₁₅ + H₁₉) ; 7.22 (m, 2H, H₂ + H₄) ; 7.71 (ddd, 1H, H₃, J = 1.9, 7.7 and 9.6 Hz) ; 8.54 (ddd, 1H, H₁, J = 1.0, 1.9 and 4.7 Hz). **¹³C NMR (DMSO-d₆, 100 MHz)** δ (ppm) = 55.0 (C₁₃) ; 56.5 (C₆) ; 60.2 (C₂₃) ; 67.1 (C₂₀) ; 68.9 (C₂₁) ; 72.4 (C₂₂) ; 113.6 (C₉ + C₁₁) ; 114.1 (C₁₆ + C₁₈) ; 121.5 (C₂ or C₄) ; 123.4 (C₂ or C₄) ; 129.9 (C₈ + C₁₂ + C₁₅ + C₁₉) ; 135.4 (C₇ or C₁₄) ; 135.5 (C₇ or C₁₄) ; 136.7 (C₃) ; 149.1

(C₁) ; 156.9 (C₁₇) ; 157.6 (C₁₀) ; 163.1 (C₅); HRMS: calcd. for C₂₃H₂₅NO₄Na (402.1676); found (402.1675).

4.2.3.3. 2-(2-(2-(4-((4-methoxyphenyl)(pyridin-2-yl)methyl)phenoxy)ethoxy)ethoxy)ethanol **4c**

From compound **3** (1.06 mmol ; 308 mg) and triethylene glycol mono-tosylate (1.06 mmol ; 322 mg) Reaction time: 4 days. FCC: (EtOAc/CH₂Cl₂ 80:20) to afford the desired compound **4c** as an oil in 40% yield (177 mg). **TLC** (EtOAc/CH₂Cl₂ 80:20; R_f : 0.14). **IR (ATR)** (cm⁻¹): ν 751 ; 807 ; 930 ; 1031 ; 1113 ; 1176 ; 1241 (ν_{C-O-C}) ; 1464 (ν_{C-OH}) ; 1507 ; 1609 (ν_{C=C}) ; 2872 (ν_{O-Me}) ; 3414 (ν_{O-H}) . **LRMS(ESI- CV=30, 35)** : 424.16 (M-H⁺) ; 446.14 (M-Na⁺). **¹H NMR (DMSO-d₆, 400 MHz)** δ (ppm) = 3.41 (m, 2H, H₂₄) ; 3.47 (m, 2H, H₂₅) ; 3.52 (m, 2H, H₂₂ or H₂₃ or H₂₄) ; 3.58 (m, 2H, H₂₂ or H₂₃ or H₂₄) ; 3.71 (m, 5H, H₁₃ + H₂₁) ; 4.03 (m, 2H, H₂₀) ; 4.58 (t, 1H, H_{OH}, J = 5.5 Hz) ; 5.54 (s, 1H, H₆) ; 6.85 (m, 4H, H₉ + H₁₁ + H₁₆ + H₁₈) ; 7.10 (m, 4H, H₈ + H₁₂ + H₁₅ + H₁₉) ; 7.20 (m, 2H, H₂ + H₄) ; 7.71 (ddd, 1H, H₃, J = 2.0, 7.7 and 9.7 Hz) ; 8.52 (m, 1H, H₁). **¹³C NMR (DMSO-d₆, 100 MHz)** δ (ppm) = 55.0 (C₁₃) ; 56.5 (C₆) ; 60.2 (C₂₅) ; 67.0 (C₂₀) ; 69.0 (C₂₂ or C₂₃) ; 69.8 (C₂₁) ; 69.9 (C₂₂ or C₂₃) ; 72.4 (C₂₄) ; 113.6 (C₉ + C₁₁) ; 114.2 (C₁₆ + C₁₈) ; 121.5 (C₂ or C₄) ; 123.4 (C₂ or C₄) ; 129.9 (C₈ + C₁₂ + C₁₅ + C₁₉) ; 135.4 (C₇ or C₁₄) ; 135.5 (C₇ or C₁₄) ; 136.7 (C₃) ; 149.1 (C₁) ; 156.8 (C₁₇) ; 157.6 (C₁₀) ; 163.1 (C₅). HRMS: calcd. for C₂₅H₂₉NO₅Na (446.1938); found (446.1933).

4.2.3.4. 2-(2-(2-(2-(4-((4-methoxyphenyl)(pyridin-2 yl)methyl)phenoxy)ethoxy)ethoxy)ethoxy)ethanol **4d**

From compound **3** (1.08 mmol ; 314 mg) and tetraethylene glycol mono-tosylate (1.08 mmol ; 376 mg) Reaction time: 5 days. FCC: (EtOAc, 100) to afford the desired compound **4d** as an oil in 33% yield (170 mg). **TLC** (EtOAc; R_f : 0.05). **IR (ATR)** (cm⁻¹): ν 751 ; 807 ; 930 ; 1062 ; 1108 ; 1176 ; 1243 (ν_{C-O-C}) ; 1464 (ν_{C-OH}) ; 1507 ; 1609 (ν_{C=C}) ; 2870 (ν_{O-Me}) ; 3443 (ν_{O-H}). **LRMS(ESI- CV=30, 35)**: 468.23 (M-H⁺) ; 490.17 (M-Na⁺). **¹H NMR (DMSO-d₆, 400 MHz)** δ (ppm) = 3.41 (m, 2H, H₂₆) ; 3.47 (m, 2H, H₂₇) ; 3.50 (m, 4H, H₂₂ or H₂₃ or H₂₄ or H₂₅) ; 3.52 (m, 2H, H₂₂ or H₂₃ or H₂₄ or H₂₅) ; 3.57 (m, 2H, H₂₂ or H₂₃ or H₂₄ or H₂₅) ; 3.71 (m, 5H, H₁₃ + H₂₁) ; 4.03 (m, 2H, H₂₀) ; 4.58 (t, 1H, H_{OH}, J = 5.4 Hz) ; 5.54 (s, 1H, H₆) ; 6.85 (m, 4H, H₉ + H₁₁ + H₁₆ + H₁₈) ; 7.09 (m, 4H, H₈ + H₁₂ + H₁₅ + H₁₉) ; 7.21 (m, 2H, H₂ + H₄) ; 7.71 (ddd, 1H, H₃, J = 1.9, 7.6 and 9.6 Hz) ; 8.54 (ddd, 1H, H₁, J = 1.0, 1.9 and 4.7 Hz). **¹³C NMR (DMSO-d₆, 100 MHz)** δ (ppm) = 55.0 (C₁₃) ; 56.5 (C₆) ; 60.2 (C₂₇) ; 67.0 (C₂₀) ; 68.9 (C₂₁) ; 69.7 (C₂₂ or C₂₃ or C₂₄ or C₂₅) ; 69.8 (C₂₂ or C₂₃ or C₂₄ or C₂₅) ; 69.9 (C₂₂ or C₂₃ or C₂₄ or C₂₅) ; 70.0 (C₂₂ or C₂₃ or C₂₄ or C₂₅) ; 72.3 (C₂₆) ; 113.6 (C₉ + C₁₁) ; 114.2 (C₁₆ + C₁₈) ; 121.5 (C₂ or C₄) ; 123.4 (C₂ or C₄) ; 129.9 (C₈ + C₁₂ + C₁₅ + C₁₉) ; 135.4 (C₇ or C₁₄) ; 135.5 (C₇ or C₁₄) ; 136.7 (C₃) ; 149.1 (C₁) ; 156.8 (C₁₇) ; 157.6 (C₁₀) ; 163.1 (C₅). HRMS: calcd. for C₂₇H₃₃NO₆Na (490.2200); found (490.2195).

4.2.3.5.14-(4-((4-methoxyphenyl)(pyridin-2-yl)methyl)phenoxy)-3,6,9,12-tetraoxatetradecan-1-ol **4e**

From compound **3** (0.83 mmol ; 232 mg) and pentaethylene glycol mono-tosylate (2.5 mmol ; 1.0 g) Reaction time: 4 days. FCC: (EtOAc/MeOH 95:5) to afford the desired compound **4e** as a yellow oil in 69% yield (280 mg). **TLC** (EtOAc/CH₂Cl₂ 80:20; R_f : 0.28). **IR (ATR)** (cm⁻¹): ν 731 ; 824 ; 943 ; 1031 ; 1063 ; 1097 ; 1177 ; 1244 (ν_{C-O-C}) ; 1297 ; 1465 (ν_{C-OH}) ; 1508 ; 1587 ; 1609 (ν_{C=C}) ; 2872 (ν_{O-Me}) ; 3449 (ν_{O-H}). **LRMS(ESI- CV=30, 35)** : 512.24 (M-H⁺) ; 534.21 (M-Na⁺). **¹H NMR (DMSO-d₆, 400 MHz)** δ (ppm) = 3.40 (m, 2H, H₂₈) ; 3.47 (m, 2H, H₂₂ or H₂₃ or H₂₄ or H₂₅ or H₂₆ or H₂₇) ; 3.50 (m, 8H, H₂₂ or H₂₃ or H₂₄ or H₂₅ or H₂₆ or H₂₇) ; 3.53 (m, 2H, H₂₉) ; 3.57 (m, 2H, H₂₂ or H₂₃ or H₂₄ or H₂₅ or H₂₆ or H₂₇) ; 3.71 (m, 5H, H₁₃ + H₂₁) ; 4.03 (m, 2H, H₂₀) ; 4.57 (t, 1H, H_{OH}, J = 5.4 Hz) ; 5.54 (s, 1H, H₆) ; 6.85 (m, 4H, H₉ + H₁₁ + H₁₆ + H₁₈) ; 7.09 (m, 4H, H₈ + H₁₂ + H₁₅ + H₁₉) ; 7.21 (m, 2H, H₂ + H₄) ; 7.71 (ddd, 1H, H₃, J = 1.9, 7.6 and 9.6 Hz) ; 8.52 (ddd, 1H, H₁, J = 1.0, 1.9 and 4.7 Hz). **¹³C NMR (DMSO-d₆, 100 MHz)** δ (ppm) = 55.0 (C₁₃) ; 56.5 (C₆) ; 60.2 (C₂₉) ; 67.0 (C₂₀) ; 68.9 (C₂₁) ; 69.8 – 69.9 (C₂₂ + C₂₃ + C₂₄ + C₂₅ + C₂₆ + C₂₇) ; 72.3 (C₂₈) ; 113.6 (C₉ + C₁₁) ; 114.2 (C₁₆ + C₁₈) ; 121.5 (C₂ or C₄) ; 123.4 (C₂ or C₄) ; 129.9 (C₈ + C₁₂ + C₁₅ + C₁₉) ; 135.4 (C₇ or C₁₄) ; 135.5 (C₇ or C₁₄) ; 136.7 (C₃) ; 149.1 (C₁) ; 156.8 (C₁₇) ; 157.6 (C₁₀) ; 163.1 (C₅). **HRMS**: calcd. for C₂₉H₃₇NO₇Na (534.2462); found (534.2455).

4.2.3.6. 2,2'-(((pyridin-2-ylmethylene)bis(4,1-phenylene))bis(oxy))diethanol **8a**

To a solution of compound **7** (1.08 mmol ; 300 mg) in anhydrous DMF (10 mL) was added K₂CO₃ (6.49 mmol ; 896 mg). The mixture was stirred at 80°C for 30 min, under argon. Then a solution of 2-bromoethanol (4.32 mmol ; 310 μL) and KI (5.41 mmol ; 898 mg) was added and the mixture was stirred for 4 days at 80°C. The mixture was diluted with water (50 mL), and extracted with CH₂Cl₂ (3 x 30 mL). The organic layer was washed with saturated solution of NaCl (50 mL) then dried over MgSO₄. The solvent was removed *in vacuo* and then the product was purified by flash column chromatography on silica gel (EtOAc/Cy 50:50) to afford the desired compound **8a** (15% ; 59 mg) as a yellow oil and a second compound **8f** (50% ; 176 mg) as a yellow oil. **TLC** (95% CH₂Cl₂/MeOH; R_f : 0.27). **IR (ATR)** (cm⁻¹): ν 664 ; 752 ; 821 ; 885 ; 928 ; 1053 ; 1126 ; 1176 ; 1240 (ν_{C-O-C}) ; 1296 ; 1356 ; 1434 (ν_{C-OH}) ; 1469 (ν_{C=C}) ; 1507 ; 1588 (ν_{C=C}) ; 1609 ; 1746 ; 2739 ; 2869 ; 2944 ; 3063 (ν_{Csp²-H}) ; 3349 (ν_{O-H}). **LRMS(ESI- CV=30, 35)** : 366.14 (M-H⁺) ; 388.10 (M-Na⁺). **¹H NMR (DMSO-d₆, 400 MHz)** δ (ppm) = 3.68 (m, 4H, H₂₀) ; 3.93 (t, 4H, H₁₉, J = 5.0 Hz) ; 4.83 (t, 2H, H_{OH}, J = 5.5 Hz) ; 5.53 (s, 1H, H₆) ; 6.84 (m, 4H, H₉ + H₁₁ + H₁₅ + H₁₇) ; 7.08 (m, 4H, H₈ + H₁₂ + H₁₄ + H₁₈) ; 7.21 (m, 2H, H₂ + H₄) ; 7.71 (ddd, 1H, H₃, J = 1.9, 7.7 and 9.6 Hz) ; 8.51 (m, 1H, H₁). **¹³C NMR (DMSO-d₆, 100 MHz)** δ (ppm) = 56.5 (C₆) ; 59.6 (C₂₀) ; 69.4 (C₁₉) ; 114.2 (C₉ + C₁₁ + C₁₅ + C₁₇) ; 121.5 (C₂ or C₄) ; 123.4 (C₂ or C₄) ; 129.9 (C₈ + C₁₂ + C₁₄ +

C18) ; 135.4 (C7 + C13) ; 136.7 (C3) ; 149.1 (C1) ; 157.1 (C10 + C16) ; 163.1 (C5). HRMS: calcd. for C₂₂H₂₃NO₄H (366.1700); found (366.1700).

4.2.37. 4-((4-(2-hydroxyethoxy)phenyl)(pyridin-2-yl)methyl)phenol **8f**

¹H NMR (DMSO-d₆, 400 MHz) δ (ppm) = 3.69 (m, 2H, H₂₀) ; 3.93 (t, 2H, H₁₉, J = 5.0 Hz) ; 4.83 (t, 1H, H_{OH} aliphatique, J = 5.5 Hz) ; 5.47 (s, 1H, H₆) ; 6.67 (d, 2H, H₉ + H₁₁, J = 8.1 Hz) ; 6.84 (d, 2H, H₁₅ + H₁₇, J = 8.1 Hz) ; 6.95 (d, 2H, H₈ + H₁₂, J = 8.1 Hz) ; 7.06 (d, 2H, H₁₄ + H₁₈, J = 8.3 Hz) ; 7.23 (m, 2H, H₂ + H₄) ; 7.71 (ddd, 1H, H₃, J = 1.9, 7.7 and 9.6 Hz) ; 8.51 (m, 1H, H₁). **¹³C NMR (DMSO-d₆, 100 MHz)** δ (ppm) = 56.7 (C₆) ; 59.6 (C₂₀) ; 69.4 (C₁₉) ; 114.1 (C₁₅ + C₁₇) ; 115.0 (C₉ + C₁₁) ; 121.4 (C₂ or C₄) ; 123.4 (C₂ or C₄) ; 129.8 (C₈ + C₁₂) ; 129.9 (C₁₄ + C₁₈) ; 133.6 (C₁₃) ; 135.6 (C₇) ; 136.6 (C₃) ; 149.1 (C₁) ; 155.7 (C₁₀) ; 157.0 (C₁₆) ; 163.4 (C₅). HRMS: calcd. for C₂₀H₁₉NO₃H (322.1438); found (322.1439).

4.2.4. General Procedure for the preparation of PEGylated symmetrical triarylmethanes

To a solution of compound **7** (1.08 mmol, 300 mg, 1 equiv) in anhydrous DMF (10 mL) was added K₂CO₃ (10 equiv). The mixture was stirred at 80 °C for 1 h, under argon. Then a solution of corresponding glycol mono-tosylate (4 equiv) in dry DMF (5 mL) was added dropwise and the mixture was stirred at 80 °C (monitoring by TLC). The mixture was diluted with water (50 mL), and extracted with EtOAc (3 x 50 mL). The organic layer was washed three times with water (25 mL). Then dried over MgSO₄, filtered and the solvent was removed in vacuo and then the product was purified by flash column chromatography on silica gel to afford the corresponding PEGylated symmetrical triarylmethanes **8b-f**. Yields: **8b**, 60% **8c**, 70%, **8d**, 50% **8f**, 45%.

4.2.4.1. 2,2'-((((pyridin-2-ylmethylene)bis(4,1-phenylene))bis(oxy))bis(ethane-2,1-diyl))bis(oxy)) diethanol **8b**

From compound **7** (1.08 mmol ; 300 mg) and diethyleneglycol mono-tosylate (4.33 mmol ; 1.13 g) Reaction time: 4 days. FCC: (CH₂Cl₂/MeOH 95:5) to afford the desired compound **8b** (60% ; 290 mg) as a colorless oil. **TLC** (CH₂Cl₂/MeOH 95:5; R_f : 0.43). **IR (ATR)** (cm⁻¹): ν 664 ; 752 ; 821 ; 885 ; 928 ; 1053 ; 1126 ; 1176 ; 1240 (ν_{C-O-C}) ; 1296 ; 1356 ; 1434 (ν_{C-OH}) ; 1469 ($\nu_{C=C}$) ; 1507 ; 1588 ($\nu_{C=C}$) ; 1609 ; 1746 ; 2739 ; 2869 ; 2944 (ν_{Csp^3-H}) ; 3063 (ν_{Csp^2-H}) ; 3349 (ν_{O-H}). **LRMS (ESI-CV= 30, 35)** : 476.25 (M-Na⁺). **¹H NMR (DMSO-d₆, 400 MHz)** δ (ppm) = 3.49 (m, 8H, H₂₁ + H₂₂) ; 3.71 (m, 4H, H₂₀) ; 4.04 (m, 4H, H₁₉) ; 4.62 (t, 2H, H_{OH}, J = 5.5 Hz) ; 5.54 (s, 1H, H₆) ; 6.85 (m, 4H, H₉ + H₁₁ + H₁₅ + H₁₇) ; 7.09 (m, 4H, H₈ + H₁₂ + H₁₄ + H₁₈) ; 7.21 (m, 2H, H₂ + H₄) ; 7.71 (m, 1H, H₃) ; 8.51 (m, 1H, H₁). **¹³C NMR (DMSO-d₆, 100 MHz)** δ (ppm) = 56.5 (C₆) ; 60.2 (C₂₂) ; 67.1 (C₁₉) ; 68.9 (C₂₀) ; 72.5 (C₂₁) ; 114.2 (C₉ + C₁₁ + C₁₅ + C₁₇) ; 121.5 (C₂ or C₄) ; 123.4 (C₂ or

C₄) ; 129.9 (C₈ + C₁₂ + C₁₄ + C₁₈) ; 135.5 (C₇ + C₁₃) ; 136.7 (C₃) ; 149.1 (C₁) ; 156.9 (C₁₀ + C₁₆) ; 163.1 (C₅). HRMS: calcd. for C₂₆H₄₃NO₆Na (476.2044); found (476.2039).

4.2.4.2. 2,2'-(((((((pyridin-2-ylmethylene)bis(4,1-phenylene))bis(oxy))bis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(oxy))diethanol **8c**

From compound **7** (1.08 mmol ; 300 mg) and triethyleneglycol mono-tosylate (4.33 mmol ; 1.32 g) Reaction time: 4 days. FCC: (CH₂Cl₂/MeOH 97:3) to afford the desired compound **8c** (70% ; 407 mg) as a colorless oil. **TLC** (97% CH₂Cl₂/MeOH ; R_f : 0.43). **IR (ATR)** (cm⁻¹): ν 665 ; 730 ; 753 ; 823 ; 885 ; 927 ; 1058 ; 1114 ; 1177 ; 1242 (ν_{C-O-C}) ; 1296 ; 1352 ; 1468 (ν_{C=C}) ; 1508 ; 1587 (ν_{C=C}) ; 1609 ; 1746 ; 2870 ; 3052 (ν_{Csp²-H}) ; 3418 (ν_{O-H}). **LRMS (ESI- CV= 30, 35)**: 542.36 (M-H⁺) ; 564.34 (M-Na⁺). **¹H NMR (DMSO-d₆, 400 MHz)** δ (ppm) = 3.41 (m, 4H, H₂₃) . 3.46 – 3.58 (m, 12H, H₂₁ + H₂₂ + H₂₄) ; 3.71 (m, 4H, H₂₀) ; 4.03 (m, 4H, H₁₉) ; 4.58 (t, 2H, H_{OH}, J = 5.5 Hz) ; 5.54 (s, 1H, H₆) ; 6.85 (m, 4H, H₉ + H₁₁ + H₁₅ + H₁₇) ; 7.09 (m, 4H, H₈ + H₁₂ + H₁₄ + H₁₈) ; 7.21 (m, 2H, H₂ + H₄) ; 7.71 (ddd, 1H, H₃, J = 1.9, 7.7 and 9.6 Hz) ; 8.51 (m, 1H, H₁). **¹³C NMR (DMSO-d₆, 100 MHz)** δ (ppm) = 56.5 (C₆) ; 60.2 (C₂₄) ; 67.0 (C₁₉) ; 68.9 (C₂₀) ; 69.8 (C₂₁ or C₂₂) ; 69.9 (C₂₁ or C₂₂) ; 72.4 (C₂₃) ; 114.2 (C₉ + C₁₁ + C₁₅ + C₁₇) ; 121.5 (C₂ or C₄) ; 123.4 (C₂ or C₄) ; 129.9 (C₈ + C₁₂ + C₁₄ + C₁₈) ; 135.5 (C₇ + C₁₃) ; 136.7 (C₃) ; 149.1 (C₁) ; 156.8 (C₁₀ + C₁₆) ; 163.1 (C₅). HRMS: calcd. for C₃₀H₃₉NO₈Na (564.2568); found (564.2561).

4.2.4.3. 2,2'-((((((((pyridin-2-ylmethylene)bis(4,1-phenylene))bis(oxy))bis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(oxy))diethanol **8d**

From compound **7** (1.08 mmol ; 300 mg) and tetraethyleneglycol mono-tosylate (4.33 mmol ; 1.51 g) Reaction time: 4 days. FCC (CH₂Cl₂/MeOH 97:3) to afford the desired compound **8d** (50% ; 342 mg) as a colorless oil. **TLC** (CH₂Cl₂/MeOH 97:3 ; R_f : 0.34). **IR (ATR)** (cm⁻¹): ν 665 ; 753 ; 823 ; 884 ; 927 ; 1060 ; 1103 ; 1178 ; 1243 (ν_{C-O-C}) ; 1296 ; 1351 ; 1468 (ν_{C=C}) ; 1508 ; 1587 (ν_{C=C}) ; 1609 ; 1746 ; 2869 ; 3060 (ν_{Csp²-H}) ; 3432 (ν_{O-H}). **LRMS (ESI- CV= 30, 35)** : 652.41 (M+Na⁺). **¹H NMR (DMSO-d₆, 400 MHz)** δ (ppm) = 3.39 (m, 4H, H₂₅) . 3.47 – 3.56 (m, 20H, H₂₁ + H₂₂ + H₂₃ + H₂₄ + H₂₆) ; 3.71 (m, 4H, H₂₀) ; 4.03 (m, 4H, H₁₉) ; 4.57 (t, 2H, H_{OH}, J = 5.5 Hz) ; 5.54 (s, 1H, H₆) ; 6.85 (m, 4H, H₉ + H₁₁ + H₁₅ + H₁₇) ; 7.08 (m, 4H, H₈ + H₁₂ + H₁₄ + H₁₈) ; 7.21 (m, 2H, H₂ + H₄) ; 7.71 (ddd, 1H, H₃, J = 1.9, 7.7 and 9.6 Hz) ; 8.51 (m, 1H, H₁). **¹³C NMR (DMSO-d₆, 100 MHz)** δ (ppm) = 56.5 (C₆) ; 60.2 (C₂₆) ; 67.0 (C₁₉) ; 68.9 (C₂₀) ; 69.8 – 69.9 (8C, C₂₁ + C₂₂ + C₂₃ + C₂₄) ; 72.3 (C₂₅) ; 114.2 (C₉ + C₁₁ + C₁₅ + C₁₇) ; 121.5 (C₂ or C₄) ; 123.4 (C₂ or C₄) ; 129.9 (C₈ + C₁₂ + C₁₄ + C₁₈) ; 135.5 (C₇ + C₁₃) ; 136.7 (C₃) ; 149.1 (C₁) ; 156.8 (C₁₀ + C₁₆) ; 163.1 (C₅). HRMS: calcd. for C₃₄H₄₇NO₁₀Na (652.3092); found (652.3089).

4.2.4.4. 14,14'-(((pyridin-2-ylmethylene)bis(4,1-phenylene))bis(oxy))bis(3,6,9,12-tetraoxatetradecan-1-ol) **8e**

From compound **7** (1.08 mmol ; 300 mg) and pentaethyleneglycol mono-tosylate (4.33 mmol ; 1.70 g) Reaction time: 4 days. FCC (CH₂Cl₂/MeOH, 97:3) to afford the desired compound **8e** (45% ; 349 mg) as a colorless oil. **TLC** (CH₂Cl₂/MeOH 95:5 ; R_f : 0.26. **IR (ATR)** (cm⁻¹): ν 609 ; 665 ; 730 ; 754 ; 824 ; 884 ; 931 ; 1061 ; 1098 ; 1178 ; 1244 (ν_{C-O-C}) ; 1296 ; 1351 ; 1463 (ν_{C=C}) ; 1509 ; 1609 (ν_{C=C}) ; 1746 ; 2868 ; 3053 (ν_{Csp²-H}) ; 3440 (ν_{O-H}). **LRMS (ESI- CV= 30, 35)** :740.47 (M+Na⁺). **¹H NMR (DMSO-d₆, 400 MHz)** δ (ppm) = 3.40 (m, 4H, H₂₇) ; 3.47 – 3.58 (m, 24H, H₂₁ + H₂₂ + H₂₃ + H₂₄ + H₂₅ + H₂₆ + H₂₈) ; 3.71 (m, 4H, H₂₀) ; 4.03 (m, 4H, H₁₉) ; 4.58 (t, 2H, H_{OH}, J = 5.5 Hz) ; 5.53 (s, 1H, H₆) ; 6.86 (m, 4H, H₉ + H₁₁ + H₁₅ + H₁₇) ; 7.08 (m, 4H, H₈ + H₁₂ + H₁₄ + H₁₈) ; 7.21 (m, 2H, H₂ + H₄) ; 7.71 (ddd, 1H, H₃, J = 1.9, 7.7 and 9.6 Hz) ; 8.51 (m, 1H, H₁). **¹³C NMR (DMSO-d₆, 100 MHz)** δ (ppm) = 56.5 (C₆) ; 60.2 (C₂₈) ; 67.0 (C₁₉) ; 68.9 (C₂₀) ; 69.8 – 69.9 (12C, C₂₁ + C₂₂ + C₂₃ + C₂₄ + C₂₅ + C₂₆) ; 72.3 (C₂₇) ; 114.2 (C₉ + C₁₁ + C₁₅ + C₁₇) ; 121.5 (C₂ or C₄) ; 123.4 (C₂ or C₄) ; 129.9 (C₈ + C₁₂ + C₁₄ + C₁₈) ; 135.5 (C₇ + C₁₃) ; 136.7 (C₃) ; 149.1 (C₁) ; 156.9 (C₁₀ + C₁₆) ; 163.1 (C₅). **HRMS**: calcd. for C₃₈H₅₅NO₁₂Na (740.3616); found (740.3616).

4.2.4. General Procedure for the preparation of PEGylated symmetrical triarylmethanes *N*-oxides

To a solution of the corresponding TAMs (1 equiv) in anhydrous CH₂Cl₂ under argon was added quickly *m*-CPBA (77%) (3 equiv). The reaction mixture was stirred at room temperature under argon. Then, the reaction mixture was quenched by the addition of a solution of KOH (40%) until pH 8-9. The medium was diluted with water (5 mL) and then extracted three times with CH₂Cl₂ (10 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated to dryness. The crude product was purified by flash column chromatography on silica gel to afford the corresponding PEGylated symmetrical triarylmethanes *N*-oxides. Yields: **5**, 83% ; **9**, 64%.

4.2.4.1.. 2-(((4-(2-hydroxyethoxy)phenyl)(4-methoxyphenyl)methyl)pyridine *N*-oxide **5**

From compound **4a** (300 μmol ; 100 mg) in anhydrous CH₂Cl₂ (2 mL) and *m*-CPBA (77%) (900 μmol ; 201 mg). Reaction time: 2 h, FCC: (CH₂Cl₂/MeOH 97:3 to 95:5) to afford the desired compound **5** as a colorless oil (83% ; 87 mg). **TLC** (3% MeOH/CH₂Cl₂; R_f: 0.15) **IR (ATR)** (cm⁻¹): ν 706 ; 758 ; 817 ; 889 ; 1025 ; 1176 ; 1240 (ν_{C-O-C}) ; 1427 ; 1508 ; 1608 (ν_{C=C}) ; 1706 ; 2931 (ν_{O-Me}) ; 3070 (ν_{Csp²-H}) ; 3292 (ν_{O-H}). **LRMS-(ESI- CV=30, 35)** : 352.14 (M+H⁺) ; 374.12 (M+Na⁺). **¹H NMR (DMSO-d₆, 400 MHz)** δ (ppm) = 3.68-3.71 (m, 5H, H₁₃ + H₂₁) ; 3.93-3.96 (t, 2H, H₂₀, J = 5.0 Hz) ; 4.86 (br, 1H, H_{OH}) ; 6.03 (s, 1H, H₆) ; 6.86-6.90 (m, 4H, H₉ + H₁₁ + H₁₆ + H₁₈) ; 6.93-6.97 (m, 5H, H₄ + H₈ + H₁₂ + H₁₅ + H₁₉) ; 7.24-7.34 (m, 2H, H₂ + H₃) ; 8.24-8.26 (m, 1H, H₁). **¹³C NMR (DMSO-d₆, 100 MHz)** δ (ppm) = 48.1 (C₆) ; 55.1 (C₁₃) ; 59.6 (C₂₁) ; 69.5 (C₂₀)

; 113.9 (C₉ + C₁₁) ; 114.5 (C₁₆ + C₁₈) ; 124.4 (C₂ or C₃) ; 124.6 (C₂ or C₃) ; 126.5 (C₄) ; 129.9 (C₈ + C₁₂ + C₁₅ + C₁₉) ; 132.7 (C₇ or C₁₄) ; 132.8 (C₇ or C₁₄) ; 139.2 (C₁) ; 153.3 (C₅) ; 157.4 (C₁₇) ; 158.0 (C₁₀). HRMS: calcd. for C₂₁H₂₁NO₄Na (374.1363); found (374.1363).

4.2.4.2. 2-(bis(4-(2-hydroxyethoxy)phenyl)methyl)pyridine *N*-oxide **9**

From compound **8a** (410 µmol; 150 mg) in anhydrous CH₂Cl₂ (2 mL) and *m*-CPBA (77%) (1.23 mmol; 277 mg). Reaction time: 3 h, FCC (CH₂Cl₂/MeOH 96:4 to 92:8) to afford the desired compound **9** as an amorphous orange solid (64%; 100 mg). **TLC** (MeOH/CH₂Cl₂ 5:95, R_f: 0.16), **IR (ATR)** (cm⁻¹): ν 725 ; 766 ; 839 ; 889 ; 915 ; 1040 ; 1174 ; 1240 (ν_{C-O-C}) ; 1428 ; 1507 ; 1608 (ν_{C=C}) ; 2868 (ν_{O-Me}) ; 2926 (ν_{Csp³-H}) ; 3075 (ν_{Csp²-H}) ; 3253 (ν_{O-H}). **LRMS (ESI- CV= 30, 35)** : 382.13 (M+H⁺) ; 404.10 (M+Na⁺). **¹H NMR (DMSO-d₆, 400 MHz)** δ (ppm) = 3.68-3.71 (m, 4H, H₂₁) ; 3.94-3.96 (t, 4H, H₂₀, J = 5.0 Hz) ; 4.84- 4.86 (m, 2H, H_{OH}) ; 6.02 (s, 1H, H₆) ; 6.87-6.90 (m, 4H, H₉ + H₁₁ + H₁₆ + H₁₈) ; 6.93-6.96 (m, 5H, H₄ + H₈ + H₁₂ + H₁₅ + H₁₉) ; 7.24-7.34 (m, 2H, H₂ + H₃) ; 8.24-8.26 (m, 1H, H₁). **¹³C NMR (DMSO-d₆, 100 MHz)** δ (ppm) = 48.1 (C₆) ; 59.6 (C₂₁) ; 69.5 (C₂₀) ; 114.5 (C₉ + C₁₁ + C₁₆ + C₁₈) ; 124.4 (C₂ or C₃) ; 124.5 (C₂ or C₃) ; 126.5 (C₄) ; 129.9 (C₈ + C₁₂ + C₁₅ + C₁₉) ; 132.7 (C₇ + C₁₄) ; 139.2 (C₁) ; 153.3 (C₅) ; 157.4 (C₁₇ + C₁₀). HRMS: calcd. for C₂₂H₂₃O₅Na (404.1468); found (404.1469).

4.2 Biological assay

4.2.1 In vitro antibacterial activity

Bacteria and growth conditions

Microorganism growth inhibition assays were performed using LB (1% Bactotryptone, 0.5% Yeast extract, 0.5% NaCl) cultures of *Gram-positive pathogens*: *Staphylococcus aureus* (ATCC 9144), *Micrococcus luteus* (LB14110) and *Gram-negative pathogens*: *Salmonella enterica* (NCTC 6017), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739). The antifungal activity was investigated against the opportunistic pathogenic yeast *Candida albicans* (ATCC 2091). All experiments were performed in triplicate.

Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

The synthesized compounds were tested in triplicate, using microplate dilution method. Minimal inhibitory concentrations (MICs) of compounds were determined according the National Committee for Clinical Laboratory Standard (NCCLS, 2002). The test was performed in sterile 96-well microplates.

The compounds were dissolved in dimethylsulfoxide (DMSO). Serial two fold dilutions of each sample to be evaluated were made to yield volumes of 100 µL per well with final concentrations ranging from 200 to 1.152 µg/mL. 100 µL of bacteria suspension with a concentration of 10⁷ CFU/mL were added to each well. Negative control wells contained bacteria only in LB broth

medium. After incubation at 37 °C for 20 h, the minimal inhibitory concentrations (MICs) were recorded as the lowest concentration of compound in the medium that showed no microbial growth. Then, 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was added to the wells to facilitate reading of the plates. If there is microbial growth, MTT turns to blue if not, the medium remains yellow. Solvent medium and positive growth controls were also run simultaneously. Then from each tube, one loopful was cultured on plate count agar and incubated for 24 h at 30 °C. The lowest concentration of the compound supporting no colony formation was defined as the MBC. The levofloxacin, a broad spectrum antibiotic and fusidic acid, a narrow spectrum antibiotic.

The inhibitory activity of the tested compounds was calculated according to the formula:

$$IA (\%) = 100 - 100 (OD\ 600\ (x) / OD\ 600\ (i))$$

where (x) is the microbial culture containing the inhibitor and (i) is the microbial culture without inhibitor.

4.2.2 Antibiofilm activity

4.2.2.1. Biofilm production:

Congo Red Agar Method (CRA)

Screening of biofilm production using Congo Red agar method was performed as described. The CRA medium was composed of Brain Heart Infusion (BHI), 37 (gL⁻¹); sucrose, 50 (gL⁻¹); agar, 10 (gL⁻¹) and Congo red indicator (Sigma) 0.8 (gL⁻¹). The plates were inoculated with different microorganisms tested, a non-biofilm producing *E. coli* DH5- α bacterium serving as a negative control. Plates were incubated at 28 °C for 24 h. Black colonies with dry crystalline consistency indicate a high production of biofilm.

96 well plate method

The ability to form a biofilm was determined using the crystal violet test. The isolated strains were inoculated into 10 mL of LB broth containing 1% glucose and incubated at 28 °C for 24 h. The culture strains were diluted to obtain OD 600 ~ 0.1. Individual wells of sterile 96-well flat-bottomed polystyrene plate (orange scientific) containing 200 μ L of fresh medium were inoculated with 50 μ L of the bacterial culture tested and a sterile broth was used as a control. The same procedure was repeated for *E. coli* DH5 α and *S. aureus* which are negative and positive biofilm respectively. After incubation of the plate at 37 °C for 24 h, the culture medium of each well was removed by tapping the lower plates. The wells were washed twice with 250 μ L of Phosphate Buffer Saline, PBS (pH 7.2) to remove floating bacteria. Adherent bacteria were fixed at 60 °C for 1 hour and stained with 150 μ L of 0.2% crystalline violet prepared in 20% (v/v) ethanol for 45 minutes. To remove excess

water, the dye wells were washed three times with sterile water. The task-related bacterium was released by adding 200 μ L of glacial acetic acid. After 1 hour at room temperature, the absorbance at 570 nm was determined using the microplate reader.

4.2.2.2. Biofilm formation inhibition (pre-treatment)

The 96-well flat bottom plates were used for biofilm cultures. *S. aureus*, a biofilm-forming strain, was grown overnight in LB medium at 37 °C and diluted with fresh medium supplemented with 1% (w/v) glucose. One hundred microliters of the bacterial culture dilution were added into each well to obtain a final OD at 600 nm of 0.1. Then, each compound was added at a concentration according to their MIC values against *S. aureus*. The final concentrations into the wells were ranged between 100 μ g/mL and 25 μ g/mL. Wells containing only LB medium supplemented with, 1% glucose and bacterial suspension were used as controls. Plates were incubated for 24 h at 37 °C. After incubation, the wells were emptied into a container by inverting the plates. Each well was gently washed twice with 250 μ L of sterile PBS in order to remove the floating bacteria. After washing, plates were dried at 60 °C for 45 min. Then, wells were stained with 150 μ L of crystal violet (0.2%) prepared in 20% ethanol for 15 min at room temperature. After staining incubation, crystal violet was removed and excess dye was washed three times with sterile water. Finally, 200 μ L of glacial acetic acid was added to each well and plates were incubated for 1 h at room temperature. The optical density (OD) was measured at 570 nm using a Varioskan microplate reader (Thermofisher). The percentage of the adhesion inhibition was determined by the following formula:

$$\% \text{ adhesion inhibition (at 570 nm)} = [(\% \text{ OD (control)} - \text{OD (treated bacterium)}) / \text{OD (control)}] * 100$$

4.2.2.3. Biofilm inhibition (post-treatment)

The stain of *S. aureus* is diluted to obtain OD 600 ~ 0.1. An individual well of sterile 96-well flat-bottomed polystyrene plate containing 150 μ L of LB medium was inoculated with 50 μ L of glucose (1%) served as a negative control. In the remainder of the plate, 150 μ L of LB medium with 50 μ L of glucose (1%) and 50 μ L of the bacterial culture were incubated. The plate was incubated for 24 h at 37 °C. The wells were washed twice with 250 μ L of PBS (pH 7.2) to eliminate the floating bacteria. Then 150 μ L of the culture medium (LB) with 50 μ L of each sample were added to obtain a concentration of 200 μ g/mL. The plate is again incubated at 37 °C for 24 h. Then culture medium of each well was removed by tapping the lower plates. The wells were washed twice with 250 μ L of PBS (pH 7.2). Adherent bacteria were fixed at 60 °C for 1 hour and stained with 150 μ L of 0.2% crystalline violet prepared in 20% (v / v) ethanol for 45 minutes. To remove excess water, the dye

wells were washed three times with sterile water. The task-related bacterium was released by adding 200 μ L of glacial acetic acid. After 1 hour at room temperature, the absorbance at 570 nm was determined using the microplate reader. The experiments were performed in triplicate. The percentage inhibition of the biofilm was determined by the following formula:

$$\% \text{ inhibition of biofilm (at 570 nm)} = [(\% \text{ OD (control)} - \text{OD (treated bacterium)}) / \text{OD (control)}] * 100$$

4.2.2.4. Microscopic observation of antibiofilm activity

The antibiofilm activity of the compound **7** against *Staphylococcus aureus* was confirmed by microscopic observation using the OLYMPUS fluorescent microscope BX50 equipped with a digital camera OLYMPUS DP70. The biofilms were grown on glass pieces (\varnothing 10 mm) placed in 24-well polystyrene plates treated with the compound **7**. Non-treated wells, containing LB supplemented with 10% (v/v) of DMSO, served as controls [36, 37]. The compound **7** was added according to MIC values against *Staphylococcus aureus* (50 μ g/mL) for the test of antiadhesion and at 200 μ g/mL for eradication in LB with 10% (v/v) of DMSO. The bacterial inoculation was adjusted to an OD 600nm of 0.1. Plates were incubated at 37 °C for 24 h. The wells were then carefully emptied with pipetting and glass slides were washed with sterile PBS (1X) before the treatment with 500 μ L of acridine orange (0.1%, w/v, dissolved in PBS1X). Visualization was performed through a 40 \times objective using U-MWB2 filter with excitation at 460–490 nm and emission at 520 nm.

MTT assay of metabolic activity

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay is a colorimetric assay that measures the enzymatic reduction of MTT, a yellow tetrazole, to formazan. After biofilm formation, 150 μ L of MTT dye (0.5 mg/mL MTT in PBS) was added to the microplate containing biofilm treated with compound **7** and incubated at 37°C for 1 h and bacterial suspension were served as controls. During this process, metabolically active bacteria reduced the MTT to purple formazan. After 1 h, the disks were transferred to a new 24-well plate, 1 mL of dimethyl sulfoxide (DMSO) was added to solubilize the formazan crystals, and the plate was incubated for 20 min with gentle mixing at room temperature, in the dark. After mixing via pipetting, 200 μ L of the DMSO solution from each well was transferred to a 96-well plate, and the absorbance at 540 nm (optical density OD 540) was measured via a microplate reader (Varioskan, Thermofisher). A higher absorbance is related to a higher formazan concentration, which indicates a higher metabolic activity in the biofilm on the disk [38].

4.2.3 Antiproliferative activity

Cell culture

Human colorectal cancer (CRC) cell lines (HT-29 and HCT116) were purchased from the American Type Culture Collection (ATCC - LGC Standards, Molsheim, France). Cells were grown in DMEM medium for HT-29 cells and RPMI 1640 medium for HCT116 cells. Cells were supplemented with 10% FBS, 1% *L*-glutamine and 100 U/mL penicillin and 100 µg/mL streptomycin. Cultures were maintained in a humidified atmosphere containing 5% CO₂ at 37 °C. Stock solutions of molecules were diluted in culture medium to obtain the appropriate final concentrations (10 to 100 µM). The same amount of vehicle (percentage of DMSO did not exceed 0.6%) was added to control cells.

***In vitro* antiproliferative effect of molecules**

Antiproliferative effect of molecules was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays as previously described by Bretin *et al.*[39]. Human CRC cells were seeded at 4x10³ cells/well and 7x10³ cells/well for HCT116 and HT-29 cells, respectively, in 96-well culture plates and grown for 24 h in culture medium prior to exposure or not to molecules. MTT assay were performed 24, 48 and 72 h after treatment and cell viability was expressed in percentage of each condition of treatment by normalizing to untreated cells. Experiments were performed in triplicate.

4.2.4 Statistical analysis

All quantitative results are expressed as the mean ± standard error of the mean (SEM) of separate experiments. Statistical significance was evaluated by the two-tailed unpaired Student's t-test and expressed as: *p < 0.05.

4.3. Docking studies

The 3D structures of *Staphylococcus aureus* Dihydrofolate Reductase (DHFR), *Escherichia coli* DNA gyrase B and *Staphylococcus aureus* heptaprenyl diphosphate synthase (HepPPS) were extracted from the PDB database [40] (PDB IDs : 2W9H, 4DUH and 5H9D respectively). Co-crystallized ligands were removed from 2W9H and 4DUH structures (no ligand was co-crystallized in the 5H9D structure). The 3 structures (2W9H chain A ; 4DUH chain ; 4DUH chains A and C) were then prepared using Chimera [41] and Molecular Graphics Laboratory (MGL) tools [42] . Three dimensional structures of compounds BSL, **4a**, **4d**, **7**, **8a** and **8d** were generated using iCon, the LigandScout v.4.3. conformer generator [43] (defaults settings of the BEST option were used, except for the maximum number of conformations that was setted to 2000). The number of conformations obtained for each ligand was 19 for BSL, 12 for compound **4a**, 354 for compound **4d**, 7 for compound **7**, 13 for compound **8a** and 1624 for compound **8d**. Protein-ligand docking of all these conformations of the 6 compounds was performed using AutoDock Vina v.1.1.2 [44]. The x, y, z AutoDock Vina grid center coordinates used are 8.960, -5.922, 16.425 for 2W9H ; 2.002,

2.167, 37.295 for 4DUH ; 27.226, 24.204, 24.816 for 5H9D. The size of the search space was set to 20 Å x 20 Å x 20 Å in the 3 cases. The pose associated with the best score was considered for each conformation and was re-scored using Hydrogen bonds and Dehydration (HYDE) scoring function [45] as implemented in SeeSAR v.9.2 [46].

4.4. ADME and drug-likeness profiles

The physicochemical and pharmacokinetic properties of the PEGylated triarylmethanes were predicted using the QikProp Panel of Schrodinger software v11.9 with a prior force field energy minimization of the structures.

Conflict of interest

All authors contributed to the drafting and revision of the article and approved the final version. The authors declare no conflict of interest

Acknowledgements

Authors thank CAMPUS France (PHC Program, UTIQUE 2017 37094W) and the Ministère de l'Enseignement Supérieur et de la Recherche Scientifique of Tunisia for financial support. FA thanks Miss Mouna JLIDI for her help during the evaluation of antibiofilm activity. The authors also acknowledge the website SMART (Servier Medical Art) <https://smart.servier.com/> for image supply used in the Graphical Abstract.

Supplementary material

Supplementary data associated with this article is available free of charge at <http://www.....>

LIST OF ABBREVIATIONS

BSL: Bisacodyl

CRA: Congo Red Agar Method

CRC: Human colorectal cancer

Cy: Cyclohexane

DHFR: Dihydrofolate Reductase

equiv: Equivalent

FCC: Flash column chromatography

FA: Fusidic acid

HepPPS: heptaprenyl diphosphate synthase

HRMS: High-resolution mass spectra

HYDE: Hydrogen bonds and Dehydration

LRMS: Low-resolution mass spectra
LvF: Levofloxacin
MBC: Minimal Bactericidal Concentration
MIC: Minimal Inhibitory Concentration
MGL: Molecular Graphics Laboratory
MTT: 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide
PBS: Phosphate Buffer Saline
TAMs: Triarymethanes
RT: room temperature
SMART: Servier Medical Art

REFERENCES

- [1] R.E. Kirk, D.F. Othmer, Encyclopedia of Chemical Technology, Copper Alloys to Distillation, Wiley-Interscience, 1979.
- [2] E.R. A. Rose, F.M. Turner, The condensed chemical dictionary, Reinhold publishing corporation, New York, 1961.
- [3] G. Vijay Nair, S. Thomas, K. Abhilash, C. Smitha, Recent advances in the chemistry of triaryl- and triheteroarylmethanes, (2006).
- [4] S.J. Culp, F.A. Beland, Malachite green: a toxicological review, Journal of the American College of Toxicology, 15 (1996) 219-238.
- [5] M. Nambo, D. Kurihara, T. Yamada, T. Nishiwaki-Ohkawa, N. Kadofusa, Y. Kimata, K. Kuwata, M. Umeda, M. Ueda, Combination of Synthetic Chemistry and Live-Cell Imaging Identified a Rapid Cell Division Inhibitor in Tobacco and Arabidopsis thaliana, Plant and Cell Physiology, 57 (2016) 2255-2268.
- [6] R. Palchaudhuri, V. Nesterenko, P.J. Hergenrother, The Complex Role of the Triphenylmethyl Motif in Anticancer Compounds, Journal of the American Chemical Society, 130 (2008) 10274-10281.
- [7] J.L. Arbiser, Triarylmethane analogs and their use in treating cancers, in, vol. 10376522/ WO2009088838A2, 2009, pp. 64.
- [8] G. Panda, Shagufta, J.K. Mishra, V. Chaturvedi, A.K. Srivastava, R. Srivastava, B.S. Srivastava, Diaryloxy methano phenanthrenes: a new class of antituberculosis agents, Bioorg. Med. Chem., 12 (2004) 5269-5276.
- [9] G. Panda, M.K. Parai, A.K. Srivastava, V. Chaturvedi, Y.K. Manju, S. Sinha, Design, synthesis and antitubercular activity of compounds containing aryl and heteroaryl groups with alkylaminoethyl chains, Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem., 48B (2009) 1121-1127.

- [10] J.L. Douglas, M.L. Panis, E. Ho, K.-Y. Lin, S.H. Krawczyk, D.M. Grant, R. Cai, S. Swaminathan, T. Cihlar, Inhibition of respiratory syncytial virus fusion by the small molecule VP-14637 via specific interactions with F protein, *Journal of virology*, 77 (2003) 5054-5064.
- [11] J.L. Douglas, M.L. Panis, E. Ho, K.-Y. Lin, S.H. Krawczyk, D.M. Grant, R. Cai, S. Swaminathan, X. Chen, T. Cihlar, Small molecules VP-14637 and JNJ-2408068 inhibit respiratory syncytial virus fusion by similar mechanisms, *Antimicrobial agents and chemotherapy*, 49 (2005) 2460-2466.
- [12] V. Maité Sylla-Iyarreta, M. Dany Siverio, L. Vanessa, M. Marta, M.G. Rosa, M. Liliana Vicet, G. Yankier Rivero, D. Françoise, F. Clotilde, A.M.d.W. Peter, D.C. Alexander, J.A. Vicente, P. Yovani Marrero, Fishing Anti-Inflammatories from Known Drugs: In Silico Repurposing, Design, Synthesis and Biological Evaluation of Bisacodyl Analogues, *Current Topics in Medicinal Chemistry*, 17 (2017) 2866-2887.
- [13] A.J. Kowaltowski, J. Turin, G.L. Indig, A.E. Vercesi, Mitochondrial effects of triarylmethane dyes, *Journal of Bioenergetics and Biomembranes*, 31 (1999) 581-590.
- [14] M. Wainwright, S.M. Burrow, S.G. Guinot, D.A. Phoenix, J. Waring, Uptake and cell-killing activities of a series of Victoria blue derivatives in a mouse mammary tumour cell line, *Cytotechnology*, 29 (1999) 35-43.
- [15] G.L. Indig, G.S. Anderson, M.G. Nichols, J.A. Bartlett, W.S. Mellon, F. Sieber, Effect of molecular structure on the performance of triarylmethane dyes as therapeutic agents for photochemical purging of autologous bone marrow grafts from residual tumor cells, *Journal of pharmaceutical sciences*, 89 (2000) 88-99.
- [16] F. Guerin, V. Nicou, M. Nieto, Dyeing composition at acidic pH comprising a direct dye of triarylmethane structure, in, L'Oreal, Fr. WO2017108830A1, 2017, pp. 83.
- [17] D. Seneca, B. Boche, Hair cosmetic water-in-oil emulsions comprising triarylmethane direct dyes, in, L'Oreal, Fr. FR3059547A1, 2018, pp.76.
- [18] M. Görmén, M.S.-I. Veitía, F. Trigui, M. El Arbi, C. Ferroud, Ferrocenyl analogues of bisacodyl: Synthesis and antimicrobial activity, *Journal of Organometallic Chemistry*, 794 (2015) 274-281.
- [19] W. Li, P. Zhan, E. De Clercq, H. Lou, X. Liu, Current drug research on PEGylation with small molecular agents, *Progress in Polymer Science*, 38 (2013) 421-444.
- [20] A. Thakur, S. Ranote, D. Kumar, K.K. Bhardwaj, R. Gupta, G.S. Chauhan, Synthesis of a PEGylated Dopamine Ester with Enhanced Antibacterial and Antifungal Activity, *ACS omega*, 3 (2018) 7925-7933.
- [21] P. Mishra, B. Nayak, R. Dey, PEGylation in anti-cancer therapy: An overview, *asian journal of pharmaceutical sciences*, 11 (2016) 337-348.

- [22] A.I. Lopez, R.Y. Reins, A.M. McDermott, B.W. Trautner, C. Cai, Antibacterial activity and cytotoxicity of PEGylated poly (amidoamine) dendrimers, *Molecular BioSystems*, 5 (2009) 1148-1156.
- [23] B.Y. Park, T.P. Montgomery, V.J. Garza, M.J. Krische, Ruthenium catalyzed hydrohydroxyalkylation of isoprene with heteroaromatic secondary alcohols: Isolation and reversible formation of the putative metallacycle intermediate, *Journal of the American Chemical Society*, 135 (2013) 16320-16323.
- [24] A.J. O'Neill, I. Chopra, Preclinical evaluation of novel antibacterial agents by microbiological and molecular techniques, *Expert opinion on investigational drugs*, 13 (2004) 1045-1063.
- [25] M. Seto, Y. Aramaki, T. Okawa, N. Miyamoto, K. Aikawa, N. Kanzaki, S.-i. Niwa, Y. Iizawa, M. Baba, M. Shiraishi, Orally active CCR5 antagonists as anti-HIV-1 agents: synthesis and biological activity of 1-benzothiepine 1, 1-dioxide and 1-benzazepine derivatives containing a tertiary amine moiety, *Chemical and pharmaceutical bulletin*, 52 (2004) 577-590.
- [26] R. Alexander, G. Warrellow, M. Eaton, E. Boyd, J. Head, J. Porter, J. Brown, J. Reuberson, B. Hutchinson, P. Turner, CDP840. A prototype of a novel class of orally active anti-inflammatory phosphodiesterase 4 inhibitors, *Bioorganic & medicinal chemistry letters*, 12 (2002) 1451-1456.
- [27] D. Guay, P. Hamel, M. Blouin, C. Brideau, C.C. Chan, N. Chauret, Y. Ducharme, Z. Huang, M. Girard, T.R. Jones, Discovery of L-791,943: a potent, selective, non emetic and orally active phosphodiesterase-4 inhibitor, *Bioorganic & medicinal chemistry letters*, 12 (2002) 1457-1461.
- [28] N. Haginoya, S. Kobayashi, S. Komoriya, T. Yoshino, T. Nagata, Y. Hirokawa, T. Nagahara, Design, synthesis, and biological activity of non-amidine factor Xa inhibitors containing pyridine N-oxide and 2-carbamoylthiazole units, *Bioorganic & medicinal chemistry*, 12 (2004) 5579-5586.
- [29] H.S. Nagendra Prasad, H.M. Manukumar, C.S. Karthik, L. Mallesha, P. Mallu, A novel copper (II) PAmPiCaT complex (cPAmPiCaTc) as a biologically potent candidate: A contraption evidence against methicillin-resistant *Staphylococcus aureus* (MRSA) and a molecular docking proof, *Bioorganic & Medicinal Chemistry*, 27 (2019) 841-850.
- [30] M. Durcik, P. Tammela, M. Barančoková, T. Tomašič, J. Ilaš, D. Kikelj, N. Zidar, Synthesis and Evaluation of N-Phenylpyrrolamides as DNA Gyrase B Inhibitors, *ChemMedChem*, 13 (2018) 186-198.
- [31] J. Desai, Y.-L. Liu, H. Wei, W. Liu, T.-P. Ko, R.-T. Guo, E. Oldfield, Structure, Function, and Inhibition of *Staphylococcus aureus* Heptaprenyl Diphosphate Synthase, *ChemMedChem*, 11 (2016) 1915-1923.
- [32] B.I. Schweitzer, A.P. Dicker, J.R. Bertino, Dihydrofolate reductase as a therapeutic target, *The FASEB Journal*, 4 (1990) 2441-2452.
- [33] T. Tihomir, M. Lucija Peterlin, Prospects for Developing New Antibacterials Targeting Bacterial Type IIA Topoisomerases, *Current Topics in Medicinal Chemistry*, 14 (2014) 130-151.

- [34] S.S. Leung, J. Mijalkovic, K. Borrelli, M.P. Jacobson, Testing physical models of passive membrane permeation, *Journal of chemical information and modeling*, 52 (2012) 1621-1636.
- [35] S.S. Leung, D. Sindhikara, M.P. Jacobson, Simple predictive models of passive membrane permeability incorporating size-dependent membrane-water partition, *Journal of chemical information and modeling*, 56 (2016) 924-929.
- [36] A.R. Padmavathi, S.K. Pandian, Antibiofilm activity of biosurfactant producing coral associated bacteria isolated from gulf of mannar, *Indian Journal of Microbiology*, 54 (2014) 376-382.
- [37] F. Abdelli, M. Jardak, J. Elloumi, D. Stien, S. Cherif, S. Mnif, S. Aifa, Antibacterial, anti-adherent and cytotoxic activities of surfactin(s) from a lipolytic strain *Bacillus safensis* F4, *Biodegradation*, 30 (2019) 287-300.
- [38] L. Cheng, W.M. D, X.H.K. Hockin, J.M. Fau - Antonucci, A.J. M, N.J. Lin, S. Gibson-Lin, S.M. Xu, X. Zhou, Effect of amorphous calcium phosphate and silver nanocomposites on dental plaque microcosm biofilms, *journal of biomedical materials research Part B, applied biomaterials*, 100 (2012) 1378-1386.
- [39] L. Bretin, A. Pinon, S. Bouramtane, C. Ouk, L. Richard, M.-L. Perrin, A. Chaunavel, C. Carrion, F. Bregier, V. Sol, Photodynamic Therapy Activity of New Porphyrin-Xylan-Coated Silica Nanoparticles in Human Colorectal Cancer, *Cancers*, 11 (2019) 1474.
- [40] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, The Protein Data Bank, *Nucleic Acids Research*, 28 (2000) 235-242.
- [41] E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, T.E. Ferrin, UCSF Chimera—A visualization system for exploratory research and analysis, *Journal of Computational Chemistry*, 25 (2004) 1605-1612.
- [42] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson, AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility, *Journal of Computational Chemistry*, 30 (2009) 2785-2791.
- [43] G. Wolber, T. Langer, LigandScout: 3-D Pharmacophores Derived from Protein-Bound Ligands and Their Use as Virtual Screening Filters, *Journal of Chemical Information and Modeling*, 45 (2005) 160-169.
- [44] O. Trott, A.J. Olson, AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, *Journal of Computational Chemistry*, 31 (2010) 455-461.
- [45] N. Schneider, G. Lange, S. Hindle, R. Klein, M. Rarey, A consistent description of HYdrogen bond and DEhydration energies in protein–ligand complexes: methods behind the HYDE scoring function, *Journal of Computer-Aided Molecular Design*, 27 (2013) 15-29.

[46] SeeSAR version 9.2; BioSolveIT GmbH, Sankt Augustin, Germany, 2019, www.biosolveit.de/SeeSAR, s. d., in.