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Abstract: Emergence of multidrug resistant pathogens was responsible for microbial infections and inefficacy of numerous antimicrobial therapies has induced a need for the research of new classes of antibiotics. In this review, we will focus our interest towards the biological properties of polyamino antimicrobial agents.

Keywords : Gram-negative bacteria – Multidrug resistance – Antibiotic enhancers – Polyamines – Antimicrobial agents

Each year in the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics and at least 23.000 people die annually as a direct result of these infections. The introduction in the Mid-20th century of efficient antibiotic therapies for infectious diseases has completely modified clinical practices in the development of life-threatening conditions leading to reduce the incidence of death resulting from bacterial infections. However, the rise of antibiotic resistance since few decades has resulted in a very pressing need for the discovery of novel antibiotics or treatment strategies [1]. In this context, numerous active avenues of research on-going to develop the next generation of antibacterial drugs are under current investigations such as the combination of two active antibacterial agents into one hybrid compound or synthetic peptide mimics [2].

Some strains have become resistant to practically all of the commonly available agents. A notorious case is the methicillin-resistant *Staphylococcus aureus* (MRSA), which is resistant not only to methicillin but usually also to aminoglycosides, macrolides and cyclines. Such strains are also resistant to disinfectants, and can act as a major source of hospital-acquired infections. An even more serious threat may be the emergence of Multidrug resistant (MDR) Gram-negative pathogens that are a global public health concern as therapeutic options for treating such infections are dwindling. Thus, Multidrug resistance in bacteria occurs by the presence of plasmids or transposons, of genes, with each coding for resistance to a specific agent, and/or by the action of multidrug efflux pumps, each of which can pump out more than one drug type.

Furthermore, the emergence of "pan-resistant" Gram-negative strains, notably those belonging to *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, occurred more recently, after most major pharmaceutical companies stopped the development of new antibacterial agents. Hence, there are almost no agents that could be used against these strains, in which an

outer membrane barrier of low permeability is combined with multitudes of specific resistance mechanisms.

On the basis of such observations, there is a need for the development of new strategies and a revival for the use of neglected polyamino derivatives has emerged as antimicrobial agents able to fight resistant Gram-negative bacteria. Polyamines are small aliphatic hydrocarbon molecules with quaternary nitrogen groups that have a net positive charge at physiological pH. During the 60's and 70's polyaminated molecules were identified in all forms of life such as bacteria, fungi, plants and all types of eukaryotic cells. They were described to be critical for all types of cellular proliferation by determining the metabolic pathways of synthesis and degradation. Thus the apparent critical influence of polyamines on cell development and survival and their recognition by the polyamine transporters have both led to polyamines being increasingly considered for the design of a range of chemotherapeutic agents [3].

On the other hand, it has been widely demonstrated that polyamines can act as endogenous modulators of outer membrane permeability [4] of bacteria inducing resistance to cationic peptide, aminoglycoside or quinolone antibiotics [5].

Polyamines are old polycationic molecules widely distributed in nature described for very first time in 1677 in seminal fluid [6]. It has been widely demonstrated that some of them such as cadaverine [7] or spermine [4] could decrease bacteria outer membrane permeability by being natural regulators of porin activity and subsequently reduce bacterial susceptibility to antibiotic treatments [8-10].

Surprisingly, it has been found that polyamines at millimolar levels can increase the susceptibility of *P. aeruginosa* to a variety of antibiotics (Table 1) [11] whereas Vaara *et al.* by using submillimolar concentrations have observed a discrepant outcome [12]. It has appeared that polyamines might be potentially useful in antipseudomonal therapies by increasing the effectiveness of numerous β-lactam antibiotics.

Table 1. MICs of antibiotics to *P. aeruginosa* PAO1 in the presence of polyamines

Antibiotic	MIC (µg/mL) in presence of				
	No compound	Spd	Spn	Put	Cad
Ampicilline	>1024	64	64	128	128
Aztreonam	4	0.5	0.5	1	0.5
Ceftazidine	2	0.5	0.5	1	0.5
Chloramphenicol	128	32	32	64	64
Nalidixic acid	128	64	64	64	64
Erythromycine	128	128	64	128	128

Compound concentrations were as follows: 20 mM spermidine (Spd), putrescine (Put), cadaverine (Cad); 1 mM spermine (Spn).

Thus, these results suggested the development of a new approach involving polyamino derivatives as potent either antimicrobial agents or chemosensitizers of ineffective antibiotics against MDR bacteria.

Development of new polyamino antimicrobial agents

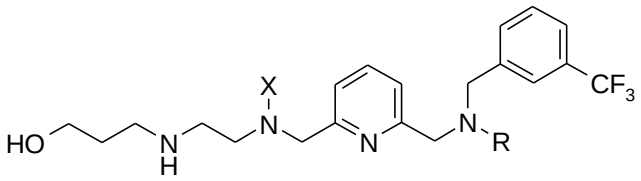
Different polyamino antimicrobial agents have been designed and we can classify these derivatives according two major classes belonging to a steroidal or a non-steroidal family.

Non-steroidal derivatives

In 1998, Cook *et al.* have prepared a 1638-member meta-substituted benzyl pyridinopolyamine library by solution-phase chemistry. Twelve compounds **1-12** exhibit potent, highly selective activity against Gram-positive bacteria over Gram-negative bacteria and very high specificity for bacteria compared with the fungus *C. albicans*. Thus, *S. pyogenes*, *S. aureus*, and *E. faecalis* were inhibited at MICs of 1-12 µM, whereas MICs for *E. coli*, *K. pneumoniae*, *P. vulgaris*, and *P. aeruginosa* were >100 µM. It clearly appeared that

functional groups in the meta-positions of the benzyl functionality set do indeed provide sufficient differentiating diversity to allow biological activities to be separated and identified from a library by iterative and positional scanning deconvolution processes (Table 2) [13].

Table 2. Structures and antimicrobial activities of meta-substituted benzyl pyridinopolyamine derivatives **1-12**.



R_1 = CH_2Ph R_4 = $CH_2C_6H_4NO_2$-m

R_2 = $CH_2C_6H_4F$-m R_5 = $CH_2C_6H_4COOCH_3$-m

R_3 = $CH_2C_6H_4CH_3$-m R_6 = $CH_2C_6H_4CF_3$-m

X = $CH_2C_6H_4CH_3$-m

1, R = R_1; **3**, R = R_3; **5**, R = R_5

2, R = R_2; **4**, R = R_4; **6**, R = R_6

X = $CH_2C_6H_4CF_3$-m

7, R = R_1; **9**, R = R_3; **11**, R = R_5

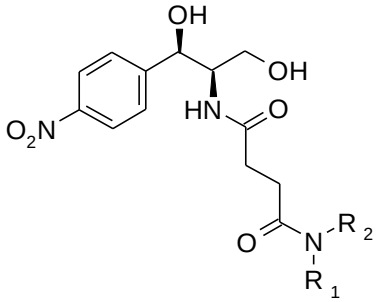
8, R = R_2; **10**, R = R_4; **12**, R = R_6

	MIC (μg/mL)							
Cpd	*S. aureus*	*E. faecalis*	*E. coli*	*S. pyogenes*	*P. aeruginosa*	*P. vulgaris*	*K. pneumoniae*	*C. albicans*
1	3-6	3-6	12-25	1-5	>100	>100	6-12	<12.5
2	3-6	3-6	>100	1-5	>100	>100	12-25	<12.5
3	3-6	3-6	>100	1-5	>100	>100	6-12	<12.5
4	3-6	3-6	>100	1-5	>100	>100	>100	25
5	6-12	3-6	>100	1-5	>100	>100	>100	<12.5
6	6-12	3-6	>100	1-5	>100	>100	>100	>100
7	12-25	6-12	>100	1-5	>100	>100	25-50	>100
8	6-12	3-6	>100	3-6	>100	>100	>100	>100
9	6-12	3-6	>100	1-3	>100	>100	50-100	>100
10	3-6	3-6	>100	1-3	>100	>100	>100	>100
11	6-12	3-6	>100	1-3	>100	>100	>100	>100

12	3-6	6-12	>100	2.5-5	>100	>100	>100	>100

On the other hand, a series of chloramphenicol (CAM) amides with polyamines **13-17** were recently synthesized either by direct attachment of the PA chain on the 2-aminopropane-1,3-diol backbone of CAM, previously oxidized selectively at its primary hydroxyl group, or from chloramphenicol base (CLB) through acylation with succinic or phthalic anhydride and finally coupling with a PA. In this context, conjugates **16** and **17** possessing a dibenzylated spermidine moiety through the succinate linker were the most potent antibacterial agents against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacterial strains (Table 3) [14].

Table 3. Determination of IC_{50} for chloramphenicol and its polyamino parent derivatives against wild-type and mutant *S. aureus* and *E. coli*.

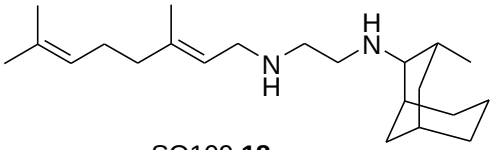


13 : R_1 = H, R_2 = $(CH_2)_4NH_2$
14 : R_1 = H, R_2 = $(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$
15: R_1 = $(CH_2)_3NH_2$, R_2 = $(CH_2)_4NH_2$
16: R_1 = $(CH_2)_3NH_2$, R_2 = $(CH_2)_4N(CH_2Ph)_2$
17: R_1 = H, R_2 = $(CH_2)_3NH(CH_2)_4N(CH_2Ph)_2$

	IC_{50} (μM)				
Cpd	MRSA (GRE2272)	*S. aureus* (WT)	*E. coli* (A2058G)	*E. coli* (WT)	*E. coli* (ΔtolC)
CAM	8.0	3.1	15.5	6.2	2.0
13	>200	>200	>200	>200	>200
14	>200	45.3	>100	>100	>100
15	>100	12.7	>150	>150	>100
16	7	4.7	9.4	9.4	19.0
17	>100	13.7	32.3	35.5	42.5

Among infectious diseases, tuberculosis still remains one of the leading single agent killer in the world with aound 2 million of deaths each year. Polyamino derivatives N-geranyl-N'-(2-adamantyl)ethane-1,2-diamine SQ109 **18** (Figure 1), a second generation agent from the first line drug ethambutol demonstrated interesting activities against both *M. tuberculosis* and *M. smegmatis* with MICs of 0.5 and 2 µg/mL, respectively [15].

Figure 1. Structure of derivative SQ109 **18**

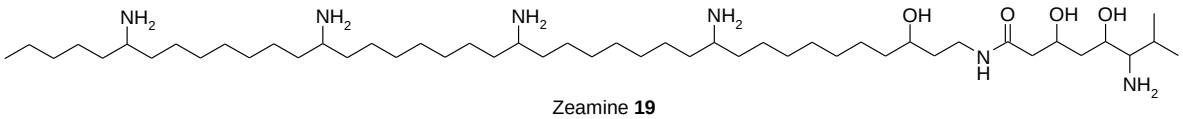


SQ109 **18**

Nevertheless, the bioavailability of this product remains low limiting its development and therapeutical use [16].

In 2010, Wu *et al.* isolated from the bacterial pathogen *Dickeya Zeae* strain DZ1 a new antibacterial compound namely zeamine **19**. Of peculiar note is that numerous MDR bacteria such as *P. aeruginosa* and *B. cenocepacia* are susceptible to zeamine with excellent to moderate MIC values varying from 0.3 to 50 µg/mL depending on the nature of the considered bacterial strain (Table 4) [17].

Table 4. Structure and antimicrobial activities of zeamine **19**

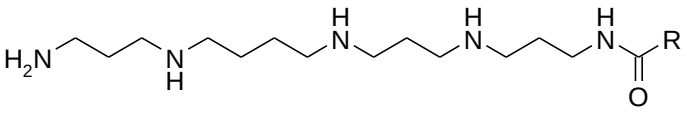


Zeamine **19**

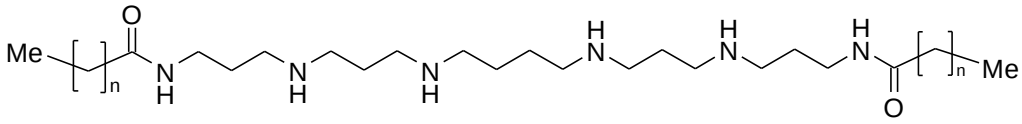
Strain	MIC (µg/mL)						
	S. Aureus ATCC25531	*B. Cereus* XJ8	*E. Coli* CFT073	*B. Cepacia* H111	*P.Aeruginosa* PAO1	*S. enterica*	*K. pneumoniae*
19	0.3	3	3	50	5	3	6

Finally, homologous series of mono- and bis-acyl polyamines **20-32** with varying acyl chain lengths were also designed and presented moderate MICs against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacterial strains (Table 5) [18].

Table 5. Structure and antimicrobial activities of mono- and bis-acyl polyamines **20-32**



20, R= CH_3; **21**, R = C_8H_{17}
22, R= C_9H_{19}; **23**, R = $C_{12}H_{25}$
24, R= $C_{14}H_{29}$; **25**, R = $C_{16}H_{33}$
26, R= $C_{18}H_{37}$



27, n =7; **28**, n = 8; **29**, n = 10;
30, n = 13; **31**, n = 15; **32**, n = 17

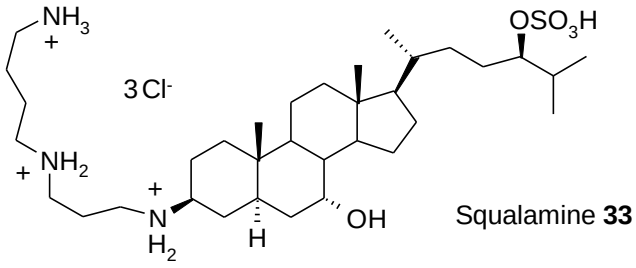
	MIC (µM)				
Cpd	*S. aureus* (WT)	*E. coli* (A2058G)	Cpd	*S. aureus* (WT)	*E. coli* (A2058G)
20	250	62.5	**27**	15.6	31.25
21	125	62.5	**28**	3.9	31.25
22	62.5	62.5	**29**	15.6	31.25
23	15.6	31.25	**30**	250	62.5
24	15.6	31.25	**31**	250	62.5
25	15.6	62.5	**32**	125	62.5
26	15.6	62.5			

Steroidal derivatives

On the other hand, numerous compounds possessing a sterol core were identified to possess interesting antibacterial activities. Among them, Squalamine **33**, a natural polyaminosterol derivative isolated from the shark *Squalus acanthias* was reported to be active against a large

panel of microorganisms with MICs ranging from 1 to 8 µg/mL against Gram positive and Gram-negative bacteria (Table 6) [19].

Table 6. Antibacterial activities of squalamine **33** (MICs mg/L).



Squalamine **33**

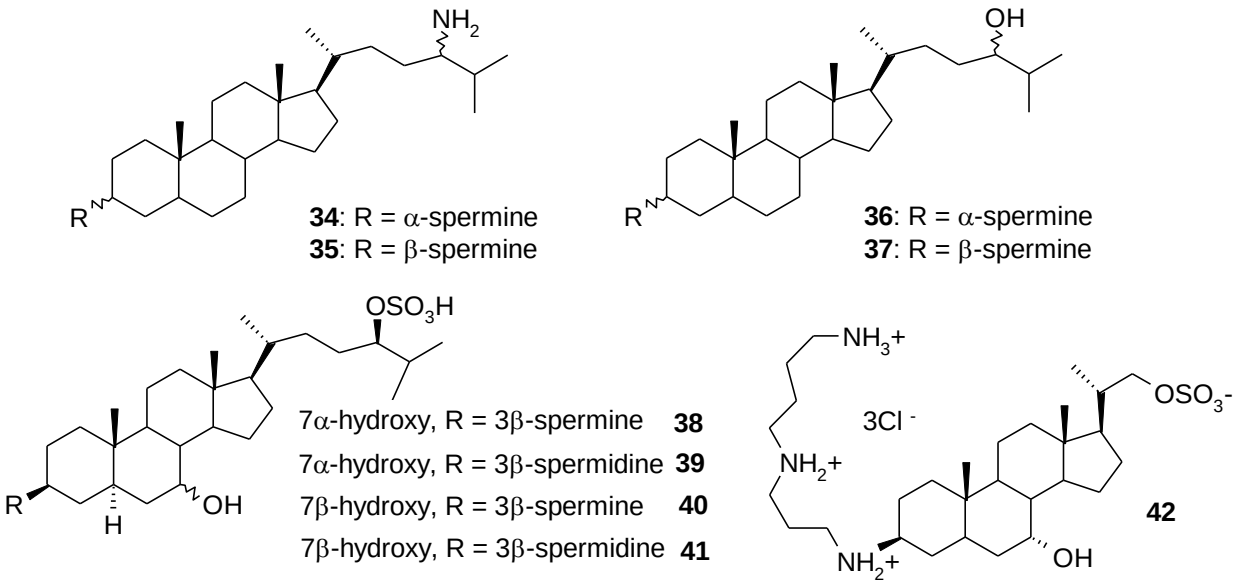
Cpd	Gram positive bacteria			Gram negative bacteria			
	S. aureus	*S. pneumoniae*	*E. faecalis*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*E. aerogenes*
33	2-8	32	-	8	2-8	8	32

On the basis of such results, the synthesis of numerous derivatives **34-37** from stigmasterol was achieved by Shu et *al.* (Figure 2) demonstrating that 3β analogs exhibit better activity than 3α ones [20]. From his part, Selinsky *et al.* reported that squalamine analogues **38-41** differing in the identity of the polyamine attached at C3 of the sterol, and the stereochemistry of a hydroxyl substituent at C7 possessed different antimicrobial activities [21]. Thus, analogs with a tetraammonium spermine polyamine appear to be somewhat more active than analogs with a shorter trisammonium spermidine polyamine, and analogs with an axial (α) hydroxyl substituent at C7 are more active than analogs with the corresponding equatorial (β) hydroxyl one. Otherwise, 7β-OH spermine analog is the most active compound against *E. coli*, but the least effective against *P. aeruginosa* (MICs varying from 1 to 32 µg/mL, respectively).

On the other hand, Khan et *al.* reported the synthesis of a series of 3β-hydroxy-7α-amino 23,24-bisnor-5α-cholan-22-ol derivatives such as compound **42** which demonstrates a good

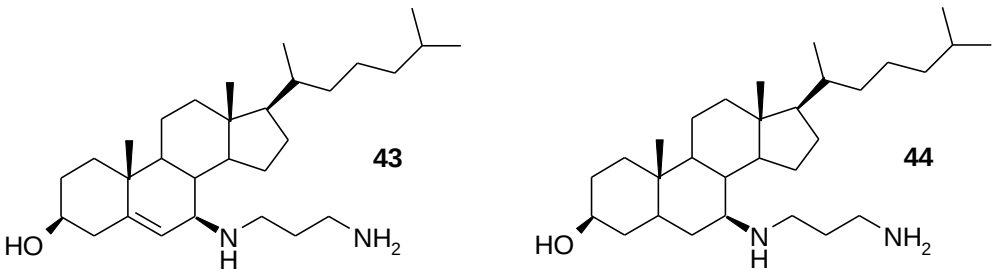
activity against Gram positive bacteria (MICs values 1.6-25 µg/mL) with respect to Gram negative ones (MICs value 6.3 to > 100 µg/mL) [22], [23]. Similar results were encountered by Kim et *al.* by using 3-polyamino-23,24-bisnorcholane derivatives [24].

Figure 2. Structures of polyaminosterol derivatives **34-42**



Amino deoxycholic acid derivatives were also reported to be active against numerous bacteria and even against vancomycin and methicillin resistant strains suggesting a high correlation between the cationic charge of the polyamine chain group and the biological activity [25]. Recently, numerous 3 and 7-polyaminosterol squalamine analogues such as **43-44** (Figure 3) were synthesized and demonstrated good activities against both Gram-positive and Gram-negative bacterial strains with MICs varying from 2.5 to 10 µg/mL [26][27] even against multi drug resistant strains recovered from cystic fibrosis patients (137 strains) [28].

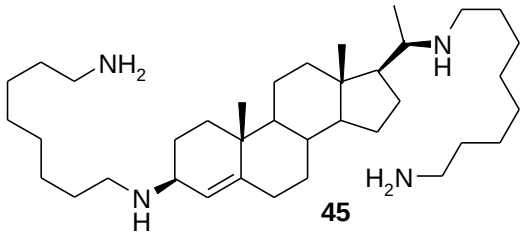
Figure 3. Structure of 7-polyaminosterol derivatives **43-44**.



In the case of Gram positive bacteria, Alhanout et *al.* demonstrated that the activity of squalamine or its parent derivatives is due to a strong depolarization of the membrane with drained cytoplasmic content suggesting a "detergent like" mechanism [29]. On the opposite, in the case of Gram-negative bacteria these derivatives are able to disturb the membrane integrity of Gram negative bacteria by interaction with negative charges of phosphate groups of the LPS at the surface of the outer membrane [30].

On the basis of such a mechanism, 3,20-Amino- and polyaminosteroid analogs of squalamine such as derivative **45** were synthesized and evaluated for their *in vitro* antimicrobial properties against reference and clinical bacterial strains exhibiting MICs ranging from 2.5 to 40 µg/mL (Table 7) [31].

Table 7. Antibacterial activities of the 3,20-polyaminosterol derivative **45** (MICs µg/mL).



45

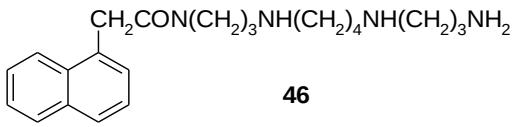
Minimum Inhibition Concentration (MIC (µg/mL))					
Compound	*S. aureus*	*E. coli*	*P.aeruginosa*	*I. limosus*	*B. cepacia*
45	2.5	5	2.5	10-20	>40

Development of polyamino chemosensitizers for antibiotic activity enhancement

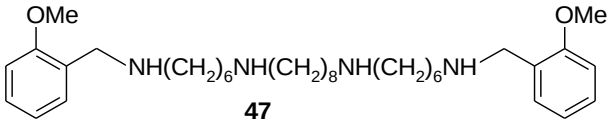
One of the first studies was realized by Yasuda *et al.* in 2004 dealing with the synthesis and use of naphtylacetylspermine **46** and methoctramine **47** as chemosensitizer agents increasing *E. coli* membrane permeability. No intrinsic antimicrobial properties were encountered for these compounds but they are able to highly potentiate novobiocine or erythromycin antimicrobial activities (Table 8). It has been suggested that these compounds could alter

membrane integrity by displacing divalent cations leading to an enhancement of noviobiocine and eryhtromycine entrance [32].

Table 8. Chemical structure of naphtylacetylspermine **46** and methoctramine **47** and their use as chemosensitizers against *E. coli*



46



47

Antibiotic	**MIC (µg/mL)**											
	Used concentrations (µg/mL) of polyamines 46 and 47											
		Naphtylacetylspermine **46**								Méthoctramine **47**			
		1	2	4	8	16	32	64	128	1	2	4	8
Novobiocine	128	128	64	32	16	8	4	1	0.5	128	128	32	16
Erythromycine	64	64	64	64	31	16	8	4	4	64	64	32	16

Otherwise, due to its previously described properties**,** an approach using squalamine as a chemosensitizer agent has been envisioned against resistant strains by using it at 1/5 and 1/10 of its MIC value to enhance significantly the activity of numerous antibiotics against isogenic multidrug *E. coli* AG100 and AG100Atet strains (Table 9) [33].

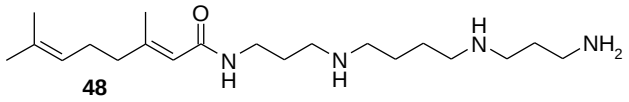
Table 9. Effect of sub-inhibitory concentrations of squalamine **33** on the antibiotic susceptibility of various Gram-negative bacteria

		MIC (µg/mL)				
Bacteria / Strains	**Addition**	**CHL**	**CIP**	**TET**	**FEP**	**ERY**
E. coli AG100	0	8	0.25	2	0.5	512
	+ 1/5 Sq	0.5	0.03	0.125	0.06	256
	+ 1/10 Sq	1	0.015	0.25	0.12	256
E. coli AG100A (*acrAB-)*	0	2	0.06	2	0.5	128
	+ 1/5 Sq	1	0.06	0.12	0.12	64
	+ 1/10 Sq	2	0.03	0.12	0.12	64
E. coli AG100tet (*acrAB*-, over-producing other pumps)	0	16	1	64	1	256
	+ 1/5 Sq	2	0.03	8	0.12	256
	+ 1/10 Sq	4	0.03	16	0.25	128

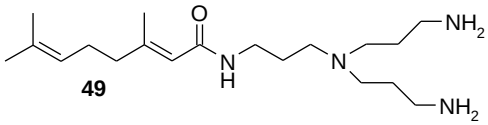
Sq, squalamine **33** was used at 1/10 and 1/5 of its MIC determined for each strain. CHL, chloramphenicol; CIP, ciprofloxacin; TET, tetracycline; FEP, cefepime, ERY, erythromycin.

Such a result appears to be of great interest for the development of new drug combinations against MDR bacteria. Due to all of these considerations, LPS assembly became recently an interesting target due to the fact that its disruption could enhance the entrance of antibiotics through Gram-negative outer membranes. Thus, geraniol, a natural monoterpene encountered in many plant extracts, was demonstrated to act in a synergistic manner with several antibiotics against Gram-negative bacterial species. Numerous geraniol derivatives including geranylamine and polyaminogeranic acid molecules **48-50** were prepared and successfully investigated as chemosensitizers of chloramphenicol and nalidixic acid against Enterobacter and Salmonella strains (Table 10). It has been also demonstrated that they can alter the activity of a drug transporter and inhibit the major *Enterobacteriaceae* efflux pump, AcrAB-TolC [34].

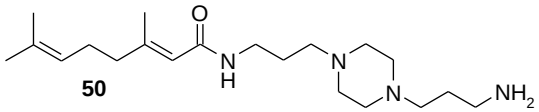
Table 10: Decrease of chloramphenicol and nalidixic acid resistance in the presence of compounds **48**-**50**.



48



49



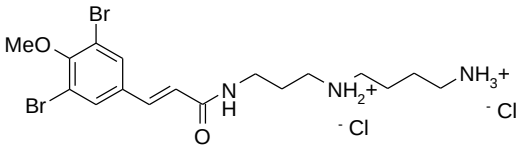
50

	Enterobacter			*Salmonella*		
Cpd	Concentration (µM)	MIC ratio CHL(*)	MIC ratio NAL(*)	Concentration (µM)	MIC ratio CHL(*)	MIC ratio NAL(*)
PAßN	38	16	8	38	8	64
48	31	16	4	31	8	32
49	62.5	8	8-16	125	16	64
50	250	8	4	250	8	64

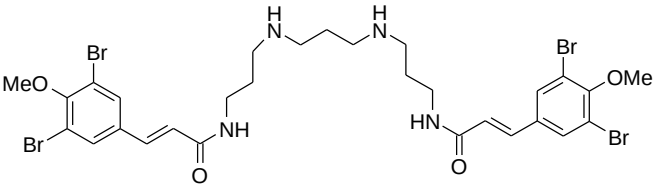
(*) MIC ratio is determined as the MIC of the antibiotic alone for each strain (ie chloramphenicol or nalidixic acid) to the MIC of the same antibiotic in the presence of compounds **48**-**50**. A ratio up to 1 indicates an improvement of the activity of the antibiotic in the presence of the considered compound.

In this context, another original chemical strategy was developed to prepare non cytotoxic ianthelliformisamine derivatives **51-56** which dramatically affected the antibiotic susceptibility of *E. aerogenes*, *P. aeruginosa*, and *K. pneumoniae* MDR strains (Table 11) [35].

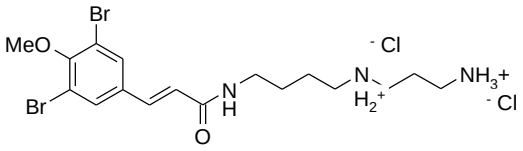
Table 11. Concentration of Ianthelliformisamine derivatives **51-56** necessary to restore doxycycline activity (2 µg/mL) against Ea289, PAO1 and KPC2 ST258 Gram-negative bacterial strains.



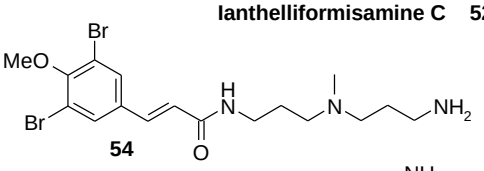
Ianthelliformisamine B 51



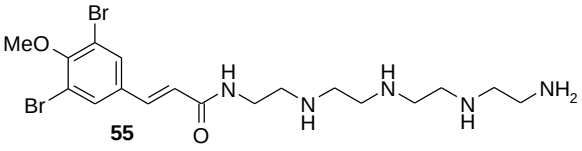
Ianthelliformisamine C 52



Tokaridine C 53



54



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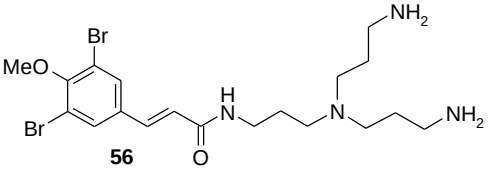

56

Compounds	Concentration of Ianthelliformisamine derivative used (µM)		
	Ea289	PAO1	KPC2 ST258
51	25	12.5	3.12
52	12.5	3.12	12.5
53	6.25	3.12	3.12
54	12.5	6.25	3.12
55	12.5	6.25	3.12
56	6.25	3.12	3.12

Ea289 : *Enterobacter aerogenes* 289 ; PAO1 : *Pseudomonas aeruginosa* ; KPC2ST258 : *Klebsiella pneumoniae ST2558*

This efficiency was correlated with the inhibition of a dye transport, suggesting an action of these molecules on the activity of drug transporters. Due to their low cytotoxicity these kind of molecules could open the way for the development of new therapeutical strategies.

Conclusion

During the last decades, the search and commercial development of new antibiotics did not follow the rhythm of emergence of Multidrug resistant bacteria. An alternative to this strategy

is the finding of active molecules (that we will call antibiotic potentiators or chemosensitizers), preferably with a weak antibiotic activity and that in combination with antibiotics are able to enhance or synergize the antimicrobial activity of the latter. Antibiotic adjuvants can function either by reversing resistance mechanisms in naturally sensitive pathogens or by sensitizing intrinsic resistant strains. Thus, the use of antibiotic adjuvants has two beneficial outcomes: enhancement of the antimicrobial effect and reduction of the occurrence of mutations that results in resistance. In this context and as underlined in this review the search for molecules such as polyamine derivatives which act by membrane depolarization and/or integrity membrane disruption could constitute an efficient alternative since their mechanism of action may significantly reduce the development of resistance. Finally, we can envision that the continuous advances in the development of new and potent high-throughput technologies will definitively allow the discovery of new compounds with antibiotic adjuvant activity which is a less expensive alternative to the problem of Multidrug resistance.

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