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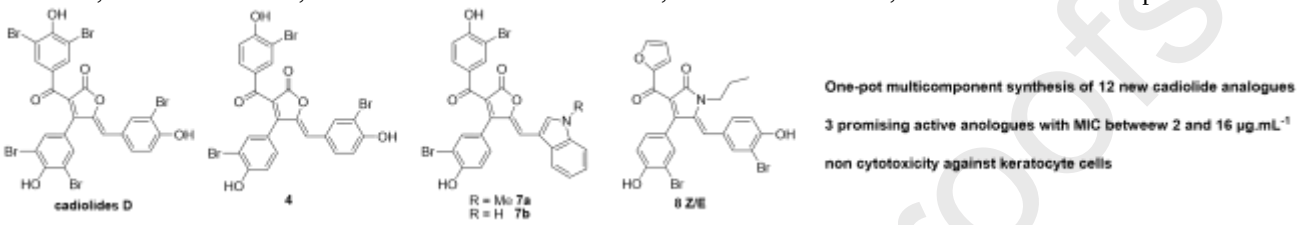

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New Antibacterial Cadiolide Analogues active Against Antibiotic Bacterial Resistant Strains

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ABSTRACT

The synthesis of new cadiolide analogues was carried out using a one-pot multi component synthesis. The antibacterial activity of these molecules was evaluated on standard and antibiotic resistant bacterial strains chosen for their involvement in human health or in food-born poisoning. Four molecules have shown good activities with MICs of 2 $\mu g/mL^{-1}$. The introduction of an indole group or the conversion of the lactone into lactam have highlighted two new families of molecules with promising antibacterial activity. In addition, most of these active molecules are devoid of cytotoxic activity against keratinocyte cells.

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significantly increased life expectancy. However, their intensive and repeated use, or even misuse, for both human and animal treatment in the hospital or agricultural environments has contributed to the development of resistant strains. The fight against this phenomenon is becoming a major public health issue. For this reason, numerous research groups and pharmaceutical industries have focused their work on the design and the discovery of new antibacterial compounds.

The most critical antibiotic-resistant strains requiring urgent new drug discovery have been recently reassessed by The World Health Organization (WHO).[1] Among these strains *Acinetobacter baumannii* (carbapenem-resistant), *Pseudomonas aeruginosa*, (carbapenem-resistant), *Enterobacteriaceae* (carbapenem-resistant), *Enterococcus faecium*, (vancomycin-resistant), *Staphylococcus aureus* (methicillin-resistant (SARM) and vancomycin-intermediate and resistant) represent the strains frequently encountered in hospital whose are responsible of hospital-acquired infection.

In this global context of the race to discover new antibacterial compounds, numerous ways have been investigated, such as antimicrobial peptides produced by mammals or insect[2] or chemically modified antimicrobial peptides[3] or natural product from the marine world.[4]

Since the late 1990s, new natural marine brominated butenolides were isolated from ascidian or tunicates, representing the cadiolide family (Figure 1).[5] The cadiolides (A-F), rearranged cadiolide I and hydroxylated cadiolide derivatives (G-H, J-M) have shown potential antibacterial activities on a large range of bacterial strains.[6]

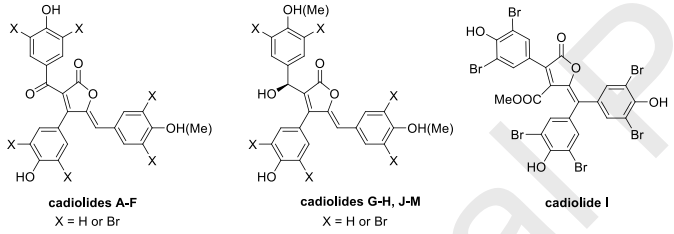
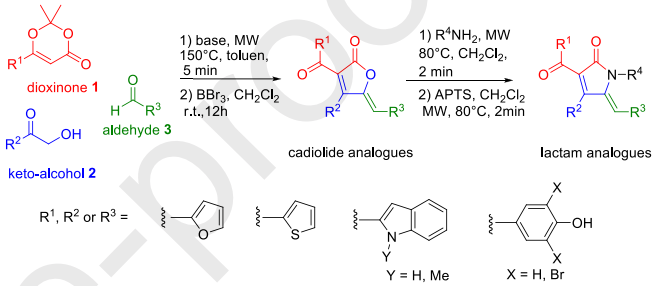


Figure 1: natural cadiolides A-M

Since their discovery, few research groups have tackled their synthesis as well the synthesis of analogues. In 2005, Pouliot and Boukouvalas[7] were the first to publish the synthesis of cadiolide B followed by us[8] in 2013 with a one-step multicomponent strategy. In 2015, Boukouvalas reported an elegant step-economical strategy by Diels−Alder cycloaddition−cycloreversion towards these molecules.[9] While using a multicomponent synthetic strategy, we have been able to obtain a large number of cadiolide analogues to study their structure-activity relationship,[10] in particular by obtaining more or less brominated molecules or/and by introducing new functional groups like furoyl instead of benzoyl. Thus, some cadiolide analogues showed greater antibacterial activity than natural products with non-resistant gram positive or negative strains like *Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Salmonella typhi*, *Escherichia coli* and *Erwinia carotovora*. In some cases, the obtained activities were similar or better than broad-spectrum antibiotics such as tetracycline. This structure-activity relationship study also revealed that the phenolic hydroxyl group plays an important role and that the overall optimal bromine atom number is 3 to 4. Importantly, we showed that the substitution of a benzoyl group by a furoyl group allowed improving their synthesized and evaluated other cadiolide analogues that showed activities in inhibiting bacterial biofilm formation.

Thus, in view of these promising results, we have decided to extend this structure-activity relationship study with resistant or not bacterial strains by varying the position of the furyl group around the furanonic skeleton, and by increasing the structural diversity with the introduction of new heterocyclic moieties such as thiophene and indole in replacement of the furyl group that we introduced earlier.[10] Indeed, during a study of structure-activity relationship with heterocyclic chalcone analogues, Tran *et al.* have shown that the substitution of a furyl group by a thiophenyl or pyriridinyl group could bring, in some cases, an improvement of the antibacterial activity.[12] But, rather than pyridine we have preferred to introduce an indole group which is more easily functionalizable and can play an important role in antibacterial activity.[13] Finally, we wanted to check if the central furanonic skeleton has a great importance on antibacterial activities and made the choice to convert the lactone ring into a lactam.



Scheme 1: Synthetic route to cadiolide derivatives

From suitable dioxinones, ketoalcohols and aldehydes (prepared according to reported procedure, Figure 2),[8,10] more than ten cadiolide analogues were synthesized using our one-step multicomponent reaction (Scheme 1) including the family of furan **5a-c** (yields ranging from 18 to 28 %), thiophene **6a-d** (yields ranging from 25 to 96 %), indole **7a-b** (yields ranging from 6 to 9 %), lactam **8** (total yield 27 %, Z/E mixture: 55/45),[14] and bis-furan **9a-b** (yields ranging from 16 to 88 %), cadiolide analogues (yield 16 %) (Table 1).

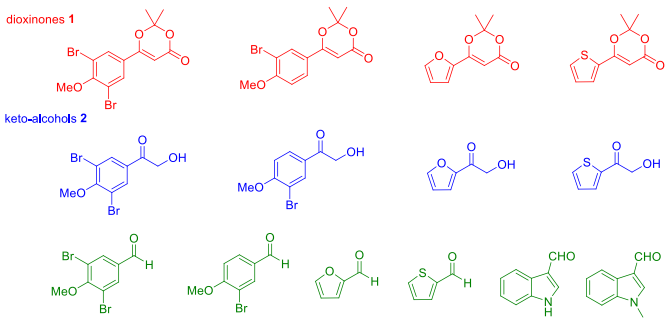


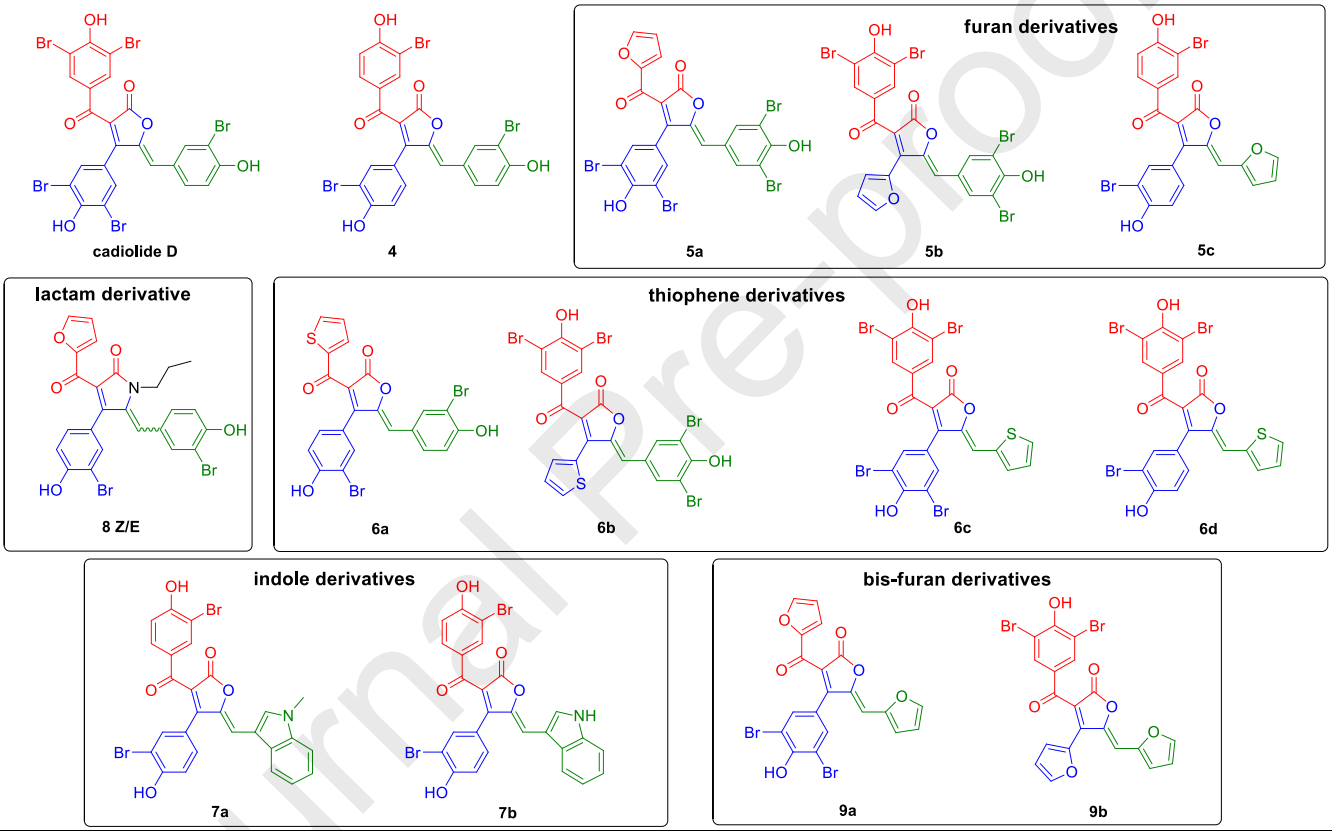
Figure 2 : list of dioxinones, ketoalcohols and aldehydes used

In order to have a point of comparison with our previously obtained results,[9] we included in the evaluation of the antibacterial activities the caliolide derivative **4** which had shown the best activities, and also synthetized for the first time the natural cadiolide D. In the present study, we have evaluated both the anti-bacterial activity of natural product cadiolide D and 13 new cadiolide analogues using both standard and antibiotic resistant-bacterial strains and, for the most active compounds, their cytotoxicity against keratinocyte cells.

Biological activity results

All [illegible] n of *vitro* for their potential antibacterial activity against 4 Gram negative bacteria and 7 Gram positive bacteria. These bacteria have been chosen for their implications either in human health or in food-born poisoning (see material and methods). No cadiolides have exhibited activity against Gram negative bacteria until 256 µg.mL^{-1} (Data not shown). In contrast, in the case of Gram positive bacteria, antibacterial activity has been observed against almost all target bacteria with MICs values between 2 and 256 µg.mL^{-1}. The best activity is obtained with the indole derivatives **7a,b** with MICs of 2 µg.mL^{-1}, below the reference compound **4** for most of the strains. When a phenol group is substituted by a furan group **5a-c**, the activity is greatly reduced with respect to the strains tested, more strongly for bis-furan analogues **9a,b**. On the other hand, when a phenol group is substituted by a thiophene **6a-d**, the activity becomes similar or even better than the substituted group-activity relationship, when the furan group is in the methylidene position such as in **5c**, the activity observed is slightly reduced compared to furan derivatives **5a,b**. This effect is more strongly accentuated when it is a thiophene group which is in this same position such as in **6c** compared to the other positions of thiophen group **6a,b**. As underlined in our previous report,[8] we can see an effect of the number of bromine atom on the activity by comparing the compounds **6c** and **6d**. Overall, we can pay particular attention to the influence of the phenol group substitution combined with the influence of a comparable number of bromine (i.e. **4**, **5c** and **7a)**. Thus, we note that the substitution of the methylidene position by an indole group **7a** compensates for the negative effect of the decrease in the number of bromine atoms on the activity.

Table 1. Structures of synthetized cadiolide analogues and antibacterial activities of synthetized cadiolide analogues and cadiolide D. MIC results of three independent experiments (µg.mL^{-1}). Bold numbers show the values obtained for the four most active compounds.



Analog	*B. cereus*	*B. subtilis*	*M.luteus*	*E. faecalis* NCTC 12697	*S. aureus* NCTC12493	*S. aureus* EMRSA-17	VRE
cadiolide D	32	64	64	64	32	64	64
4	**8**	**16**	**8**	**16**	**8**	**8**	**32**
5a	16	32	32	128	32	64	64
5b	16	16	16	32	32	64	64
5c	32	32	32	128	32	64	128
6a	4	8	8	32	8	16	32
6b	8	8	8	16	16	16	8
6c	64	32	128	64	32	256	32
6d	8	4	32	32	8	32	32
7a	**2**	**4**	**2**	**16**	**2**	**8**	**8**
7b	**4**	**8**	**8**	**8**	**2**	**4**	**8**
8	**4**	**8**	**16**	**16**	**8**	**16**	**16**
9a	128	256	256	256	64	128	256
9b	16	32	32	64	16	64	64
Gentamycin	0.25	0.25	0.25	8	0.5	>64	8
Vancomycin	1	0.5	≤0.13	4	1	2	>64

observed, comparable to the reference compound **4** thus opening the way towards the development of a new family of nitrogenated cadiolide derivative with a serious advantage of significant substitution variability on the nitrogen atom.

Furthermore, it is interesting to note that the indole derivatives **7a,b** are more potent against the two clinical resistant isolates tested (EMRSA-17 and VRE) than the compound **4** suggesting the absence of crossed resistance with cadiolide derivatives. N-methylated indole **7b** has similar activity than free indole **7a**, suggesting that further functionalization of cadiolide analogues via the indole position is possible without loss of activity.

The result of the antibacterial activity, described above has allowed us to select four compounds (i.e. **4**, **7a,b** and **8**) at a concentration of 160 µl.ml^{-1} to evaluate their impact on cultured keratinocytes cells in order to identify any cytotoxic effect of these selected compounds. Since these compounds have been dissolved in DMSO, a same concentration (0.64 %) of DMSO needed for a good solubility has been used as a control (Cells + DMSO; Figure 3). We first observed that keratinocytes cells are sensitive to DMSO alone. Our result show that the three compounds **4**, **7a** and **7b** are devoid of cytotoxic activity against keratinocyte cells. In contrast, the compound **8**, used in the same condition, provoked an increase in term of cytotoxicity towards keratinocyte cells (55.7 ± 2.7 % of LDH release versus 23.7 ± 1.4 % of LDH release in control cells with DMSO). However, it is important to keep in mind, that cultured cells such as keratinocyte used in this study, are particularly sensitive, suggesting that it would be efficient, in the future, to test the toxicity of **8** against complex organisms such as the worm *Caenorhabditis elegans* or mice. In any case, our cytotoxic approach clearly demonstrated that **4**, **7a** and **7b** have no toxic activity towards keratinocyte cells.

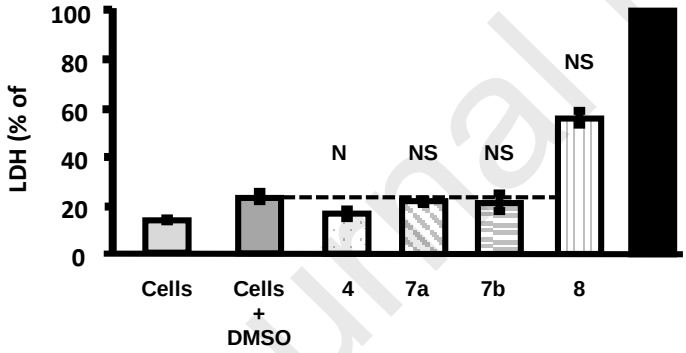


Figure 3 : Cytotoxicity evaluation. Effect of **4**, **7a,b** and **8** cardiolides derivatives on LDH release by keratinocytes HaCaT cells after 24h of contact. Data are the mean of seven measurement from two independent experiment. Dotted line corresponded to the control condition (cells exposed to DMSO). Black bar represented the maximum of cells death (100 % of LDH release), NS : Not significantly different.

All together, our combined biological data (antibacterial activity and cytotoxicity) strongly suggest that the compounds **4**, infection, while maintaining host cells integrity. In will be interesting in the future to modify slightly these compounds to obtain even more active molecules in particular by introducing a water-solubilizing function on the nitrogen atom of the indole group despite a higher cytotoxicity, the antibacterial activity of compound **8** is very encouraging and opens a way towards a new family of antibacterial molecules inspired by the cadiolide skeleton.

Acknowledgments

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Supplementary Material

Supplementary material of this article can be found online.

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