İstanbul Ecz. Fak. Derg. / J. Fac. Pharm. Istanbul
45(1) 2015 pp.19-28

Antibacterial and anti-biofilm activities of new chiral and racemic 1,3-Dioxolanes

Sibel Döşler[1,*], Emel Mataracı[1], Hatice Başpınar-Küçük[2], Ayşe Yusufoğlu[2]

[1] Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey.
[2] Department of Chemistry, Faculty of Engineering, Istanbul University, Istanbul, Turkey.

Abstract: We investigated the potential anti-microbial and anti-biofilm activities of previously synthesized 1,3-dioxolane derivatives against several pathogen microorganisms. In vitro antibacterial activities of new compounds against *Staphylococcus aureus* ATCC 29213, *S. epidermidis* ATCC 12228, *Enterococcus faecalis* 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Proteus mirabilis* ATCC 14153 and antifungal activities against *Candida albicans* ATCC 10231 were investigated. The minimum inhibitory concentrations (MIC), and minimum biofilm eradication concentrations (MBEC) were determined by microbroth dilution technique. As a result, two molecules were found active against the planktonic and biofilms of the *E. faecalis* and *S. epidermidis*. The MIC and MBEC values of those two agents were 9.76 - 39.06 mg/L and 2000 - 5000 mg/L, respectively. In addition, they inhibited the biofilm attachment according to time, and they showed the significant inhibitor activity against biofilm formation at 24 h according to their concentrations. According to these results, two 1,3-dioxolane derivatives were active against the planktonic and biofilm cells of some bacteria.

Key words: 1,3-dioxolane derivatives, antimicrobial, anti-biofilm, in-vitro activity.

Introduction

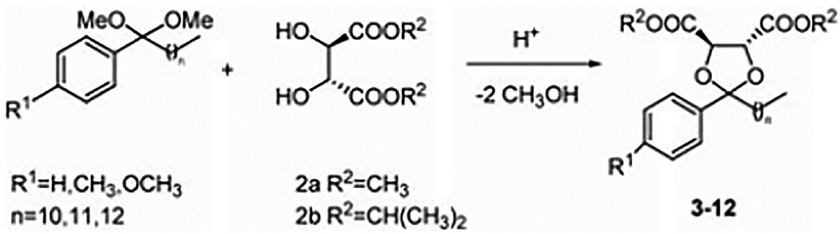
Compounds with 1,3-dioxolane's structure are of interest in the industry for the preparation of many pharmaceuticals and fragrances (Bucsi et al., 2001). They are also appreciated as starting chiral pools for the synthesis of different natural compounds, described as chiral inhibitors of leukotrienes,

***Correspondence:** sibeldosler@hotmail.com

exert pharmacological effects on the central nervous system, including anxiogenic, sedative, and anticonvulsant activities, or antimicrobial activity against several microorganisms (Crawley & Brigs, 1995; Genta et al., 2002; Özkanlı et al., 2003; Takabe et al., 2005; Zapata-Sudo et al., 2007).

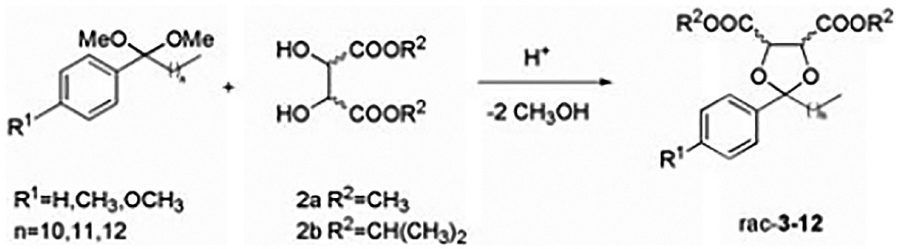
In a previous study (Küçük & Yusufoğlu, 2012), ten different chiral (4R, 5R)-1,3-dioxolanes 3–12 and also 10 racemic 1,3-dioxolanes rac-3–12, were synthesized as original 1,3-dioxolanes for the first time (Figure 1 and 2). These chiral and racemic 1,3-dioxolanes were characterized by infrared, NMR (1H, 13C), mass spectrometry, elemental analysis, optical rotation, and chiral HPLC.

In this study, we investigated the potential anti-microbial and anti-biofilm activities of previously synthesized 1,3-dioxolane derivatives against several pathogen microorganisms.



Entry	Ketone	Diol	Product	*ee*[b] (%)	Yield[c] (%)
1	**1a**	**2a**	**3**	>99	82
2	**1a**	**2b**	**4**	>99	75
3	**1b**	**2a**	**5**	>99	80
4	**1b**	**2b**	**6**	>99	77
5	**1c**	**2a**	**7**	>99	84
6	**1c**	**2b**	**8**	>99	75
7	**1d**	**2a**	**9**	>99	84
8	**1d**	**2b**	**10**	>99	75
9	**1e**	**2a**	**11**	>99	68
10	**1e**	**2b**	**12**	>99	65

Figure 1. Ketalization of orthoesters with chiral diols (2a, 2b) by montmorillonite K10 catalyst.



Entry	Ketone	Diol	Product	$(t_R^R)^b$ (min)	$(t_R^S)^b$ (min)	Yield[c] (%)
1	1a	2c	rac-3	4.225	4.965	85
2	1a	2d	rac-4	4.210	6.010	77
3	1b	2c	rac-5	4.331	6.091	82
4	1b	2d	rac-6	4.370	5.270	79
5	1c	2c	rac-7	4.160	5.420	85
6	1c	2d	rac-8	4.540	6.210	77
7	1d	2c	rac-9	5.327	6.377	87
8	1d	2d	rac-10	5.650	6.880	76
9	1e	2c	rac-11	5.400	6.930	70
10	1e	2d	rac-12	5.620	7.320	68

Figure 2. Ketalization of orthoesters with racemic diols (2c, 2d) by montmorillonite K10 catalyst.

Materials and methods

Molecules: A series of chiral and racemic 1,3-dioxolanes with >99% ee values which had been synthesized in a previous study were used in this study. Chiral dioxolanes 3–12 (Figure 1) were obtained by the reaction of orthoesters with chiral diols (2a, 2b) using Mont.K10 catalyst. Racemic dioxolanes rac-3–12 (Figure 2) were synthesized by the reaction of orthoesters with racemic diols (2c, 2d) in the presence of Mont.K10 catalyst.

Microorganisms: The American Type Culture Collection (ATCC) standard strains of *Staphylococcus aureus* ATCC 29213, *S. epidermidis* ATCC 12228, *E. faecalis* ATCC 29212, *Enterococcus faecalis* ATCC

29212, *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Proteus mirabilis* ATCC 14153.and as a representative of fungi, the yeast, *C. albicans* ATCC 10231 were used in the experiments. Inoculums of bacteria and *C. albicans* were prepared with overnight cultures for producing a concentration of $1x10^8$ colony-forming units (cfu/ml) and 1×10^7 cfu/ml, respectively.

Media: Tryptic soy broth supplemented with 1% glucose (TSB-glucose, Difco Laboratories, Franklin Lakes, NJ) was used for biofilm production, cation-adjusted Mueller-Hinton broth (CAMHB, Difco Laboratories) was used to determine the minimum inhibitory concentration (MIC) and minimum biofilm-eradication concentration (MBEC) values, and tryptic soy agar (TSA, Difco Laboratories) was used for colony counts.

Determination of minimum inhibitory concentrations (MIC): In vitro antibacterial activities of new compounds against *S. aureus* ATCC 29213, *S. epidermidis* ATCC 12228, *E. faecalis* 29212, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 4352, *P. mirabilis* ATCC 14153 and antifungal activities against *C. albicans* ATCC 10231 were investigated. MICs of compounds were determined by microbroth dilution technique as described by the Clinical and Laboratory Standards Institute (CLSI, 1997; CLSI, 2006). Serial two fold dilutions of molecules ranging from 5000 to 4.8 μg/mL were prepared in Mueller-Hinton broth (MHB) for bacteria and RPMI-1640 medium for yeast. Each well was inoculated with 50 μL of a 4-6 h broth culture that gave a final concentration of 5×10^5 cfu/mL for bacteria and 5×10^3 cfu/mL for yeast in the test tray. The trays were covered and placed in plastic bags to prevent evaporation. The trays containing MHB were incubated at 37°C for 18–20 h, those containing RPMI-1640 medium at 37°C for 46–50 h. The MIC was defined as the lowest concentrations of compounds producing complete inhibition of visible growth. Ciprofloxacin and fluconazole were used as reference antibiotics for bacteria and yeast, respectively.

Determination of anti-biofilm activities: Measurements of the antimicrobial susceptibilities of the bacterial and *C. albicans* biofilms were assessed using an MBEC assay, which was performed as previously described with the following modifications (Mataracı & Döşler, 2012). The 24 h biofilms in a 96 well tissue culture microtitre plates were washed

3x with 250 µl PBS solutions and air-dried. Serial 2-fold dilutions ranging from 10.000 to 625 mg/L for the molecules were prepared in CAMHB. Next, 200 µl of each concentration was added to each corresponding well and plates were incubated 24 h at 37°C. After incubation, the antibiotics were gently aspirated, the plates were washed, thoroughly scraped, and the contents of each well were incubated in a sonicating water-bath for 5 minutes to disrupt the biofilms. 100 µL samples were plated on TSA and the colonies were counted after 24 h of incubation at 37°C. MBEC was defined as the lowest concentration of molecules which microorganism fail to regrow after exposure.

Biofilm attachment and inhibition of biofilm formation assays were performed as previously described method with some modifications (Mataracı & Döşler, 2012). 1/10 x MIC concentrations of molecules were added to the 24 h biofilm and plates were incubated 1, 2 and 4 h at 37°C; Molecules at 1 x, 1/10 x and 1/100 x MIC concentrations were added to the 24 h biofilm and plates were incubated 24 h at 37°C, respectively. Six wells were used for each molecules. The positive controls were microorganisms in TSB-glucose without molecules. After the incubation, wells were washed with PBS solutions and measured at OD_{595} nm.

Statistically analysis: All experiments were performed in two independent assays. In MIC, and MBEC determinations, when the results were different in both experiments, we made another test for final result. In biofilm attachment and inhibition of biofilm formation assays, results are presented as mean ± standard deviation of two independent experiments. One way ANOVA-Bonferroni's multiple comparison test was used to compare differences between control and antimicrobials treated biofilms. P value < 0.001 was considering as statistically significant.

Results

Susceptibility: The in vitro activities of the studied molecules against planktonic cells, and biofilms are summarized in Table 1. According to these results, two molecules (1.2: Entry 2 in Figure 1, 2.2: Entry 2 in Figure 2) were found active against the planktonic and biofilms of the *E. faecalis* and *S. epidermidis*. The MIC values of the ciprofloxacin and fluconazole were within the accuracy range in CLSI throughout the study (CLSI, 2014).

Table 1. In vitro antibacterial and anti-biofilm activities of molecules (mg/L)

	MIC		MBEC	
Bacteria	2.1	2.2	2.1	2.2
S. epidermidis ATCC 12228	39.06	39.06	-	-
E. faecalis ATCC 29212	19.53	9.76	5000	2000

2.1: Entry 2 in figure 1, 2.2: Entry 2 in figure 2, -: Not determined

All other molecules does not have neither antimicrobial nor anti-biofilm activities.

Anti-biofilm activities: Because of the only biofilm which susceptible to studied active molecules was *E. faecalis*'s, we performed both biofilm attachment and inhibition of biofilm formation assays with it. When we carried out these tests, both two agents inhibited the biofilm attachment according to time, and they showed the significant inhibitor activity against biofilm formation at 24 h according to their concentrations (Figure 3).

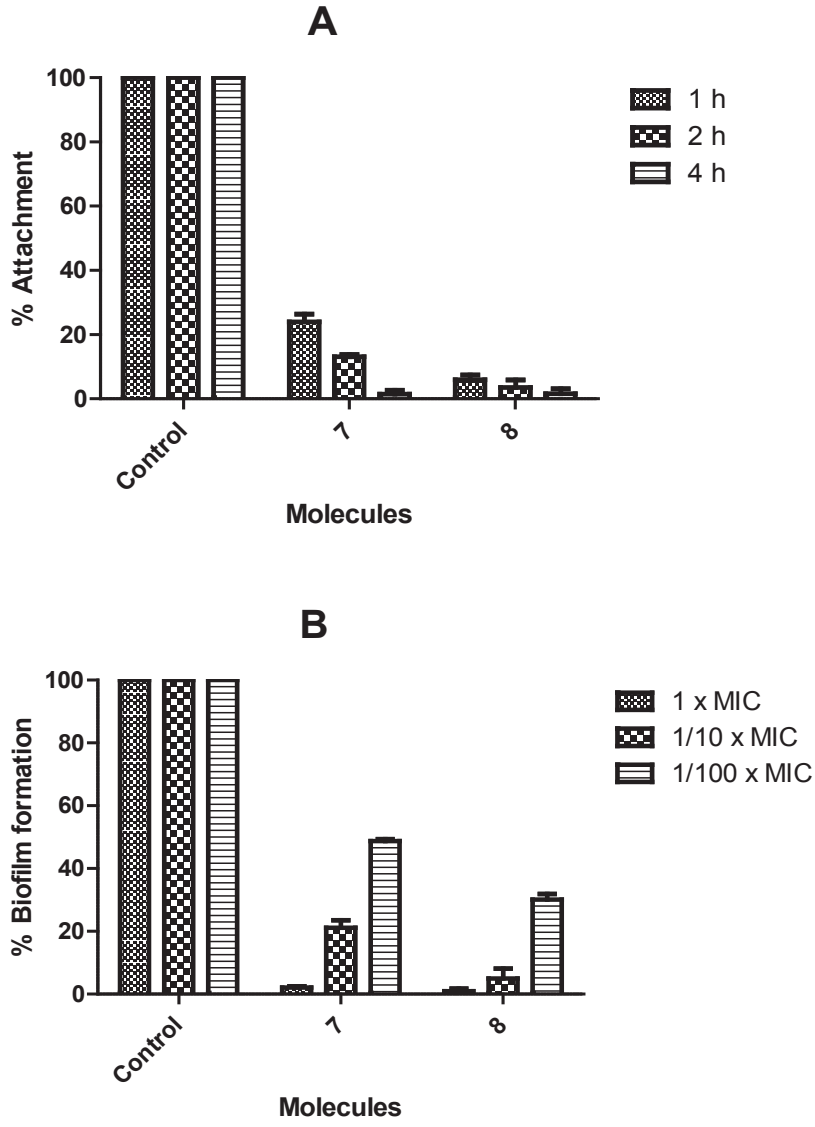


Figure 3. Inhibition of *E. faecalis* A: surface attachment to the wells contained 1/10 x MIC of molecules and an inoculum of 1×10^7 cfu/200 µl, incubated for 1, 2, or 4 h at 37°C; B: biofilm formation in each well contained 1 ×, 1/10 ×, or 1/100 × MIC of molecules and an inoculum of 5×10^5 cfu/200 µl, incubated for 24 h at 37°C. Control bars indicate bacterium without molecules, accepted as 100%. Six wells were used for each molecules. Each experiment is representative of two independent tests, and the error bars indicate the standard deviations. All differences between the control and molecules-treated biofilms were statistically significant ($P < 0.001$).

Discussion

The misuse of antibiotics in antimicrobial therapy has led to the development of widespread resistance in microorganisms. With the increasing in antibiotic-resistant strains, treating the infections become more difficult or even impossible. The failure of existing antibiotics to control the infections, especially caused by multidrug resistance bacteria, makes it crucial to find new and alternative agents with new mechanisms of action. There are many researchers such as chemists, pharmacists, biologists, and medical scientists have inevitably produced many synthetic or semi-synthetic molecules to be a candidate for new and useful drugs. A lot of new molecules which occurs from those studies, allows for the construction of unlimited libraries for drug screening. One of the major research area is searching the antibacterial or antifungal activities of new molecules to development of potent antimicrobial agents (Calderone et al., 2014; Nigam et al., 2014).

In this study, when we investigated the in vitro antimicrobial activities of 20 previously synthesized 1,3-dioxolane derivatives, two molecules had a strong antibacterial efficacy against two Gram positive bacteria (*S. epidermidis* and *E. faecalis*) which considered as common human originated pathogens. Especially *E. faecalis* causes not only community acquired but also serious and life threating nosocomial infections (Van tyne et al., 2013).

A biofilm is a microbial community that is attached to abiotic surfaces and produces extracellular polysaccharides. Microbial cells that grow in biofilms are physiologically distinct from planktonic cells of the same organism. Bacteria on biofilm structures are protected from environmental conditions, antimicrobial agents, and host immune responses, and they also exhibit up to 1000-fold increased antibiotic resistance to the wide range of antimicrobial agents (Donlan & Costerton, 2002; Ghonnoum & O'Toole, 2004; Haussler & Fuqua, 2012). When we considered the anti-biofilm activities of antibacterial molecules 1.2 and 2.2 against the *E. faecalis* biofilms, MBEC values were 5000 and 2000 mg/L, respectively. According to these results those two molecules were active against the biofilm of *E. faecalis*. The MBEC/MIC ratio which is one of the important parameter for choosing the antibiotic in the treatment of biofilm associated

infections, were found 256 and 200 fold, respectively, in contrast to many antibiotics (up to 10.000 fold).

Because biofilm-associated bacteria are not affected by therapeutically achievable concentrations of antimicrobial agents, anti-biofilm therapies have generally focused on the inhibition of biofilm formation (Jorge et al., 2012). For this purpose, we investigated the inhibition of bacterial attachment to the surfaces, as well as the inhibition of biofilm production by MIC or subMIC values of molecules. Studied molecules 1.2 and 2.2 were able to significantly inhibit the attachment of bacteria at 1/10 × MIC in 1-4 h, and 24 h biofilm formation up to 100%, especially at 1 × or 1/10 × MIC ($p < 0.001$). Even though inhibiting mature biofilm is very difficult, inhibition of biofilm formation in early critical stages seems to be more applicable.

New drug development is a long and difficult process that involves many in vitro and in vivo experiments, formulation with taking the parameters such as stability, toxicity, immunogenity, pharmacokinetic and pharmacodynamics into the consideration, and phase I/ II and III clinical trials. However, there is a very little chance for one molecule to being a drug, as long as the researchers creates more new molecules that had an antimicrobial or other beneficial activities, the chance is always exists. Our data confirm the antibacterial and anti-biofilm activities of some 1,3-dioxolane derivatives against Gram positive bacteria and these findings strengthen the opinion in terms of some molecules may take a place as a new and active group of antimicrobial agent in the near future.

References

Bucsi I, Meleg A, Molnar A, Bartok M (2001) Bronsted acid catalyzed formation of 1,3-dioxolanes from oxiranes and ketones, *J. Mol. Catal. A: Chem.* **168:** 47-52.

Calderone R, Sun N, Gay-Andrieu F, Groutas W, Weerawarna P, Prasad S, Alex D, Li D (2014) Antifungal drug discovery: the process and outcomes, *Future Microbiol.* **9:** 791-805.

Clinical and Laboratory Standards Institute (1997) Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard–2th ed. **M27-A2**: CLSI, Wayne, PA.

Clinical and Laboratory Standards Institute (2006) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed. Approved standard **M7-A7:** CLSI, Wayne, PA.

Clinical and Laboratory Standards Institute (2014) Performance standards for antimicrobial susceptibility testing; 24th informational supplement. **M100-S24:** CLSI, Wayne, PA.

Crawley GC, Briggs MT (1995) Chiral dioxolane inhibitors of leukotriene biosynthesis: Structureactivity relationships and syntheses using asymmetric dihydroxylation, *J. Med. Chem*. **38:** 3951-3956.

Donlan RM, Costerton JW (2002) Biofilms: Survival Mechanisms of Clinically Relevant Microorganisms, *Clin. Microbiol. Rev*. **15:** 167-193.

Genta MT, Villa C, Mariani E, Loupy A, Petit A, Rizzetto R, Mascarotti A, Morini F, Ferro M (2002) Microwave-assisted preparation of cyclic ketals from a cineole ketone as potential cosmetic ingredients: solvent-free synthesis, odour evaluation, in vitro cytotoxicity and antimicrobial assays, *Int. J. Pharm*. **231:** 11-20.

Ghannoum M, O'Toole GA (2004) Microbial Biofilms. ASM Pres, Washington, DC, USA.

Haussler S, Fuqua C (2012) Biofilms 2012: new discoveries and significant wrinkles in a dynamic field, *J. Bacteriol.* **195:** 2947-2958.

Jorge P, Lourenço A, Pereira MO (2012) New trends in peptide-based anti-biofilm strategies: a review of recent achievements and bioinformatic approaches, *Biofouling.* **28:** 1033-1061.

Küçük HB, Yusufoğlu A (2012) Synthesis of New Chiral and Racemic 1,3-Dioxolanes, *J. Heterocyclic Chem.* **49:** 1066-1070.

Mataracı E, Döşler S (2012) In vitro activities of antibiotics and antimicrobial cationic peptides alone and in combination against methicillin resistant Staphylococcus aureus biofilms, *Antimicrob. Agents. Chemother*. **56:** 6366-6371.

Nigam A, Gupta D, Sharma A (2014) Treatment of infectious disease: Beyond antibiotics, *Microbiol. Res.* **169:** 643-651.

Özkanlı F, Güney A, Calis U, Uzbay T (2003) Synthesis and anticonvulsant activities of some new dioxolane derivatives, *Aranelm-Forach/Drug Res.* **53:** 758-762.

Takabe K, Sugiura M, Asumi Y, Mase N, Yoda H, Shimizu H (2005) Practical optical resolution of dl-muscone using tartaric acid derivatives as a chiral auxiliary, *Tetrahedron Lett*. **46:** 3457-3460.

Van Tyne D, Martin MJ, Gilmore MS (2013) Structure, function, and biology of the Enterococcus faecalis cytolysin, *Toxins (Basel)*. **5:** 895-911.

Zapata-Sudo G, Pontes LB, Gabriel D, Mendes TCF, Ribeiro NM, Pinto AC, Trachez MM, Sudo RT (2007) Sedative–hypnotic profile of novel isatin ketals, *Pharmacol. Biochem. Behav.* **86:** 678-685.