World Applied Sciences Journal 17 (2): 145-147, 2012
ISSN 1818-4952

Antibacterial Effects and Genotoxicity of New Derivatives of Furanones

[1]E.V. Babynin, [1]A.R. Kurbangalieva, [1]Nguyen Thuy An and [1,2]M.V. Trushin

[1]Kazan Federal University, Kazan, Russian Federation
[2]Kazan Institute of Biochemistry and Biophysics, Kazan, Russian Federation

Abstract: New chlorine-containing furanones were tested for the presence of antibacterial activity and also for genotoxicity in Ames test. It has been established that the tested compounds had antibacterial properties. Antibacterial activities of all four compounds differed in rich broth and minimal glucose media. While in rich broth the minimal inhibitory concentration (MIC) of the compounds varied from 150 to 600 mcg /ml, then in the minimal glucose solution (MIC=0.75 mcg/ml) the activity of compounds were significantly increased. The estimation of genotoxicity of the tested compounds has revealed a weak mutagenic activity for two compounds. Other compounds were not mutagenic. The role of furanones structure in genotoxicity is discussed.

Key words: Antibiotics · Furanone · Genotoxicity · *Salmonella*

INTRODUCTION

The discovery of antibiotics allowed hoping that infectious diseases might be successfully treated. However, a broad application of antibiotics led to antibiotic resistance and even to multiple ones. Unfortunately, the development of resistance to antibiotics outstrips the creation and introduction of new antibacterial drugs [1, 2]. The identification of compounds for new targets and design of new approaches present itself an important area of modern scientific studies. Apart from the distribution of resistance genes, microorganisms have natural nonspecific ways for the formation of the resistance like biofilms that present itself bacterial communities enveloped with polysaccharide matrix and strongly adhered to a substrate. Biofilm formation at the surfaces of the medicinal implants that is dependent of the intercellular interactions (particularly, quorum sensing) may enhance resistance to antibiotics even 1000-fold in comparison with planktonic bacteria [3].

The selection of the quorum sensing system as a target for antibacterial therapy needs for searching compounds among inhibitors of microbial intercellular communication. The furanones take a special place among these compounds. Many of them may be produced by algae and higher plants [4, 5].

Any compound used for the prevention of biofilm formation at the implants should not have unfavorable effects to the host. Previously, some cytotoxic and mutagenic effects were reported for furanones [6-8]. By this reason, before clinical usage, these compounds should be tested for the possible mutagenic effects. The aim of the present work was to detect antibacterial effects and genotoxicity of new derivatives of furanones.

MATERIALS AND METHODS

Bacterial Strain: Histidine-dependent strain of *S. typhimurium* TA100 (hisG46/rfa/DuvrB/pKM101) was used as the tester strain in this study. The tester strain was confirmed prior to use for different requirements and characteristics according to the methods given by Maron and Ames [9]. Overnight cultures of *S. typhimurium* TA100 was grown at 37°C in nutrient broth supplemented with 80 mcg/ml ampicilin.

The Determinations of the MICs (The Minimum Inhibitory Concentrations) and the MBCs (The Minimum Bactericidal Concentrations): The MICs for the test compounds were determined using two-fold serial dilution broth method [10]. The 96-well plates were scanned with Tecan Infinite 200 PRO reader at 550 nm. The MICs were taken as the lowest concentration of

Corresponding Author: Dr. Maxim V. Trushin, Department of Genetics, Kazan Federal University, 420008 Kazan, Russian Feeration.

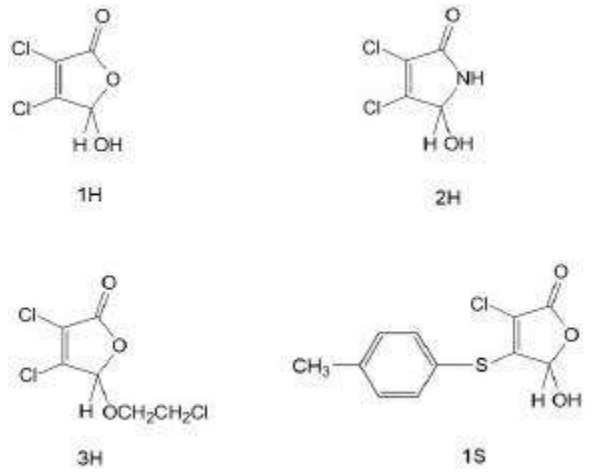

Fig. 1: Chemical structures of the furanone compounds used in the present study: 5-hydroxy-3,4-dichloro-2(5H)-furanone *(*mucochloric acid) (marked as 1H); 5-hydroxy-3,4-dichloro-3-pyrroline-2-on (marked as 2H); 3,4-dichloro-5-(2-chlorethoxy)-2(5H)-furanone (marked as 3H); 5-hydroxy-4[(4-methylphenyl)thio]-3-chloro-2(5H)-furanone (marked as 1S).

compounds that caused optical density reduction by more than 90% compared with control growth results. All the MIC wells, which did not show any turbidity, were streaked on LB agar plates. The lowest concentrations of compounds that did not permit any visible growth on the plates after 48 h of incubation at 37°C were recorded as the MBCs.

Mutagenicity Test: The Ames test was conducted to examine the mutagenicity of the test compounds. Briefly, a mixture of the following components were added in sequence: 0.1 ml of the *S. typhimurium* TA100 strain broth culture, 0.1 ml the test compound or of ethylmethane sulfonate (EMS) as positive control without S9 mix. DMSO was used as a negative control. The mixture was incubated at 37°C for 20 min. After incubation, 2 ml of melted top agar (supplemented with 0.05 mM histidine/0.05 mM biotin and maintain at 43°C to 48°C) were added to each test tube. The contents were mixed gently and poured onto the minimal glucose medium plates. When the top agar has hardened (2-3 min), the plates were inverted and incubated at 37°C for about 48 h. The plates for spontaneous reversions did not contain any chemical. After 48 hours incubation, the colonies in the control and test compound plates were counted. Spontaneous revertants were also scored.The chemicals were jugged to be mutagenic if the maximum number of revertants were 2 folds or more relative to the negative control.

The Tested Compounds: The used compounds of furanones were synthesized in A.M. Butlerov Chemical Institute, Kazan Federal University, Kazan, Republic of Tatarstan, Russian Federation. Figure 1 presents structural formulas of new derivatives of furanones.

RESULTS AND DISCUSSION

It was revealed in our experimental work that all tested compounds had antibacterial properties. The bacteriostatic activity depended on the type of growth medium: it was different in rich broth and minimal glucose medium. The most evident bacteriostatic activity was revealed for 1H furanone: MIC was 150 and 0.75 mcg/ml for rich broth and minimal glucose medium, respectively. Furanone 3H presented minimal bacteriostatic activity of 600 and 0.75 mcg/ml for rich broth and minimal glucose medium, respectively.

The difference in antibacterial activity on those two types of growth media may be related with the fact that furanones may inactivate ribonucleotide reductase-enzyme that catalyzes the synthesis of deoxyribonucleotides [10]. The inhibition of DNA synthesis at minimal medium, where a cell have to produce by itself all necessary compounds, may mediate more evident antibacterial action of the halogenated furanones toward bacterial cells.

The assessment of the possible genotoxic effects of new derivatives of furanones showed the presence of a weak mutagenic activity in compounds 1H and 3H (no more than 2-fold increase) (Fig. 2). These compounds have a very similar structure and differ only by modification of lacton cycle at 3rd location. Compounds 2H and 1S are characterized by replacement of oxygen at 1st location by nitrogen (2H) and introduction of sulfur-containing group (1S) to the 3rd location of lacton cycle. Since these compounds did not demonstrate any effects on mutation frequency, we may suggest that structural properties are important for the development of genotoxic effects of furanones.

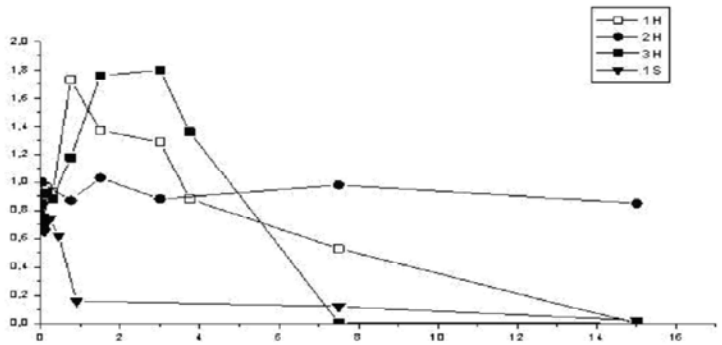

Fig. 2: Genotoxicity assay of furanone derivatives in Ames test. Axis X-minimal inhibiting concentrations for the testing compounds. Axis X-concentrations of the compounds, mcg / ml, axis Y-relation of average number of revertants in experiment toward a number of revertants in control.

REFERENCES

1. Walsh, C.T., 2003. Antibiotics: actions, origins and resistance. Washington, DC: American Society for Microbiology, pp: 345.
2. Oskay, M., N. Yenil and K. Ay, 2008. Antimicrobial activity of stable spiro-endoperoxides by sunlight-mediated photooxygenation of 1,2-O-alkylidene-5(e)-eno-5,6,8-trideoxy-d-xylo-oct-1,4-furano-7-uloses. World Applied Sciences Journal, 4: 720-723.
3. Stoodley, P., K. Sauer, D.G. Davies and J.W. Costerton, 2002. Biofilms as complex differentiated communities. Annual Reviews in Microbiology, 56: 187-209.
4. Hentzer, M. and M. Givskov, 2003. Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. Journal of Clinical Investigation, 112: 1300-1307.
5. Masuda, A., K. Mori, Y. Oda and M. Miyazawa, 2010. Volatile oil compounds from corms and flowers of *Crocus vernus* L. hill and corms of *C. sativus* L. Libyan Agriculture Research Center Journal International, 1: 244-249.
6. LaLonde, R.T., L. Bu, A. Henwood, J. Fiumano and L. Zhang, 1997. Bromine-, chlorine and mixed halogen-substituted 4-methyl-2(5H)-furanones: synthesis and mutagenic effects of halogen and hydroxyl group replacements. Chemical Research in Toxicology, 10: 1427-1436.
7. Janecki, T., E. Błaszczyk, K. Studzian, M. Rózalski, U. Krajewska and A. Janecka, 2002. New stereocontrolled synthesis and biological evaluation of 5-(1'-hydroxyalkyl)-3-methylidenetetrahydro-2-furanones as potential cytotoxic agents. Journal of Medical Chemistry, 45: 1142-1145.
8. Kim, Y., N.H. Nam, Y.J. You and B.Z. Ahn, 2002. Synthesis and cytotoxicity of 3,4-diaryl-2(5H)-furanones. Bioorganic and Medicional Chemistry Letters, 12: 719-722.
9. Maron, D. and B.N. Ames, 1983. Revised methods for the Salmonella mutagenicity test. Mutation Research, 113: 173-215.
10. National Committee for Clinical Laboratory Standards, 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically-fifth edition. Approved standard. NCCLS document M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
11. Cerqueira, N.M.F.S.A., P.A. Fernandes and M.J. Ramos, 2006. Enzyme ribonucleotide reductase: unraveling an enigmatic paradigm of enzyme inhibition by furanone derivatives. Journal of Physical Chemistry B., 110: 21272-21281.