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Antimicrobial Activity and Docking Study of Synthesized Xanthen-3-on Derivatives.

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ABSTRACT

Twelve previously synthesized biologically active 2,6,7-trihydroxy-9-aryl-3*H*-xanthen-3-one derivatives (**1-12**) were evaluated *in vitro* for their antimicrobial activity against four bacteria, *S. aureus*, *B. subtilis P. aeruginosa* and *E. coli*, and two fungi strains, *C. albicans* and *S. cerevisiae*. The most potent compound were derivatives **1** which possess hydroxyl group and bromine as substituent and **11** with bromine as substituent on phenyl ring. The results indicate that bromine increase antimicrobial activity of 2,6,7-trihydroxy-9-aryl-3*H*-xanthen-3-one derivatives. Compound **7** with ethoxy substituent on phenyl ring showed the least activity against tested bacteria and fungi strains, which is in line with an earlier observation that ethoxy substitution decreases antimicrobial activity. The most and the least potent compounds were subjected to molecular docking simulations to preliminary find out the potential molecular target and at the same moment further support the experimental antimicrobial test of xanthen derivatives.

Keywords: xanthene, antimicrobial activity, docking study

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INTRODUCTION

Xanthen derivatives are compounds with important biological activities such as immunomodulating [1], antitumor [2], antimalarial [3], antioxidant [4], antimalarial [5], antifungal [6] and antibacterial activity [7]. Xanthens have also been reported for thair antileukemic [8], antiinflammatory [9] and apoptic effects [10]. Due to their wide range of applications, xanthene derivatives have received a great deal of attention regarding their synthesis and research as potential pharmacological active agents. In recent years, because of their antimicrobial activity, these compounds are in focus of research. Also, researches try to explain mechanism of their antimicrobial activity. Related to the problem of microbial resistance, a number of enzymes and receptors important for microbial growth may be considered as potential targets for new drugs.

Cytochrome P450 isoenzyme 51 is a family of phylogenetic highly conserved monooxygenases found in mycobacteria, fungi, plants, animals and humans. The ideal antifungal agent should be a potent inhibitor of fungal CYP51 while leaving the human CYP51 unaffected [11, 12].

As such validated targets are recognized as major pillars in the drug discovery and drug development process, a number of *in silico* methods are used to identify potential targets and interactions of small molecules with target proteins in the cell. Among them, molecular docking is a popular method to study binding of small molecules (ligands) to macromolecules (receptor). In particular, the efficiency of Autodock program has been well demonstrated for that purpose in several studies [13, 14, 15].

In our previous work [16, 17] we synthesized 9-aryl substituted 2,6,7-trihydroxyxanthen-3-one derivatives using reliable one-pot synthesis followed by structure elucidating measurements, and performed antioxidant and antiplatelated potency evaluation. Antimicrobial and antiproliferative studies on similar xanthenes were reported [18, 19, 20]. Presented study therefore, was aimed to evaluate antimicrobial effects of synthesized 9-aryl substituted xanthen-3-ones. The most potent compound was subjected to molecular docking simulations.

EXPERIMENTAL

In our previos work we synthesized and confirmed structure of twelve xanthen-3-one derivatives (**1–12),** Figure 1, from 1,2,4-triacetoxybenzene and different aromatic aldehydes under acidic alcoholic conditions. After a two-fold Friedel-Crafts alkylation intermediate **A** was obtained. For accomplishing the transformation, a single trihydroxy benzene moiety of **A** had to be oxidized using potassium peroxodisulphate to the corresponding *p*-benzoquinone. To avoid decomposition of potassium peroxodisulphate, the reaction of oxidation occurred at 80°C. Benzoquinone intermediate (**B**) subsequently underwent a cyclocondensation reaction to the xanthenone fragment. To remove potassium peroxodisulphate after completed oxidation, refluxed suspension was poured onto ice water and filtered. The residue was dried under vacuum at 60 °C [16, 21].

Antimicrobial activity

Antibacterial activity was tested by the diffusion method against *Bacillus subtilis* ATCC 6051, *Staphylococcus aureus* ATCC 6538P, *Escherichia coli* ATCC 9027 and *Pseudomonas aeruginosa* ATCC 9027, while antifungal activity was tested against *Candida albicans* ATCC 1023 and *Saccharomyces cerevisiae* ATCC 9763. Test samples were dissolved in 99.5% dimethyl sulfoxide (DMSO) to obtain a 1 mg/mL stock solutions which were then applied to Müller-Hinton and Sabouraud nutritious bases. The inhibition zones for bacteria were measured in millimetres at the end of an incubation period of 18 h at 37 °C, and for fungal strains after 48 h at 25 °C. Compounds were further tested by dilution method. For this, Casein soya bean digest broth (Triptic soya bujon) was used. As referent compounds chloramphenicol and fluconazole were used in concentration of 500 µg/mL. Test solution of the compound was prepared, followed by formation of a series of 12 dilutions with liquid nutritious base. After the incubation for 24 h, the last tube with no growth of microorganisms was taken to represent minimum inhibitory concentration (MIC) expressed in mg/mL. The concentrations of the prepared solutions were in range 0.5–0.00024 mg/mL.

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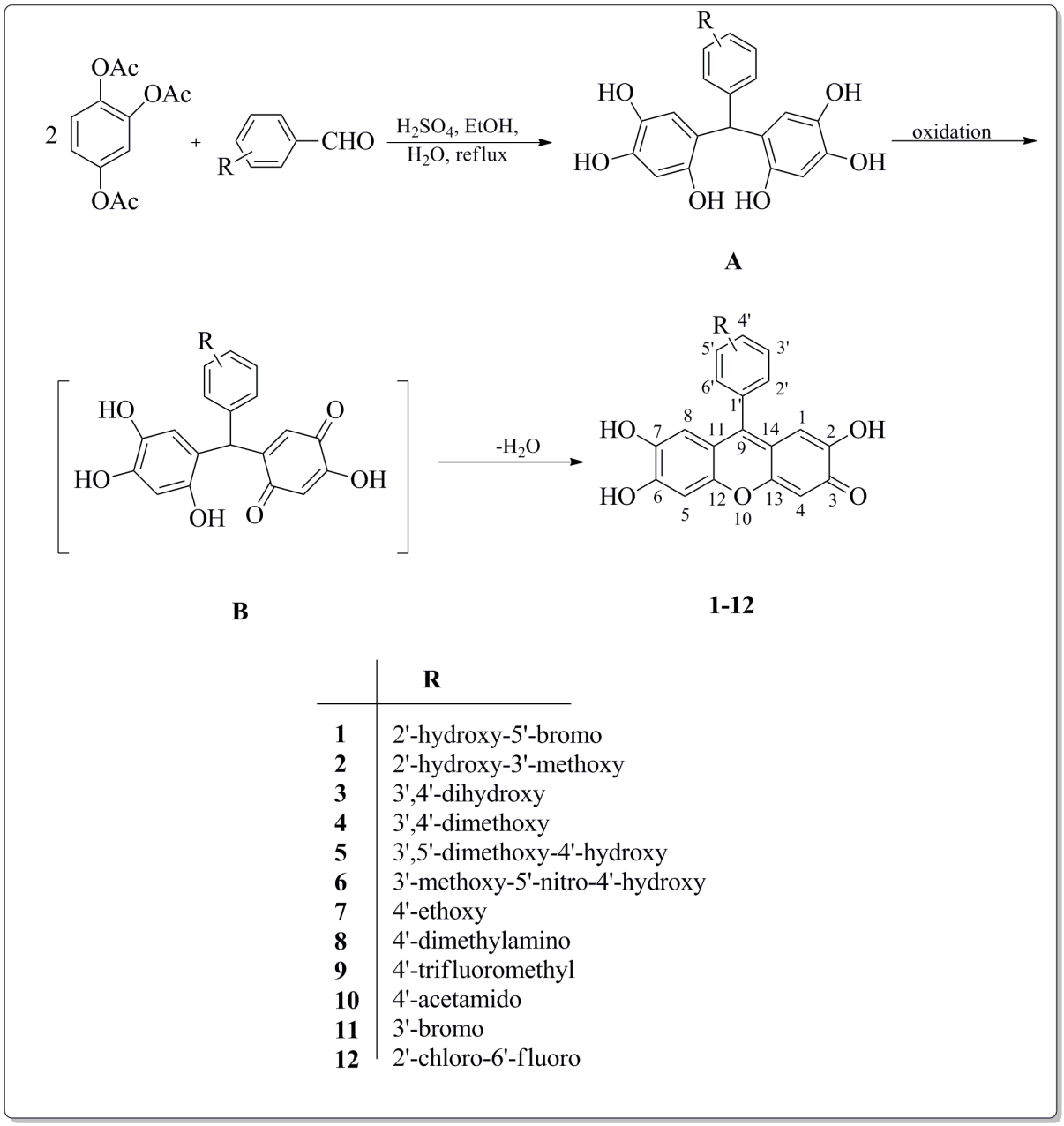

1-12

	R
1	2'-hydroxy-5'-bromo
2	2'-hydroxy-3'-methoxy
3	3',4'-dihydroxy
4	3',4'-dimethoxy
5	3',5'-dimethoxy-4'-hydroxy
6	3'-methoxy-5'-nitro-4'-hydroxy
7	4'-ethoxy
8	4'-dimethylamino
9	4'-trifluoromethyl
10	4'-acetamido
11	3'-bromo
12	2'-chloro-6'-fluoro

Figure 1: Synthesis and structure of xanthen-3-on derivatives

Docking study and physicochemical properties calculations

Lamarckian Genetic Algorithm of the AutoDock 4.0 program was used to perform the flexible-ligand docking studies [22]. Receptors' X-ray crystal structures obtained from the Brookhaven protein data bank were applied in docking studies (http://www.pdb.org/).

Prior to actual docking run, AutoGrid 4.0 was introduced to precalculate grid maps of interaction energies of various atom types. In all dockings, a grid map with 126*126*126 points, a grid spacing of 1.000 Å. In an AutoGrid procedure, the protein is embedded in a 3D grid and a probe atom is placed at each grid point. The energy of interaction of this single atom with the protein is assigned to the grid point. Autodock 4.0 uses these interaction maps to generate ensemble of low energy conformations. It uses a scoring function based on AMBER force field, and estimates the free energy of binding of a ligand to its target. For all dockings, 10 independent runs with step sizes of 0.2 Å for translations and 5 Å for orientations and torsions, an initial

population of random individuals with a population size of 150 individuals, a maximum number of 250000 energy evaluations and 27000 maximum generations.

Bindings between docked potent agents and related macromolecule were analysed using Autodock tools program (ADT, Version 1.5.4) and PyMol-1.1 software that was used for graphical visualization, analysing interactions of ligands and receptors and producing quality of images [23].

As receptors in docking simulations topoisomerase II (pdb: 1BGW) and CYP51 (pdb: 2WX2) were used.

RESULTS AND DISSCUSION

Results of antimicrobial activity

Synthesized xanthene derivatives were tested for microbiological activity by diffusion and microdilution method on four strains of bacteria and two strains of fungi. The tests were performed on two gram negative bacteries *Escherichia coli ATCC 9027* and *Pseudomonas aeruginosa ATCC 15442* and two gram positive bacteries, *Staphylococcus aureus ATCC 6538P* and *Bacillus subtilis ATCC 6051*. The antifungal activity of synthetized xanthene compounds was tested on fungi *Candida albicans ATCC 1023* and *Saccharomyces cerevisiae ATCC 9763.* As standard in the study, chloramphenicol (for antibacterial testing) and fluconazole (for the examination of antifungal activity) were used. The results of antimicrobial activity are shown in Tables 1 and 2.

Table 1: Results of antimicrobial activity

Compound	Minimum Inhibitory Concentration mg/ml (Millimeters of zone inhibition)			
	Escherichia coli (ATCC 9027)	*Pseudomonas aeruginosa (ATCC 15442)*	*Staphylococcus aureus (ATCC 6538P)*	*Bacillus subtilis (ATCC 6051)*
1	0.156 (20,5)	0.626 (18.0)	0.078 (22.5)	0.156 (21.5)
2	0.313 (16.0)	1.250 (13.0)	0.626 (16.5)	0.626 (14.0)
3	0.313 (16.0)	1.250 (14,5)	0.626 (15.0)	1.250 (11.5)
4	0.626 (11.0)	1.250 (16.0)	0.626 (16.0)	2.500 (14.0)
5	0.626 (10.0)	1.250 (12.0)	1.250 (18.5)	1.250 (12.0)
6	0.626 (11.0)	1.250 (14.5)	0.626 (18.5)	1.250 (11.5)
7	1.250 (8.0)	1.250 (16.0)	1.250 (14.0)	1.250 (11.5)
8	0.626 (12.0)	1.250 (13.0)	1.250 (11.0)	0.626 (13.5)
9	0.626 (10.0)	1.250 (13.5)	0.313 (14.5)	0.626 (11.0)
10	0.626 (12.5)	1.250 (16.5)	0.626 (19.5)	1.250 (19.0)
11	0.156 (18,5)	0.626 (18.5)	0.078 (20.0)	0.313 (20.0)
12	0.626 (14.5)	1.250 (15.0)	1.250 (16.0)	0.626 (19.5)
Chloramphenicol	0.078 (23.5)	-	0.010 (24.0)	0.010 (22.5)

Table 2: Results of antifungal activity

Compound	Minimum Inhibitory Concentration mg/ml (Millimeters of zone inhibition)	
	Candida albicans (ATCC 1023)	*Sacharomyces cerevisiae (ATCC 9763)*
1	**0.313 (18.5)**	**0.626 (20.0)**
2	-	0.626 (18.5)
3	-	1.250 (16.0)
4	-	1.250 (16.5)
5	-	1.250 (16.0)
6	-	0.626 (16.5)

7	-	1.250 (14.0)
8	1.250 (17.5)	0.626 (16.5)
9	-	-
10	-	0.626 (19.5)
11	0.626 (18.0)	0.313 (20.0)
12	-	0.626 (18.0)
Fluconazole	0.313 (20.5)	0.313 (21.0)

The *in vitro* antimicrobial activity of the compounds against gram positive *Staphylococcus aureus* and *Bacillus subtilis* bacteria strains and gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* was studied. Also, synthesized 2,6,7-trihydroxy-9-aryl-3*H*-xanthen-3-ones derivatives were tested for their antifungal activity against *Candida albicans and Sacharomyces cerevisiae* strains. The compounds were tested at a concentration of 1mg/mL using Triptic soya bujon. The diameter of growth inhibition zone were measured in millimeters of zone inhibition in diffusion method and in mg/mL in dilution method.

The results indicate that all synthesized 2,6,7-trihydroxy-9-aryl-3*H*-xanthen-3-ones derivatives (**1-12**) showed significant antibacterial activity against tested bacterial strains. Also, only derivatives **1**, **8** and **11** of tested compounds exhibited activity toward *Candida albicans ATCC 1023*. According to results the most potent antimicrobial agents were compounds **1** and **11.** These compounds contain bromine substituent on phenyl ring, which correlates with literature described effect of bromine as substituent on antimicrobial activity [24].

Described *in vitro* antibacterial activity of tetrahydrobenzoxanthene-11-one derivative against bacteria strains suggests that antibacterial activity increased with increasing number of hydroxy groups in molecule [25]. In our research the most potent antibacterial agents was compound **1** with bromine and hydroxy group substituted on phenyl ring. Thus, suggesting that the activity against these bacterial strains is dependent on the nature of substituent on phenyl ring and that bromine and hydroxy group in structure of 2,6,7-trihydroxy-9-aryl-3*H*-xanthen-3-ones derivatives increase antimicrobial activity. The least potent was compound **7** with ethoxy substituent on phenyl ring. In literature are described that ethoxy substitution decrease antimicrobial activity of similar compound [26], which correlate with our results.

Results of docking study

Docking studies for the antibacterial activity of synthesized xanthene compounds were made for the most potent compound. As a receptor in docking simulations, topoisomerase II DNA gyrase was used, which is known to be the target site for the action of most antimicrobial agents, such as ciprofloxacin and chloramphenicol [27].

For compound **1**, that was the most potent compound, docking studies have shown that this compounds bind to an enzyme topoisomerase II energy of -2.52 kcal/mol, thereby constructing three hydrogen bonds, one by oxygen from OH group in position C-2 and protons from the NH group of arginine (ARG 483), the other *via* the proton from the OH group at the C-2 position with oxygen glutamine topoisomerase II (GLU 484) and the third *via* the carbonyl group at the C-3 position with oxygen glutamine (GLU 484) enzyme topoisomerase II DNA gyrase (Figure 2).

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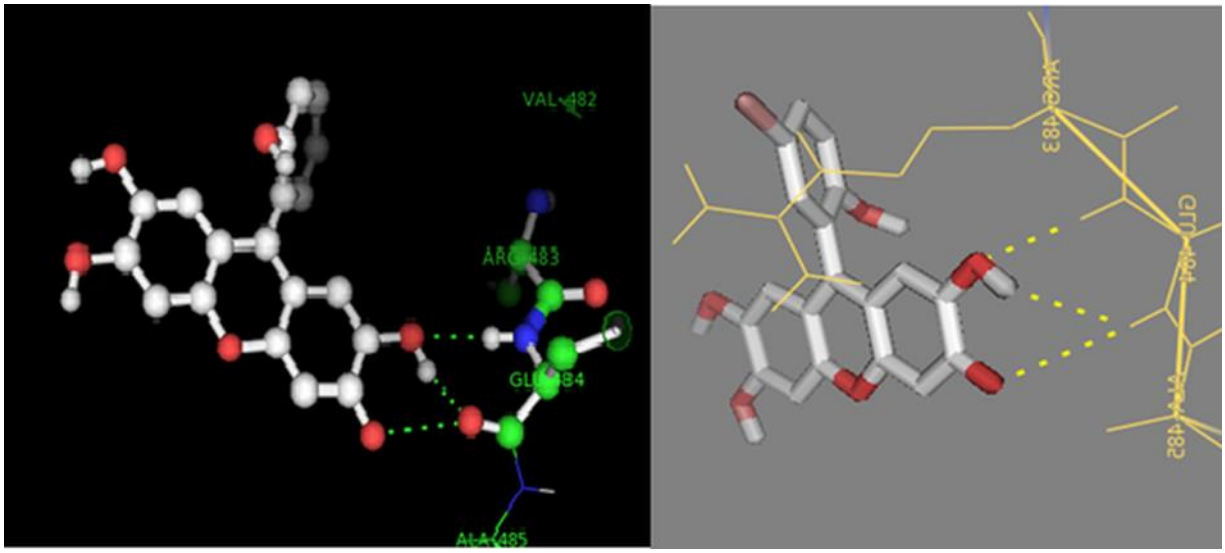

Figure 2: Binding compound 1 on Topoisomerase II

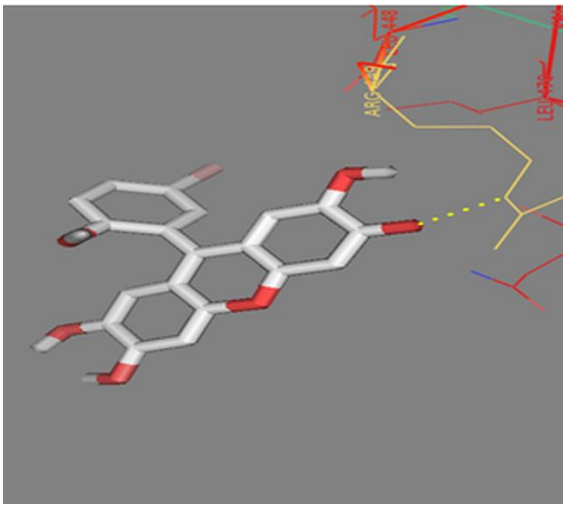

Figure 3: Binding compound 1 on CYP 51

In order to explain why compound **1** shows better action than other synthesized xanthen-3-derivatives, docking studies were also performed on the least potent xanthen-3-one derivative (compound **7**). The results showed that compound **7** has the same binding sites on topoisomerase II as compound **1**, but the binding energy is greater and was -2.05 kcal/mol.

As a receptor in docking studies for antifungal activity, CYP 51 was used as a receptor, which is the target site for the action of most antifungal agents, such as fluconazole and ketoconazole [12].

The best antifungal activity among synthetized xanthen-3-one derivatives showed compound **1**, which is bound to CYP 51 by a single hydrogen bond, *via* a carbonyl group in the C-3 of compound and NH group of the ARG 449 receptor group (Figure 3).

The binding energy of compound **1** for CYP 51 was -5.85 kcal/mol, while for compound **9**, which showed the lowest antifungal activity, was -0.61 kcal / mol.

CONCLUSION

Xanthen-3-one derivatives showed moderate antibacterial and antimycotic activities. Introduction of hydroxy group and bromide as substituents increased antimicrobial activity, while introduction of ethoxy

group decreased it. Docking study revealed correlation between activity *in vitro* and binding energy *in silico* with topoisomerase II. Also, binding energies of synthesized compounds with receptor relevant for antifungal activity showed good correlation with antifungal activity *in vitro*.

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