Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2016

46

33-38

Print ISSN: 0367-4444
Online ISSN: 2232-7266

UDC: _____

Original Scientific Paper

Solvent-free Synthesis and Antibacterial Activity of 14-Aryl Substituted Dibenzoxanthene Derivatives

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Article info
Received: 16/04/2016
Accepted: 01/06/2016

Keywords:
Dibenzoxanthenes,
Solvent-free synthesis,
Antibacterial activity,
Docking study.

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Abstract: Xanthene derivatives are important compounds because of their proven biological activities. Seven 14-aryl-14*H*-dibenzoxanthene derivatives were synthesized by reliable solvent-free synthesis procedure using iron (III) chloride hexahydrate as a catalyst. Three synthesized derivatives possess antibacterial activities against different bacteria. Compound 14-(2',5'-dimethoxyphenyl)-14*H*-dibenzo[*a,j*]xanthene (**3**) showed best activity against *Escherichia coli* and *Staphylococcus aureus* with MIC 0.616 mg/mL. Docking study for the most potent compound was carried out by taking amino terminal domain of enzyme I as a target for antibacterial activity against *Escherichia coli* and it was shown that binding energy of **3** was similar to amikacin's (around –4.2 kcal/mol) used as a referent drug, although bound on a different sites on enzyme.

INTRODUCTION

Xanthenes derivatives are interesting due to their wide range of biological and pharmacological properties, such as agricultural bactericide (Hideo, Teruomi, 1981), anti-inflammatory (Poupelin, Saint-Rut, Foussard-Blanpin, et al., 1978) and antiviral activities (Lambert , Martin, Merrett, et al., 1997). In addition to their biological applications they are also used in industry as dyes, in laser technology and as fluorescent materials for visualization of biomolecules (Knight and Stephens, 1989). Xanthenes are also available from natural sources, such as Santalin pigments wich have been isolated from a number of plant species (Kinjo, Uemura, Nohara; 1995). Thus, the synthesis of xanthenes is of continuing interest. Many synthetic methods exist for the synthesis of xanthenes and dibenzoxanthenes such as the cyclocondensation reaction of 2-hydroxyaromatic aldehydes and 2-tetralone (Jha and Beal, 2004), the reaction of benzaldehydes and acetophenones (Kuo and Fang, 2001), 2-naphtol with formamide (Papini and Cimmarusti, 1947), 2-hydroxynaphthyl carbinol with resorcinol (Sen and Sarkar, 1925) and from the reaction of hot alkali on 2-naphthyl oxide (Ota and Kito, 1976). Furthermore, 14-aryl-14*H*-dibenzoxanthenes are synthesized by cyclocondensation of β-naphtol with aldehydes in the presence of various catalysts such as silica sulfuric acid (Rajitha, Kumar, Reddy, *et al.*, 2005), AcOH-H_2SO_4 (Sarma and Baruah, 2005), p-TSA (Khosropour, Khodaei and Moghannian, 2005), $MeSO_3H$, sulfamic acid, cyanuric chloride, LiBr, $Yb(Otf)_3$ (Saini, Kumar and Sandhu, 2006), $TaCl_5$ and $BiCl_3$ (Soleimani, Khodaei and Koshvandi, 2011). However, these methods show varying degrees of success as well as limitations

such as prolonged reaction times, laborious work-up procedures, harsh reaction conditions, use of an excess of expensive reagents and use of toxic organic solvents (Kumar, Sunil Kumar and Narsimha Reddy, 2006). Thus, the development of an alternative milder and clean procedure is highly demanding for the synthesis of new and/or known compounds, wich surpasses those limitations. Convenient and solvent-free synthesis of 14-aryl-14*H*-dibenzoxanthenes catalyzed by iron (III) chloride hexahydrate has been described in literature (Liu, Zhou, Gao, *et al.*, 2013). Using this solvent-free method we synthesized seven 14-aryl-14*H*-dibenzoxanthene derivatives already reported but with iron (III) chloride hexahydrate as a catalyst.

In our work we conducted research of antimicrobial activity for synthesized 14-aryl-14*H*-dibenzoxanthenes. According to literature, this is the first time reporting on the antimicrobial activity of these dibenzoxanthene derivatives. In order to investigate potential mechanism of antibacterial activity for the most potent compound, docking study was performed. Molecular docking is a very useful method introduced to investigate molecular association and is particularly important in the drug discovery field to study the binding of small molecules (ligands) to macromolecules (receptor) (Barril and Morley 2005).

MATERIAL AND METHODS

General procedure for synthesis 14-aryl-14*H*-dibenzoxanthenes

A mixture of aldehyde (5 mmol), 2-naphthol (10 mmol) and $FeCl_3x6H_2O$ (1 mmol) was finely ground and heated at 90°C. The reaction was monitored by thin-layer chromatography (TLC). After completion, the system was cooled to room temperature, reaction mixture was washed with 60% aqueous EtOH and filtered to afford the crude product. Further purification was followed by crystallization from 96% ethanol (Liu, Zhou, Gao et al., 2013).

Melting points of synthesized compounds were determined by Melting Point Meter KSP1D, A.Krüss Optronic, Germany. Infrared (IR) spectra were recorded by Perkin Elmer BX FTIR using KBr pellets. The ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 600 and 150 MHz, respectively, in deuterated dimethyl sulfoxide (DMSO-*d*6) at 25°C using NMR spectrometer Bruker AV600, with tetramethylsilane (TMS) as internal reference. Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz.

Antimicrobial activity

Antibacterial activity was tested by the diffusion method against *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 9027. The results were presented as the inhibition zones, given in millimeters (mm). Compounds that showed good antimicrobial activity by diffusion method were further tested by dilution method. Amikacin was used as a referent drug.

For determination of antimicrobial activity (diffusion method) Müller-Hinton nutritious base was used, while Sabouraud dextrose broth was used in the dilution method. When using the diffusion method, the test samples were dissolved in 99.5% dimethyl sulfoxide (DMSO) to obtain a 1 mg/100 μL stock solution. The inhibition zones were measured in millimeters at the end of an incubation period of 24 h at 37°C. In dilution method microtiter plates with 96 sites were used. In first well on 100 μL of nutrient broth was added 100 μL solution of the test compound and the content was mixed. From this well was taken 100 μL of solution, which is transferred to the next well and the contents were mixed. The process of double dilution was repeated until they fulfill all the wells in one row. The same procedure was used when microtiter-plate was filled with other compounds. DMSO was used as blank control and amikacin as a referent compound. In each of the wells was added 10 μL of the appropriate bacterial culture ($1x10^6$ cells/mL) and thermostated for 24 hours at 37°C. After thermostating, turbidimetric method was used for reading results. Minimum inhibitory concentration (MIC) corresponds to the concentration of the compound in well, where for the first time does not occur growth of bacterial culture. The concentration is expressed in mg/mL.

Docking study

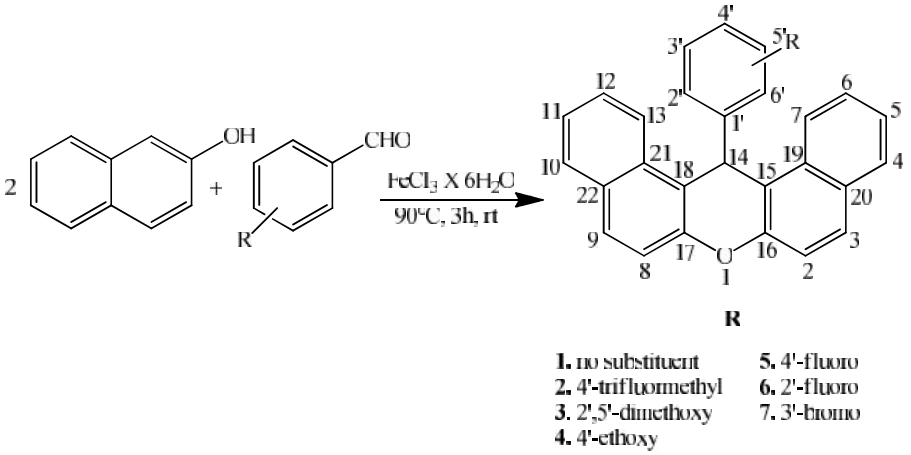
Lamarckian Genetic Algorithm of the AutoDock 4.0 program was used to perform the flexible-ligand docking studies (Morris, Huey, Lindstrom, 2009). Receptors X-ray crystal structures obtained from the Brookhaven protein data bank was applied in docking studies (http://www.pdb.org/).

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 analyzed using Autodock tools program (ADT, Version 1.5.4) and PyMol-1.1 software was used for graphical visualization, analyzing interactions of ligands and receptors and producing quality of images (Lill and Danielson 2011).

RESULTS AND DISCUSSION

Seven dibenxanthene derivatives were synthesized according to procedure given in Scheme 1



Scheme 1. Solvent-free synthesis of dibenzoxanthene derivatives.

Six different benzaldehydes with electron donating and electron withdrawing substituents along with unsubstituted benzaldehyde were used in the procedure. Syntheses lasted for 3 hours and yields ranged from 82 to 90% indicating good catalytic action of iron (III) chloride hexahydrate in this solvent-free conditions.
Analytical data of synthesized compounds are given below:

14-phenyl-14*H*-dibenzo[*a,j*]xanthene (1)

Yield: 86%; mp 186°C.

IR (KBr) n 3057, 3022, 1593, 1515, 1457, 963, 829, 744, 701 cm^{-1}.

^{1}H NMR (600 MHz, DMSO-d_6) d 6.46 (s, 1H, H-14), 6.97 (t, *J* 7.85 Hz, 1H, H-4'), 7.12 (t, *J* 7.85 Hz, 1H, H-3' and H-5'), 7.38 (t, *J* 7.55 Hz, 1H, H-5 and H-11), 7.46 (d, *J* 8.70 Hz, 1H, H-3 and H-9), 7.51 (d, *J* 7.85 Hz, 1H, H-2' and H-6'), 7.55 (t, *J* 7.55 Hz, 1H, H-6 and H-12), 7.76 (d, *J* 8.70 Hz, 1H, H-2 and H-8), 7.80 (d, *J* 7.55 Hz, 1H, H-4 and H-10), 8.37 (d, *J* 7.55 Hz, 1H, H-7 and H-13).

^{13}C NMR (150 MHz, DMSO-d_6) d 38.2 (C-14), 117.5 (C-15 and C-18), 118.2 (C-3 and C-9), 122.9 (C-7 and C-13), 124.4 (C-5 and C-11), 126.6 (C-4'), 127.0 (C-6 and C-12), 128.4 (C-2' and C-6'), 128.6 (C-3' and C-5'), 128.97 (C-4 and C-10), 129.03 (C-2 and C-8), 131.3 or 131.7 (C-19/21), 131.3 or 131.7 (C-20/22), 145.2 (C-1'), 148.9 (C-16 and C-17).

14-(4'-trifluoromethylphenyl)-14*H*-dibenzo[*a,j*]xanthene (2)

Yield: 88%; mp 259°C.

IR (KBr) n 1594, 1516, 1460, 1118, 1067, 744, 202 cm^{-1}.

^{1}H NMR (600 MHz, DMSO-d_6) d 6.55 (s, 1H, H-14), 7.39 (d, *J* 8.10 Hz, 1H, H-3' and H-5'), 7.42 (t, *J* 7.50 Hz, 1H, H-5 and H-11), 7.58 (t, *J* 7.50 Hz, 1H, H-6 and H-12), 7.62 (d, *J* 8.10 Hz, 1H, H-2' and H-6'), 7.81 (d, *J* 8.40 Hz, 1H, H-3 and H-9), 7.84 (t, *J* 7.50 Hz, 1H, H-4 and H-10), 8.32 (d, *J* 7.50 Hz, 1H, H-7 and H-13).

^{13}C NMR (150 MHz, DMSO-d_6) d 38.0 (C-14), 116.6 (C-15 and C-18), 118.2 (C-3 and C-9), 122.5 (C-7 and C-13), 124.1 (CF_3), 124.7 (C-5 and C-11), 125.7 (C-3' and C-5'), 149.03 (C-16 and C-17), 127.2 (C-6 and C-12), 128.7 (C-2' and C-6'), 129.2 (C-4 and C-10), 129.5 (C-2 and C-8), 131.3 or 131.4 (C-19/21), 131.3 or 131.4 (C-20/22), 136.2 (C-4'), 148.97 (C-1').

14-(2',5'-dimethoxyphenyl)-14*H*-dibenzo[*a,j*]xanthene (3)

Yield: 90%; mp 170°C.

IR (KBr) n 3057, 1622, 1592, 1516, 1457, 1431, 1401, 1265, 1239, 1136, 1018, 962, 822, 751 cm^{-1}.

^{1}H NMR (600 MHz, DMSO-d_6) d 4.23 (s, 3H, 6'-OCH_3), 3.46 (s, 3H, 3'-OCH_3), 6.48 (d, *J* 7.95 Hz, 1H, H-4'), 6.72 (s, 1H, H-2'), 6.78 (d, *J* 7.95 Hz, 1H, H-5'), 6.86 (s, 1H, H-14), 7.38 (t, *J* 7.50 Hz, 1H, H-5 and H-11), 7.45 (d, *J* 7.10 Hz, 1H, H-3 and H-9), 7.52 (t, *J* 7.50 Hz, 1H, H-6 and H-12), 7.75 (d, *J* 7.10 Hz, 1H, H-2 and H-8), 7.91 (d, *J* 7.50 Hz, 1H, H-4 and H-10), 8.56 (d, *J* 7.50 Hz, 1H, H-7 and H-13).

^{13}C NMR (150 MHz, DMSO-d_6) d 30.6 (C-14), 55.4 (3'-O<u>C</u>H$_3$), 56.4 (6'-O<u>C</u>H$_3$), 111.4 (C-5'), 112.2 (C-4'), 117.1 (C-2'), 118.2 (C-3 and C-9), 118.5 (C-15 and C-18), 123.5 (C-7 and C-13), 124.4 (C-5 and C-11), 126.8 (C-6 and C-12), 128.6 (C-4 and C-10), 128.7 (C-2 and C-8), 131.0 or 132.2 (C-19 and C-21), 131.0 or 132.2 (C-20 and C-22), 135.8 (C-1'), 148.4 (C-6'), 149.0 (C-16 and C-17), 154.3 (C-3').

14-(4'-ethoxyphenyl)-14*H*-dibenzo[*a,j*]xanthene (4)

Yield: 89%; mp 174°C.

IR (KBr) n 3060, 2927, 1594, 1460, 1246, 1115, 963, 812, 745 cm^{-1}.

^{1}H NMR (600 MHz, DMSO-d_6) d 8.37 (d, *J* 7.65 Hz, 1H, H-7 and H-13), 7.77 (d, *J* 9.00 Hz, 1H, H-2 and H-8), 7.81 (d, *J* 7.65 Hz, 1H, H-4 and H-10), 7.56 (t, *J* 7.65 Hz, 1H, H-6 and H-12), 7.46 (d, *J* 9.00 Hz, 1H, H-3 and H-9), 7.40 (m, 1H, H-5 and H-11), 7.40 (m, 1H, H-2' and H-6'), 6.64 (d, *J* 7.20 Hz, 1H, H-3' and H-5'), 6.37 (s, 1H, H-14), 3.82 (q, J = 7.00 Hz, 2H, OCH_2CH_3), 1.25 (t, *J* 7.00 Hz, 3H, OCH_2CH_3).

^{13}C NMR (150 MHz, DMSO-d_6) d 15.0 (OCH_2CH_3), 37.3 (C-14), 63.4 (OCH_2CH_3), 114.6 (C-3' and C-5'), 117.8 (C-15 and C-18), 118.2 (C-3 and C-9), 122.9 (C-7 and C-13), 124.4 (C-5 and C-11), 126.9 (C-6 and C-12), 128.9 (C-2 and C-8), 129.0 (C-4 and C-10), 129.3 (C-2' and C-6'), 131.3 or 131.6 (C-19/21), 131.3 or 131.6 (C-20/22), 137.4 (C-1'), 148.9 (C-16 and C-17), 157.4 (C-4').

14-(4'-fluorophenyl)-14*H*-dibenzo[*a,j*]xanthene (5)

Yield: 83%; mp 241°C.

IR (KBr) n 3071, 2361, 1624, 1594, 1503, 1460, 1433, 1401, 835, 745 cm^{-1}.

^{1}H NMR (600 MHz, DMSO-d_6) d 6.47 (s, 1H, H-14), 6.81 (t, *J* 8.57 Hz, 2H, H-3' and H5'), 7.41 (t, *J* 7.49 Hz, 2H, H-5 and H-11), 7.50-7.43 (m, 4H, H-3 and H-9), 7.50-7.43 (, 4H, H-2' and H-6'), 7.57 (t, *J* 7.73 Hz, 2H, H-6 and H-12), 7.79 (d, *J* 8.88 Hz, 2H, H-2 and H-8), 7.83 (d, *J* 8.07 Hz, 2H, H-4 and H-10), 8.33 (d, *J* 8.53 Hz, 2H, H-7 and H-13).

^{13}C NMR (150 MHz, DMSO-d_6) d 37.4 (C-14), 114.8 (C-3' and C-5'), 117.3 (C-15 and C-18), 118.2 (C-3 and C-9), 122.7 (C-7 and C-13), 124.5 (C-5 and C-11), 127.1 (C-6 and C-12), 129.1 (C-4 and C-10), 129.2 (C-2 and C-8), 129.8 (C-2' and C-6'), 131.3 (C-20 and C-22), 131.3 (C-19 and C-21), 141.0 (C-1'), 148.8 (C-16 and C-17), 161.4 (C-4')

14-(2'-fluorophenyl)-14*H*-dibenzo[*a,j*]xanthene (6)

Yield: 82%; mp 233°C.

IR (KBr) n 3080, 2209, 1624, 1594, 1516, 1483, 1460, 749 cm^{-1}.

^{1}H NMR (600 MHz, DMSO-d_6) d 6.80 (s, 1H, H-14), 7.03-6.91 (m, 1H, H-5'), 7.15-7.03 (m, 1H, H-4'), 7.15-7.03 (m, 1H, H-3'), 7.51-7.40 (m, 1H, H-6'), 7.51-7.40 (m, 2H, H-5 and H-11), 7.56 (d, *J* 8.56 Hz, 2H, H-3 and H-9), 7.64 (t, *J* 7.07 Hz, 2H, H-6 and H-12), 7.95 (d, *J* 7.45 Hz, 2H, H-4 and H-10), 7.95 (d, *J* 7.45 Hz, 2H, H-2 and H-8), 8.41 (d, *J* 7.98 Hz, 2H, H-7 and H-13).

^{13}C NMR (150 MHz, DMSO-d_6) d 30.6 (C-14), 115.3 (C-15 and C-18), 115.5 (C-3'), 117.6 (C-3 and C-9), 122.1 (C-7 and C-13), 124.5 (C-5 and C-11), 125.0 (C-5'), 127.2 (C-6 and C-12), 128.8 (C-4'), 129.3 (C-20 and C-22), 129.3 (C-19 and C-21), 129.3 (C-4 and C-10), 129.31 (C-8 and C-2), 130.8 (C-6'), 131.8 (C-1'), 148.1 (C-16 and C-17), 158.1 (C-2').

14-(3'-bromophenyl)-14*H*-dibenzo[*a,j*]xanthene (7)

Yield: 85%; mp 192°C.
IR (KBr) n 3066, 2923, 1624, 1591, 1457, 1430, 1399, 1243, 1065, 961, 811, 774, 690 cm^{-1}.

^{1}H NMR (600 MHz, DMSO-d_6) d 6.45 (s, 1H, H-14), 7.03 (t, *J* 7.85 Hz, 1H, H-5'), 7.15 (d, *J* 7.50 Hz, 1H, H-4'), 7.44 (t, *J* 7.50 Hz, 2H, H-5 and H-11), 7.54-7.49 (m, 1H, H-6'), 7.54-7.49 (m, 2H, H-3 and H-9), 7.62 (t, *J* 7.85 Hz, 2H, H-6 and H-12), 7.65 (s, 1H, H-2'), 7.81 (d, *J* 9.05 Hz, 2H, H-2 and H-8), 7.85 (d, *J* 7.50 Hz, 2H, H-4 and H-10), 8.33 (d, *J* 8.20 Hz, 2H, H-7 and H-13).

^{13}C NMR (150 MHz, DMSO-d_6) d 37.9 (C-14), 116.7 (C-15 and C-18), 118.2 (C-3 and C-9), 122.5 (C-7 and C-13), 122.9 (C-3'), 124.5 (C-5 and C-11), 127.0 (C-6'), 127.1 (C-6 and C-12), 129.1 (C-4 and C-10), 129.1 (C-4 and C-10), 129.3 (C-2 and C-8), 129.8 (C-4'), 130.1 (C-5'), 131.2 (C-20 and C-22), 131.2 (C-19 and C-21), 131.3 (C-2'), 147.3 (C-1').

Synthesized compounds differ in the substituents bound to the phenyl ring. Due similar dibenzoxanthene structure, all observed IR spectra contained absorption bands above 3000 cm^{-1}. On the IR spectrum of the compound **1**, which has no substituents on the phenyl ring, characteristic band was visible at 690 cm^{-1} indicating monosubstituted benzene derivative.
Derivative with two methoxy groups (**3**) on the phenyl ring showed bands at 1360-1390cm^{-1} characteristic for the O-CH_3 group.
IR spectra of compounds containing fluorine (**2**, **5** and **6**) showed absorption at 1000-1200 cm^{-1} originating from the C-F stretching. Compound with bromine substituent (**7**) showed characteristic band at 515-690 cm^{-1} from C-Br stretching.
^{1}H NMR spectra of synthesized compound revealed singlets from 6.37 to 6.86 ppm derived from aromatic protons from xanthene ring. ^{1}H NMR spectrum of **3** showed characteristic singlets at 4.23 and 3.46 ppm corresponding to the protons of the methoxy groups. For the derivative with ethoxy group, ^{1}H NMR spectrum showed characteristic shifts at 3.82 ppm and 1.25 ppm derived from the protons of the ethoxy group.
^{13}C NMR spectra of all compounds revelaed signals higher than 100 ppm for the aromatic carbons from xanthene molecule. Compound **3** on ^{13}C NMR showed signals of methoxy carbons and these signals, as expected, had small shifts in ppm. Signals for the secondary carbon atom at 63.4 ppm is visible only on ^{13}C NMR spectra of derivative with ethoxy group (**4**).

Antibacterial activity

Antibacterial activity of synthesized compounds was tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* (Table 1).

Table 1. Inhibition zone (mm) of the investigated compounds.

	Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus subtilis*
Compound	Inhibition zone (mm)			
1	/	/	/	/
2	18	16	18	16
3	26	10	26	10
4	12	12	12	12
5	/	/	/	/
6	/	/	/	/
7	/	/	/	/
DMSO	/	/	/	/
Amikacin	42	20	32	22

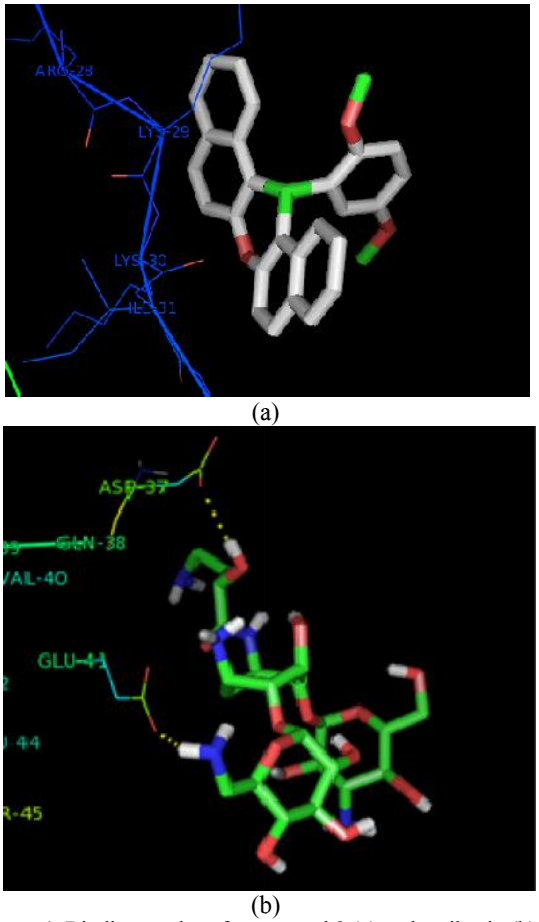
Unsubstituted compound **1** and derivatives with halogen atoms directly bound to phenyl ring (**5**, **6** and **7**) did not show any activity against tested microorganisms. The introduction of oxygen and alkyl substituents on the phenyl ring increased antimicrobial activity. 14-(4'-Trifluoromethylphenyl)-14*H*-dibenzo[*a,j*] xanthene (**2**) showed antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*, with inhibition zone of 18 mm, while against *Pseudomonas aeruginosa* and *Bacillus subtilis* inhibition zone was somewhat smaller (16 mm). The best antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* showed 14-(2',5'-dimethoxyphenyl)-14*H*-dibenzo[*a,j*]xanthene (**3**) with inhibition zone of 26 mm. The same compound against *Pseudomonas aeruginosa* and *Bacillus subtilis* showed inhibition zone of only 10 mm. The most potent antibacterial derivative **3** assessed by diffusion method was further tested by dillution method. Results are shown in Table 2.

Table 2. Minimum inhibitory concentration (MIC, mg/mL) of the most potent compound **3**.

	Escherichia coli	*Staphylococcus aureus*
Compound	MIC (mg/mL)	
3	0.616	0.616
Amikacin	0.005	0.011

Docking study

Possible target in microbial population include the phosphoenolpyruvate phosphotransferase system (PTS). PTS is ubiquitous in eubacteria and absent from eukaryotes. The system consists of two phosphoryl carriers, enzyme I (EI) and the histidine-containing phosphoryl carrier protein (HPr), and several PTS transporters, catalyzing the concomitant uptake and phosphorylation of several carbohydrates. Since a deficiency of EI in bacterial mutants lead to severe growth defects, EI could be a drug target to develop antimicrobial agents (Huang, Lin, Lin, *et al.*, 2013).



(a)

(b)

Figure 1. Binding modes of compound **3** (a) and amikacin (b) at the active site of amino terminal domain of enzyme I from *Escherichia coli* (PDB ID: 1ZYM) as assessed by molecular docking study.

In order to investigate a plausible mechanism of action of the most active compound (**3**) against *Escherichia coli*, docking study was performed using amino terminal domain of enzyme I as a target for antibacterial activity (pdb: 1ZYM). Binding mode of compound **3** into the active site of amino terminal domain of enzyme I from *Escherichia coli* is shown in Figure 1.

Compound **3** binds at the acitve site of enzyme with binding energy of -4.23 kcal/mol while forming no hydrogen bonds. Binding energy of amikacin on the same receptor is -4.20 kcal/mol and forms two hydrogen bonds with Glu 41 and Asp 37.

CONCLUSIONS

Using solvent-free method, catalyzed by iron (III) chloride hexahydrate, seven 14-aryl-14*H*-dibenzoxanthene derivatives were synthesized. Derivatives with CF_3, OCH_3 and OC_2H_5 groups showed good antimicrobial activity, while none of the synthesized derivatives with halogen substituents on phenyl ring showed activity against tested microorganisms. Docking study showed that the most potent compound binds at the active site of investigated enzyme with binding energy similar to amikacin's, but with no hydrogen bonds. These compounds are interesting in future investigations for antimicrobial agens.

REFERENCES

Barril, X., Morley, S.D. (2005). Unveiling the full potential of flexible receptor docking using multiple crystallographic structures. *J. Med. Chem*. 48, 4432-4443.

Hideo, T., Teruomi, J., (Sankyo Co.). (1981). Benzopyrano[2,3-b]xanthene derivativesand its preparation. *Jpn. Patent* 56005480.

Huang, K.J., Lin, S.H., Lin, M.R., Ku, H., Szkaradek, N., Marona, H., Hsu, A., Shiuan, D. (2013). Xanthone derivatives could be potential antibiotics: virtual screening for the inhibitors of enzyme I of bacterial phosphoenolpyruvate-dependent phosphotransferase system. *J. Antibiot.*, 66 (8), 453-458.

Jha, A., Beal, J. (2004). Convenient synthesis of 12H-benzo[a]xanthenes from 2-tetralone. *Tetrahedron Lett*., 45, 8999- 9001.

Khosropour, A.R., Khodaei, M.M., Moghannian, H. (2005). A facile, simple, and convenient method for the synthesis of 14-alkyl or aryl-14H-dibenzo[a,j]xanthenes catalyzed by p-TSA in solution and solvent free conditions. *Synlett,* 955–958.

Kinjo, J., Uemura, H., Nohara, T., Yamashita, M., Marubayashi, N., Yoshihira, K. (1995). Novel yellow pigment from Pterocarpus santalinus: biogenetic hypothesis for santalin analogs. *Tetrahedron Lett.,* 36, 5599-5602.

Knight, C.G., Stephens, T. Biochem, J. (1989). Xanthene-dye-labelled phosphatidylethanolamines as probes of interfacial pH. Studies in phospholipid vesicles. 258(3): 683-687.

Kumar, P. S., Sunil Kumar, B., Narsimha Reddy, P., Sreenivasulu, N., Thirupathi Reddy Y. (2006). A novel one pot synthesis of 14-aryl-14H dibenzo (a,j) xanthenes catalyzed by Selectfluor^{TM} under solvent free conditions, *Arkivoc,* (xii), 46-50.

Kuo, C.W., Fang, J. M. (2001). Synthesis of Xanthenes, Indanes, and Tetrahydronaphthalenes via Coupling Reactions. *Synth. Commun*, 31, 877-892.

Lambert, R. W., Martin, J. A., Merrett, J. H., Parkes, K. E. B., Thomas, G. J. (1997). PCT Int. Appl. WO 9706178. *Chem. Abstr.,* 126, 212377y.

Lill, M.A., Danielson, M.L. (2011). Computer-aided drug design platform using PyMOL. *Journal of computer-aided molecular design*, 25(1): 13-19.

Liu, D., Zhou, S., Gao, J., Li, L., Xu, D. (2013). Solvent-free Synthesis of 5H-dibenzo [b, i] xanthene-tetraones and Aryl-14H-dibenzo [a, j] xanthenes Using Ferric Chloride Hexahydrate as Catalyst. *Journal of the Mexican Chemical Society*, *57*(4), 345-348.

Morris, G.M., Huey, R., Lindstrom, W., Sanner, M.F., Belew, R.K., Goodsell, D.S., Olson, A.J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of computational chemistry,* 30(16): 2785-2791.

Ota, K., Kito, T. (1976). An improved synthesis of dibenzoxanthene. *Bull. Chem. Soc. Jpn*. 49, 1167–1168.

Papini, P., Cimmarusti, R. (1947). Action of formamide and formanilide on naphthols and on barbituric acid. *Gazz. Chim. Ital.*, 77, 142-143.

Poupelin, J. P., Saint-Rut, G., Foussard-Blanpin, O., Narcisse, G., Uchida-Ernouf, G., Lacroix R. (1978). Synthesis and antiinflammatory properties of bis (2-hydroxy-1-naphthyl)methane derivatives I. *Eur. J. Med. Chem*.13, 67-71.

Rajitha, B., Kumar, B.S., Reddy, Y.T., Reddy, P., Sreenivasulu, N. (2005). Sulfamic acid: A novel and efficient catalyst for the synthesis of aryl-14H-dibenzo[a,j]xanthenes under conventional heating and microwave irradiation. *Tetrahedron Lett*., 46, 8691–8693.

Saini, A., Kumar, S., Sandhu, J.S. (2006). A New LiBr-Catalyzed, Facile and Efficient Method for the Synthesis of 14-Alkyl or Aryl-14H-dibenzo[a,j]xanthenes and Tetrahydrobenzo[b]pyrans under Solvent-Free Conventional and Microwave Heating. *Synlett*,1928–1932.

Sarma, R.J., Baruah, J.B. (2005). One step synthesis of dibenzoxanthen. *Dyes Pigments*, 64, 91–92.

Sen, R.N., Sarkar, N. (1925). The condensation of primary alcohols with resorcinol and other hydroxy aromatic compounds. *J. Am. Chem. Soc*., 47, 1079–1091.

Soleimani, E., Khodaei, M.M., Koshvandi, A.T.K. (2011). The efficient synthesis of 14-alkyl or aryl 14*H*-dibenzo[*a,j*]xanthenes catalyzed by bismuth(III) chloride under solvent-free conditions. *Chin. Chem. Lett*, 22 (8), 927-930.

Summary/Sažetak

Ksantenski derivati predstavljaju značajne spojeve zbog svojih mnogostrukih bioloških djelovanja. Prema pouzdanoj proceduri suhe hemije sintetizirano je sedam 14-aril-14*H*-dibenzoksantenskih derivata uz željezo (III) hlorid heksahidrat kao katalizator. Tri sintetizirana derivata pokazala su antibakterijski učinak prema testiranim bakterijskim sojevima. Spoj 14-(2',5'-dimetoksifenil)-14*H*-dibenzo[*a,j*]ksanten (**3**) pokazao je najbolje djelovanje prema *Escherichia coli* i *Staphylococcus aureus* s minimalnom inhibitornom koncentracijom (MIC) od 0.616 mg/mL. U doking studiji za najpotentniji spoj prema *Escherichia coli* kao receptor je korišten amino terminalni dio enzima I. Rezultati doking studija pokazali su da najpotentniji spoj **3** i amikacin, korišten kao referentni antibiotik, imaju slične energije vezivanja za receptor (oko –4.2 kcal/mol), pri čemu se vežu na različitom dijelu enzima.