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Pages 1-246 (2016)

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Previous vol/issue Next vol/issue

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Recombinant LipL32 Protein for Leptospirosis Detection in Indonesia

Sumarningsih, Simson Tarigan, Susanti, Kusmiyati

Pages 18-25

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Research article *Open access*

Enhancing Stability and Purity of Crude Chitinase of *Achatina fulica* by Crystallization

Afaf Baktir, Nira Ambar Arum, Suyanto, Bambang Suprijanto

Pages 26-30

Download PDF Article preview

Research article *Open access*

Application of Cassava Peel and Waste as Raw Materials for Xylooligosaccharide Production Using Endoxylanase from *Bacillus subtilis* of Soil Termite Abdomen

Anak Agung Istri Ratnadewi, Agung Budi Santoso, Erma Sulistyaningsih, Wuryanti Handayani

Pages 31-38

Download PDF Article preview

Research article *Open access*

Mutation Analysis of the pK_a Modulator Residue in β-D-xylosidase from *Geobacillus Thermoleovorans* IT-08: Activity Adaptation to Alkaline and High-Temperature Conditions

Lanny Hartanti, Ali Rohman, Ami Suwandi, Bauke W. Dijkstra, ... Ni Nyoman Tri Puspaningsih

Pages 39-48

Download PDF Article preview

Research article *Open access*

Autolytic Isolation of Chitin from White Shrimp (*Penaues Vannamei)* Waste

Achmad Sjaifullah, Agung Budi Santoso

Pages 49-52

Download PDF Article preview

Research article *Open access*

Effects of Fermentation and Storage on Bioactive Activities in Milks and Yoghurts

Irma Sarita Rahmawati, Worapot Suntornsuk

Pages 53-62

Download PDF Article preview

Research article *Open access*

Scanning Electron Microscope Analysis of Rice Straw Degradation by a Treatment with α-L-arabinofuranosidase

Anita Kurniati, Handoko Darmokoesoemo, Ni Nyoman Tri Puspaningsih

Pages 63-68

Download PDF Article preview

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Secretion of *Geobacillus Thermoleovorans* IT-08 α-L-Arabinofuranosidase (AbfA) in *Saccharomyces Cerevisiae* by Fusion with HM-1 Signal Peptide

I Nengah Wirajana, Tetsuya Kimura, Kazuo Sakka, Eddy Bagus Wasito, ... Ni Nyoman Tri Puspaningsih

Pages 69-74

Download PDF Article preview

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Hydrolysis of Corncob Xylan using β-xylosidase GbtXyl43B from *Geobacillus Thermoleovorans* IT-08 Containing Carbohydrate Binding Module (CBM)

Ni Nyoman Purwani, Handoko Darmokoesoemo, Ni Nyoman Tri Puspaningsih

Pages 75-81

Download PDF Article preview

Research article *Open access*

Biochemical Potential of α-L-Arabinofuranosidase as Anti-Tuberculosis Candidate

One Asmarani, Much Zaenal Fanani, Ni Nyoman Tri Puspaningsih

Pages 82-89

Download PDF Article preview

Research article *Open access*

Shortening of Amino Acids from C-terminal of PZase as Basis of Pyrazinamide Resistance in P14 Isolate of *Mycobacterium Tuberculosis* Strain

Purkan, Redianti Galuh Novarizka, Rizka Aziz Ayuningsih, Presty Nurdiana, Wiwin Retnowati

Pages 90-95

Download PDF Article preview

Research article *Open access*

Elimination of SCMV (*Sugarcane Mozaik Virus*) and Rapid Propagation of Virus-free Sugarcane (*Saccharum officinarum* L.) Using Somatic Embryogenesis

Parawita Dewanti, Laily Ilman Widuri, Choirul Ainiyati, Purnama Okviandari, ... Bambang Sugiharto

Pages 96-102

Download PDF Article preview

Research article *Open access*

Antimicrobial Activities and In silico Analysis of Methoxy Amino Chalcone Derivatives

Hery Suwito, Ni'matuzahroh, Alfinda Novi Kristanti, Salwa Hayati, ... Ni Nyoman Tri Puspaningsih

Pages 103-111

Download PDF Article preview

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Potential Application of Oleylamine-encapsulated $AgInS_2$-ZnS Quantum Dots for Cancer Cell Labeling
Mochamad Zakki Fahmi, Jia-Yaw Chang
Pages 112-121

Download PDF Article preview

Research article *Open access*
Macrophage Activity and Capacity Following Oral Administration of Cocoa Extract to Mice
Ariza Budi Tunjung Sari, Teguh Wahyudi, Misnawi, Diana Chusna Mufida, I Wayan Suardita
Pages 122-126

Download PDF Article preview

Research article *Open access*
The Role and Efficiency of Ammonium Sulphate Precipitation in Purification Process of Papain Crude Extract
Maria Goretti M. Purwanto
Pages 127-131

Download PDF Article preview

Research article *Open access*
Isolation and Antibacterial Activity Test of Lauric Acid from Crude Coconut Oil (*Cocos nucifera* L.)
Febri Odel Nitbani, Jumina, Dwi Siswanta, Eti Nurwening Solikhah
Pages 132-140

Download PDF Article preview

Research article *Open access*
Effect of Butyric Acid on p53 Expression and Apoptosis in Colon Epithelial Cells in Mice after Treated with 9,10-dimethyl-1,2-benz(a)anthracene
Cherry Siregar, Eddy Bagus Wasito, I Ketut Sudiana
Pages 141-146

Download PDF Article preview

Research article *Open access*
Deep Eutectic Solvent (DES) as a Pretreatment for Oil Palm Empty Fruit Bunch (OPEFB) in Sugar Production
Nur Atikah Md Nor, Wan Aida Wan Mustapha, Osman Hassan
Pages 147-154

Download PDF Article preview

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Pretreatment of Oil Palm Empty Fruit Fiber (OPEFB) with Aquaeous Ammonia for High Production of Sugar
Nursyafiqah Zulkiple, Mohamad Yusuf Maskat, Osman Hassan
Pages 155-161

Download PDF Article preview

Research article *Open access*

Antibacterial Activity of Pyrogallol, a Polyphenol Compound against *Vibrio parahaemolyticus* Isolated from The Central Region of Thailand

Tran Huu Tinh, Taiyeebah Nuidate, Varaporn Vuddhakul, Channarong Rodkhum

Pages 162-168

Download PDF Article preview

Research article *Open access*

AntiHepatitis C Virus Activity of *Alectryon serratus* Leaves Extract

Lidya Tumewu, Evhy Apryani, Mei Ria Santi, Tutik Sri Wahyuni, ... Hak Hotta

Pages 169-173

Download PDF Article preview

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Toxicity Test n-Hexane: Ethyl Acetate (3:7) Fraction of Sudamala (*Artemisia vulgaris L.*)

Ira Arundina, S. Theresia Indah Budhy, Intan Nirwana, Retno Indrawati, Muhammad Luthfi

Pages 174-178

Download PDF Article preview

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Activities of *Ficus fistulosa* Leave Extract and Fractions against Hepatitis C Virus

Achmad Fuad Hafid, Adita Ayu Permanasari, Lidya Tumewu, Myrna Adianti, ... Hak Hotta

Pages 179-184

Download PDF Article preview

Research article *Open access*

Characterization of Tryptophanase from *Vibrio cholerae* O1

Taiyeebah Nuidate, Natta Tansila, Kanda Panthong, Varaporn Vuddhakul

Pages 185-189

Download PDF Article preview

Research article *Open access*

Curcuminoid Prevents Protein Oxidation but not Lipid Peroxidation in Exercise Induced Muscle Damage Mouse

Bambang Purwanto, Harjanto, I. Ketut Sudiana

Pages 190-193

Download PDF Article preview

Research article *Open access*

The Correlation between Pulmonary Function Tests and the Salivary MMP-9 Activity among Chronic Obstructive Pulmonary Disease (COPD) Patients

Mulyadi, Sunnati, Mulkan Azhary

Pages 194-198

Download PDF Article preview

Research article *Open access*

Multiple Intracranial Tuberculomas: Diagnosis Difficulties in a Clinical Case

Evita Mayasari, Sufida

Pages 199-204

Download PDF Article preview

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Study of Tree-sparrow (*Passer montanus*) as Natural Spreader of H5N1 Virus

Emmanuel Djoko Poetranto, Anna Lystia Poetranto, Aldise Mareta Nastri, Adhitya Yoppy Ro Candra, ... Kazufumi Shimizu

Pages 205-212

Download PDF Article preview

Research article *Open access*

The Effect of Spirulina as Feed Additive to Myocardial Necrosis and Leukocyte of Chicken with Avian Influenza (H5N1) Virus Infection

Widya Paramita Lokapirnasari, Andreas Berny Yulianto, Djoko Legowo, Agustono

Pages 213-217

Download PDF Article preview

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Potency of Attenuated *Eimeria tenella* in Protective Immunity Induction on Homologous and Heterologous Challenges

Muchammad Yunus, Endang Suprihati

Pages 218-224

Download PDF Article preview

Research article *Open access*

Sequence Analysis of the Gene Region Encoding ESAT-6, Ag85B, and Ag85 C Proteins from Clinical Isolates of *Mycobacterium tuberculosis*

Ni Made Mertaniasih, Didik Handijatno, Agnes Dwi Sis Perwitasari, Desak Nyoman Surya Suameitria Dewi, ... Ika Qurrotul Afifah

Pages 225-230

Download PDF Article preview

Research article *Open access*

Detection of *Mycobacterium leprae* in Formalin-Fixed Paraffin-Embedded Sample by Fite-Faraco Staining and Polymerase Chain Reaction

Willy Sandhika, Dinar Adriaty, Indropo Agusni

Pages 231-236

Download PDF Article preview

Research article *Open access*

Immunogenicity and Specificity of Anti recombinant Protein Fim-C-*Salmonella typhimurium* Antibody as a Model to Develop Typhoid Vaccine

Muktiningsih Nurjayadi, Dea Apriyani, Umar Hasan, Imam Santoso, ... Wibowo Mangunwardoyo
Pages 237-245

ISSN: 1876-6196

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20

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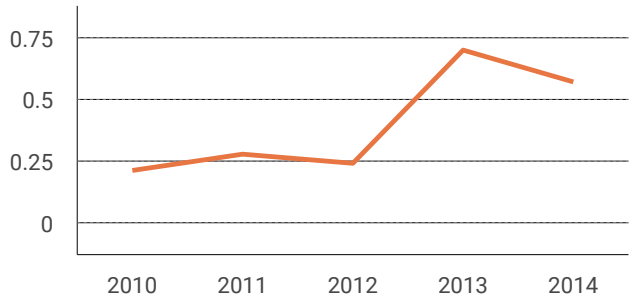

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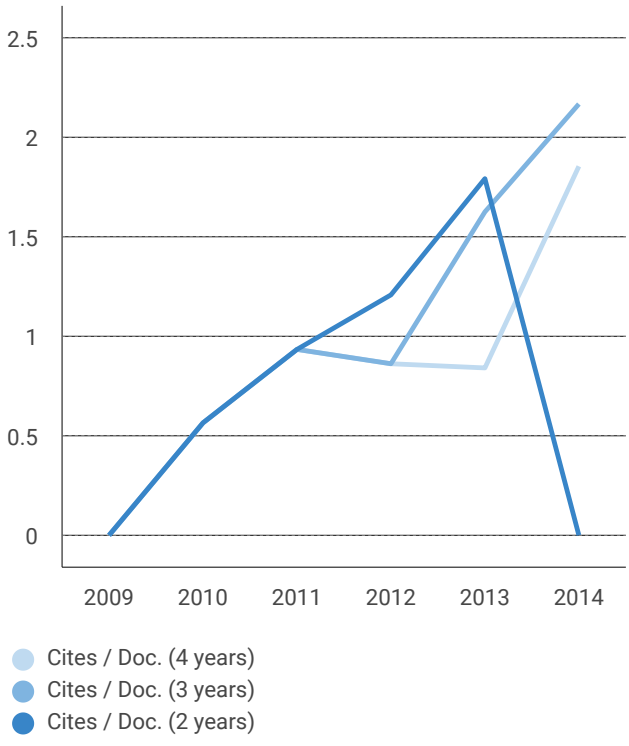

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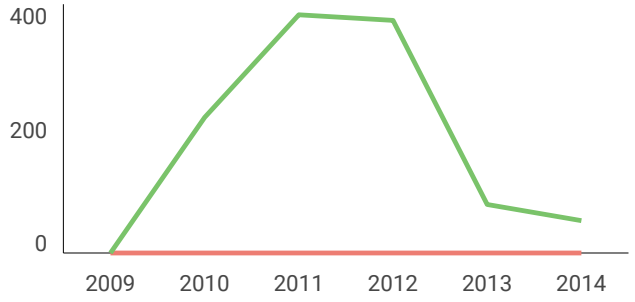

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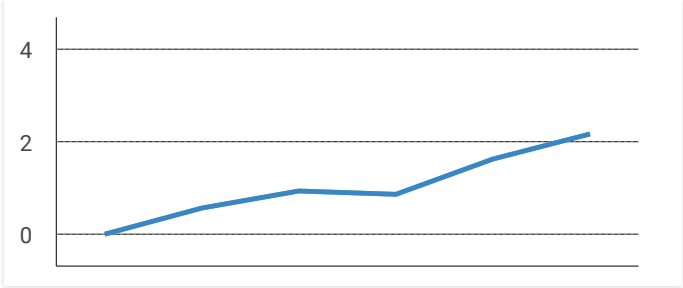

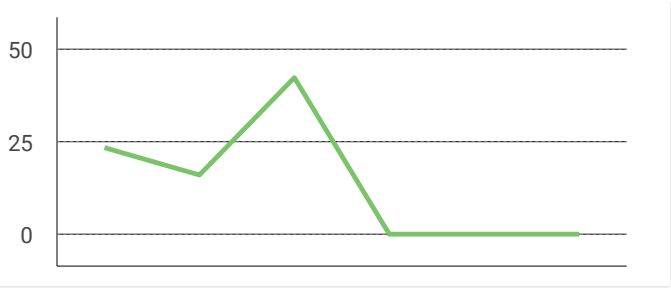

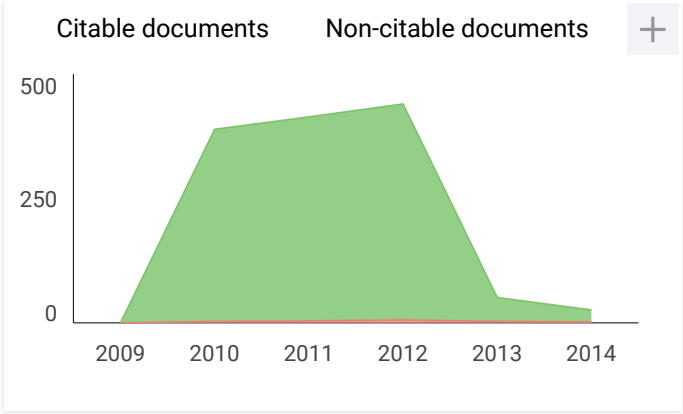

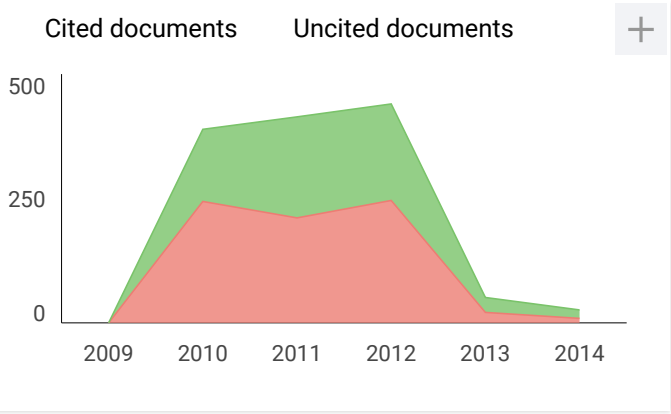

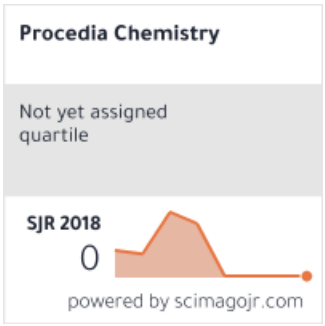

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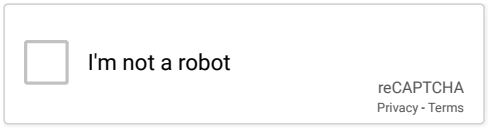

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Antimicrobial Activities and In silico Analysis of Methoxy Amino Chalcone Derivatives

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Abstract

A series of methoxy-4'-amino chalcone derivatives were tested for their antimicrobial activities against *Escherichia coli ATCC 25923*, *Staphylococcus aureus ATCC 25922* and *Candida albicans ATCC 10231*. Furthermore, their molecular interactions with dihydropteroate synthase (DHPS) of *E. coli* and *S. aureus* were studied with a docking experiment. Compound 4 ((*E*)-1-(4-aminophenyl)-3-(2,3-dimethoxyphenyl)prop-2-en-1-one) exhibited the strongest activity, in which its activity was equal to sulfamerazine and sulfadiazine used as positive controls. In addition, it showed a good potential to be used as a wide spectrum antimicrobial agent. The in silico experiment showed that the prepared compounds had higher affinity to DHPS of *S. aureus* than to DHPS of *E. coli*. The tested compounds showed high similarity interaction with hydroxymethylpterin pyrophosphate (natural substrate of DHPS) in building intermolecular interactions.

Peer-review under responsibility of the organizing committee of the Molecular and Cellular Life Sciences: Infectious Diseases, Biochemistry and Structural Biology 2015 (MCLS 2015)

Keywords: Methoxy amino chalcones; antimicrobial; dihydropteroate synthase

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1. Introduction

Besides their simple preparation procedures, the increasing interest in chalcones is due to their various pharmacological potentials, such as antitumor[1], anticancer[2,3], antimalarial[4], antihepatotoxic[5], topoisomerase I inhibitor[6], antinflammation[7], antioxidant[8] and antimicrobial activities[9].

Overuse and misuse of antimicrobial agents increases cases of antimicrobial drug resistance[10]. Sulfanilamide derivatives as the first generation of antibiotics and penicillin as one of the next generation (Patrick, 2001) have been replaced gradually by new generations of antibiotics[11]. However, the needs for more potential antibiotics are still to be explored due to the global health problems worldwide caused by antimicrobial resistance, especially in the developing countries. Suwito *et al.*[14] have designed and synthesized a series of methoxy-4'-amino chalcone derivatives as antimicrobial agents mimicking the structure of PABA (*p*-amino benzoic acid) in the 4-amino benzoyl moiety[12]. PABA is a substrate of dihydropteroate synthase (DHPS) in the biosynthesis of 7,8-dihydropteroate, which is very important in folic acid biosynthesis needed for cell proliferation[13]. The rationale of the design was that the synthesized compounds could act as a competitive inhibitor of PABA.

In this work we report the study of intermolecular interactions between the prepared compounds with DHPS to develop better understanding of the molecular interactions, so that in the future we can design compounds possessing better anti-microbial activities.

2. Methods

2.1. Synthesis of chalcone derivatives

The target molecules (compound 1-7) were synthesized using the Claisen-Schmidt reaction as reported by Suwito *et al.*[12] Structure characterization of the prepared compounds was based on spectroscopic evidence.

2.2. Antimicrobial activity assay

Seven prepared compounds were tested for their antimicrobial activities against *Escherichia coli* ATCC 25923, *Staphylococcus aureus* ATCC 25922 and *Candida albicans* ATCC 10231. The antimicrobial test was performed using the disc diffusion method and the diameter of the inhibition zone was observed. The data obtained were then analyzed statistically using the Kolmogorov-Smirnov test and the Kruskal-Wallis test. Sulfadiazine and sulfamerazine were used as positive controls.

2.3 Software and program

The ligand structures were drawn in ChemBioDraw Ultra 11.0. DS visualizer 2.5 (Accelerys, Inc., USA) and PyMOL (DeLano Scientific LLC, USA) were used to modify the ligand and to visualize the receptor structure and docking results. The preparation of the DHPS *pdbqt* file and determination of the grid box size and position were carried out using AutoDock Tools version 1.5.6. AutoDock 4 was the sole docking program employed in this work[15].

2.4 Preparation of the receptors and ligand structure

The three-dimensional structure of DHPS of *E. coli* complexed with sulfanilamide was retrieved from the Protein Data Bank [PDB:1AJ0], while the three-dimensional structure of DHPS of *S. aureus* complexed with 6-hydroxymethylpterin-diphosphate was downloaded from Protein Data Bank [PDB:1AD4]. The DHPS structure of *E. coli* was prepared for molecular docking by removing the sulphate ions and saved as a *pdb* file for the docking experiment. The DHPS three-dimensional structure of *S. aureus* was prepared for the docking experiment by removing K^+ and Mn^{2+} ions and then saved as *pdb* file for molecular docking. The ligand structures were drawn using ChemBioDraw Ultra 11.0, subsequently modified using DS visualizer 2.5 and saved as a *pdb* file.

2.5 Docking experiment

Molecular docking was carried out using AutoDock 4[15]. AutoDock Tools 1.5.6 was utilized to prepare the input *pdbqt* files of DHPS of *E. coli* and *S. aureus* and to set the size and center of the grid box. The *E. coli* DHPS binding site was set at 41.903 x 8.143 x 2.045 Å, while the *S. aureus* binding site was set at 33.106 x 8.125 x 41.335 Å in the dimensions of x, y, z using 1.000 Å spacing for *E. coli* and 3.75 Å spacing for *S. aureus*. The *pdbqt* input file required for AutoDock4 was prepared by AutoDock Tools 1.5.6. The predicted binding affinity (kcal/mol), which describes the binding strength between ligand and receptor, is calculated based on the scoring function employed in AutoDock4. A more negative binding affinity indicates stronger binding. The docking experiment was performed according to the procedures provided by the AutoDock4 protocol.

3. Results and discussion

3.1. Antimicrobial activity

The compounds used in this work were methoxy-4'-amino chalcone derivatives, the molecular structures of which are displayed in Table 1. Their synthesis and structure characterization have been reported by Suwito *et al.*[12]

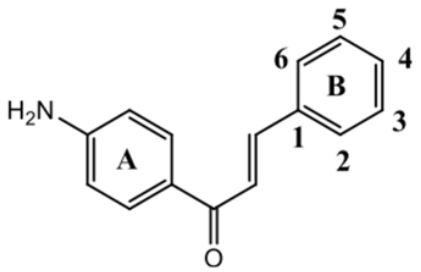

Table 1. Structure of the tested chalcones

Comp	Ring B					
	1	2	3	4	5	6
1	-	OMe	-	-	-	-
2	-	-	OMe	-	-	-
3	-	-	-	OMe	-	-
4	-	OMe	OMe	-	-	-
5	-	OMe	-	OMe	-	-
6	-	OMe	-	-	OMe	-
7	-	-	-	-	-	-

The data of antimicrobial tests of the entire compounds against *E. coli* ATCC 25923, *S. aureus* ATCC 25922, and *C. albicans* ATCC 10231 have been reported.[14] Dose dependence of the antimicrobial activity of the tested compound was observed. The higher the concentration of the tested compound, the larger the inhibition zone diameter.[14] The effect of the substituent of the prepared chalcone derivatives without considering the variation of concentration toward its activity was studied and is reported in this article. Based on the Kolmogorov-Smirnov test, it was shown that the diameters of the inhibition zones were not normally distributed ($\alpha = 0.05$). The test was then followed with the Kruskal-Wallis test. The results of the tests are presented in Fig. 1.

Based on the analysis using the Kruskal-Wallis test toward *E. coli*, sulfadiazine showed the strongest inhibition activity, followed by sulfamerazine, compound 7 and then compound 4. However, the inhibition activity of compound 7 did not differ significantly from sulfamerazine and compound 4. Statistical analysis of the antimicrobial test toward *S. aureus* showed that compound 4 exhibited the strongest activity. However its activity did not differ significantly from sulfamerazine. The inhibition activity of compounds 7, 1, 5, 3 and 2 also did not differ significantly from sulfadiazine. The inhibition activity analysis toward *C. albicans* showed that sulfadiazine possessed the strongest activity, although its activity did not differ significantly from compounds 5, 3 and 4. Structure-activity relationship analysis employing Kruskal-Wallis test gave us information that the amino group played an important role in the antimicrobial activity, while the methoxy group on ring B played a less significant role. Among the tested compounds, compound 4, (*E*)-1-(4-aminophenyl)-3-(2,3-dimethoxyphenyl)prop-2-en-1-one, exhibited a promising wide spectrum of antimicrobial activity as strong as the positive controls.

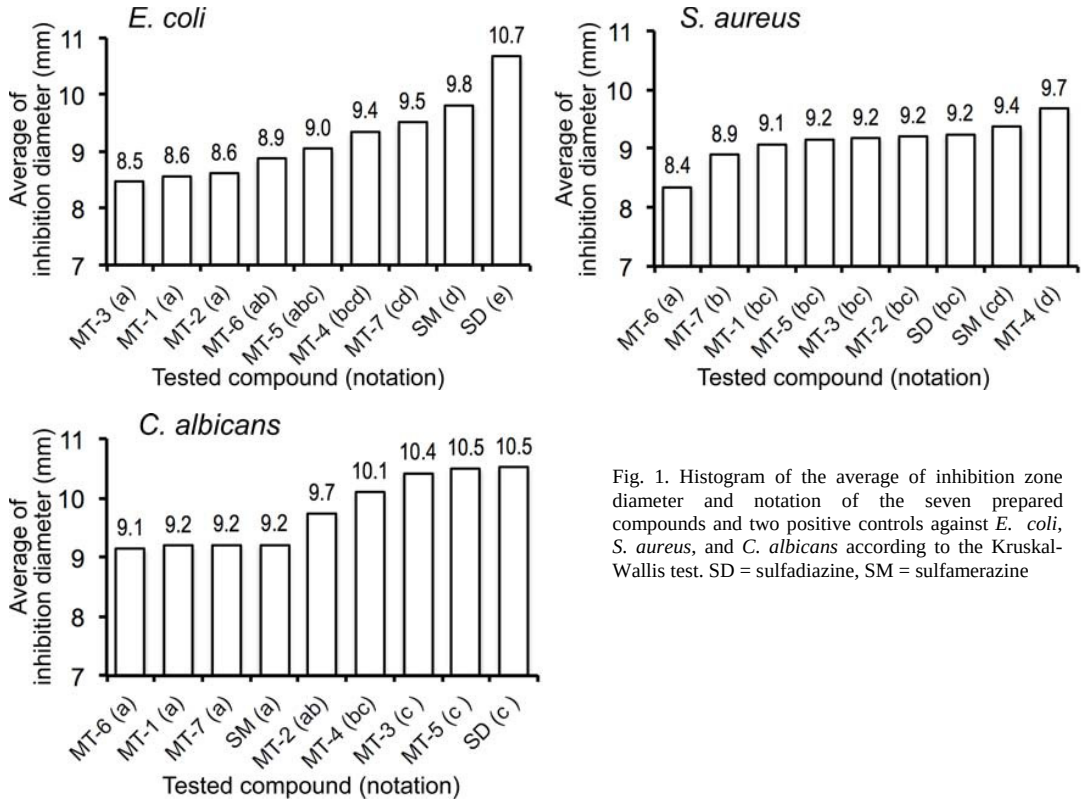

Fig. 1. Histogram of the average of inhibition zone diameter and notation of the seven prepared compounds and two positive controls against *E. coli*, *S. aureus*, and *C. albicans* according to the Kruskal-Wallis test. SD = sulfadiazine, SM = sulfamerazine

3.2. In silico analysis

This article discusses the molecular interaction between DHPS of *E. coli* and *S. aureus* with the title compounds, as determined by in silico analysis, while the interaction with DHPS of *C. albicans* could not be discussed because the three dimensional structure of DHPS of *C. albicans* was not available in the PDB.

3.3. Validation of the docking

In the molecular docking experiment, to ensure that ligand conformations bound correctly with the binding pocket of the target protein, the size and position of the grid box had to be validated. In this work, docking validation was carried out by redocking the co-crystallized DHPS-sulfanilamide complex (PDB:1AJ0)[16] for *E. coli*, and the co-crystallized DHPS-6-hydroxymethylpterin diphosphate complex for *S. aureus* (PDB:1AD4).[17] We found that the binding conformations of both redocked complexes reproduced the binding modes of the co-crystallized complexes with binding affinities –2.91 kcal/mol (RMSD = 1.58 Å) for *E. coli*, and –7.33 kcal/mol (RMSD = 0.83 Å) for *S. aureus*.

According to Achary *et al.*[16], the 7,8-dihydropterin pyrophosphate substrate bound in a deep cleft in the barrel, while sulphanilamide bound closer to the surface. Precisely, the sulphanilamide in the *E. coli* DHPS binding site was sandwiched between the main chain of Arg220 and the side chain of Lys221 on one side and the side chain of Arg63 on the other. The sulphonamide NH_2 formed a hydrogen bond to the carbonyl of Ser219, while sulphonamide oxygen accepted a hydrogen bond from the guanidinium of Arg63. Our redocking experiment provided the following observation: the sulphanilamide was flanked between the side chain of Lys221 and the main chain of Thr62. The hydrogen of aminophenyl moiety of sulphanilamide NH_2 donated a hydrogen bond with the carbonyl of Thr62, while the hydrogen of sulphonamide donated a hydrogen bond with the carbonyl of Ser219. Hydrophobic interactions were observed between sulphanilamide and Thr62, Phe190, Arg220, and Lys221.

According to Hampele *et al.*[17], the interactions of hydroxymethylpterin pyrophosphate with amino acid residues of the *S. aureus* binding site occurred through polar and hydrophobic interactions. Amino acid residues involved in the polar interactions with the pterin moiety of the ligand were Asp167, Asn103, Lys203 and Asp84. The primary amine on C-2 and the protonated nitrogen at N-3 donated hydrogen bonds to the carboxylate oxygen atom of Asp167. Again the 2-NH_2 group donated a hydrogen bond with the side-chain oxygen of Asn103, while the NH group of Asn103 built a hydrogen bond with N-1 of the pterin. Lys203 donated its hydrogen atom to form a hydrogen bond with the oxygen atom of the carbonyl group of the pterin and with the N-5. Asp84 donated a hydrogen bond to the N-8 of the pterin moiety. Our redocking experiment conferred the following observation: the interactions observed between *S. aureus* DHPS binding site with 6-hydroxymethylpterin diphosphate are explained as follows: 6-hydroxymethylpterin diphosphate built hydrophobic interactions with Ile9, Asn11, Arg52, Asp84, Asn103, Met128, Asp167, Phe172, Lys203, Arg239 and His241, while hydrogen bonds existed between the pyrophosphate moiety and four amino acid residues, which were Arg52, Lys203, Arg239, and His241; Arg52 with 4-OH; Asp167 with NH_2, Asn103 with 1-N-pterin; Arg52 with 4-N-pterin; and Asp84 with H of the N-8-pterin moiety.

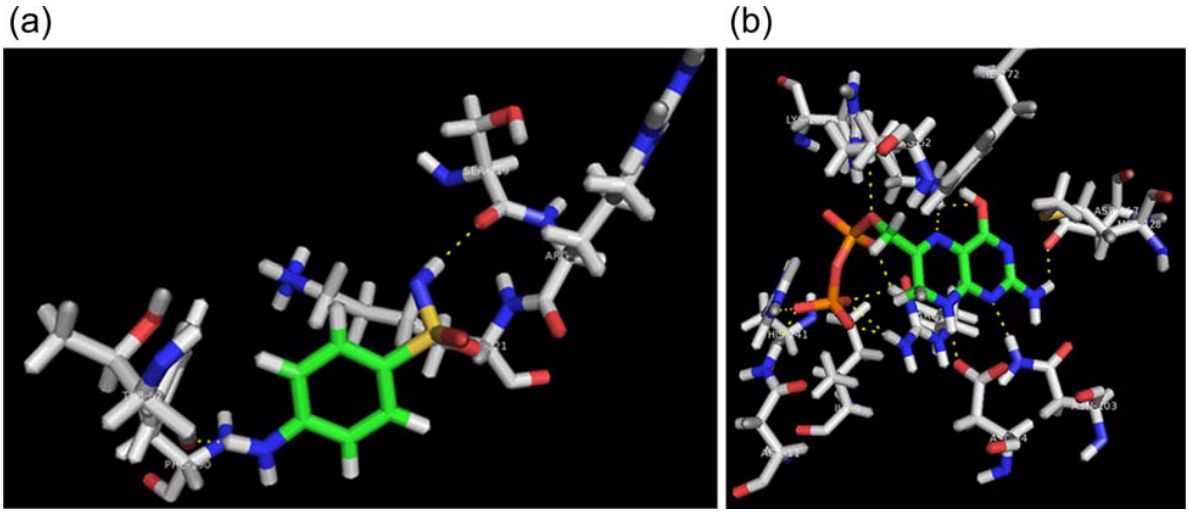

Fig. 2. Redocking of sulphanilamide into *E. coli* DHPS (a) and 6-hydroxymethylpterin diphosphate into *S. aureus* DHPS (b). The docking poses of the ligand are shown in green carbons. Residues with hydrophobic contacts with sulphanilamide and 6-hydroxymethylpterin diphosphate are labelled in grey while hydrogen bonds are shown in yellow with dashed lines.

3.4. Docking of the title compounds

In this work we performed molecular docking on 9 compounds, which were 7 derivatives of methoxy-4'-amino chalcone, sulfadiazine, and sulfamerazine. The results presenting the docking poses are displayed in Fig. 2, while the docking results and molecular interactions between tested compounds with amino acid residues of DHPS binding site are tabulated in the Table 2.

General observation of the docking results provided that the tested chalcone derivatives exhibited preponderant affinity with DHPS of *S. aureus* than with *E. coli* (based on the data of binding affinity). Three dimensional structural complementarities between the protein binding site and the ligands is also one of the important factors determining the binding affinity. Based on the sequence alignment of DHPS of *E. coli* and *S. aureus*, only 36% of homology was identified. This results provided that DHPS of both microbes were similar but not identical (Fig. 3), and the three dimensional structures of their binding sites differed consequently. In addition, all the tested compounds were bound in the deep cleft of the binding site, which was in accordance with the binding location of hydroxymethylpterin pyrophosphate. This information gave us an understanding that the prepared compounds, which were designed as competitive inhibitors of PABA, were more suitable to dock into a hydroxymethylpterin pyrophosphate binding location than into a sulphanilamide binding location.

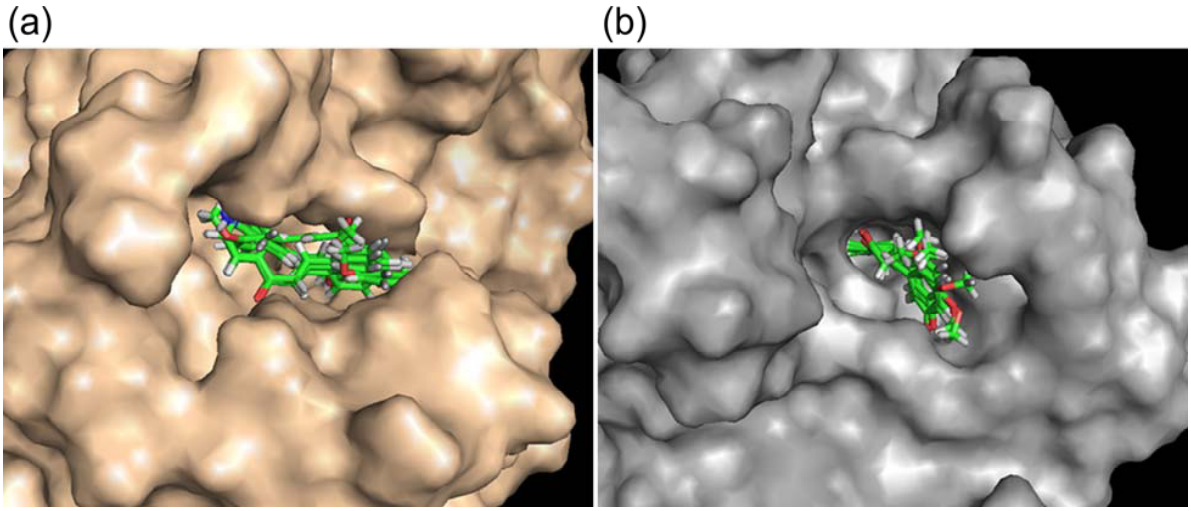

Fig. 3. Docking poses of the tested compounds in the DHPS binding site, (a) *E. coli*, (b) *S. aureus*.

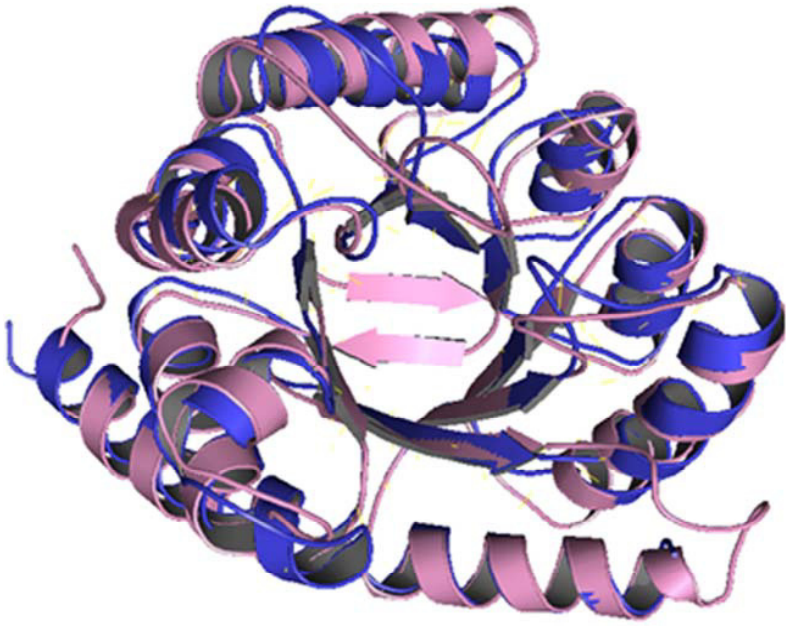

Fig. 4. Superimposed 3D-structure between DHPS of *E. coli* (pink) and *S. aureus* (blue).

In this article we discuss the docking results of compounds 4, 2 and 7. The reasons for the choice of these compounds were shown by the antimicrobial test, in which compound 4 (a dimethoxy-4'-amino chalcone derivative) displayed good potential to be used as a wide spectrum antimicrobial agent, compound 2 representing a monomethoxy-4'-amino chalcone derivative, and compound 7 representing 4'-amino chalcone derivative.

The factors considered for the scoring function in AutoDock4 were van der Waals interactions, electrostatic interactions, hydrogen bonds, desolvation, and rotations[16]. Besides hydrogen bonds, from the docking experiment we also obtained data about van der Waals and electrostatic interaction, which are not displayed in the Table 2.

Table 2. Docking results and molecular interactions between tested compounds with amino acid residues of DHPS.

Comp	DHPS *E. coli* ΔG (kcal/mol)	Hydrogen bond interactions	DHPS *S. aureus* ΔG (kcal/mol)	Hydrogen bond interactions
NS	-2.91	Thr62, Ser219	-7.33	Asn11, Asp84, Asn103, Lys203, Arg239, His241
1	-3.13	Pro145	-6.23	Asn103, Asp167, Lys203
2	-2.19	Thr62, Pro145	-6.48	Asn103, Asp167, Lys203
3	-1.05	Thr62, Pro145	-6.19	Asn103, Asp167, Lys203
4	-2.05	Thr62, Pro145	-6.10	Asn103, Asp167, Lys203
5	-1.09	Thr62, Pro145	-6.11	Asn103, Asp167, Lys203, Arg239
6	-0.66	---	-6.47	Asn103, Asp167, Lys203
7	-3.57	Pro145	-6.23	Asn103, Asp167, Lys203
SD	-3.25	Thr62, Arg63	-6.31	Val49, Asp84, Lys203
SM	-3.12	Thr62, Arg63	-6.08	Asn103, Asp167, Arg239

SD = sulfadiazine, SM = sulfamerazine, NS = natural substrate (as in PDB).

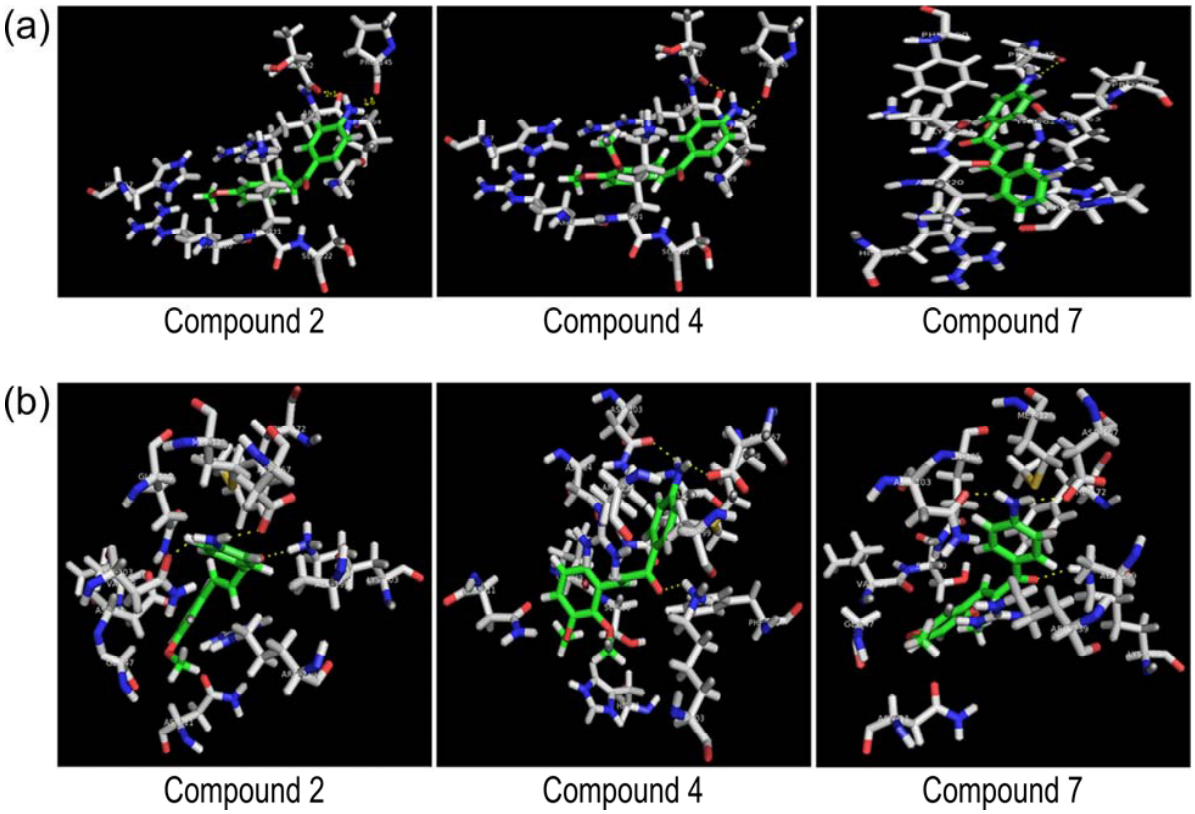

Fig. 5. Docking poses and intermolecular interactions of compound 2, 4 and 7 in DHPS binding sites. (a) *E. coli*, (b) *S. aureus*. The docking poses of the ligand are shown in green carbons. Residues with hydrophobic contacts with tested compounds are labelled in grey while hydrogen bonds are shown in grey with dashed lines.

3.4.1. Docking into E. coli DHPS (Figure 4a)

Compound 2: The location of ring A of compound 2 was flanked by the side chain of Gly189 and Arg63, whilst the ring B was located near the side chain of Lys221. The primary amino group of compound 2 donated hydrogen bonds to the carbonyl groups of Thr62 and Pro145. The position of the methoxy group was pointing to the

guanidinium moiety of Arg220. The amino acid residue Thr62, Arg63, Pro64, Pro145, Gly189, Arg220, Lys221, Ser222, and His257 contributed to hydrophobic interactions.

Compound 4: The position of ring A of compound 4 was flanked by the side chain of Arg63 and Pro64, whilst ring B was near the side chain of Lys221. The primary amino group of compound 4 made a bifurcated hydrogen bond with the carbonyl groups of Thr62 and Pro145. One methoxy group pointed to the guanidinium moiety of Arg63 and another pointed to the guanidinium moiety of Arg220. Amino acid residues Thr62, Arg63, Pro64, Pro145, Gly189, Arg220, Lys221, Ser222 and His257 contributed to hydrophobic interactions.

Compound 7: The side chain of Phe190 and Arg63 flanked the ring A of compound 7, while ring B was flanked by the side chains of Pro232 and Arg220. The primary amino group of compound 7 donated a hydrogen bond to the carbonyl group of Pro145. The amino acid residues involved in the formation of hydrophobic interactions were Thr62, Arg63, Pro64, Pro145, Phe190, Arg220, Lys221, Pro232 and His257.

3.4.2. Docking into S. aureus DHPS (Figure 4b)

Compound 2: The ring A of compound 2 was in a twisted conformation relative to ring B and was flanked by the side chains of three amino acid residues, Gln105, Asp167, and Ala199, whilst ring B was flanked by Asn103 and Arg239. The methoxy group pointed to the side chain of Asn11. The primary amino group of compound 2 made a bifurcated hydrogen bond with the carbonyl group of side chains of Asp167 and Asn103, while the carbonyl group of compound 2 acted as a hydrogen acceptor in the hydrogen bond with the amino group of the side chain of Lys203. Non-polar interactions were contributed by Asn11, Gly47, Val49, Asp84, Asn103, Gln105, Met128, Asp167, Phe172, Ala199, Lys203 and Arg239.

Compound 4: The ring A of compound 4 was flanked by the side chains of the amino acid residues Gln105, Arg239, and Ala199, whilst the ring B was flanked by Val49 and Ser40. The position of the two methoxy groups was pointing to Asn11 and His55. The amino group of compound 4 donated its two hydrogen atoms to the formation of hydrogen bonds with the side chain carbonyl groups of Asn103 and Asp167, while the carbonyl group of compound 4 acted as a hydrogen acceptor of the side chain amino group of Lys203 in hydrogen bond formation. The following amino acid residues Asn11, Val49, Ser50, His55, Asp84, Asn103, Gln105, Met128, Asp167, Phe172, Ala199, Lys203 and Arg239 contributed to hydrophobic interactions.

Compound 7: The ring A of compound 7 was located in the area flanked by Phe172, Asn103, and Ala199, while ring B was flanked by Val19 and Arg239. The amino group of compound **7** donated hydrogen bonds with the side chain carbonyl group of Asn103 and Asp167. The carbonyl group of compound **7** acted as a hydrogen acceptor of the side chain amino group of Lys203 in hydrogen bond formation. The hydrophobic interactions were contributed by the following amino acid residues: Asn11, Val49, Asp84, Asn103, Gln105, Met128, Asp167, Phe172, Ala199, Lys203 and Arg239.

The docking results showed that the amino acid residues involved actively in the molecular interaction from the docking experiment were in accordance with the results of protein-ligand complexes that were co-crystallized.

4. Conclusions

The antimicrobial activities of the methoxy amino chalcone derivatives have been assayed and their molecular interaction with the *E. coli* and *S. aureus* DHPS were studied with a docking experiment using the AutoDock4 program. Compound 4, (*E*)-1-(4-aminophenyl)-3-(2,3-dimethoxyphenyl)prop-2-en-1-one), exhibited a promising wide spectrum antimicrobial activity. The docking experiment gave us information that the prepared compounds were docked in the binding location of hydroxymethylpterin pyrophosphate in the DHPS binding site.

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