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Antibacterial activity and QSAR of homoisoflavanones isolated from six Hyacinthaceae species

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Abstract

Antibacterial activity, as detected in the bioautographic and microplate assays against *Staphylococcus aureus*, was shown for thirteen homoisoflavanones isolated from six Hyacinthaceae species. Four of these homoisoflavanones were isolated from *Eucomis comosa*, one from *Eucomis schijffii*, one from *Albuca fastigiata*, one from *Drimia delagoensis*, five from *Drimiopsis maculata* and one from *Drimiopsis burkei*. Extracts from most of these plants are employed as traditional medicines in South Africa. Biological screening was followed by a computer-based quantitative structure–activity relationship (QSAR) study. Stepwise multiple linear regression analysis of the data yielded a statistically significant two-component model ($R^2=0.81$, $p<0.003$), depicting log*P* and electron potential at O-1 as effective descriptors of antimicrobial activity against *S. aureus*. The derived model provides valuable parameter guidelines for those properties influencing the antibacterial activity of these homoisoflavanones.

Keywords: Antibacterial activity; Homoisoflavanones; Hyacinthaceae; Physicochemical descriptors; Quantitative structure–activity relationships

1. Introduction

The development of antimicrobial agents for clinical use has brought unquestionable benefit to individuals and society. Infectious diseases that were formerly often fatal became curable (Lerner, 1998). However, mankind is now confronted with new and re-emerging infections for which no effective treatments are available (Cragg et al., 1997). In contrast to other types of medication, antibiotics ultimately lose their effectiveness as they are used over time and resistant strains of bacteria develop (Lerner, 1998). One example is that of the Gram-positive, methicillin-resistant *Staphylococcus aureus*. Incidence figures in some hospitals have shown that more than 40% of *S. aureus* strains are now resistant to methicillin (Lesse, 1995). There is thus an urgent need to identify novel, active chemotypes as leads for drug development (Cragg et al., 1997). Natural products could play a crucial role in meeting this demand (Cragg and Newman, 2001). Of the drugs approved between 1983 and 1994 by either the United States Food and Drug Administration (FDA) or comparable entities in other countries, drugs of natural origin predominated (78%) in the area of antibacterials (Cragg et al., 1997).

The presence of antibacterial activity in plants is important from an ecological as well as pharmacological viewpoint (Cox and Balick, 1994). Hyacinthaceous plants have been employed in Xhosa and Zulu remedies for many years. The remedies are used for purposes ranging from the treatment of rheumatic fever and hangovers to syphilis (Pohl et al., 2000). Extracts from *Drimia delagoensis* are incorporated in a protective cream (Hutchings et al., 1996) and are also used to protect animal skins from dogs (Watt and Breyer-Brandwijk, 1962) whilst *Drimiopsis maculata* is frequently used in enema preparations for

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young children suffering stomach ailments (Hutchings et al., 1996). The bulbs of the much sought-after *Eucomis* species are used in Xhosa, Tswana, Zulu and Sotho medicine in decoctions prepared from shaved bulbs and roots boiled in water or milk. These decoctions are then used in infusions for the treatment of pain and fever (Watt and Breyer-Brandwijk, 1962).

A large number of homoisoflavanones have been isolated from several hyacinthaceous genera including *Eucomis* L'Hér., *Merwilla* Speta, *Ledebouria* Roth, *Veltheimia* Gled. and *Drimiopsis* Lindl. and Paxton (Pohl et al., 2000). Homoisoflavanones belong to a small homogeneous group of naturally occurring oxygen heterocycles which, within the Hyacinthaceae, are largely but not exclusively restricted to the subfamily Hyacinthoideae. The few reports on the biological activity of homoisoflavanones describe anti-inflammatory, antibacterial, antihistaminic, antimutagenic and angioprotective properties, and potent phosphodiesterase inhibition (Della Loggia et al., 1989; Amschler et al., 1996; Heller and Tamm, 1981).

The aim of this study was to determine the antibacterial activity of the isolated homoisoflavanones against *S. aureus* and also to develop a set of physicochemical parameters that would describe antibacterial activity for these and future compounds.

2. Experimental

2.1. Test compounds

The structures of the homoisoflavanones are given in Fig. 1. The plant species from which these compounds were isolated as well as the references of publications describing the isolation and confirming the identity of the compounds isolated are listed in Table 1. The structures and purity of all compounds isolated were confirmed using NMR, MS and IR techniques and the physical data was compared with that in the literature.

2.2. Antibacterial assays

The test compounds were screened against *S. aureus* (ATCC 12600) using the bioautographic and microplate assays (Eloff, 1998).

Test compounds were spotted on thin layer chromatography (TLC) plates (Merck, Kieselgel 60 F254). The plates were then developed in ethyl acetate/hexane (4:1) and left to dry overnight. An overnight culture of *S. aureus* was sprayed onto the TLC plate and incubated at 37 °C in an oven at 100% humidity

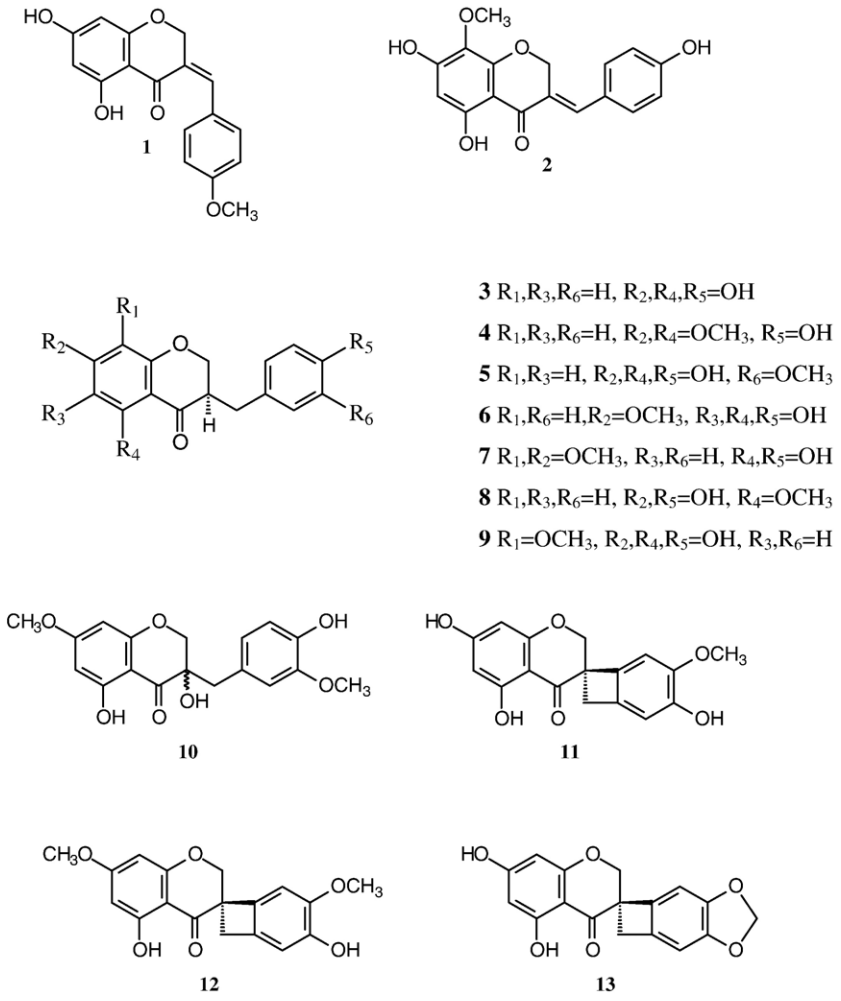


Fig. 1. Structures of compounds tested for antibacterial activity against *Staphylococcus aureus*.

Table 1
Comparison of minimum inhibitory concentration and bacteriostatic concentration (where applicable) values of homoisoflavanones tested against *S. aureus* with the microplate assay

Compound number	Reference	Plant source	MIC (mM)	BC (mM)
1	Koorbanally et al. (2006a)	*E.comosa* (Houtt.) Wehrh.	0.52	
2	Koorbanally et al. (2006a)	*E.comosa* (Houtt.) Wehrh.	0.24	
3	Koorbanally et al. (2006b)	*D.maculata* Lindl.	*	
4	Koorbanally et al. (2006b)	*D.burkei* Baker	*	
5	Koorbanally et al. (2005a)	*D.delagoensis* (Baker) Jessop	1.97	
6	Koorbanally et al. (2006b)	*D.maculata* Lindl.	3.95	
7	Koorbanally et al. (2006b)	*D.maculata* Lindl	0.47	
8	Koorbanally et al. (2006a)	*E.comosa* (Houtt.) Wehrh.	4.15	2.07
9	Koorbanally et al. (2006a)	*E.comosa* (Houtt.) Wehrh.	0.98	
10	Koorbanally et al. (2005b)	*A.fastigiata* Dryand.	*	
11	Koorbanally et al. (2001)	*D.maculata* Lindl.	3.95	1.97
12	Koorbanally et al. (2001)	*D.maculata* Lindl.	7.60	
13	Koorbanally et al. (2006a)	*E. schijffii* Reyneke	0.50	
Neomycin			0.0025	

*Compound showed antibacterial activity in the microplate assay but was not available for further testing.

for 18 h. Plates were sprayed with a 2 mg/ml solution of *p*-iodonitrotetrazolium violet (INT) (Sigma). Bacteria reduce the tetrazolium salt through dehydrogenase activity and produce an intensely coloured formazan (Eloff, 1998). Inhibition of growth is indicated by clear zones against a dark pink background.

Compounds that showed antibacterial activity with the bioautographic method, were further tested to establish their MIC values using the initial concentration of 10 mg/ml in ethanol or DMSO. Sterile water (100 µl) was added to each well of the microplates. For each dissolved compound a two-fold serial dilution was made down the microplate starting at a concentration of 5.0 mg/ml. An overnight bacterial culture was diluted (1:100) in Mueller–Hinton (MH) broth and 100 µl added to each well. This gives a further dilution to 2.5 mg/ml. The following day 40 µl of 0.2 mg/ml INT was added to each well and the plates incubated at 37 °C for 30 min. With INT the bacterial suspension turned red where bacterial growth was not inhibited. Where bacterial growth was inhibited, the suspension in the well remained clear. Bacteriostatic activity caused the wells to become a faint pinkish brown colour. Neomycin (50 µg/ml) was used as a positive control and solvent and bacteria free wells were used as negative controls. Experiments were repeated twice and the results of the antibacterial activities of the test compounds are summarized in Table 1.

2.3. Physicochemical characterization

Modeling and structural optimization were accomplished using PC Spartan Pro® 1.0 modeling software. MM^+ and AM1 minimization models were used for molecular and electronic calculations. Ground state energies were optimized using MMFF94 (Merck Molecular Force Field) calculations. Strain energy (SE) was determined from molecular mechanics calculations and heat of formation (EF) from semi-empirical calculations.

PC Spartan Pro® 1.0, was further used to calculate the following parameters from the energy minimized structures: volume of a space-filling model (SV), surface area (SA), aqueous phase energy (ES), dipole moment (DP), vibrational enthalpy (VEL), and vibrational entropy (TE) (Table 2). Descriptors measuring electron potential at atomic level were also calculated using Spartan Pro® and are summarized in Table 3. Advanced Chemistry Development's ACD program was used to calculate parameters for molar refractivity (MR), parachor (P), density (D), refractive index (RI), surface tension (ST), polarizability (POL) and log*P* (Table 4). The Insight II program was used to calculate parameters for Van der Waals interaction energy (VE), Coulombic interaction energy (CE) and total non-bonded interaction energy (NIE) (Table 5).

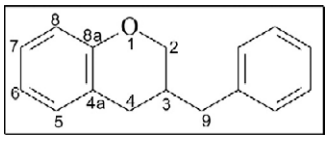
Microsoft Excell® spreadsheets were generated containing biological activity values as well as physicochemical property values for the different compounds. The sets of data were of

Table 2
Summary of computational descriptors employing the Spartan Pro® program

Comp	MIC (mM)	SV	SA	SE	EF	DP	ES	VEL	TE
1	0.52	321.46	329.05	42.031	−129.009	4.695	−13.354	191.155	61.656
2	0.24	331.42	337.53	49.029	−170.755	4.170	−14.321	194.730	66.123
5	1.975	338.39	346.04	30.690	−199.494	3.721	−15.554	209.547	69.950
6	3.95	338.16	344.93	42.450	−194.216	4.949	−14.896	209.379	71.084
7	0.47	359.62	367.50	57.020	−187.646	0.960	−12.612	228.349	76.782
8	4.15	329.21	337.70	35.520	−148.494	1.005	−16.351	205.540	65.131
9	0.98	338.17	344.93	39.233	−197.384	3.747	−14.052	209.447	68.748
11	3.95	327.12	332.57	52.600	−144.490	2.480	−17.000	194.641	62.952
12	7.60	347.79	352.83	64.400	−139.098	1.380	−13.520	213.689	68.908
13	0.50	316.71	321.41	44.580	−124.439	3.380	−16.820	180.704	54.808

SV = volume of a space-filling model ($Å^3$), SA = surface area of a space-filling model ($Å^2$), SE = strain energy (kcal/mol), EF = heat of formation (kcal/mol), DP = dipole moment (debyes), ES = aqueous phase energy (kcal/mol), VEL = vibrational enthalphy (kcal/mol), TE = vibrational enthropy (cal/mol.K).

Table 3
Summary of computational descriptors measuring electron potential at atomic level utilizing the Spartan Pro® program



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Comp	MIC (mM)	C8	C5	C8a	C7	C6	C4a	O1	C2	C3	C4
1	0.52	−0.590	0.699	0.582	0.598	−0.622	−0.775	−0.408	0.212	−0.354	0.798
2	0.24	−0.198	0.670	0.426	0.539	−0.610	−0.738	−0.303	0.111	−0.309	0.793
5	1.97	−0.578	0.707	0.565	0.599	−0.626	−0.794	−0.408	0.198	−0.287	0.773
6	3.95	−0.470	0.570	0.478	0.386	−0.140	−0.760	−0.401	0.175	−0.288	0.820
7	0.47	−0.228	0.678	0.540	0.484	−0.584	−0.829	−0.360	0.156	−0.275	0.817
8	4.15	−0.467	0.489	0.504	0.481	−0.480	−0.610	−0.403	0.180	−0.233	−0.155
9	0.98	−0.240	0.737	0.458	0.601	−0.676	−0.820	−0.306	0.103	−0.249	0.800
11	3.95	−0.573	0.718	0.574	0.597	−0.626	−0.835	−0.409	0.189	−0.155	0.820
12	7.60	−0.600	0.644	0.635	0.576	−0.560	−0.819	−0.412	0.230	−0.331	0.854
13	0.50	−0.565	0.725	0.570	0.600	−0.629	−0.844	−0.402	0.182	−0.171	0.824

**This structure does not represent any specific molecule. It is used to show which atom (if present) is represented in the table.

reasonable size and had minimum diversity of structures which increased the accuracy of the models. Individual correlation trends between biological activity and physicochemical descriptors of compounds were evaluated by means of linear regression analysis as well as polynomial least square fit procedures (the nature of the curve was assessed by the linear correlation coefficient R^2), using the Statistica® data analysis software system. Models produced in this study were validated by evaluating the residues for normality. The data was analyzed for indications of high colinearity between property parameters which would overly influence the calculation of the regression function. In these cases only one member of each cluster showing colinearity was used to increase independent variation in physical properties.

3. Results and discussion

3.1. Antibacterial assay

Compounds that showed antibacterial activity in the bioautographic method, were chosen, depending on availability, for determination of Minimum Inhibitory Concentration (MIC) and Bacteriostatic Concentration (BC) values.

Significant inhibitory activity against *S. aureus* was shown by compounds 1, 2, 7 and 13 with MIC values of ≤0.52 mM. The only compounds exhibiting bacteriostatic activity were 8 and 11. Although compounds 3, 4 and 10 also exhibited antibacterial activity in the bioautographic assay, quantities available were not sufficient for further testing.

Although little is known about the antimicrobial activity of the specific *Eucomis* species from which the test compounds were isolated, the ethnomedicinal usage of all *Eucomis* species in terms of antimicrobial activity was broadly considered. *E. autumnalis* (Mill.) Chitt. (Hutchings et al., 1996) and *E. regia* (L.) L'Hér. (Watt and Breyer-Brandwijk, 1962) are used for coughs and respiratory problems. Leaves of *E. autumnalis* (syn. *E. undalata* Aiton) are sometimes used as a poultice on suppurating sores and boils and the juice of the stems are used to soothe sores and rashes (Roberts, 1990). Secondary infections of *S. aureus* are associated with pneumonia, bronchitis and suppurative infections like boils (Venter, 1997). The homoisoflavanones present in *Eucomis* species (Pohl et al., 2000) could

Table 4
Summary of computational descriptors employing the ACD® program

Comp	MIC (mM)	MR	P	RI	ST	D	POL	Log*P*
1	0.52	81.24	604.8	1.69	65.7	1.40	32.20	4.01
2	0.24	83.12	620.1	1.71	74.7	1.49	32.95	3.85
5	1.97	81.65	634.1	1.66	67.3	1.43	32.36	2.75
6	3.95	81.65	634.1	1.66	67.3	1.42	32.36	2.79
7	0.47	86.44	677.5	1.62	56.6	1.33	34.27	3.12
8	4.15	79.77	618.9	1.63	59.3	1.34	31.62	2.23
9	0.98	81.65	634.1	1.65	67.3	1.42	32.36	2.43
11	3.95	78.70	601.2	1.74	92.4	1.62	31.20	2.64
12	7.60	83.53	644.6	1.68	75.2	1.50	33.11	2.82
13	0.50	76.86	575.0	1.77	95.7	1.69	30.47	3.54

MR=molar refractivity (Å^3), P=parachor (Å^3), RI=refractive index, ST=surface tension (dyn/cm), D=density (g/cm^3), POL=polarizability (Å^3), Log*P*=1-octanol-water partition coefficient.

Table 5
Summary of computational descriptors employing the Insight II® program

Compound no	MIC (mM)	VE	CE	NIE
1	0.52	72.40	−4.31	68.09
2	0.24	71.10	−2.23	68.87
5	1.97	73.91	−14.29	59.61
6	3.95	74.21	−0.54	73.66
7	0.47	74.51	−5.41	69.09
8	4.15	71.32	−14.29	57.03
9	0.98	72.34	−9.41	62.93
11	3.95	73.04	−5.47	67.58
12	7.60	75.64	0.64	76.28
13	0.50	70.64	−3.09	67.55

VE=Van der Waals interaction energy (kcal/mol), CE=coulombic interaction energy (kcal/mol), NIE=non-bonded interaction energy (kcal/mol).

thus account for the reported effectiveness of some of these species against respiratory problems, as well as sores and boils.

Compound 7 isolated from *D. maculata* showed antimicrobial activity with a MIC value of 0.47 mM. Compounds 6, 11 and 12 from *D. maculata* also exhibited antimicrobial activity and the activity of the compounds isolated from this plant validates its popularity amongst traditional healers. *D. maculata*, considered non-toxic (Hutchings et al., 1996), is rated amongst the twenty-three most popular plants grown by Zulu traditional healers at their homes (Mander, 1997).

3.2. QSAR models for compounds with antibacterial activity

Simple linear regression analysis revealed a statistically significant correlation between antimicrobial activity and log*P* ($R^2=0.75$, $p<0.050$). Stepwise multiple linear regression analysis of all data (Tables 2–5), yielded a five-component model ($R^2=0.95$, Adj.$R^2=0.89$, $p<0.009$, $n=10$, Eq. (1)), depicting log*P*, Coulombic interaction energy (CE) and electron potential at C-4a, C-2 and O-1 as effective descriptors of antimicrobial activity (MIC).

$$-\log\mathrm{MIC} = -2.45-4:90(\mathrm{C}-2)+0:99(\log P)-0:07(\mathrm{CE}) \\ -6.36(\mathrm{C}-4\mathrm{a})+7.44(\mathrm{O}-1) \quad (1)$$

The ratio between the parameters in the above equation, and the number of test compounds may however result in a overfitting of data and a three-component model ($R^2=0.89$, $p<0.002$) was obtained by omitting the electron potential of C-2 and C-4a. Removing Coulombic interaction energy (CE) as well gave a two-component model ($R^2=0.81$, Adj.$R^2=0.75$, $p<0.003$, Eq. (2)).

$$-\log\mathrm{MIC} = 0.39+0.59(\log P)+6.05(\mathrm{O}-1) \quad (2)$$

Predicted MIC values derived from this equation were correlated against experimental MIC values and it was found that Eq. (2) has a significant descriptive value (see Fig. 2).

From the equations above, the influence of log*P* is clear. The significant role that log*P* plays in the antimicrobial activity of homoisoflavanones suggests that the compounds have to cross a biological membrane in order to interact with the intra-bacterial active site to exhibit biological activity.

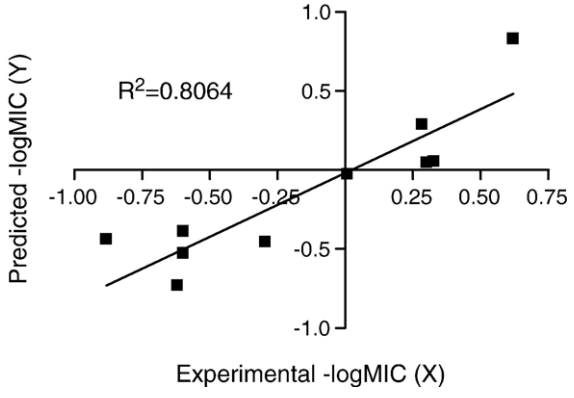

Fig. 2. Correlation between the predicted MIC values (from Eq. (2)) and that obtained experimentally.

Although lipophilicity is very important for the partitioning of compounds the hydrogen bond acceptor/donor properties of compounds also influence the diffusion through membranes. Roberts et al. (1996) found that the introduction of one hydrogen-bonding group caused a dramatic reduction in diffusion, second and third groups caused further reduction (although non-linear), and additional groups had no effect. Thus above a saturable number of H-bonding groups diffusion of compounds seems to be constant (Roberts et al., 1996). Since the homoisoflavanones and related compounds studied are highly oxygenated with at least two hydroxyl groups attached, most of them probably reached the saturation point in terms of H-bonding.

The oxygen of the carbonyl group at position 4 forms an intramolecular hydrogen bond with the proton of the hydroxyl group at position 5 in most of the homoisoflavanones studied. This intramolecular hydrogen bond decreases the possibility of intermolecular hydrogen bonding with water and should decrease water solubility (Malan et al., 2002). Inductive effects due to electron-withdrawing groups (the oxygen atoms in the case of homoisoflavanones) will also increase the log*P* value when hydrogen-bonding groups are involved (Nogrady, 1988).

Resonance effects are also important as delocalization of non-bonded electrons into aromatic systems will decrease their availability for hydrogen bonding with water and increase the log*P* value (Nogrady, 1988). The extra 3,9-double bond in the 3-benzylidene-4-chromanone-type homoisoflavanones, compounds 1 (log*P*=4.01; MIC=0.52 mM) and 2 (log*P*=3.85; MIC=0.24 mM), will increase the number of resonance structures (Loudon, 1988) for delocalization of non-bonded electrons, decreasing the availability of these electrons for hydrogen bonding and increasing the log*P* value.

The inclusion of parameters like Coulombic interaction energy (CE) that takes into account the interaction of charges and electron potential at different carbon atoms (C-4a, C-2 and O-1) describe electronic characteristics and give a strong indication of the importance of these descriptors for antibacterial activity. Though they may have an effect on the partitioning of the compounds, a role in the actual activity (binding at the active site) of the compounds cannot be ruled out.

The regression models for compounds with antimicrobial activity indicated that the active compounds must be able to penetrate cell membranes as log*P* played an important role in the activity of these compounds. Lipophilic groups like halogens substituted at non-sensitive positions will increase the lipophilicity of these compounds (Wermuth, 1996). Fluorine has the advantage of its small size and will pose less steric effects (Wermuth, 1996). Thus, homoisoflavanones and related compounds can be synthesized with chlorine or fluorine atoms in non-sensitive positions to increase the log*P* value. Another way of increasing the log*P* value would be to replace methoxy groups with ethoxy groups (Wermuth, 1996). Electrostatic interaction energy impacts on the binding abilities of these compounds to the active site and care must be taken not to

replace important substituents that influence the electron potential as expressed in the equations.

4. Conclusion

A statistically significant two-component model ($R^2 = 0.81$, $p < 0.003$), depicting log*P* and electron potential at O-1 as effective descriptors of the activity of the studied homoisoflavanones against *S. aureus*, was obtained during this study. It must be emphasized that the different parameters are influenced by different factors and cannot be taken account of as single parameters as this will be an oversimplification of the actual state. The prediction capabilities of the model is also limited to the compounds and physicochemical parameters studied. However, the derived model provides valuable parameter guidelines in terms of the properties influencing the activity of the studied homoisoflavanones against *S. aureus*. Information concerning the activity of these compounds can thus be predicted with confidence after identification of structures isolated/synthesized.

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