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Review

REVIEW

A survey on the characterization and biological activity of isatin derivatives

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Abstract: The derivatives of isatin have already been known to display a variety of biological activities. Therefore, the studies on their activity and its relation to structure have recently become a popular subject for investigation. The examined compounds were synthesized by the reaction of isatin and substituted primary amines and characterized by spectroscopic methods. The investigation of the antimicrobial and antioxidative activity of the synthesized compounds was performed by broth microdilution method. As for the characterization of the investigated isatin based Schiff bases, the linear solvation energy relationships (LSER) were used to analyze the solvent influence on the UV absorption maxima shifts (ν_{max}), using the well known Kamlet–Taft model and taking geometrical isomers into consideration when possible. Linear free energy relationships (LFER) were used to analyze substituent effect on pK_a, as well as NMR chemical shifts and ν_{max} values. The antimicrobial activity and characterization were related using both experimental and theoretical methods.

Keywords: antimicrobial activity; *E*/*Z* isomers; solvent effects; substituent effects; TD-DFT; 3D QSAR.

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1. INTRODUCTION

The derivatives of isatin (indole-2,3-dione), its Schiff and Mannich bases, have been reported to show a variety of biological activities, such as antibacterial,[1] antifungal[2] and anti-HIV[3,4] activities. The wide spectrum of isatin derivatives and their various chemical properties have led to their expanded use as precursors for the preparation of many biologically active compounds.[5–11] Isatin and its metabolites are components of many natural substances and display a wide range of the already mentioned activities such as antiviral, anticancer, antibacterial, antituberculosis, antifungal and anticonvulsants.[5–11] Our recently published study on the antimicrobial activity, as well as on the antioxidative activity of isatin derivatives showed their moderate to significant antimicrobial activity.[12]

To the best of our knowledge not much has been reported on the solvatochromic effects of isatin derivatives, therefore the studies were performed by our team recently.[13,14] It is already known that the absorption spectra in different solvents are often used for investigation of the solvatochromic effect of organic molecules. When absorption spectra are measured in solvents of different polarity, it is usually found that the positions, intensities, and shape of absorption bands are influenced by the solvent. Mostly used spectra which can provide information about solute-solvent are: UV–Vis, IR, ^{1}H- and ^{13}C-NMR spectra.[15] In here presented studies,[13,14] UV–Vis and NMR data were analysed by the use of LSER and LFER models, in order to evaluate the influence of the solvent/ /solute interactions and substituent effects, respectively. Quantification of the solvent effects: dipolarity/polarizability and the hydrogen-bonding ability on the UV spectral shifts (ν_{max}), were interpreted by means of the Kamlet–Taft (LSER)[16] Equation:

$$\nu_{max} = \nu_0 + s\pi^* + a\alpha + b\beta \quad (1)$$

Later on, LFER analysis was applied to the UV and NMR data in of studied compounds. The transmission of substituent effects from the substituent R (see Fig. 1) to the carbon atoms of interest were studied using equation:[17–19]

$$s = \rho\sigma + h \quad (2)$$

Again, the transmission of substituent effects, *i.e.*, LFER study, was discussed in relation to the geometry of the molecules obtained optimized by density functional theory (DFT) calculations.[13]

Furthermore, it is known that Schiff bases show *E/Z* isomerisation caused by the presence of imino group, which was analysed in the next part of our investigation.[14] For example, β-phenylethylamines in the reaction with isatin form the mixtures of the two stereoisomers *E* and *Z*, which can be thermodynamically or kinetically controlled, but their ratios may vary, depending on compounds structure and conditions.[20–24] It was of interest to find out which of all the mentioned

characteristics of the isatin derivatives have the impact on their biological activity.

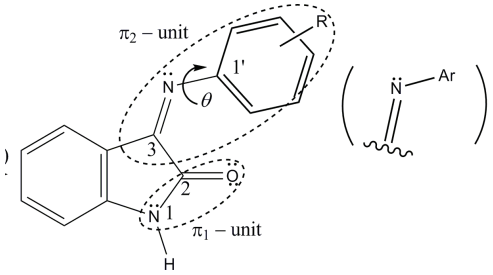
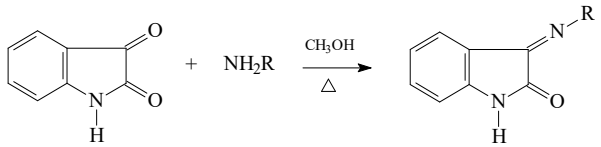


Fig. 1. The chemical structure of isatin derivatives with the labelling of π-electronic units.[13]

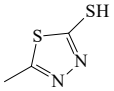
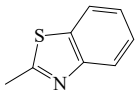
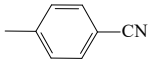
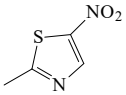
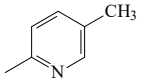
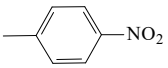
2. ANTIMICROBIAL AND ANTIOXIDATIVE ACTIVITY OF ISATIN SCHIFF BASES

The examined Schiff bases were synthesized by the reaction of isatin and a corresponding primary amine,[12] Scheme 1, and the general formula is given in Fig. 1.[13] The list of synthesized compounds is given in Table I.



Scheme 1. Isatin derivatives synthesis.

TABLE I. The synthesized derivatives of isatin[12]

No.	R	Compound name
2.1		1,3-Dihydro-3-[(2,4,5-thiadiazolone-2(*3H*)-thione)imino]-*2H*-indol-2-on
2.2		1,3-Dihydro-3-[(2-benzothiadiazole) imino]-*2H*-indol-2-on
2.3		1,3-Dihydro-3-[(4-cyanophenyl)imino]-2*H*-indol-2-one
2.4		1,3-Dihydro-3-[(5-nitro-2-thiazolyl)imino]-2*H*-indol-2-one
2.5		1,3-Dihydro-3-[(4-methyl-2-pyridyl)-2-imino]-*2H*-indol-2-on
2.6		1,3-Dihydro-3-[(4-nitrophenyl)imino]-2*H*-indol-2-one

The compounds **2.1** and **2.5** were not synthesized before.[2] Compound **2.2** has already been used for metal complexes synthesis,[25] compound **2.3*** is commercially available, compounds **2.4**[26] and **2.6**[27] have also already been used for research of their antimicrobial activity. The structure of all synthesized compounds (**2.1**–**2.6**) from Table I was confirmed by melting points, FTIR, ^{1}H- and ^{13}C-NMR spectra and also by elemental analysis for new compounds (**2.1** and **2.5**).[12]

The antimicrobial activity of all synthesized compounds **2.1**–**2.6** was determined on a wide range of different microorganisms (Table II) by the broth microdilution method.[28]

TABLE II. The examined bacteria and fungi types[12]

No.	Microorganism	ATCC No.
1	*Staphylococcus aureus*	6538
2	*Lysteria monocytogenes*	19115
3	*Enterococcus faecalis*	29212
4	*Shigella sonnei*	29930
5	*Salmonella enteritidis*	13076
6	*Yersinia enterolitica*	27729
7	*Escherichia coli*	35150
8	*Proteus hauseri*	13315
9	*Pseudomonas aeruginosa*	27853
10	*Candida albicans*	10259

All examined compounds have shown considerable activity against all tested microorganisms except for compound **2.3**, which has shown rather weak activity against *E. coli*, *P. aeruginosa* and *C. albicans*, in the range of investigated concentrations. Generally, the examined activity can be described as moderate with some selectivity against Gram-positive (G+) or Gram-negative (G–) strains of bacteria, or yeast *C. albicans.* The selectivity to G– bacteria is of importance, as it enables the antibiotic agent based on a G– selective compound to be taken without the support of an agent that recovers the gastrointestinal tract, because it contains G+ natural bacteria. The activity of certain examined isatin derivatives against fungi is also important for they can be applied as antifungal agents. The overall results of the antimicrobial screening are given in Table III. Some of the isatin derivatives synthesized in this research have displayed significant activity against various examined bacteria and fungi, while the others were only moderately or even weakly active.

Compound **2.1** has shown the most prominent overall activity on both G+ (*S. Aureus* and *L. monocytogenes*) and G– bacterial strains, (*Y. enterolitica* and *P. hauseri*). The highest activity of this compound was noticed against the *S. Aureus*

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(*MIC* value of 0.16 mM) and close to it against *L. monocytogenes* (*MIC* value of 0.32 mM). However, compound **2.1** has displayed only moderate activity on *Y. enterolitica*, unlike compound **2.5**, which was significantly active against the same strain (*MIC* value of 0.33 mM). Except the above mentioned, the overall antimicrobial activity of compound **2.5** is not as strong as that of compound **2.1**. Besides *Y. enterolitica*, it has also shown some moderate activity on *S. sonei* and somewhat slighter on *P. hauseri*. Compound **2.6** behaved somewhat similarly to compound **2.5** but without any really prominent antimicrobial activity. It has displayed only moderate activity to *Y. enterolitica* and *S. sonei*. Compounds **2.2** and **2.3** have generally shown comparably weak antimicrobial activity. The only observation that can be of interest is the relative selectivity of compound **2.3** to *L. monocytogenes* (*MIC* value of 0.63 mM). Compound **2.4** can be noticed for certain moderate activity against *S. aureus, E. Faecalis* and also *S. sonei*.[12]

TABLE III. Antimicrobial activity of examined compounds (*MIC* / mM)[12]

Microorganism	Compound					
	2.1	**2.2**	**2.3**	**2.4**	**2.5**	**2.6**
1. *S. aureus*	0.16	2.24	2.53	0.57	5.27	4.68
2. *L. monocytogenes*	0.32	1.12	0.63	2.28	2.64	1.17
3. *E. faecalis*	2.56	2.24	2.53	0.57	2.64	4.68
4. *S. sonnei*	1.28	1.12	5.06	0.57	0.66	0.59
5. *S. enteritidis*	2.56	1.12	>5.06	1.14	5.27	4.68
6. *Y. enterolytica*	0.64	1.12	2.53	1.14	0.33	0.59
7. *E. coli*	2.56	1.12	>5.06	1.14	2.64	4.68
8. *P.Hauseri*	0.64	1.12	5.06	1.14	0.99	1.17
9. *P.Aeruginosa*	5.12	2.24	>5.06	2.28	2.64	2.34
10. *C. Albicans*	2.56	2.24	>5.06	2.28	2.64	1.17

Furhermore, all the synthesized compounds were screened for antioxidative activity by the diphenylpycrilhydrazyl radical (DPPH).[29] The results of DPPH analysis have shown that the most prominent antioxidative activity displays compound **2.1**, while the other investigated specimens, including pure isatin, have shown very slight if any activity.

With the increase of the concentration of compound **2.1**, the absorbance of DPPH was decreased, displaying linear dependence of $DPPH_{red}$ in the range of examined concentrations (c / mM), which are described by the following equation:[12]

$$DPPH_{red} / \% = 5.099 + (101.02 \pm 5.24)c \quad (3)$$
$$r = 0.995,\ s = 3.17,\ n = 6$$

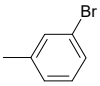
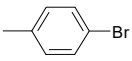
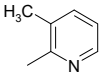
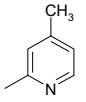
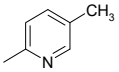
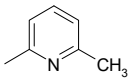
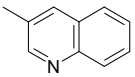
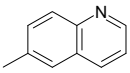
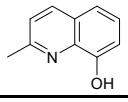
$DPPH_{red}$ is actually the percent of DPPH reduction and c is the concentration of compound **2.1**, given in mM. This equation enables the precise determination of the concentration which reduces 50 % of DPPH concentration

(DC_{50}). Compound **2.1** showed prominent antioxidative activity, with DC_{50} value of 0.444 mM.[12]

3. SOLVATOCHROMISM OF ISATIN BASED SCHIFF BASES: LSER AND LFER STUDY

Another series of eleven isatin derivatives (1,3-dihydro-3-arylimino-2*H*--indol-2-one), including the compound **2.5** from the previous one (compound **3.7** in this series) was synthesized and the list of compounds is given in Table IV.[13] The characterisation of the synthesized isatin derivatives is described in the original paper.[13]

TABLE IV. The list of synthesized isatin derivatives[13]

Compd.	Compound	Substituent R
3.1	1,3-Dihydro-3-(phenylimino)-2*H*-indol-2-one	
3.2	1,3-Dihydro-3-[(2-bromophenyl)imino]-2*H*-indol-2-one	
3.3	1,3-Dihydro-3-[(3-bromophenyl)imino]-2*H*-indol-2-one	
3.4	1,3-Dihydro-3-[(4-bromophenyl)imino]-2*H*-indol-2-one	
3.5	1,3-Dihydro-3-[(3-methyl-2-pyridinyl)imino]-2*H*-indol-2-one	
3.6	1,3-Dihydro-3-[(4-methyl-2-pyridinyl)imino]-2*H*-indol-2-one	
3.7	1,3-Dihydro-3-[(5-methyl-2-pyridinyl)imino]-2*H*-indol-2-one	
3.8	1,3-Dihydro-3-[(6-methyl-2-pyridinyl)imino]-2*H*-indol-2-one	
3.9	1,3-Dihydro-3-(3-quinolinylimino)-2*H*-indol-2-one	
3.10	1,3-Dihydro-3-(6-quinolinylimino)-2*H*-indol-2-one	
3.11	1,3-Dihydro-3[2-(8-hydroxy)quinolinyl imino]-2*H*-indol-2-one	

The UV absorption spectra were recorded in the range from 200 to 600 nm in 22 solvents of different polarity, in order to study substituent effect on the

solvatochromism of the investigated compounds. The absorption spectra of the isatin derivatives **3.1**–**3.11**, indicate the presence of two bands, corresponding to different electronic transition of the investigated compounds. The examples of the UV–Vis absorption spectra of compounds **3.1**–**3.11** in acetone, acetonitrile (AcN), benzyl alcohol (BzOH) and *N*,*N*-dimethylformamide (DMF) are given in Fig. S-1 of the Supplementary material to this survey,[13] and the complete results are given in the original paper.[13] The shifts of ν_{max} in UV absorption spectra showed relatively weak dependence on the both solvent and substituent effects compared to those of the parent compound **3.1**. The data[13] indicate that the values of absorption frequencies of the investigated compounds depend on the electronic effect of the substituent present on C3 position of indole-2-one core. The introduction of Br as a substituent in C4 position of the phenyl ring (compound **3.4**) and all quinoline derivatives (compounds **3.9**–**3.11**) bring about the positive solvatochromism, comparing to the unsubstituted compound. The other compounds showed ν_{max} shifting to higher wavelength in 2-chloroethanol, 2-methoxyethanol, DMF, DMAc and AcN, while in other solvents no rule could be observed (irregular behaviour regarding both substituent and solvent). The highest batochromic shifts was found for compoubd **3.9** in almost all solvents used.

The Kamlet–Taft solvent parameters are taken from literature[30] as well as substituent constants used in LSER and LFER correlations.[19] The presented data confirm that the positions of the UV–Vis absorption frequencies depend on the nature of the solvent used and the substituent present at the aryl or phenyl ring at the imino group of the examined isatin derivatives.[13] The results are presented in the original paper.[13]

According to the correlation results, obtained by the use of Kamlet–Taft equation,[13] alternation of solvatochromic coefficients with respect to solvent/substituent effects exists. The positive sign of *s* and *a* coefficients for isatin derivatives (compounds **3.1**, **3.3**–**3.5** and **3.9**–**3.11**) indicates a hypsochromic shifts with increasing solvent dipolarity/polarizability and hydrogen-bond donor capability. Largest hypsochromic shift of the absorption maxima, regarding coefficient *s* and *a*, were found for the compounds **3.5** and **3.9**, respectively. The positive values of *s* and *a* coefficients suggest better stabilization of the ground state relative to the electronic excited state with the increasing solvent polarity, *i.e.*, higher dipolar properties of the molecule in the ground state, with more pronounced HBA (hydrogen bond acceptor) properties of solvated molecules.[13] The percentage contribution of solvatochromic parameters for all isatin derivatives, showed the highest P_π value of compound **3.5** (71.01 %), with lowest P_α value (2.28 %). Oppositely could be noticed for compound **3.1** with highest value for P_β (70.72 %) and lowest P_π value (8.88 %). Highest P_α value was obtained for comp. **3.4** (51.28 %).[13] From the correlation analysis obtained by the Kamlet–Taft equation, the negative sign of *s* and *a* coefficients, except for comp. **3.9** (positive values of

1.57 and 1.42, respectively), were found indicating a batochromic shift with the increasing solvent dipolarity/polarizability.[13] This suggests better stabilization of the electronic excited state relative to the ground state, *i.e.*, higher dipolarity/polarizability and HBD (hydrogen bond properties of solvated molecules in the excited state. Alternation of the sign of coefficients *b*, positive values found for compounds **3.3**, **3.4**, **3.8** and **3.11** indicate better stabilization of the isatin derivatives in the ground state, in other words higher HBA solvent interaction with solute molecule was found. For the other compounds the opposite behaviour was noticed. The non-specific solvent effect is a factor of the highest contribution to UV–Vis spectral shifts of all the investigated compounds, except for compound **3.9** with the highest contribution of HBA solvent effect (45.04 %), and compound **3.11** with the highest contribution of P_α (44.15 %). The highest value of P_π was obtained for compound **3.5** (65.41 %). HBA solvent effect is of lower contribution and the highest values of solvent HBA effect was found for compound **3.9** (–2.45) and exceptionally low values were found for compounds **3.6**–**3.8** (–0.05, –0.06 and 0.02, respectively). The presented results, obtained using the Kamlet–Taft model, indicated that the solvent effects on UV–Vis absorption spectra of the investigated isatin derivatives are rather complex, due to the diversity of the contribution of both solvent and substituent effect in studied compounds. This also indicated that the electronic behaviour of the nitrogen atoms in indol-2-on moiety is significantly different between derivatives with high contribution of localized HBA effect, that originates from the electron-accepting quinoline and the methyl substituted pyridyl groups.[13]

The LFER concept was applied to the ν_{max} and SCS values of isatin derivatives with the aim to get an insight into substituent electronic effect on the absorption maxima shifts and NMR chemical shifts. An analysis, using LFER principles in the form of the Eq. (2) was performed. The Hammett substituent constants are given in the literature,[13] and the obtained correlation results are presented in the original paper.[13] The observed ρ values indicate different susceptibilities of the chemical shifts to substituent effects. The correlations were of reasonably good to high quality, which means that the *SCS* values reflect the electronic substituent effects correctly. Evidently the chemical shifts of C1' showed an increased susceptibility and normal substituent effect.

The presented results indicate that the contribution of both substituent effects, electron-donating and electron-accepting, have significant influence on electron-density shift in overall investigated molecule. The effectiveness of the transmission of substituent effects was determined by the conformational change of the investigated molecules which originate from the out-of-plane rotation of the aryl substituent, with respect to indole-2-one plane, defined by the torsion angle θ (Fig. 1). The reverse substituent effect was observed at H1 and C2 carbon. The existence of these correlations was interpreted as an evidence of sub-

stituent effect on the change of the electronic density over investigated molecule. The presence of an electron-accepting –C=O group at C2 position, and electron-donating amino group (N1-H), as a part of π_1-electronic system of amide group, contribute to the intensive electronic interactions which exists in 3-(substituted imino)-2-pyrrolidone moiety.[31] The correlations for C2 showed negative values of correlation coefficients ρ. The negative sign of the reaction constant, ρ, indicates a certain reverse behaviour, *i.e.*, the value of *SCS* decreases when the electron-withdrawing ability of the substituents, measured by σ, increases. Such finding clearly reflect the behaviour opposite to normal polarization in keto group which comes from the contribution of two opposite effects: electron-accepting C2=O group and aryl substituents with the participation of the electron-donating effect of the indole nitrogen. Magnitude of this effect strongly depends on the electron-accepting character of aryl substituents which cause the adequate/proportional reverse π-electron density shift from C2=O keto group. The contribution of the resonance interaction depends on spatial arrangement of the aryl moiety, and thus, the favourable conformation provides the effective transmission of the resonance effect to C2 and C3 carbons. According to that, it is expected that the presence of the electron-donating group support normal polarization of C2=O, and the electron-accepting group exert opposite effect, which is reflected in the reverse polarization. The normal polarization at C3 carbon is a reflection of the contribution of electronic effects in both π-electronic units (Fig. 1), and primarily dictated by polarization of C=N imino bond which is more or less disturbed by change of substituent at phenyl (aryl) moiety. It is obvious that the chemical shifts of C1' show an increased susceptibility and normal substituent effect related to the substituent constant.[13]

The correlation results of UV–Vis absorption maxima of the investigated compounds, obtained by the use of LFER principles indicate the influences of both solvent and substituent effect on UV–Vis absorption maxima shift. These results indicate that the solvent effects: dipolarity/polarizability, HBD and HBA abilities cause the appropriate sensitivity of the position of absorption maxima (ν_{max}) to substituent effect.[13]

Highly dipolar aprotic solvent DMF, and solvent with low HBD effect, AcN, as well as those with high HBD effect, like ethanol, contribute to the opposite behaviour of the absorption frequencies shift at higher wavelength with respect to the substituent effects. Aprotic solvents do not stabilize anions well, while they usually stabilize larger and more dispersible positive charges better. Lower contribution of substituent effects in a solvent with higher relative permittivity can be explained by the fact that highly dipolar surrounding medium suppresses electron density shift inducing lower susceptibility of the absorption maxima shift to electronic substituent effects. Similar behaviours were found for AcN and ethanol, and it could be observed that solvent dipolarity/polarizability is the most sig-

nificant effect which contribute to the reverse relationship of ν_{max} to substituent effects. The opposite substituent effect on ν_{max} change was found in THF.[13]

In the two solvents, which showed HBD properties, namely AcN and ethanol, the negative correlation slopes of lower wavelength peak was obtained for the former. Somewhat higher sensitivity of ν_{max} to substituent effect was found for AcN (first set of solvents), and THF. In the DMF, ethanol and THF negative solvatochromism was obtained, indicating better stabilization of ground state of investigated compounds. Somewhat lower values and similar trend of correlation slope was found for ethanol, due to lower contribution of solvent dipolarity/polarizability effect. This result suggests that the transmission of electronic substituent effects significantly depends on the conformation of studied molecules and solvent properties. The presented results showed that the transmission of substituent electronic effects through π-resonance units takes place by balanced contribution of two modes: through localized π-electronic unit and overall conjugated system of investigated compounds. Their contribution depends on substitution pattern, as well as solvent properties. This fact implies that the electron density change is of localized/extended delocalization phenomena in compounds with the electron-acceptor substituents. The consequence of this is a lower substituent effect in a solvent with higher hydrogen bond accepting ability.[13]

An additional analysis of solvent and substituent effects on the measured absorption frequencies and the conformational changes of the studied compounds, needed theoretical calculations, *i.e.*, geometry optimization and therefore the molecular electrostatic potential (MEP) analysis were performed.[13] The ground state geometries of compounds **3.1**–**3.11** were fully optimized with DFT method. The theoretical absorption spectra of compound **3.1** were calculated in acetone, acetonitrile, ethanol, tetrahydrofuran, dimethylsulfoxide, formamide and toluene with time-dependent density functional theory (TD-DFT) method.[13]

Geometry optimization of the investigated molecules was performed using B3LYP functional with 6-311G(d,p) basis set.[13] The most stable conformations of compounds **3.1**–**3.11** are presented in Fig. S-2 of the Supplementary material. The elements of optimized geometries of calculated compounds are given in the original paper. The theoretical absorption spectra of compound **3.1**[13] were calculated in acetone, acetonitrile, ethanol, tetrahydrofuran, dimethylsulfoxide, formamide and toluene by TD-DFT method, and showed very good agreement with the experimental data.

The calculation of the optimal geometry, with the focus on determination of the value of torsion angle θ (Fig. 1), gives valuable results required for better understanding of the transmission of substituent effects, *i.e.*, electron density distribution. In the investigated molecules, these values are different and mostly depend on substituent present. Somewhat larger deviation of θ was noticed for compounds **3.2**, **3.5**–**3.8** and **3.11**, indicating the significance of the extended

resonance interaction in the electron-donor substituted compounds. Oppositely, in electron-acceptor substituted compounds the appropriate contribution of *n,π*--conjugation (nitrogen lone pair participation) to overall electronic interaction with π-electronic system of pyridone unit causes the perturbation of *π*-electron density. The geometric elements of the substituted isatin derivatives turned out to be similar to those for the unsubstituted one. The introduction of mainly electron-accepting substituent causes a decrease of C3=N bond length, *i.e.*, a substituent supports normal polarization in the imino group, causing the appropriate shift of electron density to nitrogen. The presence of an electron-donating group attached to a complex structure of aryl moiety contributes to the electron density shift from the phenyl ring to the indol moiety, causing the increased molecule planarization. A decrease of the C2=O lengths is caused by the superposition of two effect: normal polarization in C2=O bond and the opposite effect of the electron--withdrawing group at C3 position; the consequence of this is a slight shifting of *π*-electron density to carbonyl C atom. Therefore the length of N1–C2 gets longer, comparing to compound **3.1** as a result of the suppression of amide type of resonance.[13] The larger deviation from the planarity was found for compounds **3.2**, **3.7**, **3.8** and **3.11** which is associated with *ortho*-effect of 2-Br in compound **3.2**, presence of the electron-donating methyl group situated at pyridyl in different position (compounds **3.7** and **3.8**) as well as the strong electron-donating hydroxyl group in compound **3.11**. The two opposite electron accepting effects operate all in investigated compounds: the electron-accepting aryl substituents and indole-2-one core which influence the appropriate geometrical adjustment of these molecules, as a response to the electronic demand of the electron deficient environment. Except for the vicinity of the electron density at C2=O and nitrogen atom in compounds **3.5**–**3.8** and **3.11,** which are also contributing factor to the increased deviation from planarity, due to the repulsion of negative potential present at these molecular fragments.[13] The variation of substituent properties clearly indicates that the contributions of both conformations and donor–acceptor character are involved in the electronic transition of investigated compounds. The electron density of the investigated compounds is presented using MEP analysis, which was used to evaluate the charge distribution over investigated compounds and to illustrate the three dimensional charge distributions overall the investigated molecules. MEP potential, at a point in space around a molecule, gives the information about the net electrostatic effect produced at that point by the total charge distribution (electron + proton) of the molecule and correlates with dipole moments, electro-negativity, partial charges and chemical reactivity of the molecules. An electron density iso-surface mapped with electrostatic potential surface depicts the size, shape, charge density and the site of chemical reactivity of the molecules. MEP shown in Fig. S-3 of the Supplementary material illustrates the three dimensional charge distributions overall the investigated molecules. As it

can be seen from the Fig. S-3, the different values of the electrostatic potential at the surface are represented by different colours; red represents regions of the most electronegative electrostatic potential, it indicates the region of high electron density, *i.e.*, the sites favourable for electrophilic attack; blue represents regions of the most positive electrostatic potential, *i.e.*, region of low electron density favourable for nucleophilic attack, and green represents regions of zero potential. The potential increases in the order: red<orange<yellow<green<blue. The blue colour indicates the strong attractive potential, the region favourable for HBA solvent interaction, while red colour indicates the repulsive potential, and includes the sites favourable for HBD solvent interactions.[13] There is an obvious negative potential at the oxygen atom, a part of the keto group (C2=O) in the indole-2-one structure, and nitrogen atom present in the structure of the aryl substituent. Thus, most of the HBA capabilities of the investigated compounds could be assumed to be from keto group (C2=O) and aryl nitrogen which are electron--acceptor groups and cause the increase of electron density at these two sites, therefore creating a favourable interaction with proton-donating solvents.[13]

In the molecules with heterocyclic structure the nitrogen present acts as a centre that shows significant proton-acceptor interaction with solvent molecules. Non-planarity of the investigated molecules significantly contributes to the electron density distribution and therefore the electron density transfer occurs due to the both solvents and substituent influence. The resonance effect of nitrogen atom increases the substituents negative potential, which causes the significant contribution of HBD solvent effect. In cases where nitrogen heteroatoms are in the *ortho* position, relative to the C=N bond (compounds **3.6**–**3.8**), some steric hindrance is observed, and increase of the electron density of nitrogen as a result of the obtained conformation of the investigated compounds which results in reduction of the electron density in the rest of the heterocyclic ring. These factors contribute to the increase of the proton-accepting capability with solvent molecules that have pronounced HBD effect.[13] The influence of the substituents electronic effects when they are in a different position on the geometry and electron density distribution was observed in compounds **3.2**–**3.4**. The effect of a bromine atom, which shows a negative inductive and a positive resonance effect on the electron density distribution over the compounds **3.2**–**3.4.** significantly depends on the substituent position, *i.e.*, *ortho*, *meta* or *para*-position. The electron-acceptor effect of the bromine atom affects the shifting of the electron density to a substituent which causes significant change in the geometry of the *ortho*, *meta* and *para*-derivatives. The consequences are the differences in electron density distribution and therefore some different interactions with a solvent.[13]

4. DETAILED STRUCTURAL AND QUANTUM CHEMICAL STUDY RELATED TO ANTIMICROBIAL ACTIVITY

The list of compounds synthesized in this part of the research (the general formula given in Fig. 1), namely the third series, and their elemental analysis are given in Table V.[14] Only the compounds **4.1** and **4.16** were used before (labeled as the compound **3.1** the third Chapter and the compound **2.6** in the second Chapter, respectively).

TABLE V. The list of synthesized isatin derivatives[14]

No.	Compound	Substituent
4.1	1,3-Dihydro-3-(phenylimino)-2*H*-indol-2-one	H
4.2	1,3-Dihydro-3-[(2-hydroxyphenyl)imino]-2*H*-indol-2-one	2-OH
4.3	1,3-Dihydro-3-[(3-hydroxyphenyl)imino]-2*H*-indol-2-one	3-OH
4.4	1,3-Dihydro-3-[(4-hydroxyphenyl)imino]-2*H*-indol-2-one	4-OH
4.5	1,3-Dihydro-3-[(2-methoxyphenyl)imino]-2*H*-indol-2-one	2-OCH_3
4.6	1,3-Dihydro-3-[(3-methoxyphenyl)imino]-2*H*-indol-2-one	3-OCH_3
4.7	1,3-Dihydro-3-[(4-methoxyphenyl)imino]-2*H*-indol-2-one	4-OCH_3
4.8	1,3-Dihydro-3-[(2-chlorophenyl)imino]-2*H*-indol-2-one	2-Cl
4.9	1,3-Dihydro-3-[(3-chlorophenyl)imino]-2*H*-indol-2-one	3-Cl
4.10	1,3-Dihydro-3-[(4-chlorophenyl)imino]-2*H*-indol-2-one	4-Cl
4.11	1,3-Dihydro-3-[(2-iodophenyl)imino]-2*H*-indol-2-one	2-I
4.12	1,3-Dihydro-3-[(3-iodophenyl)imino]-2*H*-indol-2-one	3-I
4.13	1,3-Dihydro-3-[(4-iodophenyl)imino]-2*H*-indol-2-one	4-I
4.14	1,3-Dihydro-3-[(2-nitrophenyl)imino]-2*H*-indol-2-one	2-NO_2
4.15	1,3-Dihydro-3-[(3-nitrophenyl)imino]-2*H*-indol-2-one	3-NO_2
4.16	1,3-Dihydro-3-[(4-nitrophenyl)imino]-2*H*-indol-2-one	4-NO_2

Imino compounds can arrange themselves into *E*- or *Z*-configuration around the C=N double bond and the chemical environment of the azomethine carbon (C3) is different for such isomers.

The investigated isatin derivatives showed chemical shift of the azomethine carbon in the range of 150.43–156.72 for *E* isomer, and 150.90–155.16 for *Z* isomer.[14]

The stereochemistry of the imines was established using 2D homonuclear (COSY and NOESY) and heteronuclear (HSQC and HMBC) NMR techniques.[14] As an example, the chemical shifts of compound **4.10** were assigned by a combined use of one-dimensional (^{1}H and ^{13}C proton decoupled)[14] and two-dimensional NMR experiments (COSY, NOESY, HSQC and HMBC)[14]. In order to assess conformation of **4.10** in a solution, two-dimensional NOESY sequence was applied.[14] The *E* stereochemistry turned out to be the major isomer in DMSO-d_6 solutions as determined by the NOESY experiments that showed proximity of C4-H and C2'-H atoms.[14]

In addition, the signal for H1 (see Fig. 1) of the *E*-isomer was shifted considerably up-field relative to the H1 signal of the parent compound isatin. More-

over, the chemical shifts of the proton present in *Z*-isomer showed little difference to those found in parent compound.[14] The 7.5:2.5 isomer ratio for compound **4.10** in DMSO-d_6 was obtained from ^{1}H-NMR spectrum.[14] Analogously, the analyses of ^{1}H-NMR data of N1–H1 (see Fig. 1) proton were used for the calculation of *E*/*Z* isomer ratio of other studied compounds.[14] The obtained results showed that *E* form dominate in DMSO solution, without regard to the electronic influences of substituents. Four compounds with 2- and 4-NO_2, and 3-OH and 3-OCH_3 group showed presence of only *E* isomer. On the other hand, the influence of electron-donating groups causes the stabilization of *Z* isomer. Such properties could contribute to the change of physico-chemical characteristics and to the differences in biological activity, within these compounds as a function of varying isomer compositions.

As a continuation of the presented work, the determination of acidity constants was carried out using UV–Vis spectroscopy. In hydroxy substituted compounds two p*Ka* values were obtained corresponding to the dissociation of proton on oxygen (pH 8.00–9.00) and isatin nitrogen (pH 9.68–12.11). Acidity constants, K_a, K_{a1} and K_{a2} values, were calculated according to the literature method.[32] The obtained pK_a, pK_{a1} and pK_{a2} values are given in the original paper.[14] K_a and K_{a2} represent the dissociation of the amide N-H hydrogen, while K_{a1} corresponds to the dissociation of the hydroxy substituted isatins.[14] The variation of the values of ionization constants showed the dependence on electronic substituent effects, as well as an appropriate contribution of steric effect in compounds **4.2**, **4.5**, **4.8** and **4.11**. Similar pK_a and pK_{a2} values of compounds **4.15** and **4.16**, with respect to **4.3** and **4.4**, showed unexpected behaviour, when the transmission mode of substituent effects through π_1- and π_2-units (Fig. 1) is considered. The electron-donating capability of hydroxy group in compounds **4.2**–**4.4** contribute to the slight decrease of pK_a values. Unexpectedly high pK_a values, *vice versa* lower acidity, of compounds **4.14**–**4.16**, in comparison to **4.2**–**4.4**, is a result of appropriate degree of the attenuation of the transmission of substituent effect through azomethine bond and competitive π-conjugative interactions in π_1- and π_2-units.[14] On the other hand, the values of ionization constants of compounds **4.5**–**4.13** behave in a regular fashion indicating that the position-dependent influences of substituent effects are the main contributing factor to changes of pK_a. The trends of increasing of the pK_a in compounds **4.5**–**4.13**, are followed by pK_a values increase as the electron-withdrawing capability of substituent increase.[14]

In order to understand and quantify the influence of structural effect and ionization constants of prospective sites at investigated molecules LFER analysis, the already mentioned Hammett's SSP method was performed. The obtained correlation results[14] clearly pointed out that the relationship pK_a *vs*. σ showed a significant influence, of both electron-accepting and electron-donating substi-

tuent effects, on the change of acidity of N-H hydrogen on isatin ring. The reverse substituent effect on pK_a values change means that the increased electron-accepting power of a substituent cause the increase of pK_a value, although σ increases. Higher sensitivity of pK_a to substituent effect was found in second series of compounds due to the contribution of both steric and strong electron-accepting character of substituents.[14]

In order to get some deeper insight into solvent and substituent effects on the conformational changes of the isatin based compounds, the quantum-chemical calculations were performed. Geometry optimization and charge density analysis was performed by the use of MP2 method with 6-311G(d,p) basis set. The significant stability of the compounds **4.1**–**4.3**, **4.6**–**4.9** and **4.11**–**4.13** in *E* form were obtained and in addition, the geometries of the investigated molecules were fully optimized by the use of MP2 method, the detailed elements of optimized geometries are given in the original paper.[14]

Non-consistent substituent dependent variation and some noticeable differences in the trend of the elements of the optimized geometries of both forms of isatin derivatives were registered. In general, low/moderate influences of electronic substituent effects could be noticed. Small variation of N1–C2 bond length indicates a low influence of substituent effect, *i.e.*, a low contribution of resonance in interaction in amide group (π_3-unit), and low cross-interaction with π_1-unit. Simultaneously, C2–C3 bond length increase in compounds containing strong/moderate electron-donating hydroxy/methoxy substituent in 2- and 4-position of *E* and *Z* isomers, excluding compound **4.5** in *E*, and **4.2** and **4.5** in *Z* form.[14] Contrary to that, low increase was observed for *meta*-substituted derivatives. Such trend of C2–C3 bond length change indicates that two opposite electron accepting effects operate in the π_2-unit: the electron accepting phenyl substituted ring and C2=O carbonyl groups, as a response to the electronic demand of the electron deficient environment. The normal carbonyl groups polarization is suppressed and causes a slight bond length decrease.[14]

The introduction of both electron-donor and electron-acceptor substituents causes the increasing of C3=N3 bond length in all *E* isomers, except compound **4.14** (2-NO_2). The results are clearly opposite for the compounds in *Z* isomers where the highest increase of bond length were found for 2- and 4-OH and for 2- and 4-OCH_3 substituted compounds.[14] The opposite trend is displayed when electron-acceptor (such as NO_2 group) is present. The compounds with *ortho*-, *meta*- and *para*-halogen substituent showed similar values for N1-C2 and C3=N3 bond length in derivatives in *E* isomers, except compound **4.11**. Presence of a halogen cause the decrease of the N3-Ph bond length in all *E* isomers with the largest effect found for *ortho*-substituted compounds. This result reflect the effect of the extended conjugation operative in the π_1- and π_2-unit on the π-electron density shifts towards C2=O carbonyl group and supporting n,π-conjugation in

the π_3-unit.[14] A decrease in the N3-Ph bond length, which is a part of the aniline π-electronic system (π_1-unit), contribute to the greater extent of the *n*,π-conjugation. This bond is slightly longer in the electron-donor 3-OH/3-OCH_3 substituted derivatives. On the other hand, in *Z* isomer generally, all N3-Ph bond length are lower for *ortho*- and *para*-substituted ones than in compound **4.1**, while the bond increase were observed for all nitro substituted compounds. These results implied that the attenuation of the resonance interaction was the process of lower significance in compounds **4.14**–**4.16** as a result of competitive cross--interactions of π_1- and π_2-units.[14]

Better understanding of the transmission of substituent effects was based on the value of torsion angle θ (Fig. 1) which contributes to the extended conjugation. More planar structure provides higher contribution of the extended π-conjugation which in turn produces bathochromic shift in UV spectrum. The values of the torsional angle θ for *E* isomers are fairly similar, except in 2- and 4-substituted compounds, *e.g.*, 2-OH and 2-OCH_3, indicating the significance of the extended resonance interaction in electron-donor substituted compounds. An electron-donor substituent (hydroxy and methoxy) supports the electron density shift from the π_1-unit (substituted phenyl ring) to the isatin moiety, causing the whole molecule planarization in a greater extent. Also, the deviation from the planarity rises with the increasing steric effect of phenyl substituent, except for compound **4.7** in *Z* isomer (4-OCH_3) with almost planar geometry (θ = 0.01). Comparing to the unsubstituted *Z* isomer, larger values of θ were obtained for halogen and nitro-substituted compounds. In these compounds the contribution of *n*,π-conjugation (nitrogen lone pair participation) to overall electronic interaction cause perturbation of π-electron density in the molecule as a whole. The deviation from the planarity increases with the decreasing electron acceptor ability of the arylidene substituent.[14]

In the next step, the mechanism of electronic excitations and the electron density distribution in ground and excited states was studied by the calculation of HOMO/LUMO energies (E_{HOMO}/E_{LUMO}) and E_{gap} values. TD-DFT results indicated a significant contribution of a single particle HOMO to LUMO excitations in ground to the first excited state transition, higher than 60 % for all calculated compounds, except for compound **4.15** (47 %) and **4.16** (48 %), for isatins in *Z* form. Similar results were obtained for compounds in *E* form (higher contribution than 59 % for all compounds) except compound **4.11** (40 %).[14] A lower E_{gap} values were observed for all compounds in *Z* form, except of the compound **4.2**. Concerning electron-donor substituents, small influences on E_{gap} changes could be observed, and the lowest values were found for hydroxy and methoxy-substituted compound, *i.e.*, compounds **4.4** (5.59) and **4.7** (5.60), respectively. According to TD-DFT analysis, the solvent effects on the change of

E_{HOMO}/E_{LUMO} and E_{gap} showed a little difference between the results obtained for the gas phase and the calculations involving solvent DMSO, EtOH and AcN.[14]

An illustration of the differences of atomic charges in the excited and in the ground state (Δ_{charge}) for the appropriate atoms are given in Fig. S-4a (*E* isomers) and S-4b (*Z* isomers) of the Supplementary material.[14] The presence of an electron-donor substituent (hydroxy and methoxy) in *E* isomers (Fig. S-4a), cause a decrease of electron density on C1' (except compound **4.2**, 2-OH), imino carbon C3 and imino nitrogen N3, compared to unsubstituted compound **4.1**. Otherwise, the presence of halogen substituents cause an increase of the amount of charge (Δ_{charge}) on C1' and imino nitrogen N3, while imino carbon C3 has the similar values as compound **4.1**. Effect of electron-accepting character of nitro group in *E* isomers induce reduction of the electron density on C1' as well as imino on nitrogen N3 and increasing electron density on imino carbon C3 relative to compound **4.1**.[14]

Relative to *Z* isomer of the unsubstituted compound **4.1** (Fig. S-4b) value of the amount of charge (Δ_{charge}) on C1' and imino carbon C3 is decreasing if the hydroxy group is in *ortho* and *para* position, as well as for methoxy group in *para* position. Oppositely, the electron density on imino nitrogen N3 in presence of 2-OH and 4-OMe, compounds **4.2** and **4.7**, increases respectively. All chloro--substituted *Z* isomers, including compound **4.13** (4-I), have a larger amount of charge (Δ_{charge}) on C1', in comparison to compound **4.1**. Therefore, if the halogens are in *ortho* position, the imino carbon C3 and the nitrogen N3 gain Δ_{charge}. The presence of the electron-accepting nitro group causes the decreasing of electron density on C1' and imino carbon C3, while opposite is true for the imino nitrogen N3. The electronic charge change upon transition, presented separately for *E* and *Z* isomers, give a picture on local/overall electron densities shift.[14]

Additionally, the intramolecular charge transfer (ICT) was interpreted with the aid of the TD-DFT calculations related to the distance between two centres of the density depletion and the density increment zones (D_{CT}) and amount of transferred charge (Q_{CT}) in the course of excitation, the detailed results are given in the original paper.[14] According to the results, it could be seen that in both forms the distinct ICT processes were observed with electron density transfer from the isatin ring to the substituted phenyl in the course of transition. It is observed that the strongest ICT take place in molecules **4.14** (2-NO_2) for *Z* isomers and **4.6** (3-OMe) for *E* isomers, demonstrating the transfer of 0.732 e^- over 2.131 Å and of 0.776 e^- over 2.608 Å, respectively. On the other hand, in molecule **4.7** (4-methoxy substituent), the intramolecular charge transfer of 0.703 e^- over 0.156 Å becomes a local process which occurs within *imino* bridging group (Fig. S-5 of the Supplementary material).[14]

The variation of substituent patterns clearly indicate that the contributions of both conformational arrangement and donor–accepting character are involved in

the ICT mechanism of the investigated molecules. The decreasing trend, in order from *ortho* to *para* position, of the Q_{CT} in *Z* isomers, for halogen (chloro and iodo) and nitro substituted isatin derivatives, can be observed from the results.[14] On the other hand, Q_{CT} values change of nitro substituted isatin derivatives in *E* isomers showed the increasing trend.[14] For hydroxy and methoxy substituted derivatives in both *E* and *Z* isomers, and chloro- and iodo-substituted compounds in *E* isomers the value of calculated Q_{CT} for *ortho*, *meta* and *para* position showed non-linear relationship. The specific behaviour of hydroxy substituted isatin derivatives in *E* and *Z* forms and methoxy in *E* form was reflected in higher Q_{CT} values, which are found for *meta* substituted compounds. On the other hand, the methoxy substituted compounds in Z form and chloro and iodo substituted ones in *E* form showed the lowest value for *meta* substituted isatin derivatives.[14]

In our research,[14] as the continuation of the previous paper,[12] the isatin derivatives were tested for their antibacterial activity against *Staphylococcus aureus* +, *Listeria monocytogenes* +, *Shigella sonnei* –, *Yersinia enterocolitica* –, *Escherichia coli* –, *Proteus Hauseri* –, *Pseudomonas aeruginosa* –, *Cryptococcus neoformans* and *Candida albicans*, by the already used broth microdilution method. The obtained results are presented in Table VI. Compounds **4.7** (4--OMe), **4.12** (3-I) and **4.13** (4-I) were excluded from the antimicrobial analysis due to poor solubility in 10 % DMSO. All the other compounds showed activity on some of the microbial strains used in the study.[14] The activity of the investigated isatin derivatives against the same microbial strains is similar in most cases, with few exceptions. However, there is a trend of considerable activity of *ortho*-substituted hydroxy and chloro derivatives and *ortho*- and *para*-nitro deri-

TABLE VI. *MIC* values (μg mL^{-1}) of isatin derivatives against the investigated microbial strains;[14] compounds **7**, **12** and **13** are excluded from the Table VI due to not having antimicrobial activity

Microorganism	Compound												
	1	**2**	**3**	**4**	**5**	**6**	**8**	**9**	**10**	**11**	**14**	**15**	**16**
S. aureus	1250	1250	1250	>1250	1250	1250	1250	1250	>1250	1250	1250	>1250	1250
L. monocytegenes	1250	313	>1250	>1250	1250	1250	313	1250	1250	1250	313	1250	313
S. sonnei	625	313	1250	625	625	625	313	625	1250	>1250	313	1250	156
Y. enterocolytica	1250	156	>1250	313	1250	625	313	625	1250	1250	156	1250	156
E. coli	1250	1250	>1250	>1250	>1250	1250	1250	>1250	>1250	>1250	1250	1250	1250
P. hauseri	1250	313	>1250	1250	1250	1250	313	1250	1250	156	313	1250	313
P. aeruginosa	625	1250	>1250	>1250	>1250	>1250	1250	>1250	1250	1250	625	1250	625
C. neoformans	156	19	39	39	156	>1250	39	156	156	19	39	156	39
C. albicans	1250	313	>1250	>1250	>1250	>1250	313	625	1250	1250	1250	1250	313

vatives against most of the strains. While former are electron-donors and latter an electron-acceptors, they are all electron-negative groups, which could be an important property for the antimicrobial activity. The difference is most pronounced in the case of compound **4.2** (2-OH) and compound **4.3** (3-OH) activity against *Y. enterocolitica*, which is more than 10 times greater in former (*MIC* = 156 mM for 2-OH and *MIC* > 1250 mM for 3-OH).[14]

There is no difference in activity against G+ and G– bacteria in general, but some strains are more susceptible, as *L. monocytogenes*, *S. sonnei*, *Y. enterocolitica* and *P. hauseri.*[14]

As for fungal strains, *C. neoformans* was shown to be far more sensitive to the tested compounds than *C. albicans*, while the *MIC* values for *C. albicans* were similar to those for bacterial strains. The *MIC* values obtained for *C. neoformans* were, in some cases, even 100 times greater (compound **4.2**; 2-OH and compound **4.11**; 2-I, *MIC* = 0.019 mM). Compounds **4.3** (3-OH), **4.4** (4-OH), **4.8** (2-Cl), **4.14** (2-NO_2), **4.16** (4-NO_2) also showed prominent antifungal activity against this human pathogen (*MIC* = 0.038 mM).[14] The overall activity of the compounds was most pronounced against the fore mentioned *C. neoformans*. Compounds **4.2** (2-OH) and **4.8** (2-Cl) showed the best activity against *C. albicans* (*MIC* = 0.313 mM) of all investigated compounds. The obtained results suggest that the other substituents present on phenyl or heterocyclic ring of the isatin derivatives should be tested.[14]

In order to explore the structural properties important for the antimicrobial activity of compounds **4.1**–**4.16**, QSAR models were generated, taking the *MIC* values for *S. sonnei*, *Y. enterocolitica* as well as *C. neoformans* but the statistically significant model was obtained only for the activity of compounds toward *C. neoformans*.[14] Principal component analysis (PCA) was performed using the whole set of GRIND-2 descriptors. Model with 3 principal components (PC) explained 77.78 % of *X* sum of squares (SSX_{acc}), and 70.22 % of *X* variance ($VarX_{acc}$).[14] Partial least squares regression model (PLS) was created in order to correlate he structural features of compounds with antimicrobial potency toward *C. neoformans*. After filtering of descriptors through 2 cycles of FFD, two latent variables (LV) model with $r^2 = 0.89$, q^2 (leave-one-out, LOO cross-validation) = = 0.69, and standard deviation of error of prediction (*SDEP*) = 0.29.[14]

From the PLS coefficients plot (Fig. S-6 of the Supplementary material) several variables important for the activity were identified. The most informative variables in PLS model are the one that clearly separate the more potent compounds from the less. Structural motifs associated with the most informative variables were found.[14] The positively correlated variables are depicted on compound **4.2** as a model for the more potent compounds (Fig. S-7 of the Supplementary material). The variables with the negative influence on potency are

depicted on the least potent compound **4.6** (Fig. S-8 of the Supplementary material).[14]

5. CONCLUSIONS

A series of 30 isatin derivatives, some of them new compounds, were synthesised in order to examine their properties and the application potential. Their structures were confirmed by spectral methods. The antimicrobial screening was performed, and some compounds showed relative selectivity to certain bacterial strains. In order to test antioxidative activity, DPPH reduction test was performed on some of the compounds, which should be continued. From the solvatochromic and characterization point of view UV–Vis and NMR data were analysed by the use of LSER and LFER principles and it could be concluded that solvent effects have significant influence on the transmission mode of substituent effects. The quantum chemical calculations, performed next, showed that the present substituents significantly change the extent of conjugation. Then, the *E*/*Z* isomer ratios of different investigated isatin compounds were estimated from the point of NMR data, theoretical calculations and UV–Vis spectra. The inclusion of solvent effects in the TD-DFT calculations demonstrated that substituents, depending on their position in molecules, and solvent effects significantly affect the ICT character. The testing of antimicrobial activity was continued further on isatin derivatives, with the aim of relating the activity to the compound structure. The most of the investigated isatin derivatives exhibited moderate activity against bacterial strains, but compounds with hydroxy, nitro group and chlorine in *ortho* position as well as *para* nitro showed considerable activity. The 3D QSAR model created pointed on hydroxyl groups as substituents important for the potency of compounds toward fungal strain *C. neoformans*.

This complex research was performed to study the examined compounds for their potential biological activity as well as to link activity with molecular structure. The aim was to determine compounds that should be submitted to further investigation, since they have a considerable chance for practical use, particularly as active compounds of medicines.

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SUPPLEMENTARY MATERIAL

Additional data are available electronically at the pages of journal website: https:// //www.shd-pub.org.rs/index.php/JSCS/index, or from the corresponding author on request.

ИЗВОД

ПРЕГЛЕД КАРАКТЕРИЗАЦИЈЕ И БИОЛОШКЕ АКТИВНОСТИ ДЕРИВАТА ИСАТИНА

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Деривати исатина су познати по потенцијалној биолошкој активности, па су постали интересантни за проучавање везе између наведене активности и њихових структурних карактеристика. Наш тим их је годинама истраживао са оба аспекта и у овом прегледном раду су приказани сви резултати од значаја до којих смо дошли. Испитивани деривати исатина су синтетисани реакцијом између исатина и различитих примарних амина, окарактерисани су спектроскопским методама (FTIR, NMR) као и елементалном анализом. На њима су извршена и разна теоријска проучавања као и одређивање антимикробне активности, како би се стекла што потпунија слика о вези између њихове структуре и активности и одредила једињења за даља испитивања због њиховог потенцијала за примену.

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