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Synthesis, antimicrobial and antioxidative activity of some new isatin derivatives

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Abstract: The isatin derivatives, Schiff bases, were synthesized by the reaction of isatin and various substituted primary amines and characterized by several spectroscopic methods. Investigation of the antimicrobial activity of the synthesized compounds was performed by the agar dilution method, against different strains of bacteria and one fungus. The antioxidative activity of the synthesized compounds was also determined. Some of the compounds showed significant activity against the selected strains of microorganisms and antioxidative activity.

Keywords: isatin derivatives; Schiff bases; antimicrobial activity; antioxidative activity.

INTRODUCTION

The derivatives of isatin (indole-2,3-dione), as well as its Schiff and Mannich bases, have already 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 has led to their increasingly expanded use as precursors for the preparation of many biologically active compounds.[5–11] Hydrazine derivatives of isatin were found to be active against Walker carcinosarcoma 256.[10,12] Similarly, acetone- and ketone-derivatives of isatin exhibited anticonvulsant activity.[13] Another class of thiosemicarbazone derivates of isatin was found to exhibit interesting applications as research

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tools in physiological studies.[14–18] Similarly, many other isatin-derived compounds possess a wide spectrum of medicinal properties and have thus been studied for activity against tuberculosis,[19,20] leprosy,[21] fungal,[22–26] viral[26] and bacterial[18,27] infections, trypanosomiasis[28] and as anticonvulsants.[29–31] Therefore the antimicrobial activity determination of this class of compounds is of significance and it has already been performed by the diameter of zone of inhibition method.[32,33]

Besides antimicrobial activity, the antioxidative capacities of a compound have become more and more significant nowadays. Oxidation reactions can produce free radicals, which, in turn, can initiate chain reactions. Antioxidants terminate these chain reactions by being oxidized themselves, thereby removing free radical intermediates and inhibiting other oxidation reactions. Such are thiols, ascorbic acid and polyphenols that are also reducing agents.[34] Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, or coronary heart disease.[34] Antioxidants also have many industrial uses, *i.e.*, as preservatives in food and cosmetics and even to prevent the degradation of rubber and gasoline.[35]

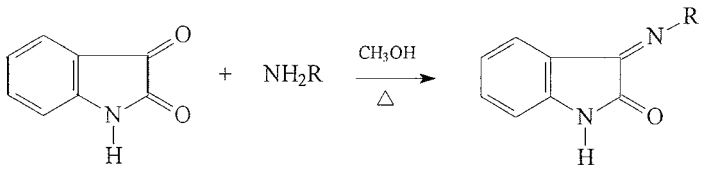
Taking all this into consideration, it is deemed of interest to test the antimicrobial and antioxidative activity of synthesized isatin derivatives, in order to estimate their activity potential.

In this study, a series of six isatin derivatives, two of them new, which could be classified as Schiff bases, was synthesized, characterized and tested for their antimicrobial and antioxidative activity.

EXPERIMENTAL

Chemistry

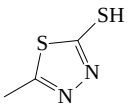
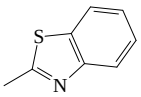
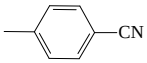
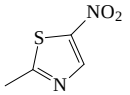
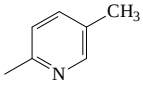
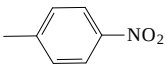
The examined Schiff bases were synthesized by the reaction of isatin and the required primary amine, Scheme 1. All employed chemicals were of *p.a.* quality (Fluka–Alrich). The list of synthesized compounds is given in Table I.



Scheme 1. The synthesis of the isatin derivatives.

Synthesis. Isatin (5 mmol) was dissolved in methanol (40 mL), and corresponding reactants (amine (R–NH_2, 5 mmol) and glacial acetic acid (1 mL) in the given order) were added. Reaction mixture was refluxed at 70 °C for 6 h under stirring at atmospheric pressure. ubsequently, the mixture was left overnight without stirring at room temperature. The obtained crystals were filtered off, dried and recrystallized from methanol. On average, the yield was about 70–79 % (details are given in the Supplementary material).

TABLE I. The synthesized isatin derivatives

Compound	R	Compound name
1		1,3-dihydro-3-[(5-mercapto-1,3,4-thiadiazol-2-yl)imino]-*2H*--indol-2-one
2		1,3-dihydro-3-(2-benzothiazolylimino)-*2H*-indol-2-one
3		1,3-dihydro-3-[(4-cyanophenyl)imino]-2*H*-indol-2-one
4		1,3-dihydro-3-[(5-nitro-2-thiazolyl)imino]-2*H*-indol-2-one
5		1,3-dihydro-3-[(5-methyl-2-pyridyl)imino]-*2H*-indol-2-one
6		1,3-dihydro-3-[(4-nitrophenyl)imino]-2*H*-indol-2-one

Characterization

Melting points were determined on a Stuart SMP 30 melting point apparatus. The FTIR spectra were recorded on Bomem MB100 spectrometer, using the standard KBr pellet technique. The ^{1}H-NMR and ^{13}C-NMR spectral measurements were performed on a Bruker AC 250 spectrometer at 250 MHz for the ^{1}H-NMR and 62.89 MHz for the ^{13}C-NMR spectra. The spectra were recorded at room temperature in DMSO-d_6. Elemental analysis was realized using an Elemental Vario EL III micro-analyzer.

In vitro *antimicrobial activity*

The antimicrobial activity of all synthesized compounds **1**–**6** was determined on a wide range of different microorganisms by the broth micro-dilution method.[36] The advantage of this method, in comparison to the technique of the diameter of inhibition zones,[32,33] lies in its capability to quantitatively determine antimicrobial activity and give a more precise insight into the effect of every examined compound on the applied bacterial strains.

The broth micro-dilution method[36] was applied to determine the minimal inhibitory concentrations (*MIC*) of the investigated compounds against nine American Type Cell Collection (ATCC) bacterial strains and one strain of yeast, *Candida albicans* (Table II). The method was performed in agreement with Clinical and Laboratory Standard Institute (CLSI 2005).

The active microbial cultures were prepared from lyophilized standard strains by transferring them to test tubes with the appropriate broth. The nutrient broth was used for bacterial strains, except for *L. monocytogenes*, for which the soya triptose broth was used. The malt broth was used for *C. albicans.* The density of microbial suspensions was set approximately at 10^5 CFU (Colony Forming Units), using the appropriate broth.

All examined compounds were first dissolved in 5 % dimethyl sulphoxide to a concentration of 2.5 mg mL^{-1}, and then series of concentrations were prepared by two-fold dilution,

using the appropriate broth. The serial concentrations were prepared directly in micro-titre plates and the final volume of specimens was 50 μL. The investigated concentrations were in the range from 0.0024 to 1.25 mg mL^{-1}. In the last column only the appropriate broth was added. Then 50 μL of each microbial suspension were added in each well, so that the final concentrations of the examined extracts were half of those at the beginning, and the final volume was 100 μL in each well. Triphenyltetrazolium chloride (TTC), in concentration of 0.75 vol. % was used as the growth indicator. If growth of a microbial strain occurs, this indicator gives a rosy–red colour to the broth. The plates with bacteria were incubated at 37 °C and that with candida at 32 °C, for 24 h. The results were read the following day and the *MIC* value of each compound on every strain was taken as the concentration at which there was no development of a red colour. All tests were performed in triplicate and the *MIC* values were constant.

TABLE II. The examined bacteria and fungus types

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 enterocolitica*	27729
7	*Escherichia coli*	35150
8	*Proteus hauseri*	13315
9	*Pseudomonas aeruginosa*	27853
10	*Candida albicans*	10259

Antioxidative activity

All the synthesized compounds were screened for their antioxidative activity by the 2,2-diphenylpicrylhydrazyl (DPPH) assay.[37]

DPPH method. A methanolic DPPH solution (0.037 mg mL^{-1}) was prepared and kept in the dark before analysis. Methanolic solutions of the compounds were prepared in various concentrations, depending on the examined compound. 200 μL of each sample was added to 2.8 mL of DPPH solution and reaction mixture was kept in the dark for 20 min. A blind test was performed by adding 200 μL of methanol in 2.8 mL of DPPH solution and the absorbance of both the blind check and the investigated samples was measured at 517 nm. The percent of DPPH reduction, $DPPH_{red}$, was calculated from the equation:

$$DPPH_{red} = 100\frac{(A_{BT} - A_{SX})}{A_{BT}}$$

where A_{BT} is the absorbance in the blind test, and A_{SX} corresponds to absorbance of a specific sample.

RESULTS AND DISCUSSION

Characterization of the synthesized compounds

The structures of all synthesized compounds **1**–**6** from Table I were confirmed by melting points, FTIR, ^{1}H-,^{13}C-NMR spectra and also by elemental anal-

ysis for compounds **1** and **5**. The data for compound characterization are given in the Supplementary material to this paper.

To the best of our knowledge, there are no literature data that compounds **1** and **5** had been hitherto synthesized, therefore they could be classified as new compounds. Compound **2** has already been used for the synthesis of metal complexes,[32] commercial sources exist for compound **3***, and the antimicrobial activities of compounds **4**[38] and **6**[33] have also already been researched. However, no available FTIR or NMR spectra of them could be found in the mentioned studies.

Antimicrobial screening

All the examined compounds showed considerable activity against all the tested strains of microorganism except for compound **3**, which exhibited rather weak activity against *E. coli*, *P. aeruginosa* and *C. albicans* in the range of the investigated concentrations. The overall activity could be described as moderate with some selectivity against Gram-positive (G+) or Gram-negative (G–) strains of bacteria, or the yeast *C. albicans.*

The selectivity to G– bacteria, which is an important property for the pharmacological activity, also appeared in a few cases. This property enables the antibiotic agent based on a G– selective compound to be taken without the support of an agent that recovers the gastrointestinal tract, as the natural bacteria it contains are G+. The activity of certain examined isatin derivatives against the fungus was also important because they could be applied as antifungal agents.

The overall results of the antimicrobial screening are given in Table III.

TABLE III. Antimicrobial activity of the examined compounds (*MIC* / mM)

Cmpd.	Microorganism									
	S. aureus	*L. monocytogenes*	*E. faecalis*	*S. sonnei*	*S. enteritidis*	*Y. enterocolytica*	*E. coli*	*P. hauseri*	*P. aeruginosa*	*C. albicans*
1	0.16	0.32	2.56	1.28	2.56	0.64	2.56	0.64	5.12	2.56
2	2.24	1.12	2.24	1.12	1.12	1.12	1.12	1.12	2.24	2.24
3	2.53	0.63	2.53	5.06	>5.06	2.53	>5.06	5.06	>5.06	>5.06
4	0.57	2.28	0.57	0.57	1.14	1.14	1.14	1.14	2.28	2.28
5	5.27	2.64	2.64	0.66	5.27	0.33	2.64	0.99	2.64	2.64
6	4.68	1.17	4.68	0.59	4.68	0.59	4.68	1.17	2.34	1.17

Some of the isatin derivatives synthesized in this research displayed significant activity against the various examined bacteria and fungus, while some others were only moderately or even weakly active.

Compound **1** exhibited the most prominent overall activity on both G+ (*S. aureus* and *L. monocytogenes*) and G– (*Y. enterocolitica* and *P. hauseri*) bacterial strains. The highest activity of this compound was registered against *S. aureus*

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(*MIC* value of 0.16 mM), followed closely by *L. monocytogenes* (*MIC* value of 0.32 mM).

However, compound **1** displayed only moderate activity on *Y. enterocolitica*, unlike compound **5**, which had a rather significant activity against this specific strain (*MIC* value of 0.33 mM). Except for this example, the overall antimicrobial activity of compound **5** was not as strong as that of compound **1**. Besides against *Y. enterocolitica*, it showed moderate activity on *S. sonei* and somewhat slighter on *P. hauseri*.

Compound **6** behaved somewhat similar to compound **5** but without any really prominent antimicrobial activity. It displayed only moderate activity against *Y. enterocolitica* and *S. sonei*.

Compounds **2** and **3** generally showed comparably weak antimicrobial activity. The only observation that could be of interest was the relative selectivity of compound **3** to *L. monocytogenes.* (*MIC* value of 0.63 mM)

Compound **4** exhibited certain moderate activity against *S. aureus*, *E. faecalis* and *S. sonei*.

Antioxidative activity

The results of DPPH analysis showed that compound **1** displayed the most expressed antioxidative activity, while the other investigated compounds, including pure isatin, showed very slight, if any, activity.

With increasing concentration of compound **1**, the absorbance of DPPH decreased, displaying a linear dependence of % $DPPH_{red}$ in the range of examined concentrations (c, mM), which is described by the following equation:

$$DPPH_{red}\ [\%] = 5.099 + (101.02 \pm 5.24)c$$

$$R = 0.995,\ s = 3.17,\ n = 6$$

$DPPH_{red}$ is actually the percent of DPPH reduction and c is the concentration of compound **1**, given in mM. The equation enables the precise determination of the concentration which reduces 50 % of the DPPH concentration (DC_{50}).

Compound **1** showed prominent antioxidative activity, with a DC_{50} value of 0.444 mM. Ascorbic acid was used as a standard, with a DC_{50} value of 0.341 mM (see Fig. S-1 of the Supplementary material), which is quite comparable.

CONCLUSIONS

Six isatin derivatives were synthesized, of which two were new, in order to test their antimicrobial activity. Their structure was confirmed by melting points, and FTIR and NMR spectra. Antimicrobial screening was performed on nine bacterial strains and one yeast strain, by the broth micro-dilution method. Some compounds showed relative selectivity to certain bacterial strains. In several cases, slight selectivity could be noticed against certain bacterial strains. Com-

pounds **1**, **5** and **6** exhibited relative selectivity against the G– bacteria *S. sonei*, *Y. enterocolitica* and *P. hauseri*, shown by the *MIC* values being approximately 4 to 16 times lower than for other examined G– bacteria, which means that the effects of the mentioned compounds were considerably stronger on these three bacterial strains, than on the others tested. Compounds **3** and **6** showed relative selectivity to *L. monocytogenes* – if only the other G+ bacteria were taken into consideration, with approximately 4 times better activity on *L. monocytogenes* than on the other examined G+ bacteria. Compound **1** showed significant activity against both *S. aureus* and *L. monocytogenes.*

Overall effect of the examined compounds on selected bacterial and yeast strains could be described as moderate, with the exception of compounds **1** and **5** which exhibited prominent activity to *S. aureus* and *L. monocytogenes* (**1**) and *Y. enterocolitica* (**5**) and would be of interest for further analysis. Compounds **5** and **6** showed somewhat better activity to certain G– strains than to G+ strains, which makes them interesting from the medicinal point of view.

The DPPH reduction test was performed to investigate the antioxidative activity of the synthesized compounds and compound **1** displayed significant activity, which makes it of highest interest for further study.

SUPPLEMENTARY MATERIAL

Characterization data of the synthesized compounds and graph for antioxidative activity of compound **1** are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА, АНТИМИКРОБНА И АНТИОКСИДАТИВНА АКТИВНОСТ ДЕРИВАТА ИЗАТИНА

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Деривати изатина, Шифове базе, синтетисани су реакцијом између изатина и различитих супституисаних примарних амина, и затим окарактерисани са неколико спектроскопсих метода. Испитивање антимикробне активности синтетисаних једињења је изведено бујон-микродилуционом методом, на различитим сојевима бактерија и једној гљивици. Такође је испитана и антиоксидативна активност синтетисаних једињења. Нека од њих су показала значајну како антимикробну, тако и антиоксидативну активност.

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