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Synthesis, antioxidant and antimicrobial activity of carbohydrazones

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Abstract: Fourteen mono- and bis-carbohydrazone ligands have been synthesized and characterized. Antioxidant activity of the substances was investigated together with possible (*E*)/(*Z*) isomerization, and explained on the most active antioxidant compound **4** in various dimethyl sulphoxide–water mixtures. The addition of water to the system was involved in the formation of hydrated molecules which was confirmed in NMR after the addition of D_2O. The ligands were tested *in vitro* against Gram-positive and Gram-negative bacteria and fungi, and their activity was discussed in relation to the structure of investigated carbohydrazone.

Keywords: carbohydrazones; antibacterial; antifungal, antioxidant activity; isomers.

INTRODUCTION

In recent years, heterocyclic compounds have received considerable attention due to their significant importance in pharmacological and agricultural field.[1–3] Nitrogen-containing heterocycles and aromatic compounds with reactive hydroxyl radical display excellent biological activity.[4] Due to their multiple biological profiles, carbonic acid amide compounds also have found application in medicinal and pesticide chemistry as insecticides, fungicides, herbicides and plant growth regulators.[5]

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Carbohydrazides are dihydrazide derivatives of carbonic acids. As their monohydrazide analogue semicarbazide, carbohydrazides also form corresponding monohydrazones. Semicarbazone moiety and its various derivatives were studied frequently in the past time and found to have various pharmacological activity.[6] The presence of additional hydrazide group in monohydrazones, which has increased antioxidant activity,[7] allows preparation of symmetric and asymmetric dihydrazones.

Hydrazones are of great interest to researchers because of their diverse biological and clinical applications. They are usually formed easily from carbonyl compounds and hydrazines in a reversible reaction and can coordinate to metal ions through the azomethine nitrogen.[8,9] They have been reported to exhibit antimicrobial, anticonvulsant, analgesic, anti-inflammatory, antiplatelet, antitubercular and antitumor activities.[10–12] It was found that coordination of the Schiff base ligand with transition metals lead to an increase in the antimicrobial[13,14] and antioxidative activities[15,16] in most of cases.

It is little known about the biological properties of mono- and bis- carbohydrazones. Some of them were evaluated for their cytotoxic properties[17] and recently 2-acetylpyridine carbohydrazones were synthesized and tested as inactivators of HSV-1 ribonucleotide reductase.[18] Insignificant number of carbohydrazone ligands have been tested *in vitro* against some Gram-positive and Gram--negative bacteria, yeasts and moulds.[19]

Carbohydrazones might exist in keto or enol tautomeric forms[20] and can adopt (*E*)- or (*Z*)- configuration around the C=N double bond. Tautomeric equilibrium can also involve aldehyde moiety if the hydroxyl group is situated in *ortho* position with respect to the C=N double bond.[20]

In the present research, as a continuation of previous reports,[19] the synthesis, characterization of new carbohydrazones was described. The antimicrobial and antioxidant activities of synthesized ligands were evaluated and discussed. Since (*E*)/(*Z*) isomers can have diverse chelating properties as well as pharmacodynamics activities,[22] and the presence of hydroxyl groups increases the antioxidant activity of compounds[7], (*E*)/(*Z*) isomerization and keto-enol tautomerization in a mixed dimethyl sulphoxide/water mixtures of selected compounds was also considered and discussed.

EXPERIMENTAL

Chemicals

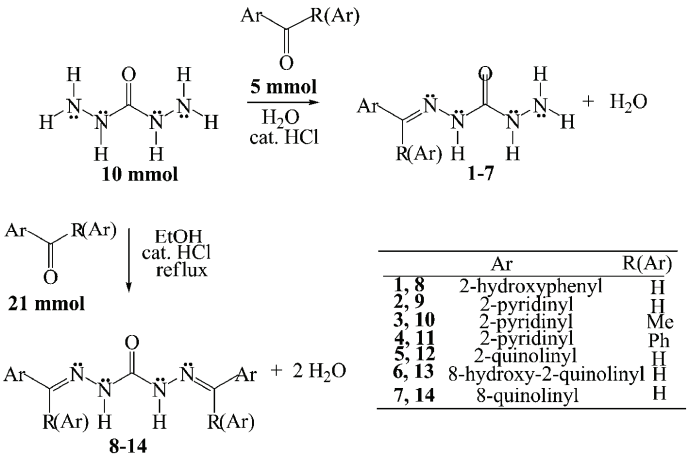
Salicylaldehyde, methyl 2-pyridyl ketone, 2-pyridinecarboxaldehyde, 2-benzoylpyridine, 2-quinolinecarboxaldehyde and carbohydrazide were obtained from Sigma–Aldrich Company. 8-Quinoline-carboxaldehyde (98%) and 8-hydroxy-2-quinolinecarboxaldehyde (98%) were obtained from Acros Organics. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma–Aldrich Company. All used solvents were of UV spectroscopic quality.

*General procedure for synthesis of carbohydrazones **1**–**14***

Carbohydrazones **1**–**7** were prepared by reactions of carbohydrazide (1,3-diaminourea, 10 mmol) and appropriate aldehydes and ketones (5 mmol) in water (10 ml) at room temperature with addition one drop of conc. hydrochloric acid. Reaction mixture was stirred for 2 h and the resulting precipitate was collected by filtration, and recrystallized from a suitable solvent.

Components **8**–**14** were prepared by condesation of carbohydrazide (10 mmol) in ethanol (40 ml) and appropriate aldehydes or ketones (21 mmol) dissolved in ethanol (10 ml) under reflux for 3 h with addition of one drop of conc. hydrochloric acid. After completion of reaction, the resulting precipitate was collected by filtration and washed successively with cold alcohol, ethyl ether, dried and recrystallized from a suitable solvent.

The compounds **1**[22], **3**, **10** and **11**[19], **5**–**7** and **12**–**14**[7], **8**[23] and **9**[24], are known compounds which have been previously synthesized. All these compounds are synthesized by our procedures and their purity was verified through melting point and elemental analysis. The synthetic route used to synthesize the compounds **1**–**14** is outlined in Scheme 1.



	Ar	R(Ar)
1, 8	2-hydroxyphenyl	H
2, 9	2-pyridinyl	H
3, 10	2-pyridinyl	Me
4, 11	2-pyridinyl	Ph
5, 12	2-quinolinyl	H
6, 13	8-hydroxy-2-quinolinyl	H
7, 14	8-quinolinyl	H

Scheme 1. General procedure for the synthesis of mono- and bis-carbohydrazones **1**–**14**.

Characterization methods

Elemental analyses (C, H, N) were performed by the standard micromethods using the Elementar Vario ELIII CHNS/O analyzer. Fourier-transform infrared (FTIR) spectra were obtained using FTIR Bomem MB 100 in the form of KBr pellets. FTIR spectra were recorded in the transmission mode between 600 and 4000 cm^{-1} with a resolution of 4 cm^{-1}. All NMR spectral measurements were performed on a Bruker Avance III 500 spectrometer equipped with a broad-band direct probe. The spectra were recorded at room temperature in deuterated dimethyl sulfoxide (DMSO-d_6). Chemical shifts are given on δ scale relative to tetramethylsilane (TMS), as internal standard for ^{1}H and ^{13}C, or relative to urea (77.0 ppm) as the external standard for recording ^{15}N-NMR spectra. Coupling constants (J) were expressed in Hz. Abbreviations used for NMR spectra: *s*, singlet; *dd*, doublet of doublets; *ddd*, double double doublet; *td*, triplet of doublets. Numeration of atoms used in NMR analysis are given in Figs. S-1 and S-2 of the Supplementary material to this paper. Solution absorption spectra were recorded on a Shimadzu

1700 UV–Vis spectrophotometer. The analytical and spectral data of the synthesized compounds are included in the Supplementary material to this paper.

Samples preparation for UV-measurements

Stock solution of all compounds were prepared by dissolution of the weighed samples in dimethyl sulfoxide (DMSO). The working solutions was prepared in a 10.0 mL volumetric flak by adding appropriate volume of stock solution and water, and diluted to 10.0 mL with DMSO. Working sample, used for the measurement of UV–Vis spectra, was prepared in such a way to obtain V(DMSO)/V(H_2O) mixed solvents ratio of 4/1,[25] and solution was UV irradiated continuously at a wavelength of absorption maximum.[26] Concentration of the hydrazones in the obtained solution was 5×10^{-5} mol L^{-1}.

Free radical scavenging antioxidant assay

The proton donating ability was assayed using a protocol for the determination of radical scavenging activity.[27] Compounds were dissolved in DMSO and diluted into ten different concentrations. Commercially available DPPH radical was dissolved in methanol at a concentration of 6.58×10^{-5} mol L^{-1}. Into a 96-well microplate, 140 μL of DPPH solution was loaded and 10 μL DMSO solution of the tested compounds was added, or pure DMSO (10 μL) as the control. The microplate was incubated for 30 min at 298 K in the dark and the absorbance was measured at 517 nm using a Thermo Scientific Appliskan. All the measurements were carried out in triplicate. The scavenging activity of the compounds was calculated using the equation (1):

$$\text{Scavenging activity}(\%) = 100\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \quad (1)$$

where A_{sample} and A_{control} refer to the absorbances at 517 nm of DPPH in the sample and control solutions, respectively. IC_{50} values were calculated from the plotted graph of scavenging activity against the concentrations of the samples. IC_{50} is defined as the total antioxidant concentration necessary to decrease the amount of the initial DPPH radical by 50 %. IC_{50} was calculated for all compounds based on the percentage of DPPH radicals scavenged. Ascorbic acid was used as the reference compound (positive control) with concentrations 50 to 500 mg mL^{-1}.

Antimicrobial activity

Antibacterial activity was evaluated using four different strains of Gram-positive bacteria: *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Clostridium sporogenes* (ATCC 19404) and *Kocuria rhizophila* (ATCC 9341), and four strains of Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Proteus hauseri* (ATCC 13315) and *Salmonella enterica* subsp. *enterica* serovar Enteritidis (ATCC 13076). Antibacterial activity was determined by the well diffusion method.[28]

To each Petri dish (90 mm diameter), 22 mL nutrient agar (HiMedia, Mumbai, India) and 100 μL bacterial suspension (10^6 cells per dish) were added. A well with a diameter of 8 mm was then punched carefully using a sterile cork borer and 100 μL test substance (1 mg/100 μL DMSO) was added to each labeled well. Amikacin (30 μg/100 μL H_2O) was used as a positive control, whereas 100 μL water and DMSO served as negative controls. The same procedure was repeated for different microorganisms. After the inoculation of the organisms, compounds and controls, the plates were incubated for 24 h at 37 °C. Zones of inhibition were recorded and presented in mm.

The tested fungi used in this study were: *Candida albicans* (ATCC 10231), *Saccharomyces cerevisiae* (ATCC 9763) and *Aspergillus brasiliensis* (ATCC 16404). Sabouraud dextrose agar (Torlak, Belgrade, Serbia) was prepared according to the manufacturer's instructions. Into each sterile Petri dish (90 mm diameter), 22 mL previously prepared agar suspension was poured and 100 μL fungi (10^5 spores per dish) was added. A well with a diameter of 8 mm was punched using a sterile cork borer. Into each well 100 μL test substance (1 mg/100 μL DMSO) was added. Nystatin (30 μg/100 μL DMSO) was used as a positive control, whereas 100 μL DMSO served as a negative control. The plates were incubated for 48 h at 24 °C. Antifungal activity was determined by measuring the diameter of the inhibition zone.

RESULTS AND DISCUSSION

Chemistry

The condensation of appropriate aldehyde or ketone and carbohydrazide at 1:0.5 and 1:2.1 mole ratio were used for the syntheses of mono- and bis-carbohydrazone, respectively (Scheme 1). The structures and results of characterization of the synthesized compounds, are reported in the Supplementary material.

In the infrared spectrum of the carbohydrazones the absorptions between 1674–1681 cm^{-1} (mono-) and 1696–1708 cm^{-1} (bis-) were attributed to the ν(C=O) stretching, δ(N–H) and ν(C–N) vibrations due to contributions of both amide(I) and amide(II) modes,[29,30] while the absorption between 1604–1640 cm^{-1} is assigned to the ν(C=N) vibration. Absorptions between 3282–3316 cm^{-1}, attributed to the ν(NH_2) vibration in the spectrum of the mono-carbohydrazones, disappears in the spectra of bis-carbohydrazones suggesting that condensation of free terminal amino group with another mol of the aldehyde or ketone was successful.

For compounds **2** and **4** proton and carbon chemical shifts were assigned by a combined use of one- (^{1}H and ^{13}C proton decoupled, Figs. S-3, S-4, S-7 and S-8) and two-dimensional NMR experiments (COSY, HSQC and HMBC). In order to assess conformation of **2** and **4** in solution two-dimensional NOESY sequence was applied.

It is known that carbohydrazones can adopt (*E*)- or (*Z*)-configuration around the C=N double bond.[22] The (*E*)-configuration of the hydrazone moiety in the **2** (Fig. S-2) can be evidenced from 2D NOESY spectrum (Fig. 1) in which protons at C7 and N3 are correlated. Also, additional evidence was obtained from 2D ^{15}N-HMBC spectrum of **2** (Fig. S-5) where no observable connectivity of proton at C7 and N1 nitrogen was found. It iss interesting to note that exposure of substance **2** to sunlight in the solid state for several days leads to *trans–cis* photoisomerization, which can be noticed by the color change from white to yellow. Analogous phenomenon was described in the literature for (*E*)-4-phenyl-1-(pyr-

idin-2-ylmethylene)semicarbazide which exhibited photochromism in both solution and solid state.[26]

2-Benzoylpyridine semicarbazone analogues of compound **4**, can have the (*E*)-[31] and (*Z*)-configuration[32] (Fig. S-6). For the compound **4** lack of correlation between proton N3 with the phenyl nucleus indicates that the compound adopt the (*Z*)-conformation (Fig. S-9). The presence of (*Z*)-conformation of compound **4** can be also confirmed from 2D ^{15}N-HMBC spectra which shows connectivity of the nitrogen N1 and N3 proton (Fig. S-10).

It is known that chemical environment of the N3 proton is different in the (*E*)- or *anti* isomer from that for the (*Z*)- or *syn* isomer. (*Z*)-isomer of phenyl hydrazones shows a peak on higher chemical shift for the N3 protons. The downfield shift of the N3 protons, found at 13.20 ppm, for the (*Z*)-izomer of compound **4** is consistent with hydrogen bonding between the N3 proton and the aromatic nitrogen in 2-pyridyl group (Supplementary material; **4**-b).[33]

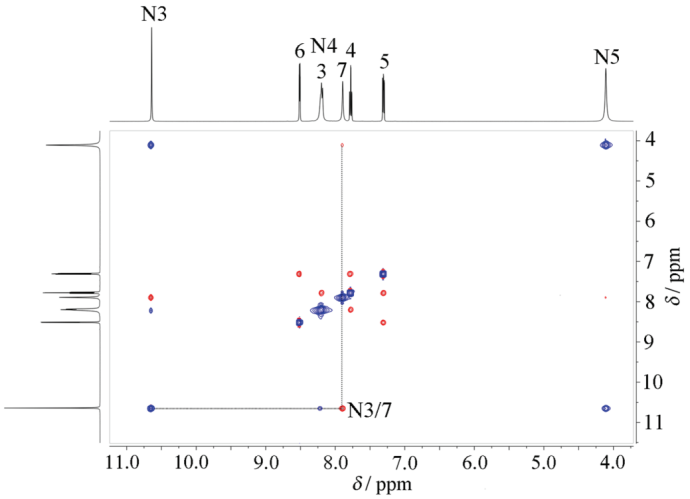


Fig. 1. 2D NOESY of **2** in DMSO-d_6 at 298 K (*x*- and *y*-axis, ^{1}H-NMR chemical shift).

In the ^{13}C-NMR spectra of all studied compounds, the number of the signals fitted exactly the number of carbon atoms. Elemental analysis results were within ±0.4% of the theoretical values for all compounds.

*Structural investigation of **4** in dimethyl sulphoxide/water mixtures*

Structural investigation of the compounds of interest, related to their conformation, which could originate from the isomerization/tautomerism phenomena, *i.e.*, stereochemistry of studied molecules, generally offered a wealth of fundamental information necessary to establish appropriate structure–properties relationships. A variety of spectroscopic methods could be used for analysis of

the state of tautomeric equilibria, *i.e.*, study of the mechanism of tautomer transformation. The simple and effective technique, usually used for studying of the tautomeric equilibria is based on advantageous characteristics of UV–Vis spectroscopy: diversity of spectral properties of the corresponding tautomeric forms, sensitivity of the tautomeric equilibria to the effect of surrounding solvent dipolarity/polarizability, solvent basicity and acidity, substituent effect present at solute molecule, as well as operational temperature.

The small free energy difference between tautomers, *e.g.*, **1**-b and **1**-c (Fig. S-1), makes them sensitive to the influence of environment, *e.g.*, pH, temperature, acidity/basicity, and solvent properties, and substituent effects, *e.g.* position and electronic effects, and inter- and intra-molecular interactions due to creation of hydrogen bonds.[34] Influences of external factors such as the addition of water could support tautomeric conversion of the existing forms, as it was shown for structurally similar equilibrium of the ketoamine/enolimine forms.[35] Analogous study performed, according to one presented in literature, did not show the formation of ketoamine tautomer upon water addition which is usually accompanied by appearance of a new band in the spectral region at ~400 nm.[35]

Except of the analysis of presence of tautomeric forms, it could be recognized possible existence of a number of conformers (rotamers) of investigated compounds due to free rotation around of any present single bond. Absorption spectra of isomers are generally different and could be useful for detection/quantification of solvent-induced isomerisation. It is known that the spectral behavior of an organic molecule is strongly related to its electronic structure in both ground and excited states, and the knowledge of the solvent effect on absorption spectra is of particular importance. Indeed, a change of solvent is accompanied by a variation of polarity, dielectric constant or polarizability of the surrounding medium. It is shown that (*E*)/(*Z*)-photoisomerization of pyridine substituted semicarbazide Schiff bases in solution take place under influence of UV irradiation. This is confirmed by shifting of the absorption maxima toward higher wavelengths. Furthermore, the position of the pyridine nitrogen atom with respect to the C=N double bond is important too, and the *ortho*-nitrogen position leads to the most photosensitivne sequence.[26,36]

For structurally related aroylhydrazones, it has been reported that (*E*)-isomers prevail in non-aqueous media, while water induces changes in conformation leading to the formation of (*Z*)-isomers,[37] and formation of hydrated molecules.[25] The (*E*)/(*Z*) isomerisation of hydrazones could be noticed in UV spectra since (*E*)- and (*Z*)-isomers have different absorption maxima. Absorption maximum wavelengths of the (*Z*)-isomer of hydrazones are 5–8 nm lower than those for the (*E*)-isomer. At the same time, a batochromic shift from 297 to 302 nm indicate a possible (*E*)/(*Z*) isomerisation of mono-substituted hydrazone in the system with changing solvent properties.[36] A study on possible isomerization (Fig. S-6) have

been performed with all investigated compounds, and example was given for **4** as the most active carbohydrazone derivatives. All the carbohydrazones in a solution of DMSO were in the form of (*E*)-isomer, except compound **4**. After addition of water absorption maximum was shifted to lower wavelengths which was in agreement with the literature data.[25] The most active antioxidant component **4** in the DMSO solution was present in the (*Z*)-form, which was also confirmed by the analysis of 2D NOESY spectrum (Fig. S-9).

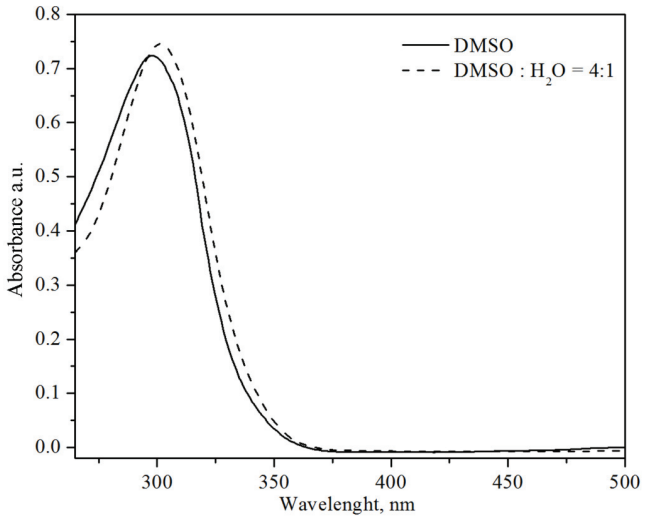


Fig. 2. UV–Vis spectra of **4** in DMSO and DMSO/H_2O mixtures: V(DMSO)/V(H_2O) = 4/1.

The absorption electronic spectra of **4** were acquired in pure DMSO and mixtures of DMSO/water. The bands in the spectral region 289–303 nm can be assigned to the azomethine C=N double bond and aromatic rings.[38] Arrows (dashed line) show the change in absorbance, *i.e.*, solvent induced (*E*)/(*Z*) isomerisation equilibria shift, with the increase in water content in DMSO/water mixtures. The addition of water into the system caused increase absorption maxima at 297 nm for approximately 0.08 a.u. (Fig. 2), and the absorption maximum wavelengths were higher for the (*E*)-isomer by 5 nm. The contribution of the (*E*)--isomer increases with the fraction of D_2O in the mixed solvents, judging whether the imino protons take part in the creation of intramolecular hydrogen bonding. These spectral changes accompanied by the occurrence of isosbestic points was assigned to the hydrogen bond formation between the -NH protons and solvent molecules.[25] As an evidence on significance of molecular conformation and hydrogen bonding the signals attributable to –NH and $–NH_2$ protons appeared as singlets at downfield δ: 4.15, 8.26 and 13.20 ppm, disappear upon adding D_2O,

which is the evidence that breakage of intermolecular hydrogen bonds take place causing conformational adaption of molecule.

DPPH free radical scavenging activity

The interaction of synthesized compounds **1**–**14** with stable DPPH free radical indicates their scavenging ability/generated free radical stability. The DPPH assay was believed to involve hydrogen atom transfer reaction[39] which cause generation of the new radical. In that way it could be postulated that higher capacity/ability of compounds to lose hydrogen, *i.e.*, creation of structurally stable radical is correlated with higher scavenging activity of studied molecule. Except of other factors, *e.g.*, solvent properties, structure of the studied compounds and electronic properties of substituents have a crucial role to the stabilization of generated radical. Appropriate structure–activity relationships (SARs) were generally established in relation to the presence and position of substituents, the presence of a pyridine ring and on the position of nitrogen atom relative to the N–H bond.[40] Also, the antioxidant properties of some diarylamines in the benzo[*b*]thiophene series were reported, and appropriate SARs was established with respect to position of arylamination, either on the benzene or on the thiophene ring, and in the presence of different substituents on both rings.[41]

In that context antioxidant activity of synthesized compounds was determined, while activity of substances **5**–**7** and **12**–**14** was already published.[7] The studied compounds showed wide variation of antiradical activity by inhibiting DPPH radical, calculated according to Eq. (1), and obtained results are given in Table I. Ascorbic acid was used as the reference compound (positive control) that of the standard (vitamin C) at a similar concentration. Presented results indicate that mono-derivatives are more active than corresponding bis-carbohydrazone analogues in DPPH radical scavenging which indicates a significance of the $–NH–NH_2$ group to the higher effectiveness of their antioxidant activity[7]. It was shown that presence of hydroxyl groups usually contribute to increases of antioxidant activity[7], and for compounds **1** and **8** lower IC_{50} indicate that intermolecular hydrogen bonding (Fig. S-1; **1**-a) contribute to higher stability of OH hydrogen, while after substraction of hydrogen generated radical was not involved in this type of stabilization.[42] Compound **4** is most active and its IC_{50} values was similar to that found for vitamin C (Table I).

Considering DPPH scavenging activity of studied compounds, the phenolic type moieties would be expected to be more active than the amino containing ones due to the lower bond dissociation energies of O–H than that of N–H. In this case of **1** similar value of antioxidative activity with respect to **6** was noticed, and significantly lower with respect to the most active compound **4**. The structure and position of the substituents in carbohydrazone plays an important role to antioxidative activity. Substituent present at azomethine carbon, capable to parti-

cipate in extended π-electron delocalization, significantly contribute to the stabilization of generated radical. The most active compound **4** contain 2-pyridyl ring substituted and phenyl group at benzylic carbon which imply that the extended π-delocalization play crucial role in a resonant stabilization of created radical. Except for this electron-withdrawing capability, the aza nitrogen contributed to the radical stabilization, e.g. higher activity of **5,** *i.e*., 2-qunolinyl derivate, in relation to **7,** *i.e.*, 8-qunolinyl derivate. It is also known, and in accordance with obtained result, that the compound with 2-napthyl group exhibited very good antioxidant activity.[43] In the other hand bis-substituted carbohydrazone showed in general lower antioxidative activity, and similar trend could be observed in relation to mono-substituted derivatives. Again it could be discussed that phenolic type of compounds, **8** and **13**, showed lower activity than **11**, while in general all bis-substituted compounds showed lower activity due to steric hindrance of active hydrogen and difference in chemistry of amino hydrogen showing lower capability in reaction with DPPH.

TABLE I. IC_{50} values (mM) determined by DPPH method

Tested compound	IC_{50} / mM	Tested compound	IC_{50} / mM
1	0.25±0.02	**8**	6.48±0.20
2	0.60±0.06	**9**	49.9±0.13
3	0.86±0.10	**10**	12.6±0.11
4	0.09±0.01	**11**	0.83±0.18
5	0.67±0.05	**12**	10.5±0.04
6	0.21±0.03	**13**	1.50±0.08
7	0.87±0.07	**14**	16.4±0.12
Acsorbic acid	0.08±0.01		

Antimicrobial evaluation

The *in vitro* antimicrobial activity of all given compounds **1**–**14** was tested against Gram-positive bacteria: *Bacillus subtilis*, *Staphylococcus aureus*, *Clostridium sporogenes* and *Kocuria rhizophila*, Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus hauseri* and *Salmonella enterica* subsp. *enterica* serovar enteritidis, as well as against fungal species: *Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus brasiliensis* by an agar well diffusion method. Some of the tested compounds show moderate activity towards Gram-positive and Gram-negative bacteria (Table II).

Compounds **6** and **7** are the best antimicrobial agents with inhibition zones ranging from 14–18 mm against Gram-positive and Gram-negative bacteria. All tested components show poor antifungal activity (Table S-I).

The relationships between the bioactivity data and the structure of studied compounds indicate that the antimicrobial properties depend on the type of condensed aldehydes or ketones present in the structure of carbohydrazones. In fact,

almost all pyridine mono- and bis-substituted carbohydrazone derivatives show lower inhibition zone than the quinoline substituted ones. Furthermore, the presence of an methyl and phenyl substituent in **3** and **4,** *i.e.*, compounds based on 2-acetylpyridine and 2-benzoylpyridine, also contributed to decreases of antimicrobial activity. It indicates that steric effect at azomethine carbon substituent cause decrease of their activity. It is interesting that some bis-substituted carbohydrazones (**8**–**10**) showed moderate activity against Gram-positive bacteria, whereas against Gram-negative bacteria exhibited no activity. Obtained activity depends on the studied compound properties (lipophile/hydrophile balance, geometry, proton accepting/donating capabilities, *etc*.) and the membrane structure/ /properties of the bacteria. The outer layer of the membrane of Gram-negative bacteria is composed primarily of lipopolysaccharide, while a thin peptidoglycan layer is situated below the lipopolysaccharide layer. In this way, Gram-negative bacteria are protected against the effect of drugs which are hydrophilic in the nature. The lack of activity of compounds **8**–**10,** observed against Gram-negative bacteria, is due to the higher permeability barrier conferred by the outer membrane. On the other hand, Gram-positive bacteria are sensitive to the effects of lysozyme and hydrophilic drugs.[44] In general compounds with quinolinyl moieties showed wide range of antimicrobial activities toward Gram-negative to Gram-positive bacteria. With regard to quinoline based compounds, those which have the nitrogen in position 8 (**7**) showed better antimicrobial and antifungal activity.

TABLE II. *In vitro* antimicrobial activity of the compounds tested by the well-diffusion agar assay expressed as the diameter (mm) of the inhibition zone (includes diameter of the wells 8 mm)

Tested compound	*E. coli*	*P. aeruginosa*	*P. hauseri*	*S. enterica*	*B. subtilis*	*S. aureus*	*C. sporogenes*	*K. rhizophila*
1	–	–	–	–	–	–	–	–
2	10	12	10	10	10	10	10	10
3	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–
5	12	12	12	12	12	12	12	12
6	14	14	16	16	14	18	14	18
7	18	16	18	16	16	18	16	18
8	–	–	–	–	10	10	10	10
9	–	–	–	–	10	10	10	10
10	–	–	–	–	12	12	12	12
11	–	–	–	–	–	–	–	–
12	12	12	12	12	12	12	12	12
13	12	12	12	12	12	12	12	12
14	12	12	12	12	14	12	14	12
Amikacin	42	20	24	24	22	32	20	22

CONCLUSIONS

In the presented work a series of mono- and bis-carbohydrazone was synthesized, and their properties, antioxidant and antimicrobial activity were investigated. An isomerization study showed that all the carbohydrazones in a solution of DMSO were in the form of (*E*)- isomer, except compound **4**. (*E*)/(*Z*) isomerization was exemplified for compound **4** in DMSO and DMSO/water mixtures, by means of the analysis of UV–Vis spectra. In pure DMSO hydrazones exist as (*Z*)-isomer in ketoamine form, with respect to the hydrazide part, while (*E*)-isomer was noticed in the mixed DMSO/water solutions. Based on the analysis of 2D NOESY spectra is was determined that **2** and **4** occupy (*E*)- and (*Z*)--conformation, respectively. The results of the antioxidant screening by the DPPH radical scavenging assay revealed that compound **4** showed excellent antioxidant activity close to that found for vitamin C. The results of the antimicrobial screening revealed that compounds **6** and **7** are the best antimicrobial agents with respect to both Gram-positive and Gram-negative bacteria. All tested components showed poor antifungal activity.

SUPPLEMENTARY MATERIAL

Spectral data of the synthesized compounds are available electronically at the pages of journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА, АНТИОКСИДАТИВНА И АНТИМИКРОБНА АКТИВНОСТ КАРБОХИДРАЗОНА

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У раду је синтетисано четрнаест моно- и ди-хидразонских лиганада. Испитивана је антиоксидативна активност супстанци као и (*E*)/(*Z*)-изомеризација и кето-енолна таутомерија, која је објашњена на примеру најактивније супстанце **4** у смеши диметилсулфоксида и воде. Додатак воде у систем проузрокује формирање хидратисаних молекула што је потврђено НМР спектрима након додатка деутерисане воде. Испитана је антимикробна активност на Грам-позитивне бактерије, Грам-негативне бактерије и гљивице и дискутована је повезаност између активности и структуре карбохидразона.

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