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Experimental and theoretical study of antioxidative properties of some salicylaldehyde and vanillic Schiff bases†

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The antioxidative capacity and structure–activity relationships of ten Schiff bases were investigated experimentally and theoretically. All compounds contain the aniline moiety, while the aldehyde part is either salicylaldehyde or vanillin. The DPPH assay was used to test the potential antioxidative activity of these compounds, and DFT study was used to investigate their electronic structures and provide insight into their structure–activity relationships. The effect of the position of the hydroxy, as well other groups present, on the antioxidative activity was examined. The possible radical scavenging mechanism was determined in polar (water and methanol), and nonpolar (benzene) solvents. Based on the experimental and computational results, compounds 7 and 8 exhibit the highest radical scavenging properties.

Introduction

Schiff bases are compounds that were first obtained in the condensation reactions of aromatic amines and aldehydes (1864).[1] They are also known as imines or azomethines.[2] A wide range of these attractive compounds, with the general formula RHC═N–R1 (R and R1 can be alkyl, aryl, cycloalkyl or heterocyclic groups), have been synthesized to date. Schiff bases are of great importance in the field of coordination chemistry because they are able to form stable complexes with metal ions.[3] The chemical and biological significance of Schiff bases can be attributed to the presence of a lone electron pair in the sp^2 hybridized orbital of the nitrogen atom of the azomethine group.[4] These imines are used in the fields of organic synthesis, chemical catalysis and analysis, medicine, pharmacy, as well as other new technologies.[5] The antitumor, antiviral, antifungal and antibacterial properties of these compounds means they have found applications in medicine and pharmacy.[6] Due to these biological properties, Schiff bases are used as basic materials for the synthesis of many drugs.[7] It has also been reported that Schiff bases of salicylaldehydes show some antimicrobial activity.[8]

The ability to scavenge free radicals is a common feature of phenolic compounds. Antioxidative activity of phenolic Schiff bases (SB–OH) is directly related to their ability to release hydrogen atoms. A few different mechanisms of free radical scavenging are known: hydrogen atom transfer (HAT), single electron transfer followed by proton transfer (SET-PT), and sequential proton loss electron transfer (SPLET).[9] All these mechanisms have the same net result, *i.e.* the formation of corresponding phenoxy radical.[10]

HAT mechanism is the only one which consists of one step in which hydrogen atom is transferred to free radical.[11]

$$\text{SB–OH} \rightarrow \text{SBO}^{\bullet} + \text{H}^{\bullet} \quad (1)$$

SET-PT and SPLET mechanisms consist of two steps. In SET-PT mechanism, the first step is characterized by process in which one electron is lost and radical cation is created, whereas in the second step radical cation is deprotonated and corresponding radical is formed.[9b,12]

$$\text{SB–OH} \rightarrow \text{SB–OH}^{\bullet+} + \text{e}^{-} \quad (2.1)$$

$$\text{SB–OH}^{\bullet+} \rightarrow \text{SB–O}^{\bullet} + \text{H}^{+} \quad (2.2)$$

In SPLET mechanism, the first step is deprotonation of parent molecule. In the second step the anion formed loses an electron and corresponding radical is formed.[13]

$$\text{SB–OH} \rightarrow \text{SB–O}^{-} + \text{H}^{+} \quad (3.1)$$

$$\text{SB–O}^{-} \rightarrow \text{SB–O}^{\bullet} + \text{e}^{-} \quad (3.2)$$

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These mechanisms are described by thermodynamical parameters: bond dissociation enthalpy (BDE) related to eqn (1), ionization potential (IP) related to eqn (2.1), proton dissociation enthalpy (PDE) related to eqn (2.2), proton affinity (PA) related to eqn (3.1), and electron transfer enthalpy (ETE) related to eqn (3.2).

It is known that some phenolic Schiff bases act as effective antioxidants and potential drugs that can prevent disease caused by free radical damage.[14] However, the antioxidative activity of this class of polyfunctional compounds deserves further investigation. Also, further advance in analysis of their structure–activity relationship, particularly how the position of the hydroxy group effects the reactivity of these phenolic compounds towards radicals is needed. In this sense, we put under consideration some salicylaldehyde and vanillic Schiff bases using experimental and theoretical tools.

The first part of this work is devoted to investigation of antioxidative capacity of ten phenolic Schiff bases, depending on substitution on the both phenyl rings – aldehyde and aniline. To fulfil this, DPPH assay is selected as method. Choice was made due to its well-known application in determination of the antioxidative activity of compounds,[15] and due to fact that it can be used in prediction of activity against reactive oxygen species present in the living cells.[16]

Polarity of solvents plays significant role and specifies which mechanism to overcome. Bearing in mind this, further important aim of this paper was to estimate the solvent effects to the reaction enthalpies. To complete this, water and methanol were used as polar, whereas benzene was used as nonpolar solvent. To our best knowledge, this type of compounds, which can be considered as imine analogues of good antioxidant resveratrol, has not been subjected to this kind of study.

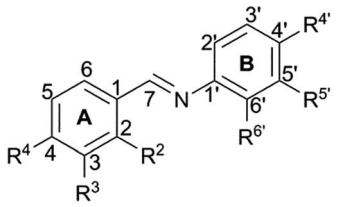
Results and discussion

In the reaction of aldehyde (salicylaldehyde or vanillin) and aromatic amine (aniline, 4-fluoroaniline, 4-nitroaniline, toluidine, 2-hydroxyaniline, 3-hydroxyaniline or 4-hydroxyaniline) in methanol, a series of ten Schiff bases was synthesized (**1–10**, Fig. 1), wherein compound **10** is newly synthesized. The selection of these compounds was based on their structural characteristics, such as positions of hydroxy and methoxy groups in rings **A** and **B**.

DPPH test

All of the obtained compounds were subjected to evaluation of their antioxidative activity in DPPH test, Tables 1 and S1 ESI.† It was found that compounds **2–6** are poor radical scavengers, while **1**, **9**, and **10** turned to be more active. Schiff bases **7** and **8** interact well with DPPH radical and exhibit high, slightly lower, activity than the reference compound NDGA. On the basis of this, compounds **7** and **8** can be considered as good antioxidants.

Obtained results in this study suggest that position of the hydroxy groups in the Schiff bases plays decisive role in the antioxidative activity. Namely, the active compounds possess



methoxy groups in rings **A** and **B**.

Fig. 1 General structural formula of the investigated Schiff bases **1–10**.

p-hydroxy group in the ring **A** (**8–10**), or *o*-hydroxy group in the ring **B** (**7** and **8**), relatively to the positions 1 and 1′, Fig. 1. Less active compounds (**2–5**) are hydroxy substituted only in *o*-position of the ring **A**, while compound **6** bears additional hydroxy group in *m*-position of the ring **B**. The only difference between compounds **1** and **7** is substitution in the ring **B**, *p*- and *o*-respectively. On the basis of the IC_{50} values, it can be concluded that *o*-hydroxy position in the ring **B** is responsible for radical scavenging of these compounds. Common for the Schiff bases **8–10** is that hydroxy group is present in *p*- and methoxy group in *m*-position in the ring **A**. Taking into account IC_{50} for these molecules, as well as for the compound **1**, one can conclude that presence of *p*-hydroxy group in the ring **A** contributes more to antiradical activity than equivalent substitution in the ring **B**. Observation that appearance of hydroxy group in the *p*-position of the ring **A**, as well as in *o*-position of the ring **B** contributes to the highest extent to the radical scavenging activity is additionally supported by the fact that the most active compound **8** possess hydroxy groups in both positions. We note in passing that substitution of the ring **B** by electron donor or acceptor functional groups, had negligible impact on the antioxidative activity.

The low activity of compounds **2–5** towards radical scavenging activity can be rationalised on the basis that only present hydroxy group in *o*-position in the ring **A** can form intramolecular hydrogen bond with nitrogen, and thus will be prevented to interact with DPPH. Although Schiff base **6**, in addition to the hydroxy group in *o*-position in the ring **A**, has another one in the *m*-position in the ring **B**, it is reasonable to expect that radical obtained in *m*-position will not be stabilised by delocalisation of its unpaired electron over the entire molecule, but only over ring **B**. On the other hand, in active compounds, this stabilisation through delocalisation over both rings is possible.

Performed DPPH test provided insight into potential antioxidative activity of the investigated compounds. However, to obtain full insight in the structure–activity relationship of the investigated Schiff bases, further investigation on the electronic structure of these compounds was necessary. For the sake of completeness, DFT study of the compounds subjected to this examination was performed.

Table 1 Calculated and experimental properties of investigated Schiff bases

Compound	HOMO (eV)	LUMO (eV)	HOMO–LUMO gap (eV)	ΔE_{iso} (kJ mol^{-1})	IC_{50} (μM)		
Methanol							
1	−0.274	−0.039	0.234	−13.038	561.3 (ref. 17)	117.4 (ref. 18)	186.3[a]
2	−0.293	−0.075	0.218	41.638			>500[a]
3	−0.280	−0.040	0.239	42.042			>500[a]
4	−0.285	−0.041	0.244	43.145			>500[a]
5	−0.285	−0.042	0.243	42.827			>500[a]
6	−0.283	−0.043	0.240	1.896	468.2 (ref. 17)	406.9 (ref. 18)	>500[a]
7	−0.280	−0.046	0.234	−3.678	27.4 (ref. 17)	98.5 (ref. 18)	18.8[a]
8	−0.269	−0.041	0.227	−11.227			5.3[a]
9	−0.273	−0.033	0.240	−13.385			86.2[a]
10	−0.273	−0.034	0.239	−13.188			68.8[a]
Water							
1	−0.274	−0.040	0.234	−13.015			
2	−0.293	−0.075	0.218	41.310			
3	−0.280	−0.041	0.239	41.656			
4	−0.285	−0.042	0.244	42.775			
5	−0.285	−0.042	0.243	42.486			
6	−0.283	−0.043	0.240	1.961			
7	−0.280	−0.046	0.234	−3.946			
8	−0.269	−0.041	0.227	−11.479			
9	−0.273	−0.033	0.240	−13.592			
10	−0.273	−0.034	0.239	−13.422			
Benzene							
1	−0.272	−0.037	0.235	−13.301			
2	−0.295	−0.073	0.221	48.283			
3	−0.278	−0.038	0.239	49.522			
4	−0.283	−0.040	0.243	50.509			
5	−0.284	−0.042	0.242	49.611			
6	−0.281	−0.042	0.240	1.050			
7	−0.279	−0.047	0.232	1.124			
8	−0.266	−0.040	0.226	−6.238			
9	−0.270	−0.029	0.240	−9.005			
10	−0.271	−0.032	0.239	−8.325			

[a] IC_{50} values obtained in this study.

Density functional theory

All geometrical and conformational isomers of the investigated Schiff bases were determined, and their energies calculated. The most stable isomers of all compounds are presented in Fig. S1 of ESI.†

To verify the quality of structures predicted by theoretical calculations, available crystal structures (those for compounds **1** and **7**)[19] were compared to their optimized structures (Tables S2 and S3,† respectively). As expected, the obtained geometrical parameters for all solvents used are in mutual, excellent agreement. Furthermore, the calculations reproduced the experimental bond lengths and angles, as well as dihedral angles very well. Some deviations between experimental and calculated structural characteristics are, certainly, a consequence of the fact that experimental values refer to the solid state, whereas calculated values refer to the solution. As the skeleton of other molecules under investigation is identical with these two, we can assume that their geometries are also well determined. Furthermore, assumption that Schiff bases **1–7** form hydrogen bond between hydroxy group in *o*-position of the ring **A** and nitrogen from C=N is confirmed. On the basis of the lengths of these intramolecular hydrogen bonds (Tables S2 and S3†) they can be considered as strong.

HOMO and LUMO

The HOMO and LUMO are delocalized through the entire molecule for all studied Schiff bases (Fig. S2†). The energies of HOMO and LUMO are very important parameter in defining the reactivity of molecules, because they usually take part in chemical reactions. The molecule which has the lower E_{HOMO} has weak electron donating ability. On the other hand, the higher E_{HOMO} implies that the molecule is a good electron donor.[20] The E_{HOMO} values for the Schiff bases are shown in Table 1. The molecules with hydroxy group in *p*-position and methoxy group in *m*-position in ring **A** show the largest E_{HOMO} values of −0.269 eV for compound **8**, and −0.273 eV for compounds **9** and **10**. In contrast, molecules with hydroxy group in *o*-position in ring **A** (compounds **2–7**) show decreased HOMO values. Consequently, these compounds have weaker electron donating ability than other Schiff bases. The results obtained in

the present work indicate that the existence of structure that resembles vanillin in **A**-ring is important for the increased energy of HOMO orbitals, and thereby better antioxidative potential of these compounds. The *o*-OH group in ring **B** contributes to the increase of E_{HOMO}, and cannot be neglected. All these results are in accordance with the experimental data for IC_{50} (Table 1).

The HOMO–LUMO gap determines chemical reactivity. This energy is directly related to the easiness of excitation of investigated molecules. Data from Table 1 for HOMO–LUMO gap also suggest that **8** has the highest antioxidative potential since it has the lowest value.

Stabilization energies (ΔE_{iso})

Calculated ΔE_{iso} values are presented in Table 1. On the basis of obtained values it is possible to find relative stability for the involved hydroxy and methoxy groups of investigated Schiff bases. Applying stabilization energy is a simple method to predict the antioxidative potential to scavenge free radicals. Obtained results in this study confirm the importance of *p*-hydroxy group in the ring **A**, and *o*-hydroxy group in the ring **B**, in the stabilization of radical species obtained after hydrogen atom abstraction. The presence of an additional methoxy group decreased ΔE_{iso} for compounds **8–10**, because of the fact that oxygen atoms can donate lone electron pairs to stabilize the corresponding semiquinone free radical.

Bond dissociation enthalpy and proton affinity

Homolytic O–H bond cleavage of the investigated molecules yields corresponding radicals. The calculated BDE values are presented in Table 2. It is obvious that stability of the obtained semiquinone radicals plays a very important role in determining the antioxidative activity of Schiff bases. Distribution of spin density provides a reliable representation of reactivity and stability of free radicals.[21] The radicals with good spin delocalization are formed more easily and they are more stable than those with localized spin density. SOMOs for the most stable radicals of investigated Schiff bases are presented in (Fig. 2) and distribution of the spin density for all radicals in methanol are presented in Fig. S3.† In all radicals formed by homolytic cleavage of the OH group in *p*-position, either in the rings **A** or **B**, spin density is delocalized over the involved oxygen, and *o*- and *p*-carbon atoms of the corresponding aromatic ring. Additional stabilization is achieved by delocalization across the double CN bond, as well as across *o*- and *p*-carbon atoms of the adjacent ring. As a consequence Schiff bases with hydroxy groups in *p*-position have the smallest BDE values (compounds **1**, **8–10**). Table 2 shows that BDE values of Schiff bases with hydroxy group in *p*-position are lower than those with hydroxy groups in *m*- and *o*-positions. The main reason for somewhat higher BDE values of *m*-hydroxy groups lies in the fact that less stable radicals are formed, and that there is less delocalization of spin density *via* CN double bond and another aromatic ring (**6** in Fig. 2). As for hydroxy groups in *o*-position in the **A** ring, relatively strong intramolecular hydrogen bond with nitrogen is also responsible for significantly higher BDE values.

Table 2 Calculated thermodynamical parameters (kJ mol^{-1}) of antioxidative mechanisms for Schiff bases

	HAT		SET-PT			SPLET			
	BDE		IP	PDE		PA		ETE	
	A	B		A	B	A	B	A	B
Methanol									
1	407	351	542	56	0	183	149	414	392
2	406		624	−28		168		428	
3	406		559	38		181		416	
4	407		584	13		180		417	
5	407		586	11		179		419	
6	407	366	566	32	−9	178	152	419	404
7	396	360	555	31	−4	167	152	419	399
8	353	364	533	11	22	140	171	403	384
9	351		542	0		146		395	
10	351		541	0		146		396	
Water									
1	412	356	522	71	16	191	159	401	379
2	411		603	−12		177		415	
3	411		539	53		189		403	
4	412		564	29		189		404	
5	412		566	27		188		405	
6	412	371	546	47	7	187	161	406	391
7	401	365	535	47	11	176	161	406	386
8	358	369	513	26	37	150	179	389	371
9	356		522	15		155		381	
10	356		521	16		155		382	
Benzene									
1	415	352	636	189	126	465	416	359	345
2	414		661	163		434		390	
3	415		650	174		464		360	
4	416		665	161		462		363	
5	415		639	186		457		368	
6	417	367	661	165	115	458	426	368	350
7	400	367	651	159	125	437	429	373	347
8	359	370	621	148	159	409	456	360	324
9	357		630	136		422		345	
10	357		633	134		418		349	

Heterolytic cleavage of the O–H bonds of Schiff bases leads to formation of the corresponding anions. Geometrical parameters for anions of Schiff bases **1** and **7** are listed in Tables S2 and S3.† In comparison to the parent molecules, there are significant changes in bond lengths in the ring where anion is formed, suggesting decrease of aromaticity of this ring. All anions, with corresponding charge distribution obtained by NBO analysis, are presented in Fig. S4.† The charge distribution shows that anions formed in the ring **A** are slightly better delocalized compared to those formed in the ring **B**. The decrease of negative charge on *o*- and *p*-oxygen atoms is a consequence of good delocalization of negative charge over the ring **A** and CN double bond, and across *o*- and *p*-carbon atoms of the adjacent ring (Fig. S3†).

Ionization potential

The ionization potential (IP) illustrates the easiness of electron donation of phenolic compounds. It is well known that

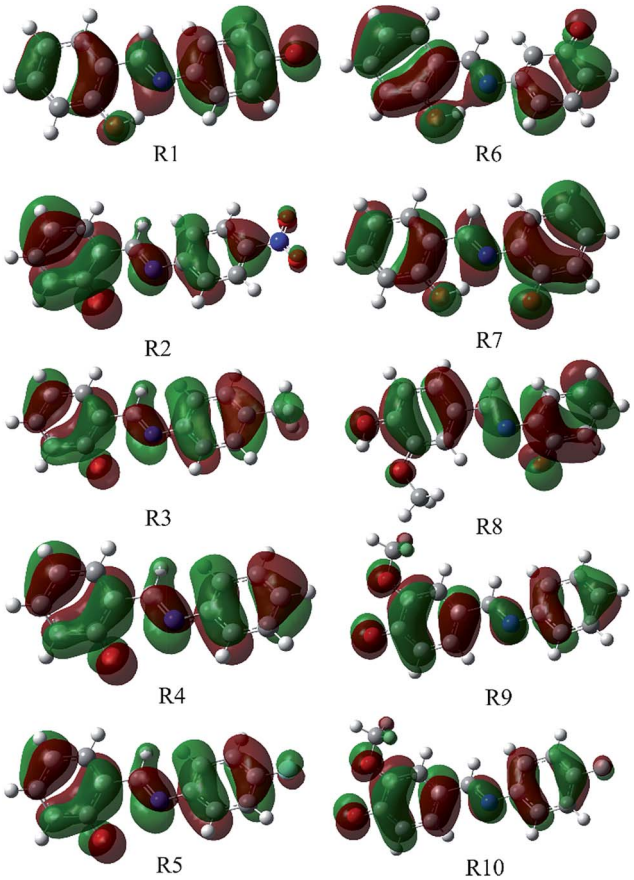


Fig. 2 SOMOs for the most stable radicals issued from the investigated Schiff bases.

molecules with lower IP values are more active. The obtained IP values of investigated compounds are presented in Table 2. Comparison of the IP values from Table 2 with IP values of other Schiff bases,[22] showed that the values obtained in this study are generally somewhat lower. This is due to using different approaches for calculating these parameters. On the basis of our results, molecules with only one *o*-hydroxy group in the ring **A** (compounds **2–5**) have generally higher IP values. Introducing another hydroxy group in the ring **B** in *m*-, *o*-, or *p*-position (compounds **6**, **7**, and **1**), additionally reduces the IP value. This is consistent with the well-known fact that position of the hydroxy group in the molecule plays a very important role for the electron donating capacity. The IP values decrease in molecules with additional methoxy group in *m*-position (compounds **9** and **10**). Moreover, introduction of another hydroxy group in ring **B** in *o*-position, additionally reduces the IP values (compound **8**). This implies that this compound has increased electron donor capacity, which facilitates the formation of the radical cation.

Antioxidative mechanisms

On the basis of values of thermodynamical parameters (BDE, IP, PDE, PA, and ETE) prevailing antioxidative mechanism of Schiff bases in a corresponding solvent can be predicted.[23] The lowest value for some parameter points out which reaction mechanism is thermodynamically more probable. All thermodynamical parameters for the investigated Schiff bases were calculated using M05-2X/6-311+G(d,p) in water, methanol, and benzene (Table 2). The obtained results show that compounds **2–6** have significantly higher BDE, IP, and PA values than other compounds under investigation. The values of thermodynamical parameters undoubtedly show that negligible antioxidative activity can be expected for these compounds. These results are in agreement with experimental IC_{50} values Table 1. Mutual comparison of BDE, IP, and PA values in Table 2 for other compounds (**1**, **7–10**) reveals that IP values are the largest, indicating that SET-PT mechanism is not a favourable reaction path in all investigated solvents.

On the other hand, in polar solvents PA values are significantly lower than the corresponding BDEs. It means that the SPLET mechanism is a dominant reaction pathway in polar medium. Low PA values show that Schiff bases can easily undergo heterolytic dissociation of OH bonds and yield the corresponding phenoxide anions. Taking this into account, it is reasonable to expect that SPLET mechanism prevails under physiological conditions (pH of 7.4). BDE and PA values in Table 2 show that HAT and SPLET mechanisms are competitive in nonpolar solvent.

Conclusions

In this study, antioxidative capacity of some phenolic Schiff bases has been examined, using experimental and theoretical tools. Two of investigated compounds, one salicylaldehyde (**7**) and one vanillic (**8**) Schiff base, can be considered as good radical scavengers. *p*-Hydroxy group in ring **A**, as well as *o*-in the ring **B**, are responsible for the antioxidative activity of these compounds. Furthermore, DFT examination showed that presence hydroxy groups in respective positions influences the increase of E_{HOMO}, lowers HOMO–LUMO gap, and in that way contributes to better antioxidative potential of these compounds. Low activity of Schiff bases **2–6** is assigned to the intramolecular hydrogen bond formation between *o*-hydroxy group in the ring **A** and nitrogen from C=N, which consequences significantly higher values for the thermodinamical parameters for these compounds. Based on the same data for the molecules which exhibit radical scavenging activity (**1**, **7–10**), SET-PT mechanism is not expected in all investigated solvents. In polar medium, SPLET mechanism prevails, while HAT and SPLET mechanisms are competitive in nonpolar solvent. Taking into account that Schiff bases **7** and **8** interact well with DPPH radical, and fact that DPPH assay may indicate activity of compounds towards reactive oxygen species present in the living cells, these compounds can be considered as good antioxidants and will be further investigated *in vitro*/*vivo*, for example on the cancer cell lines.

Experimental

Materials and reagents

The compounds salicylaldehyde, vanillin, aniline, 4-fluoroaniline, 4-nitroaniline, toluidine, 2-hydroxyaniline, 3-hydroxyaniline,

4-hydroxyaniline, nordihydroguaeretic acid (NDGA), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Aldrich Chemical Co. All common chemicals were of reagent grade. The NMR spectra were run in DMSO on a Varian Gemini 200 MHz spectrometer. Melting points were determined on a Mel-Temp capillary melting points apparatus, model 1001. Elemental microanalyse for carbon, hydrogen, and nitrogen were performed at the Faculty of Chemistry, University of Belgrade.

Synthesis of Schiff bases

Schiff bases (**1–10**) were prepared according to procedure in the literature with some modifications.[24] In our case, aldehyde (salicylaldehyde, vanillin) (1 mmol), corresponding aromatic amine (aniline, 4-fluoroaniline, 4-nitroaniline, toluidine, 2-hydroxyaniline, 3-hydroxyaniline, 4-hydroxyaniline) (1 mmol), and 3 mL of methanol were placed in flask and stirred at 70 °C for 3 h. After completion of the reaction, the solvent was evaporated, and final product was obtained by recrystallization from ethanol. Schiff bases were obtained in 90–97% yield. All Schiff bases (**1–10**) were characterized with melting point and ^{1}H NMR spectra (ESI,† compounds **1–10**).

The corresponding data for the new compound (*E*)-4-((4-fluorophenylimino)methyl)-2-methoxy-phenol (**10**) are presented here: colourless crystals – mp 144–146 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ = 3.84 (s, 3H, $-OCH_3$), 6.89 (d, J = 8.10 Hz, 1H, Ar-H), 7.16–7.28 (m, 5H, Ar-H), 7.32 (dd, J = 8.3, 1.9 Hz, 1H, Ar-H), 7.52 (d, J = 1.80 Hz, 1H, Ar-H), 8.44 (s, 1H, CH=N), 9.77 (s, 3 –OH); ^{13}C NMR (50 MHz, DMSO) δ = 56.7, 111.8, 111.4, 116.1, 116.5, 116.6, 117.2, 123.6, 127.5, 145.3, 149.21, 151.1, 153.9, 162.1; $C_{14}H_{12}FNO_2$ (FW = 245.25): C, 68.56; N, 5.71; H, 4.93%; found: C, 68.15; N, 5.68; H, 4.81%.

DPPH free radical scavenging assay

In this study, the free radical scavenging activity of the examined Schiff bases was performed using the DPPH method, according to ref. 25. In brief, 1 mL (0.1 mm) of DPPH solution in methanol was mixed with an equal volume of the tested compound (20 μL of compound solution in DMSO and 980 μL of methanol). The reaction mixture is left at room temperature for 30 and 60 min. After incubation the absorbance was measured at 517 nm. As control solution, methanol was used. IC_{50} values represent the concentration necessary to obtain 50% of a maximum scavenging capacity. NDGA was used as positive control with 96% activity at 0.1 mM.

Computational details

All calculations in this paper were performed using the Gaussian program package.[26] The equilibrium geometries of all Schiff bases and corresponding radicals, radical cations, and anions were optimized by the empirical exchange-correlation M05-2X functional[27] and split-valence basis set 6-311+G(d,p). The M05-2X functional yields reasonable results for thermochemical calculations of organic, organometallic, and biological compounds, as well as for noncovalent interactions.[11a,28] This functional has been successfully used for solution of different problems by independent authors.[11b–e]

The local and global minima were confirmed to be real minima by frequency analysis (no imaginary frequencies were obtained). To evaluate the impact of different solvents (water, methanol, and benzene) the continuum solvation model CPCM was used.[29] The solvent effects were taken into account in all geometry optimizations and energy calculations. Water and benzene were used to mimic aqueous and lipid environments, whereas methanol was selected because the experiments were performed in this solvent. The NBO analysis of all species was performed at the M05-2X/6-311+G(d,p) level of theory.[30] The NBO analysis describes a structure by a set of localized bonding, antibonding, and Rydberg orbitals. Also, this analysis provides explanation of stabilizing and destabilizing interactions between occupied and unoccupied orbitals.

BDE, IP, PDE, PA and ETE values were determined from total enthalpies of the individual species, as follows:

$$\mathrm{BDE} = H(\mathrm{SB{-}O^{\bullet}}) + H(\mathrm{H^{\bullet}}) - H(\mathrm{SB{-}OH}) \quad (4)$$

$$\mathrm{IP} = H(\mathrm{SB{-}OH^{\bullet+}}) + H(\mathrm{e^{-}}) - H(\mathrm{SB{-}OH}) \quad (5)$$

$$\mathrm{PDE} = H(\mathrm{SB{-}O^{\bullet}}) + H(\mathrm{H^{+}}) - H(\mathrm{SB{-}OH^{\bullet+}}) \quad (6)$$

$$\mathrm{PA} = H(\mathrm{SB{-}O^{-}}) + H(\mathrm{H^{+}}) - H(\mathrm{SB{-}OH}) \quad (7)$$

$$\mathrm{ETE} = H(\mathrm{SB{-}O^{\bullet}}) + H(\mathrm{e^{-}}) - H(\mathrm{SB{-}O^{-}}) \quad (8)$$

The values for solvation enthalpies of proton and electron were taken from literature.[31] All reaction enthalpies defined in eqn (4)–(8) were calculated at 298 K.

The radical stability was determined by the calculation of stabilization energies (ΔE_{iso}), as shown in eqn (9), where Ph–OH and Ph–O$^{\bullet}$ stand for the molecule of phenol and phenoxy radical.

$$\Delta E_{iso} = (H(\mathrm{SB{-}O^{\bullet}}) + H(\mathrm{Ph{-}OH})) - (H(\mathrm{SB{-}OH}) + H(\mathrm{Ph{-}O^{\bullet}})) \quad (9)$$

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