Research Article

Synthesis, experimental and theoretical antiradical activity assessment of some azomethines and phenylhydrazones

Rafik Bensegueni[1,2] · Mounia Guergouri[2] · Chawki Bensouici[3] · Mustapha Bencharif[2]

Abstract
A set of four azomethines and two phenylhydrazones, labeled SIM, SIF, SIT, SIC, SIP and NIP, were synthesized. Afterward, they were subjected to antiradical activity tests (DPPH, CUPRAC and reducing power). Moreover, molecular modeling calculations at the density functional theory level were carried out on these molecules to determine some thermodynamic molecular descriptors (BDE, IP, PDE, PA and ETE) to analyze and explain the corresponding experimental results. Antiradical activity tests were very fulfilling showing a well-established antiradical power for the studied compounds especially in the case of CUPRAC assay in which all of our molecules show a more or less pronounced activity with NIP and SIP in top positions, displaying an $A_{0.5}$ values of 3.09 ± 0.08 µg/mL and 4.26 ± 0.44 µg/mL, respectively. These values are inferior to those of the used standard antioxidants; butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). The NIP compound exhibits the highest DPPH scavenging activity, followed by SIP, which was even greater than that of BHA and BHT, with an IC_{50} of 3.81 ± 0.26 against 6.14 ± 0.41 and 12.99 ± 0.41 µg/mL, respectively. The resulting molecular descriptors values and the calculated properties are in a good agreement with the experimental results. Therefore, the attained findings showed an excellent correlation between theoretical and experimental studies, jointly confirming the antiradical power of our compounds, which could allow them to be used in pharmaceutical or in food industries.

Keywords Azomethines · Phenylhydrazones · Antiradical activity · DFT calculations

1 Introduction

The antiradical compounds searching, continues to attract interest of scientists and industrial actors from pharmaceutical to food industries. Azomethines or Schiff bases, are compounds characterized by a structure of the form RN = CR'R", that have been widely studied. Their antiradical activity was investigated and demonstrated in several works [2, 23]. Schiff bases can also form with metal ions very interesting complexes with biological activities [6, 25, 28, 32]. Phenolic Schiff bases in particular, constitute a class of drugs for oxidative stress diseases [11]. Phenylhydrazones are compounds that contain a Ph–NH–N = CH–Ph group in their structures. Antiradical activity of phenylhydrazones and their derivatives was established in various studies [17, 31]. Other applications of these compounds have been cited in some articles in a wide range of fields. Thus, they are found as conductive polymers [15], or fluorescence sensors [19], or as catalysts [12]. Theoretical studies on this compounds kind are found in literature trying to make a structure-properties relationship and to correlate experimental findings to theoretical ones [9, 24].

In this work, a set of four azomethines and two phenylhydrazones were synthesized (Fig. 1), characterized with ^{1}H NMR and then subjected to three antiradical activity tests namely, DPPH scavenging activity test, cupric reducing antioxidant capacity test (CUPRAC) and ferric reducing

✉ Mustapha Bencharif, m_bencharif@umc.edu.dz | [1]Université Mohamed Cherif Messaadia, 41000 Souk Ahras, Algeria. [2]Laboratoire de Chimie des Matériaux Constantine, Université des frères Mentouri Constantine 1, 25000 Constantine, Algeria. [3]Centre de Recherche en Biotechnologie, Ali Mendjeli Nouvelle ville UV03, Constantine, Algeria.

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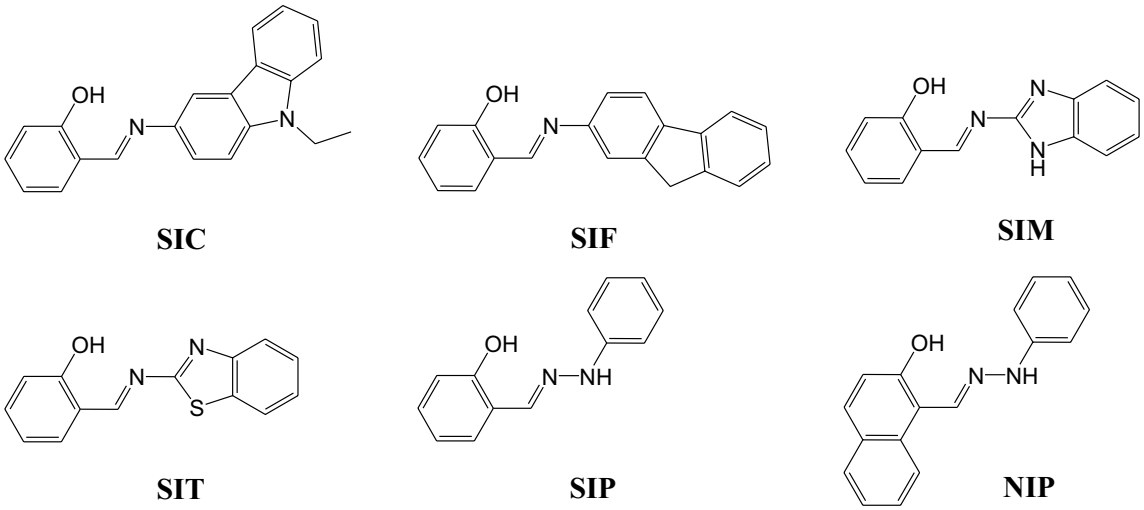


Fig. 1 Chemical structures of the studied compounds (azomethines: SIC, SIF, SIM and SIT, phenylhydrazones: SIP and NIP)

antioxidant power test (FRAP). Throughout this article, our six compounds will be labeled SIC, SIF, SIM and SIT for azomethines and SIP and NIP for phenylhydrazones. The theoretical part of this study relates to the calculation of some thermodynamic molecular descriptors of the tested molecules, with the density functional theory (DFT). The comparison of these descriptors allows proposing the most favorable mechanism for the free radicals scavenging activity. Hence, three mechanisms that may coexist could be observed. The first one is called hydrogen atom transfer (HAT), the second one is known as single electron transfer–proton transfer (SET-PT) and the third mechanism is the sequential proton loss electron transfer (SPLET) [20, 21]. The following equations illustrate the cited mechanisms:

$$\text{HAT} : RX - H \rightarrow H^{\cdot} + RX^{\cdot}$$

$$\text{SET} - \text{PT} : \begin{cases} RX - H \rightarrow (RX - H)^{+\cdot} + e^{-} \\ (RX - H)^{+\cdot} \rightarrow RX^{\cdot} + H^{+} \end{cases}$$

$$\text{SPLET} : \begin{cases} RX - H \rightarrow RX^{-} + H^{+} \\ RX^{-} \rightarrow RX^{\cdot} + e^{-} \end{cases}$$

Each mechanism is characterized by one or more descriptors such as for HAT which is related to the O–H bond dissociation enthalpy (BDE). The first step of the SET–PT mechanism is controlled by the ionisation potential (IP) while the second step is governed by the proton dissociation enthalpy (PDE) of the corresponding cation-radical. Finally, for the SPLET mechanism, the proton affinity (PA) of the anion RX^{-} and the electron transfer enthalpy (ETE) are the enthalpies that manage the two steps of the mechanism, respectively. Therefore, BDE, IP and PA are the main molecular descriptors which determine the preferred antiradical mechanism. The lower are their values, the higher is the activity.

CUPRAC and reducing power tests mechanism consists of an electron transfer from the studied molecule to the copper(II) complex and the iron(III) complex [4], respectively. That's why CUPRAC and reducing power activities are related to the IP value of the tested compound, the higher is the IP value, the lower is the antiradical activity.

Other properties as molecular orbital energies or spin density distributions, that permit to analyze and explain the obtained experimental results were calculated.

2 Materials and methods

2.1 Materials

For synthesis: Salicylaldehyde, 3-amino-9-ethylcarbazole, 2-aminofluorene, 2-aminobenzimidazole, 2-aminobenzothiazole, 2-hydroxy-1-naphthaldehyde, phenylhydrazine hydrochloride, sodium acetate and absolute ethanol were obtained from *Sigma-Aldrich*. All products were used without further purification.

For antiradical tests: 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), neocuproine, trichloroacetic acid (TCA), potassium ferricyanide ($K_3Fe(CN)_6$), were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany), iron (III) chloride ($FeCl_3$), copper (II) chloride($CuCl_2$), ammonium acetate ($ACNH_4$) were obtained from Biochem Chemopharma. All other chemicals and solvents were of analytical grade.

All calculations were performed with GAUSSIAN 09 software [14], while GaussView 5.0.9 [13] was used for results visualization and analysis.

^{1}H NMR spectra were recorded on a Bruker Avance DPX 250 spectrometer with TMS as internal reference. Measurements and calculations of the antiradical activity were carried out on a 96-well microplate reader, Perkin Elmer Multimode Plate Reader EnSpire.

2.2 Synthesis

Azomethines (SIC, SIF, SIM and SIT) were synthesized by a condensation between salicylaldehyde and the corresponding amine in ethanolic medium [18]. A solution containing salicylaldehyde (5.2 mmol) and amine (5.2 mmol) in ethanol (12 mL), was refluxed for 1 h and cooled to 5 °C. The resulting precipitate was collected by filtration and washed with cold ethanol (Scheme 1).

Identification of 2-((E)-(9-ethyl-9H-carbazol-7-ylimino)methyl) phenol (SIC) Yellow powder, yield: 65%. M.p.: 114 °C. ^{1}H RMN ($CDCl_3$, 250 MHz): 11.75 (s, 1H, –OH); 8.8 (s, 1H, –CH = N–); 8.22 (d, 1H, J = 7.77 Hz); 8.09 (d, 1H, J = 1.93 Hz); 7.56–7.43 (m, 4H); 7.37 (d; 1H, J = 8.5 Hz); 7.28 (t, 1H, J_1 = 8.11 Hz, J_2 = 6.57 Hz); 7.09 (d, 1H, J = 7.97 Hz); 6.98 (t, 1H, J_1 = 7.36 Hz, J_2 = 7.46 Hz); 4.42 (q, 2H, CH_2, J = 7.22 Hz); 1.47 (t, 3H, CH_3, J = 7.22 Hz).

Identification of 2-((E)-(9H-fluoren-7-ylimino)methyl)phenol (SIF) Yellow powder, yield: 70%. M.p.: 160 °C. ^{1}H RMN ($CDCl_3$, 250 MHz): 13.5 (s, 1H, –OH); 8.8 (s, 1H, –CH = N–); 7.86 (d, 1H, J = 7.87 Hz); 7.82 (s, 1H); 7.6–7.34 (m, 7H); 7.08 (d, 1H, J = 8.13 Hz); 7 (t, 1H, J_1 = 7.04 Hz; J_2 = 7.47 Hz); 3.9 (s, 2H, CH_2).

Identification of 2-((E)-(1H-benzoimidazol-2-ylimino)methyl) phenol (SIM) Yellow powder, yield: 94%. M.p.: 205 °C. ^{1}H RMN ($CDCl_3$, 250 MHz): 12.4 (s, 1H, –OH); 9.6 (s, 1H, –CH = N–); 7.8 (s, 1H, NH); 7.55–7.46 (m, 4H); 7.34 (d, 1H, J = 6.02 Hz); 7.31 (d, 1H, J = 5.98 Hz); 6.95–7.1 (m, 2H).

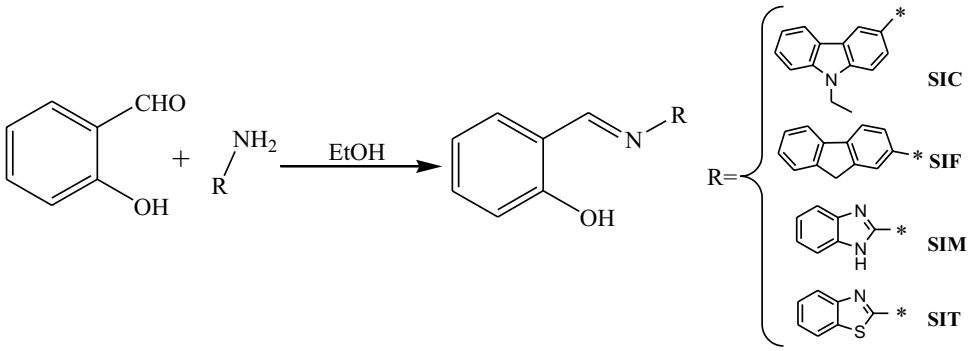
Identification of 2-((E)-(benzothiazol-2-ylimino)methyl)phenol (SIT) Yellow powder, yield: 34%. M.p.: 108–110 °C. ^{1}H RMN ($CDCl_3$, 250 MHz): 12.3 (s, 1H, –OH); 9.33 (s, 1H, –CH = N–); 8.02 (d, 1H, J = 8.02 Hz); 7.89 (d, 1H, J = 7.33 Hz); 7.37–7.55 (m, 2H); 7.01–7.15 (m, 4H).

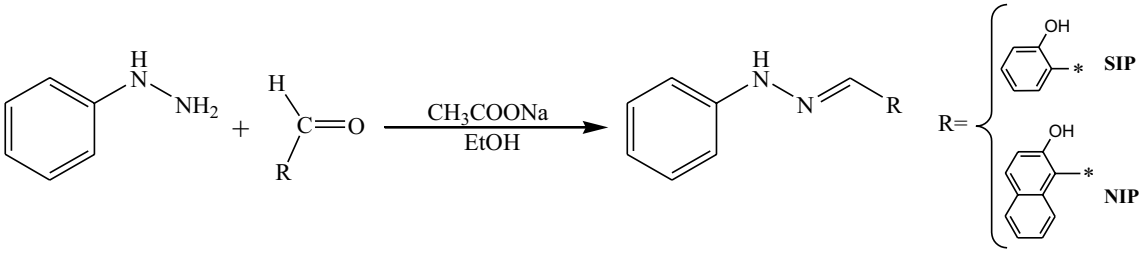
The phenylhydrazones compounds (SIP and NIP) were synthesized by the action of the hydrochloride of phenylhydrazine on an aldehyde in acetate medium (Scheme 2).

To an equimolar mixture (0.025 mol) of phenylhydrazine hydrochloride NH_2NHPh,HCl and sodium acetate CH_3COONa, dissolved in water, was added 0.02 mol of the carbonyl compound which is placed in ethanol. Afterwards, the mixture was refluxed. In the colloidal solution, the precipitate was gradually formed, filtered, and strongly washed with distilled water.

Identification of 2-hydroxy-1-naphthaldehyde phenylhydrazone (NIP) Yellow powder, yield: 75%. M.p.: 205–208 °C.

Scheme 1 Synthesis scheme of azomethine compounds





Scheme 2 Synthesis scheme of phenylhydrazone compounds

^{1}H RMN (DMSO, 250 MHz): 12.25 (s, 1H, OH); 8.88 (s, 1H, CH = N); 7.97 (d, 1H, J = 8.6 Hz); 7.69 (d, 1H, J = 8 Hz); 7.63 (d, 1H, J = 8 Hz); 7.41 (t, 1H, J = 8.31 Hz); 7.1–7.28 (m, 4H); 6.94 (d, 2H, J = 7.6 Hz); 6.78 (t, 2H, J = 7.31 Hz).

Identification of salicylaldehyde phenylhydrazone (SIP) Yellow powder, yield: 81%. M.p.: 141–142 °C. ^{1}H NMR ($CDCl_3$, 250 MHz): 10.92 (s, 1H, OH); 7.98 (s, 1H, CH = N); 7.25–7.5 (m, 5H); 7.2(d, 1H, J = 6.8 Hz); 6.9–7.15 (m, 4H).

2.3 Antiradical activity assessment

The antiradical activity of compounds NIP, SIP, SIC, SIF, SIT and SIM was tested using DPPH scavenging activity, CUPRAC and reducing power assays. BHA and BHT were used as positive standards.

2.3.1 DPPH scavenging activity method

The free radical-scavenging activity was determined by the DPPH assay [10]. Briefly, 40 µL of each sample solution prepared in ethanol at different concentrations was added to 160 µL of a DPPH ethanolic solution (0.1 mM). The mixture was left in darkness for 30 min. The absorbance was measured at 517 nm using microplate reader. BHT and BHA were used as antioxidant standards. The results were given as IC_{50} values (µg/mL) corresponding to the concentration of the compound capable of scavenging 50% of the initial DPPH. The scavenging percentage was calculated by using the formula:

$$\text{Inhibition (\%)} = \frac{A_{Sample} - A_{Control}}{A_{Sample}} \times 100$$

2.3.2 Cupric reducing antioxidant capacity (CUPRAC) method

The cupric reducing antioxidant capacity was determined according to Apak method [5]. In each well the reaction mixture containing 40 µL of our sample, 50 µL of copper (II) chloride solution, 50 µL of neocuproine alcoholic solution and 60 µL of ammonium acetate aqueous buffer at pH 7. This mixture was left 30 min before measuring absorbance at 450 nm. The results were expressed as $A_{0.50}$ values (µg/mL), corresponding to the sample concentration indicating 0.5 absorbance.

2.3.3 Ferric reducing antioxidant power (FRAP) method

Oyaizu's method [22] was used to estimate the reducing power activity of our compounds. 10 µL of each sample solution at various concentrations were added to 40 µL of 0.2 M phosphate buffer (pH 6.6) and 50 µL of potassium ferricyanide (1%). Everything was incubated for 20 min at 50 °C. 50 µL of trichloroacetic acid (10%) and 10 µL of ferric chloride (0.1%), was added to the mixture and completed with 40 µL of distilled water. The ferric reducing antioxidant power was followed spectrophotometrically at 700 nm, and the $A_{0.50}$ values (µg/mL) were calculated.

All data on antiradical activity test were the average of triplicate analyses. Data were recorded as the mean ± standard deviation. Significant differences between means were determined using Student's t test; p values < 0.05 were regarded as significant.

2.4 DFT calculations

2.4.1 Molecular modeling and geometry optimization

Molecular structures of the six molecules and the corresponding charged and radical species (RX^-, $RX^{\cdot}$ and $RX\text{-}H^{+\cdot}$) were optimized by DFT using B3LYP functional with the 6-311G(d,p) basis set. No geometrical constraints were applied. Frequency calculations were carried out by the same level of theory. Solvent effects were considered via the IEF-PCM salvation model [26]. Noting that a preliminary molecular dynamics study using Chem3D Ultra software (version 8.0) with the molecular mechanics force field MM2 was performed [(ChemOffice 2003, CambridgeSoft Corporation)].

2.4.2 Descriptors calculation

Molecular descriptors are calculated using Eqs. (1)–(5) at 298.15 K and 1 atmosphere. For this purpose, we need to know the enthalpy values of hydrogen atom ($H^{\cdot}$), proton (H^+) and electron (e^-) which are equal to − 1309.408 kJ/mol, 6.197 kJ/mol and 3.145 kJ/mol, respectively, in the gas phase [8, 16]. In ethanol, the value of − 1307.479 kJ/mol was used for the hydrogen atom enthalpy [7] whereas, the enthalpies of proton and electron, $H\left(H^+_{sol}\right)$ and $H\left(e^-_{sol}\right)$, were obtained from the corresponding solvation enthalpies [27] and were equal to − 1012.303 kJ/mol and − 102.154 kJ/mol, respectively.

$$BDE = H(RX^{\cdot}) + H(H^{\cdot}) - H(RX - H) \quad (1)$$

$$IP = H\left((RX - H)^{+\cdot}\right) + H(e^-) - H(RX - H) \quad (2)$$

$$PDE = H(RX^{\cdot}) + H\left(H^+\right) - H\left((RX - H)^{+\cdot}\right) \quad (3)$$

$$PA = H(RX^-) + H\left(H^+\right) - H(RX - H) \quad (4)$$

$$ETE = H(RX^{\cdot}) + H(e^-) - H(RX^-) \quad (5)$$

2.4.3 Frontier orbitals and spin-densities calculation

The distribution of the frontier orbitals which are the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of the optimized molecular structures was established at the B3LYP/6–311G(d,p) level of DFT calculation. Besides, radicals atoms spin densities were computed, using the unrestricted open shell level UB3LYP/6-311G(d,p), through the calculation of vibrational frequencies of the generated radicals RX° and $RX\text{-}H^{+\circ}$. It should be noted that no significant change was observed in the results obtained after geometry optimization of the latter.

3 Results and discussion

3.1 Antiradical activity assessment

Results of the three used methods are gathered in Table 1.

3.1.1 DPPH scavenging activity method

The NIP compound exhibited the highest activity (IC_{50}: 3.81 ± 0.26 µg/mL) more than the standards BHA and BHT (IC_{50}: 6.14 ± 0.41 and 12.99 ± 0.41 µg/mL, respectively) followed by SIP compound (IC_{50}: 6.59 ± 0.37 µg/mL) showing a better activity also, in comparison with standards.

Table 1 Antiradical assays (DPPH scavenging activity, CUPRAC and reducing power) results. The corresponding antioxidant standards values are mentioned

Molecule	Antiradical activity		
	DPPH assay IC_{50} (µg/mL)	CUPRAC assay $A_{0.50}$ (µg/mL)	Reducing power assay $A_{0.50}$ (µg/mL)
NIP	3.81 ± 0.26	3.09 ± 0.08	9.21 ± 1.91
SIP	6.59 ± 0.37	4.26 ± 0.44	10.37 ± 1.07
SIC	> 200	6.98 ± 0.13	> 200
SIF	> 200	4.29 ± 0.23	42.46 ± 1.01
SIT	> 200	126.22 ± 10.36	> 200
SIM	NA	20.02 ± 2.34	> 200
BHA	6.14 ± 0.41	5.35 ± 0.71	6.77 ± 1.15
BHT	12.99 ± 0.41	8.97 ± 3.94	5.39 ± 0.91

NA: No absorbance

$A_{0.50}$ (µg/mL): Sample concentration indicating 0.5 absorbance

IC_{50} (µg/mL): Sample concentration corresponding to 50% scavenging of initial DPPH

3.1.2 Cupric reducing antioxidant capacity (CUPRAC) method

The obtained results from the CUPRAC assay showed that NIP, SIP, SIF and SIC exhibited a better antiradical activity than the tested standards, with an $A_{0.50}$ of 3.09 ± 0.08 µg/mL, 4.26 ± 0.44 µg/mL, 4.29 ± 0.23 µg/mL and 6.98 ± 0.13 µg/mL, respectively. SIM and SIT have a relatively weak activity with an $A_{0.50}$ of 20.02 ± 2.34 µg/mL and 126.22 ± 10.36 µg/mL, respectively.

3.1.3 Ferric reducing antioxidant power (FRAP) method

FRAP results showed that NIP and SIP have the best reducing power activity among the tested molecules with an $A_{0.5}$ of 9.21 ± 1.91 µg/mL and 10.37 ± 1.07 µg/mL, respectively. SIF presents a less important activity. No significant activity was observed for SIC, SIT and SIM.

3.2 Molecular descriptors calculation

The comparison of the calculated molecular descriptors with the experimental results allows us to note the following facts: (1) For the DPPH scavenging assay, only the two phenylhydrazones (NIP and SIP) showed an antiradical activity. These molecules present two possibilities of hydrogen atom transfer to the DPPH molecule via one of the three possible mechanisms mentioned above. The first possibility concerns the hydrogen atom bound to the oxygen atom (O–H) while the second one is related to that connected to the nitrogen atom (N–H). Consider first the O–H bond, we can observe that for both molecules in vacuum, the following sequence: BDE > IP > PA, is respected (Table 2), which favours a HAT mechanism. Whereas the SPLET mechanism is preferred for the two molecules in ethanol, since the three descriptors are ordered in the flowing manner: PA > BDE > IP. Concerning the N–H bond, the results in Table 3 show that the mechanism preference is the same as in the first case (O–H bond). Then, when

Table 2 Molecular descriptors (BDE, IP, PDE, PA and ETE) values (kJ/mol) calculated in vacuum, for the tested molecules and standards

Molecule	BDE	IP	PDE	PA	ETE
NIP	392.62	645.62	1065.73	1491.19	220.16
SIP	397.47	672.76	655.30	1512.01	204.20
SIC	361.84	649.78	1030.79	1446.91	233.67
SIF	428.73	685.98	1061.49	1510.98	236.49
SIT	381.68	721.60	978.81	1283.59	416.82
SIM	380.28	699.58	999.44	1440.52	258.50
BHA	363.39	717.91	926.50	1488.69	156.76
BHT	349.19	722.41	943.59	1461.39	204.61

Table 3 N-H bond's BDE and PA values (kJ/mol) calculated in vacuum and ethanol, for NIP and SIP

Molecule	BDE		PA	
	Vacuum	Ethanol	Vacuum	Ethanol
NIP	320.82	330.25	1438.15	265.68
SIP	328.32	337.68	1446.41	268.11

Table 4 Molecular descriptors (BDE, IP, PDE, PA and ETE) values (kJ/mol) calculated in ethanol, for the tested molecules and standards

Molecule	BDE	IP	PDE	PA	ETE
NIP	383.88	402.22	174.69	244.73	332.17
SIP	394.02	417.08	169.95	293.54	293.49
SIC	361.99	417.99	137.02	239.22	315.79
SIF	421.43	453.52	160.93	297.21	317.25
SIT	381.53	489.90	84.64	233.77	340.78
SIM	379.41	468.19	104.24	240.61	331.82
BHA	357.61	447.17	103.46	288.79	261.84
BHT	347.45	464.94	75.53	264.36	276.11

comparing SIP and NIP PA values in ethanol phase, which was found to be the determinant descriptor in both cases, the O–H bond proton departure was favourable in NIP; however the N–H bond proton departure was favourable in SIP. Furthermore, it's known that the hydrogen atom of the N–H bond is mostly more labile than that of the O–H bond, because of their bonds strengths equal to 314 ± 17 kJ/mol and 428 ± 2.1 kJ/mol [29], respectively, but the existence of the electron attraction effect exerted by the phenyl group in NIP seems to weaken the O–H bond and further makes its hydrogen-atom abstraction more favourable. If we make the same comparison in vacuum, but this time with BDE values, we can see that the N–H bond hydrogen departure is favourable for the two molecules. Hence, in all possible cases, NIP may have a better DPPH scavenging activity than SIP. In the other hand, the structural difference between SIP and the four azomethines lies in the substitution of the NH–Ph group by other groups. This difference may be responsible for the loss of DPPH scavenging activity for our azomethines. This fact supports our hypothesis that the departure of

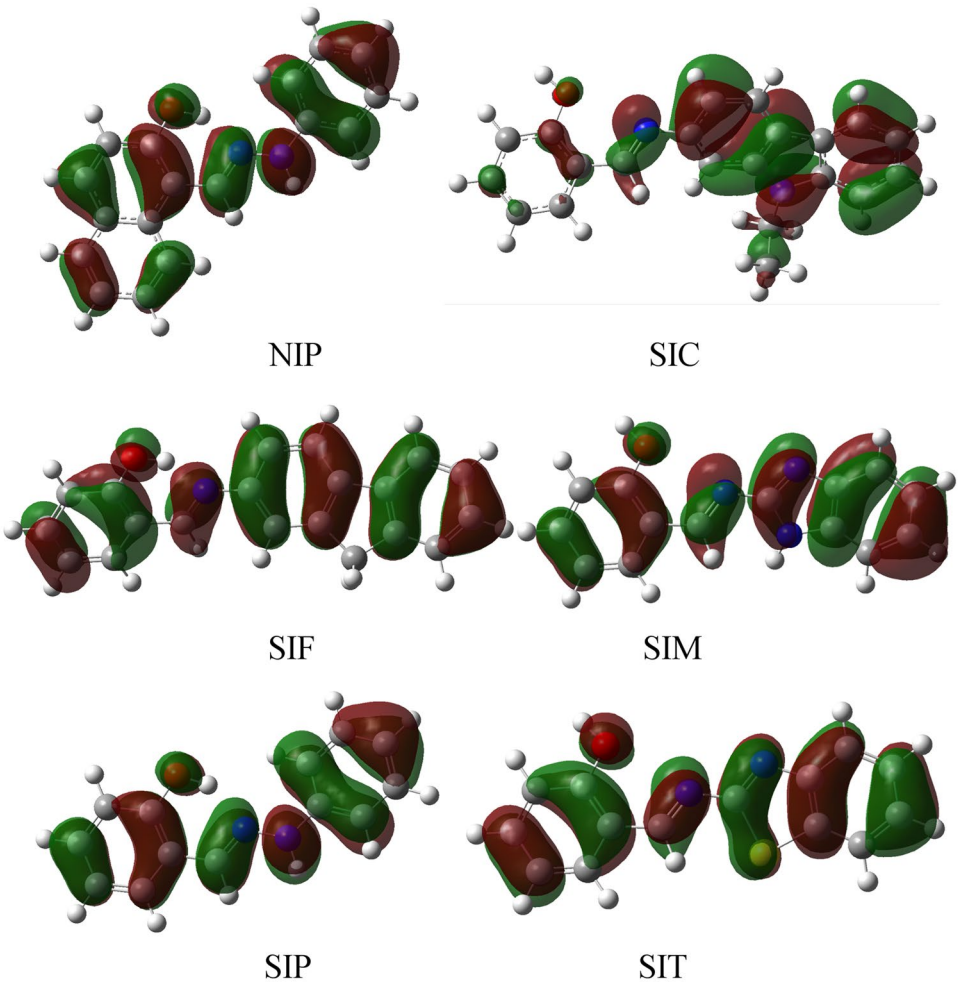


Fig. 2 SOMO isosurfaces plots of the six cation-radicals, calculated at UB3LYP/6-311G(d,p) level in ethanol phase. All of these plots are delocalized through the entire corresponding cation-radical

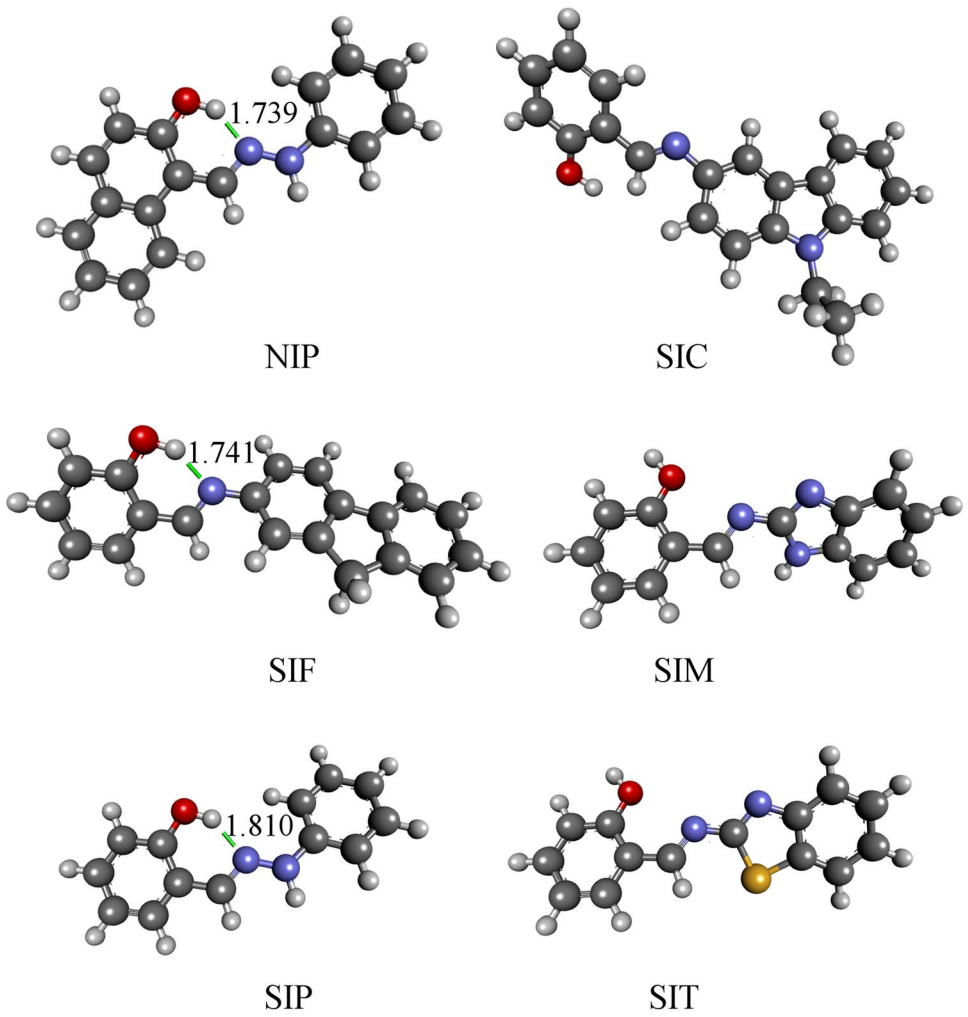


Fig. 3 3D structures of the six cation-radicals, obtained at UB3LYP/6-311G(d,p) level in ethanol phase. Only NIP, SIF and SIP cation-radicals have an intramolecular hydrogen bond (dashed green line). The mentioned values in Å are obtained by means of the Discovery Studio 4.0 software [1]

the N–H bond proton, in the case of SIP, is more favourable than that of the O–H bond.

The comparison of the antioxidant standards (BHA and BHT) descriptors with those of SIP and NIP shows that, the following order was observed in ethanol: O–H PA of NIP < N–H PA of SIP < PA of BHA < BDE of BHT. This result matches perfectly with the IC_{50} values of the DPPH assay (Table 1). Moreover, in vacuum we can note that N–H BDE of NIP < N–H BDE of SIP < BDE of BHT < BDE of BHA.

(ii) For the used electron transfer based tests, CUPRAC and reducing power, the order of antiradical activity, which is inversely proportional to the IP values of molecules, was: NIP > SIP > SIF > SIC > SIM > SIT, for CUPRAC assay and NIP > SIP > SIF, for reducing power assay. Likewise, IP values in ethanol (Table 4) are ordered as follows: SIT > SIM > SIF > SIC > SIP > NIP which constitute a nearly perfect agreement between the theoretical approach and experimental one.

3.3 Frontier orbitals calculation

HOMO energy (E_{HOMO}) is also a revealing criterion of the reactivity between the corresponding molecule and DPPH, so that the electronic transfer is made from the highest E_{HOMO} entity to the lowest one. Therefore, the electronic transfer is energetically unfavourable in the case where E_{HOMO} of the DPPH is the highest. The calculated values of E_{HOMO} in ethanol are − 513.88 kJ/mol, − 527.66 kJ/mol and − 553.71 kJ/mol for NIP, SIP and DPPH, respectively, which match well with the DPPH assay results. Concerning CUPRAC and reducing power assays, the electron donating is easier for a molecule that possesses a higher E_{HOMO} [3]. The order of that energy values in ethanol, for the tested compounds is as flows: NIP > SIP > SIC > SIF > SIM > SIT which is in a good accordance with experience.

Singly occupied molecular orbital (SOMO) isosurfaces of the six cation-radicals look to be homogeneously distributed which is a synonym of their stability (Fig. 2). Thus, the

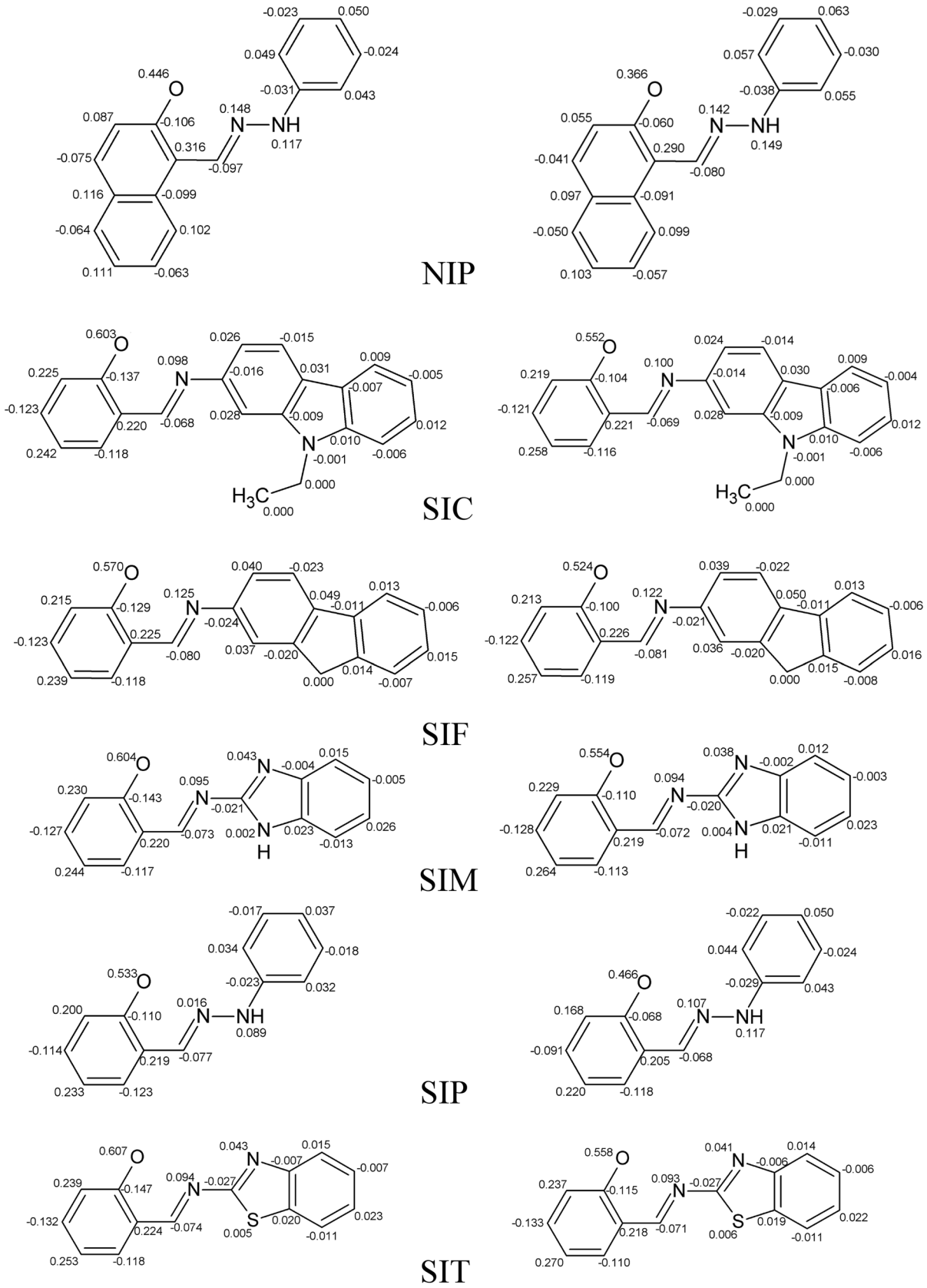


Fig. 4 Spin density distribution of the studied molecules, calculated in ethanol medium (right) and in gas phase (left), at UB3LYP/6-311G(d,p) level

electron—transfer is favourable for all of the corresponding neutral molecules. One other stabilizing factor for the three most active tested molecules (NIP, SIP and SIF) is the existence of an intramolecular hydrogen bonding interaction between the O–H hydrogen atom and the nearest nitrogen atom (Fig. 3), which is noticeable in the bounding character of the SOMO distributions of the three molecules in this region (Fig. 2).

3.4 Spin density

Frequency calculation for radicals and cation-radicals were performed and no spin contamination was found. The comparison of the spin density distribution permits to evaluate the relative stability of molecules. The more the spin density is delocalized, the more the radical is stable and its formation is favourable. We can note that the highest value of spin density in each radical is localized on the hydroxyl oxygen atom. The lower is this value, the better the spin distribution is homogeneous. The lowest values are assigned to NIP and SIP, respectively, as we expected (Fig. 4). Moreover, for all the present molecules, this value is always lower in ethanol than in vacuum which means that the formed radicals are further stabilized by the solvent molecules, as reported in literature [30].

4 Conclusion

The obtained results from the used antiradical activity methods illustrate that NIP and SIP exhibited a good radicals scavenging power, even better than the customary used antioxidant standards. Besides, the most thermodynamically favorable DPPH scavenging mechanisms were investigated theoretically and the SPLET mechanism was suggested for both cited molecules, with a difference in the nature of the outgoing proton. The O–H bond proton extraction is more favorable for NIP, whereas, the N–H bond proton departure is more suitable, in the case of SIP. In the other hand, for CUPRAC and reducing power, the tested molecules were ordered as follows: "NIP > SIP > SIF > SIC > SIM > SIT" and "NIP > SIP > SIF", respectively. These results are in excellent accordance with the calculated IP and E_{HOMO}. Further, the existence of intramolecular hydrogen bonds for the cation-radical of the most active molecules NIP, SIP and SIF constitutes a stabilizing element for these radicals. Basing on the experimental and the theoretical parts of our study, we believe that with additional tests, our compounds can be useful to prevent products oxidation in pharmaceutical or in food industries.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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