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Theoretical and experimental analysis of the antioxidant features of substituted phenol and aniline model compounds

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Abstract

Although natural polyphenols have attracted extended attention as antioxidants, there is only limited information available on their structure-activity relationship (SAR). In addition, while often having significant antioxidant activity, amino group-containing compounds have only been sporadically studied. Often, the complex structure makes studying the individual contribution of aromatic OH or NH_2 groups on the activity of these antioxidants difficult. In this work, several substituted simple phenols and anilines were selected as model compounds. Both the experimental radical scavenging activity and major structural descriptors have been determined to gain more insights into the potential SAR. Physicochemical properties pertaining to energetic and structural parameters were determined and experimental data gathered from three antioxidant assays to identify fundamental features with reasonable effect on antioxidant activity. Density functional theory (DFT) calculations were carried out at the B3LYP/6-31G(d,p) level to determine the N–H and O–H bond distances, dipole moments, logP values, highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) orbital energies, HOMO-LUMO gaps, radical spin densities, proton affinities, and ionization potentials. The compounds were screened for activity against the 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl-1picrylhydrazyl (DPPH), and peroxyl (ORAC assay) radicals. Based on the results, ABTS antioxidant activity was selected for further investigations to observe correlations with the calculated properties. The HOMO energies, bond-dissociation energy values, HOMO-LUMO gap energies, dipole moment, proton affinity, and the Hammett constants appear to show meaningful correlation with the experimental data.

Keywords Phenols · Anilines · Antioxidant capacity · Radical scavenging · DFT calculations · Structure-activity relationship

Introduction

Redox homeostasis is essential for cell survival. Free radicals are used by cells to perform physiological functions such as cell signaling and immune response [1–3]. The concentration of radical species is balanced by endogenous antioxidant systems supplemented with exogenous antioxidants from the diet [4–7]. When these systems fail, damage caused by radicals leads to rampant oxidation within the cell resulting in damage to DNA, proteins, and lipids ultimately leading to cellular death [8].

Free radical damage has been identified as a common factor in the progression of many neurological diseases, such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis (MS) [9–13]. Radicals are also reported to affect aging, cancer, and diabetes [11, 14, 15]. While oxidative stress may not be the primary reason for the progression of these diseases, it is an underlying factor that needs to be incorporated into the design of multi-target drug therapeutics.

Many exogenous small-molecule antioxidants are isolated from natural sources, most often from plants [16]. The most abundant plant-based antioxidants are polyphenols, which possess large structural variety [17, 18]. The literature is primarily focused on the extraction, identification, and quantification of their potency [19–25]. Natural polyphenols have excellent in vitro activity; however, their poor bioavailability limits their practical applications in biological systems [6, 17, 26, 27]. Several reports suggest that the polyphenol content of a plant does not equate to antioxidant functionality within the human body [17, 28, 29]. There are many variables, including solubility, absorption, stability during digestion, transport, and

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metabolic degradation of polyphenols, that need to be considered [26, 27, 30–33]. Beyond polyphenols, there are several types of natural small-molecule antioxidants, such as carotenoids or nitrogen-containing compounds that work as potent bioavailable antioxidants: amines, betaines, and betalains [18, 34, 35]. However, while often having significant antioxidant activity, these compounds have only been sporadically studied [34, 36, 37].

There are also synthetic nitrogen-containing compounds that were found to be excellent antioxidants such as diarylhydrazones reported in our earlier studies [38]. The direct analysis of the individual contribution of structural features to the antioxidant effect of these compounds, however, is challenging due to complicated geometric features (*E/Z* isomers, conformational isomers, etc.). Similar issues (e.g., multiple ring systems, electron delocalization) also hinder SAR studies on polyphenols. Several reviews summarize recent developments on antioxidants and their role in chemical and biological systems [39, 40].

The major aim of this work is to gain new insights to the fundamental mode of action of these compounds. Therefore, antioxidant activity of simple phenol and aniline model compounds will be compared and the effect of underlying structural features will be investigated. The selected compounds are simple, single-ring structures that allow a direct comparison of the electronic and steric properties. Although these molecules are not practical antioxidants, their simple structures offer a better opportunity to reveal important structural features that significantly affect their activity.

Experimental and theoretical methods

Materials

Substituted phenol and aniline derivatives, solvents for the experiment, (ethanol and DMSO), salts for buffer solutions (Na_2HPO_4, NaH_2PO_4, and NaCl), potassium persulfate for 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical generation, and radical compounds (DPPH, ABTS, and AAPH) were obtained from Sigma-Aldrich. The chemicals were used without further purification.

Determination of radical scavenging activity with the DPPH assay

The DPPH assay was carried out in a 50% aq. ethanol following an earlier procedure [41–44]. Stock solutions of the compounds were prepared at a concentration of 10 mM in DMSO. Known antioxidants were dissolved at 10 mM concentration in ethanol (Trolox, resveratrol) or water (ascorbic acid). All compounds and controls were diluted with ethanol prior to evaluation to a concentration of 200 μM. A DPPH solution was prepared by using 50% aqueous ethanol at a concentration of 105.3 μM. The DPPH was stirred and incubated at 37 °C for 45 min to ensure the DPPH was fully dissolved. Phenols, anilines, and control compounds were added to a 96-well plate and DPPH solution was added to the wells. The final concentrations of the compounds were 10 μM and the DPPH concentration was 100 μM. Readings were taken with a VersaMax plate reader (Molecular Devices) set to 37 °C and 519 nm coupled with the SoftMax Pro 5 software recording the absorbance of the plate every 15 min for 60 min. Control wells with 50% aqueous ethanol, containing the same percentage of DMSO as the samples with the compounds, were used as the blank. Percent radical scavenging was determined using the equation: Percent radical scavenging = $(Abs_c - Abs_t)/Abs_c] \times 100$ where Abs_c is the absorbance of the solution with only the radical and Abs_t is the absorbance of the solution with the radical and the test compound.

Determination of radical scavenging activity with the ABTS assay

The ABTS assay was carried out in a 75-mM phosphate buffered saline (PBS) solution at pH 7.4 with 50 mM NaCl following an earlier procedure [41–44]. Stock solutions of compounds were prepared at 50 mM in DMSO. Trolox, resveratrol, and ascorbic acid were prepared as described in the DPPH procedure. All compounds and standards were diluted to 500 μM with ethanol. The ABTS radical solution was prepared 16 h in advance by dissolving ABTS (7 mM) and $K_2S_2O_8$ (2.45 mM) in water and storing the mixture in the dark at room temperature. The ABTS solution was diluted to an absorbance of approximately 0.7 prior to use with 75 mM phosphate buffered saline solution at pH 7.4 with 50 mM NaCl. Compounds were plated in a 96-well plate to a concentration of 10 μM (4 μL of diluted compound), and 196 μL of diluted ABTS was added. Control wells of the buffer solution, containing the same percentage of DMSO as the samples with the compounds, were used as a blank. Measurements were recorded using a VersaMax plate reader (Molecular Devices) set to 37 °C and 734 nm coupled with SoftMax Pro 5 software recording the absorbance of the plate at 0, 6, and 12 min. Percent radical scavenging was determined using the equation: Percent radical scavenging = $(Abs_c - Abs_t) / Abs_c] \times 100$ where Abs_c is the absorbance of the solution with only the radical and Abs_t is the absorbance of the solution with the radical and the test compound.

Determination of radical scavenging activity with the ORAC assay

The ORAC assay was carried out in a pH 7.4, 75 mM phosphate buffered solution following an earlier procedure [41, 42,

44]. Stock solutions of the compounds and known antioxidants (Trolox, resveratrol, and ascorbic acid) were prepared as described in the DPPH protocol. All compounds were diluted to 80 μM in ethanol prior to screening the compounds. A fluorescein stock solution was prepared at a 4.19 μM concentration in a pH 7.4 75 mM phosphate-buffered solution and freshly diluted to an 81.6 nM solution every day. An AAPH solution was prepared in cold pH 7.4, 75 mM phosphate buffer at a concentration of 0.153 M prior to screening the compounds and stored on ice. The compound solutions (25 μL) were plated in a black 96-well plate and 150 μL of fluorescein was added to the wells and the plate was incubated at 37 °C for 15 min. Then, 25 μL of AAPH was added to the wells and the plate was screened. A SpectraMax i3x plate reader in kinetic mode set to 37 °C with excitation and emission wavelengths at 485 nm and 520 nm respectively was used to record the fluorescence of the plate every 2 min for 60 min. Control wells with pH 7.4, 75 mM phosphate-buffered solution containing the same percentage of DMSO as the samples with the compounds were used as the blank. The percent radical scavenging activity was calculated by the equations shown below.

$$\text{Net AUC} = 0.5 + \sum_{0-29} \frac{f_i}{f_0} + \left(0.5 * \frac{f_{30}}{f_0}\right)$$

Net AUC is the net area under the curve, f_i is the fluorescence intensity at measurement 0–29, f_0 is the fluorescence intensity at measurement 0, and f_{30} is the fluorescence intensity at measurement 30.

$$\text{Percent Radical Scavenging} = \frac{(\text{Net AUC}_c - \text{Net AUC}_t)}{\text{Net AUC}_{f\_max}} \times 100$$

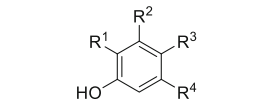
Net AUC_t is the area under the curve of the test sample with the radical and the fluorescein dye. Net AUC_c is the area under the curve of control sample with the radical and the dye but no test sample. Net AUC_{f_max} is the area under the curve of sample with the dye alone.

Computational methods

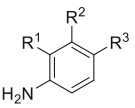
The electronic structures of the phenol and aniline derivatives were determined using density functional theory (DFT). Calculations were carried out at the B3LYP/6-31G(d,p) [45, 46] level of theory using the Gaussian09 program suite [47]. The O–H and N–H bond dissociation enthalpy (BDE) for the thirty-two compounds was determined by subtracting the sum of the enthalpies for the radical (·N or ·O) and the hydrogen atom from that of their respective neutral compounds. Additional parameters were also calculated to identify any experimental correlations between the phenols and anilines and their experimental radical scavenging activities: N–H and O–H bond distances, dipole moments, logP values, highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) orbital energies, HOMO-LUMO gap energies, radical spin densities, proton affinities, ionization potentials, and the experimentally derived Hammett constants [48].

Results and discussion

In our earlier experimental and DFT studies investigating the free radical scavenging activity of diarylhydrazones, it was established that the –NH group of the hydrazones provided significant potency for these compounds [38, 44]. Since there are several N-containing natural antioxidants (e.g., betalain, bilirubin, or uric acid) [22, 23, 27], it prompted us to evaluate the comparative activity of the OH vs. the NH groups. To avoid structural features that unnecessarily complicate (e.g., the possibility of conformational or *E-Z* isomers) the DFT studies, we decided to focus on simple substituted phenols and anilines. In this work, our major goals were to (i) compare the antioxidant activity of similar phenol and aniline derivatives and (ii) identify the effect of a broad variety of structural



	R^1	R^2	R^3	R^4
1	H	H	H	H
2	H	H	CH_3	H
3	CH_2CH_3	H	H	H
4	H	H	OH	H
5	H	H	$(CH_2)_2H_3$	H
6	H	CH_3	Br	H
7	H	H	COOH	H
8	H	H	CHO	H
9	CHO	H	OCH_3	H
10	H	H	COPh	H
11	OH	COOH	H	H
12	H	COOH	OH	H
13	COOH	OH	H	H
14	OH	H	COOH	H
15	H	OH	H	COOH
16	H	OCH_2	OH	H
17	Ph	H	OH	H



	R^1	R^2	R^3
18	H	H	H
19	F	H	H
20	H	Cl	H
21	H	CF_3	H
22	H	H	Cl
23	H	H	Br
24	H	H	F
25	H	H	NO_2
26	H	H	CH_2CH_3
27	H	H	$CH_2CH_2CH_3$
28	H	CH_3	Br

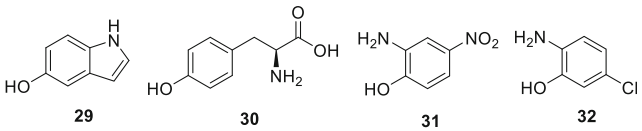


Fig. 1 The structure of phenols (**1**–**17**), anilines (**18**–**28**), and phenols with an NH group (**29**–**32**) studied in this work

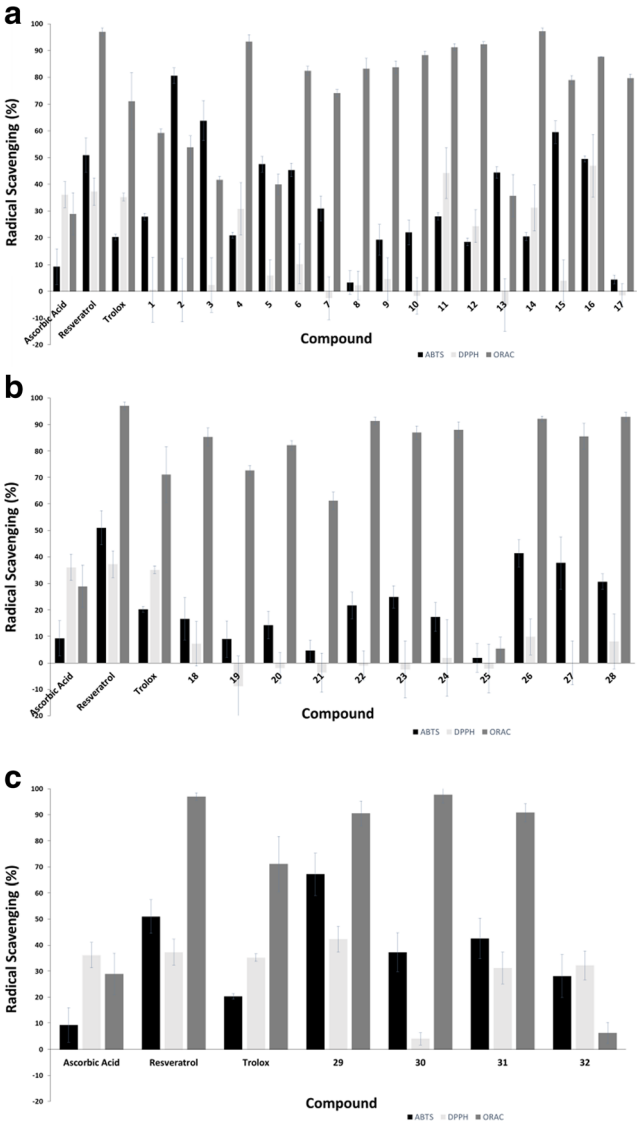


Fig. 2 Radical scavenging activity of phenols (**a**), anilines (**b**), and phenols with an NH group (**c**) determined by the ABTS (black), DPPH (light gray), and ORAC (dark gray) assays

features to better understand the antioxidants' mode of action. As the overwhelming majority of reports regarding antioxidants focus on natural polyphenols, studies on NH-containing compounds are scarce, despite the great number and variety of natural and synthetic anilines that can act as antioxidants [49–52]. The structures of the compounds studied can be seen in Fig. 1.

The compounds analyzed are commercially available and chosen to have a broad variety of substituents from the strongly electron-withdrawing (EW) to the electron-donating (ED) groups. First, the experimental activities were determined using three commonly applied and widely accepted assays: the DPPH, ABTS, and ORAC protocols. The data are depicted in Fig. 2.

The free radical scavenging data collected in the ABTS, DPPH, and ORAC experiments varied greatly between the different assays. Exogenous antioxidants that function in the body act through several different mechanisms: boosting endogenous systems, preventing radical formation, scavenging free radicals, and repairing radical-induced damage [4, 53].

There are three general mechanisms to how antioxidants scavenge free radical species: hydrogen atom transfer (HAT), single-electron transfer (SET), and sequential proton loss electron transfer (SPLET) (Fig. 3) [41, 42]. Compounds with high activity in the ORAC experiment are thought to primarily react through the HAT mechanism [40] (Fig. 3).

The data in Fig. 2 showed that almost every compound performed extremely well in the ORAC assay, with radical scavenging percentages almost always above 80% (Fig. 2) except for compounds **25** and **32** which had minimal activity in all three assays. In contrast, the compounds exhibited uniformly negligible activity in the DPPH assay (Fig. 2). Due to the uniform activity profiles that provided minimal variations, neither the DPPH nor the ORAC data were appropriate for the SAR analysis. In contrast, the radical scavenging of ABTS, which may occur through the SET and HAT mechanisms [42], provided the most viable dataset for the compounds investigated (Fig. 2). Thus, all further discussion will be based on the ABTS assay. It was observed that phenols generally performed better than anilines. Several assays, such as the ABTS assay, are known to report higher activities for compounds with multiple –OH groups [41], which does not appear to be the case with our compounds (e.g., **11**, **12**, and **14**). Although we noticed similar trends to those seen in the literature, it was found that the position of the OH on the aromatic

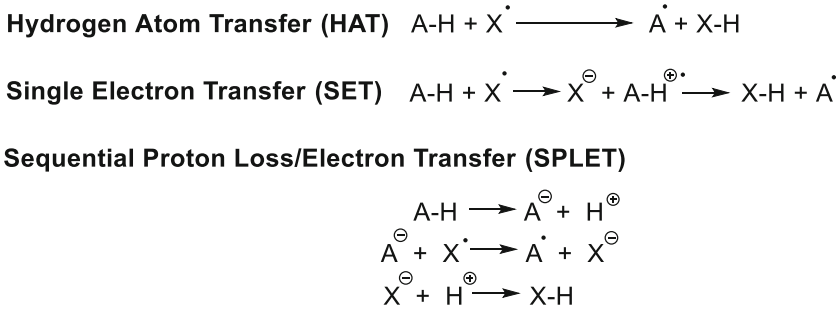


Fig. 3 Three common mechanisms for radical (X) scavenging by an antioxidant species (A-H) [14]

Table 1 Theoretical parameters of the substituted phenols and anilines investigated. Values were determined using Gaussian09

	N–H distance[a]	O–H distance[a]	μ (D)	LogP	HOMO[b]	LUMO[b]	HOMO-LUMO gap [c]	BDE $[NH_2]$[d]	BDE [OH][d]	Spin density N radical	Spin density O radical	PA[d]	Ionization potential[d]	Ionization potential[c]	σ (meta)	σ (para)
1	NA	0.966	1.335	1.45	− 0.2193	0.00054	5.98	NA	74.41	NA	0.444	182.57	184.9	8.01	NA	NA
2	NA	0.966	1.312	1.94	− 0.2111	0.0019	5.79	NA	72.67	NA	0.643	185.24	176.7	7.66	NA	− 0.17
3	NA	0.966	L079	2.11	− 0.21309	0.00753	6.00	NA	71.97	NA	0.631	185.45	177.4	7.69	NA	− 0.15
4	NA	0.965	0	0.79	− 0.19904	− 0.00286	5.33	NA	69.82	NA	0.624	182.76	170.3	7.38	NA	− 0.37
	NA	0.965						NA		NA					NA	
5	NA	0.966	1.333	2.23	− 0.21129	0.00178	5.79	NA	72.94	NA	0.642	185.81	175.5	7.61	NA	− 0.13
6	NA	0.966	2.385	2.59	− 0.2184	− 0.00933	5.68	NA	73.74	NA	0.638	178.73	178.3	7.73	− 0.07	0.23
7	NA	0.966	1.481	0.57	− 0.2357	− 0.0382	5.37	NA	76.72	NA	0.427	NA	190.7	8.27	NA	OAS
8	NA	0.966	2.491	1.34	− 0.2387	− 0.0536	5.03	NA	76.59	NA	0.412	NA	193	8.37	NA	a42
9	NA	0.966	4.099	1.16	− 0.2289	− 0.2386	− 026	NA	76.33	NA	0.645	NA	182.7	7.92	0.12	NA
10	NA	0.966	4.458	2.21	− 0.231	− 0.057	4.73	NA	75.69	NA	0.417	NA	179.9	7.8	NA	0.43
11	NA	0.986	2.796	0.84	- 0.21279	− 0.0504	4.41	NA	82.71	NA	0.587	214.26	174.8	7.57	0.37	NA
	NA	0.966						NA	70.43	NA	0.626	190.14				NA
12	NA	0.972	1.813	0.77	− 0.21016	− 0.05145	4.31	NA	77.72	NA	0.598	190.78	173.8	7.53	0.37	− 0.37
	NA	0.965						NA	71.12	NA	0.636	180.71				NA
13	NA	0.971	1.909	1.58	− 0.21749	− 0.05513	4.41	NA	78.79	NA	0.588	186.35	178.1	7.72	0.12	NA
	NA	0.985						NA	84.46	NA	0.592	206.93				NA
14	NA	0.968	4.572	0.02	− 0.22166	− 0.03815	4.99	NA	76.05	NA	0.642	183.55	181.2	7.85	NA	0.45
	NA	0.965						NA	75.55	NA	0.567	182.5			NA	NA
15	NA	0.966	2.980	− 0.03	− 022448	− 110,471	4.82	NA	75.35	NA	0.651	176.31	182.5	7.91	0.37	NA
	NA	0.966						NA		NA						NA
16	NA	0.966	3.233	1.33	− 0.21334	− 0.05039	4.43	NA	68.74	NA	0.586	179.05	175.4	7.6	0.376	NA
	NA	0.966						NA	73.14	NA	0.644	183.82				NA
17	NA	0.965	0.246	1.92	411,978	− 0.02317	4.75	NA	67.32	NA	0.597	195.76	161.8	7.01	0.109	− 0.37
	NA	0.965						NA	70.24	NA	0.621	188.03				NA
18	1.011	NA	1.270	0.90	− 0.1981	0 0085	5.63	79.34	NA	0.634	NA	214.36	167	7.24	NA	0
19	1.01	NA	1.719	0.99	− 0.2034	0.0041	5.65	78.90	NA	0.630	NA	211.95	170	7.37	NA	NA
20	0.966	NA	3.165	1.81	− 0.2105	− 0.0059	5.57	80.55	NA	0.639	NA	209.14	172.6	7.48	0.37	NA
21	1.01	NA	3.153	2.11	− 0.2145	− 0.019	5.32	80.88	NA	0.642	NA	207.55	175.5	7.61	0.43	NA
22	1.01	NA	2.251	1.88	412,046	− 0.0074	5.36	104.04	NA	0.619	NA	209.87	168.1	7.29	NA	0.23
23	1.01	NA	2.325	1.83	− 0.2036	− 0.0075	5.34	79.42	NA	0.617	NA	210.25	166.8	7.23	NA	0.23
24	1.011	NA	3.316	1.80	− 0.1992	− 0.0044	5.3	78.19	NA	0.627	NA	212.09	166.8	7.23	NA	0.06
25	1.008	NA	5.802	1.56	− 0.2294	410,716	4.29	83.4	NA	0.619	NA	199.62	185 1	8.03	NA	0.78
26	1.011	NA	0.736	1.81	− 0.1926	0.0089	5.49	78.37	NA	0.620	NA	217.2	160.1	6.94	NA	− 0.15
27	1.011	NA	1.525	2.11	− 0.1925	0.00902	5.48	78.37	NA	0.771	NA	217.52	159.3	6.90	NA	− 0.13
28	1.01	NA	3.250	2.29	− 0.20083	− 0.00186	5.41	79.29	NA	0.901	NA	212.51	163.7	7.09	− 0.07	0.23
29	1.006	0.965	3.329	1.65	− 0.191	− 0.0052	5.05	78.78	70.30	0.395	0.426	205.55	162.2	7.03	NA	NA
30	1.017	0.973	1.550	− 188	− 0.2164	− 0.0053	5.74	88.17	73.51	0.967	0.430	223.91	171	7.41	NA	NA
31	1.01	0.966	6.404	− 0.20	− 0.2105	− 0.0059	5.57	80.48	65.56	0.629	0.280	207.99	174.9	7.58	NA	0.78
32	1.01	0.965	2.750	0.83	− 0.1957	− 0.00002	5.33	77.56	63.55	0.595	0.310	215.75	161.5	7.00	0.37	NA

[a] Å

[b] hartree

[c] eV

[d] k_{cal}/mol

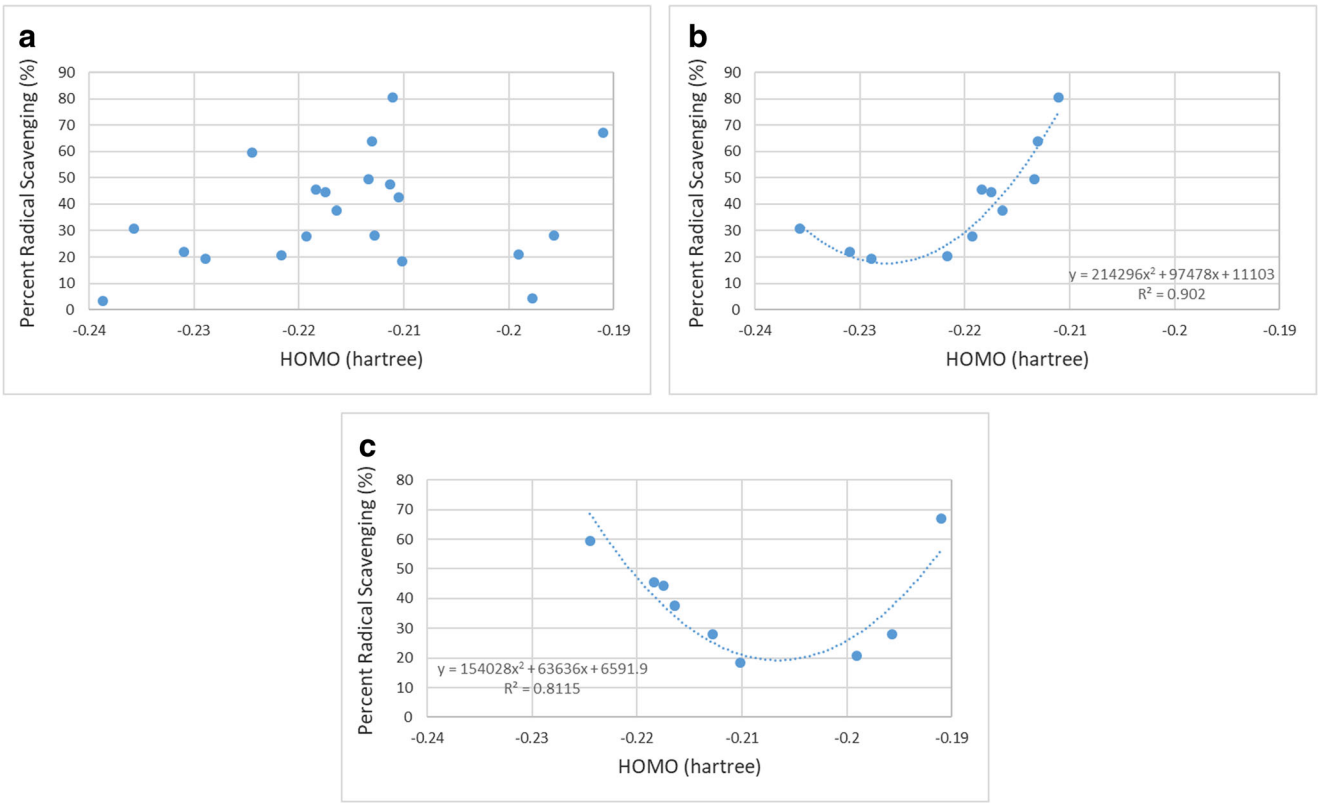


Fig. 4 **a** Effect of the HOMO energies of phenols on the radical scavenging activity of the compounds. **b** Activity vs. HOMO correlation for compounds **1**–**3**, **6**, **7**, **9**, **10**, **13**, **14**, **16**, and **30** which correspond to the left parabola on **a**. **c** Activity vs. HOMO correlation for compounds **4**, **6**, **11**–**13**, **15**, **29**, **30**, and **32** which correspond to the right parabola

ring affects the activity of the compound more than the number of the OH groups.

The ABTS data suggest a few key points. While the phenols have higher free radical scavenging activity, it is worth noting that compounds **21**, **29**, and **30** from the aniline set also have comparable activity to that of the best phenols. In addition to simple phenols, benzoic acids having OH groups in different positions were also studied. If compounds possess higher activity solely by the number of OH groups, compounds **11**–**15** should all have similarly high activity. Instead, varying values were observed across these compounds with the highest activity belonging to benzoic acids with –OH groups located in the 1,3-positions. Another interesting trend with the ABTS data suggests that electron-donating groups on the phenyl ring promote radical scavenging activity, e.g., compounds **7** (Et) and **8** (Pr). The experimental data alone suggest that it is not just the amount of OH groups present in the compound that correlate to activity, but there are other properties which significantly contribute to the activity of these phenol and aniline models.

To assess the potential relationships between physical and chemical properties versus radical scavenging of the model compounds, DFT calculations were carried out using Gaussian09 software package (Table 1) [47]. Correlating the theoretical properties of the compounds to the ABTS activity data led to several important observations. As these

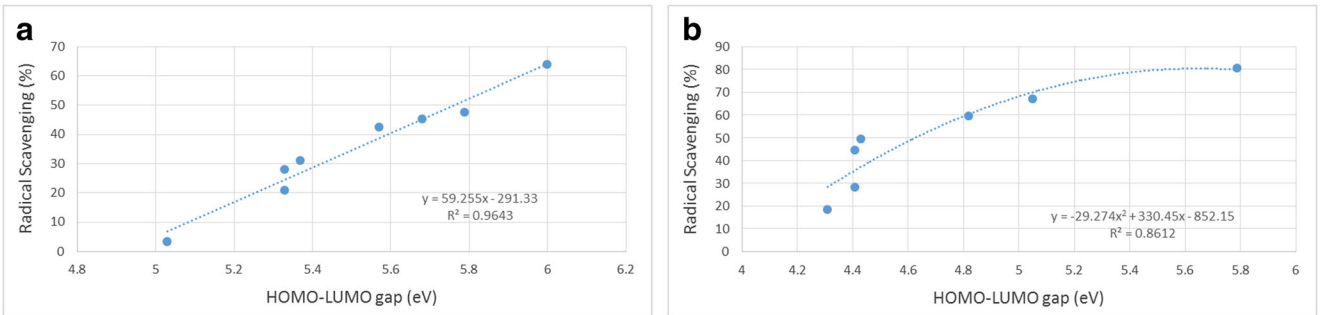


Fig. 5 Free radical scavenging activity vs HOMO-LUMO gap functions of **a** compounds **3**–**8**, **31**, and **32** and **b** compounds **2**, **11**–**13**, **15**, **16**, and **29**

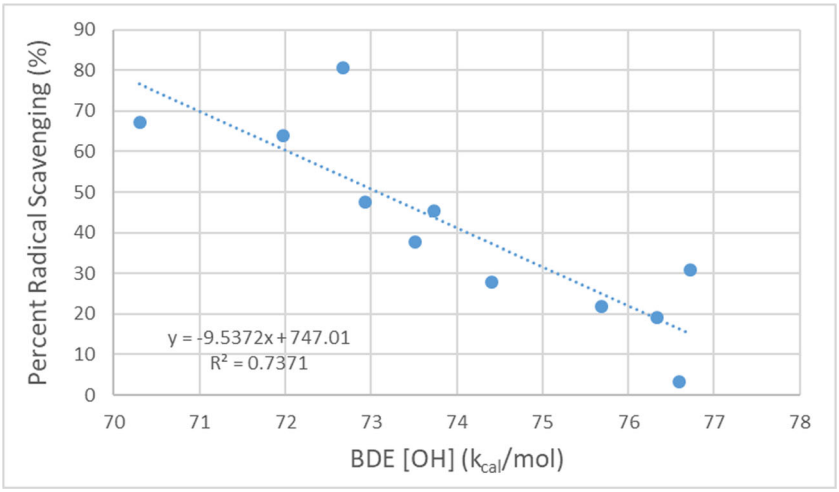


Fig. 6 Effect of bond-dissociation energy (BDE) of the phenols **1**–**3**, **5**–**10**, **29**, and **30** on their corresponding radical scavenging activity

compounds have similar chemical structures, their experimental activity data and structural parameter values were expected to be related. Thus, the experimentally determined ABTS radical scavenging data have been plotted as a function of each calculated parameter set. Then the obtained plots were analyzed in order to reveal potential relationships between the experimental activity and the physicochemical characteristics. It was observed that several properties seem to have little to no effect on the activity of the compounds. OH distance, NH distance, logP, LUMO, spin density of O radical, spin density of N radical, proton affinity, and Hammett constants (*meta*) all appeared to be irrelevant as modulators to the radical scavenging activity (data not shown). Along with this observation, correlating the calculated properties with experimental activity using the complete compound set did not reveal any reasonable correlations with the data (data not shown). It suggests that comparing phenols and anilines in a single unified set is not a sufficient approach. Thus, it was decided to analyze the compounds in two separate groups as defined by their parent compounds, phenols, and anilines.

Phenols

Phenols investigated in this set (**1**–**17** and **29**–**32**) showed several reasonable correlations between the ABTS radical

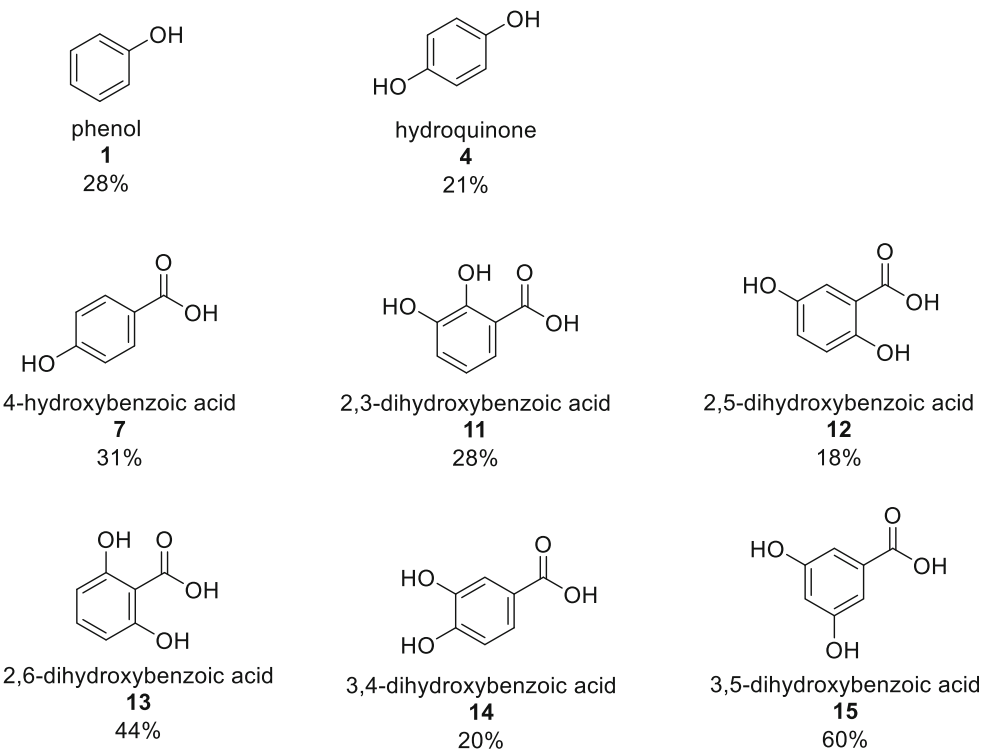


Fig. 7 The structure and ABTS activity data of compounds **1**, **4**, **7**, and **11**–**15**

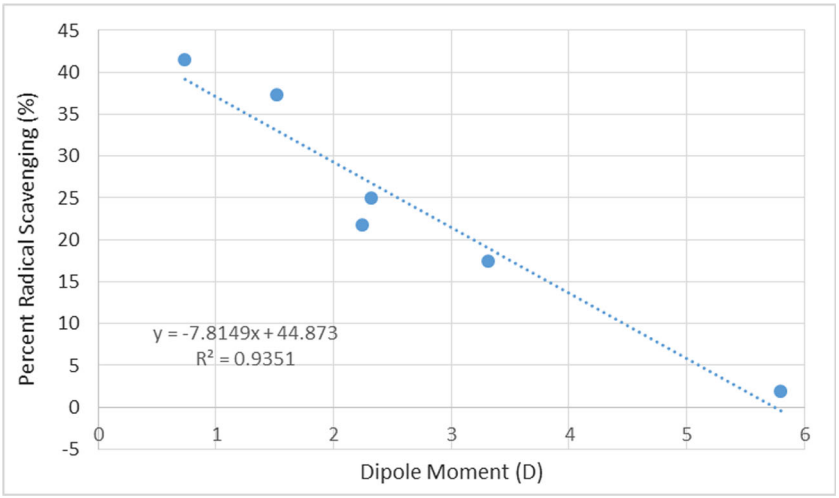


Fig. 8 Effect of dipole moment on the ABTS scavenging activity of anilines **22**–**27**

scavenging data and the physicochemical properties examined. The HOMO energies are used to determine the likelihood of an electron to be donated by a compound [33]. An elevated HOMO value indicates that the compound is likely to donate electrons [54]. The effect of the HOMO energies on the experimental activity of the phenols is depicted in Fig. 4.

Two activity wells can be identified in Fig. 4a where the activity of the phenols drops between − 0.20 and − 0.21 hartree as well as between − 0.22 and − 0.23 hartree. These minima appear as two distinct but overlapping convex parabolic functions when all phenols are included. Compounds **1**–**3**, **6**, **7**, **9**, **10**, **13**, **14**, **16**, and **30** can be seen forming the left parabola (Fig. 4b), while compounds **4**, **6**, **11**–**13**, **15**, **29**, **30**, and **32** form the right parabola (Fig. 4c). The R^2 values indicate a reasonable fit between the calculated functions and the data. Compounds **13** and **30** appear to be part of both functions. The compounds that form the left parabola (Fig. 4b) are primarily consisted of single OH-containing molecules, while compounds in the right parabola (Fig. 4c) generally include molecules with multiple OH groups.

The LUMO energies for the phenols were found to be far too similar to identify a specific trend within the data set (Table 1). Similarly to the above observations, the effect of the HOMO-LUMO gap energy on the activity yielded two distinct relationships (Fig. 5).

HOMO-LUMO gap energy is a common theoretical factor used to predict radical scavenging activity and to help determine stability of the spent antioxidant [55]. In Fig. 5a, for compounds **3**–**8**, **31**, and **32**, the activity vs. HOMO-LUMO gap energy relationship corresponds to a linear function. The molecules that are plotted in Fig. 5a are all compounds with a single OH group. In contrast, compounds with multiple OH substituents (**2**, **11**–**13**, **15**, **16**, and **29**) are plotted in Fig. 5b; the activity vs. HOMO-LUMO gap energy function shows an exponential relationship. Both functions can be characterized by reasonable R^2 values, the linear relationships being an excellent fit ($R^2 = 0.964$). Whether exponentially or linearly related, it seems clear that increasing HOMO-LUMO gap energy results in enhanced radical scavenging activity. When the HOMO-LUMO gap energy data are coupled with the HOMO

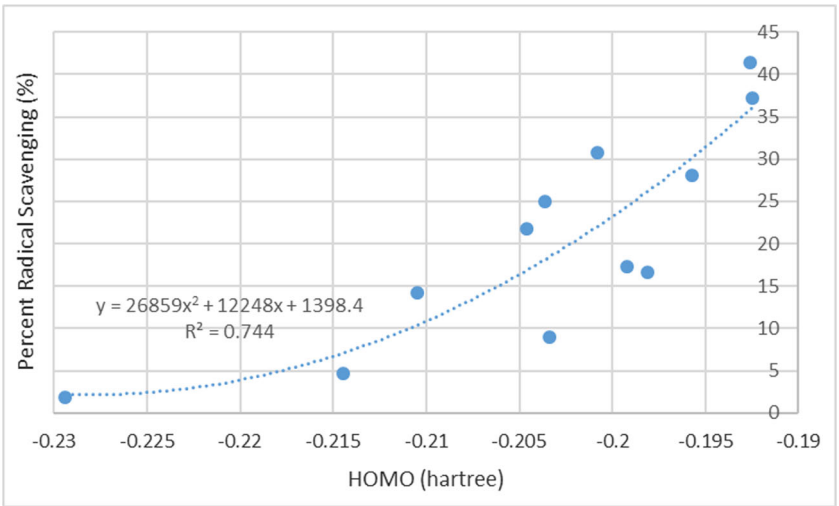


Fig. 9 The effect of HOMO energies of the anilines (**18**–**28** and **32**) on their scavenging activity

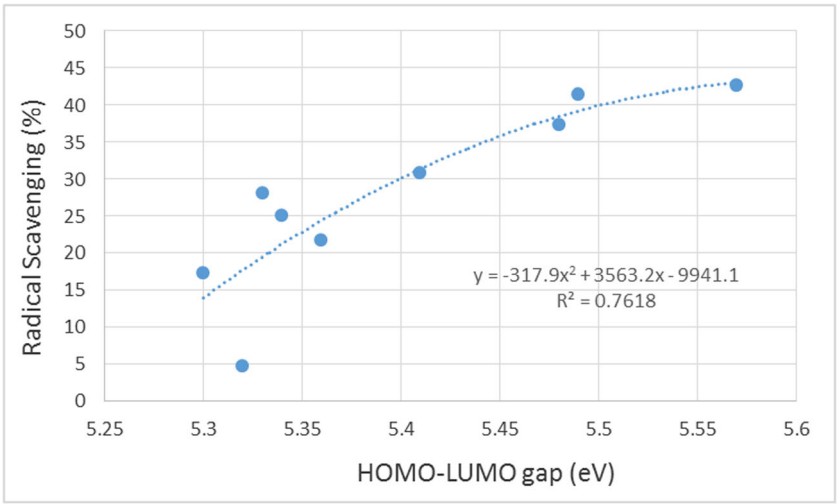


Fig. 10 Effect of HOMO-LUMO gap energy of the anilines on their radical scavenging activity with compounds

energy correlations, it suggests that phenols with high activity react via the SET mechanism where they donate an electron to the radical species.

The bond dissociation enthalpy or BDE is also a common feature to interpret the radical scavenging activity of an antioxidant [36, 37, 54, 56]. This feature is often associated with the HAT mechanism; the stronger this bond, the less likely the antioxidant will react with the radical species in solution [54]. This is probably the best predictor of compounds that will react by the HAT mechanism, which involves the transfer of a hydrogen atom to the radical. The BDE calculations are usually restricted to X–H bonds, such as O–H and N–H [27]. The activity vs. BDE data are depicted in Fig. 6.

Figure 6 shows a less characteristic relationship as compared to Figs. 4 and 5. While the general trend indicates that BDE is in inverse relationship with the activity, the low R^2 value obtained from the full set (data not shown) suggests a relatively modest correlation. This could be due to experimental limitations; compounds with a greater number of OH groups tend to be more reactive in these assays than they would be in biologically relevant systems [41]. Removing all of the compounds with more than one OH group (**11**–**18**) as well as compounds **31** and **32** (dual OH/NH) produces Fig. 6. The linear correlation of the BDE data with the ABTS scavenging activity of the single OH compounds (**1**–**3**, **5**–**10**, **29**, and **30**) shows that the lower the BDE of the OH, the better radical scavenger the compound will be. It indicates that the compound will likely donate a hydrogen atom that will terminate free radical species in solution.

Additional properties such as spin density of the O radical did not show correlation with radical scavenging activity. However, where correlation between the properties and ABTS data exist, they are typically high (HOMO, HOMO-LUMO gap energy, BDE). The combination of these factors suggests that the scavenging occurs via mixed mechanism (SET and HAT) including electron (HOMO, HOMO-LUMO gap) and H atom (BDE) transfers. There is extensive literature available about polyphenols being potent free radical scavengers because they have more OH groups present to scavenge the radical [57, 58]. However, our investigations with the

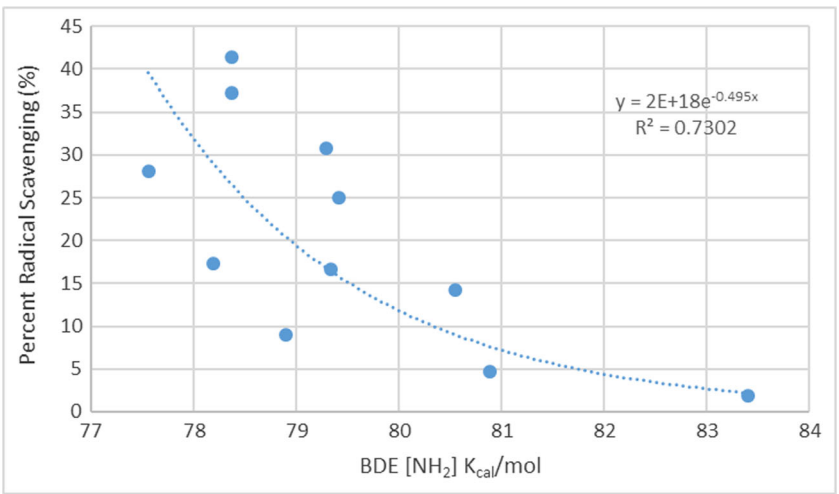


Fig. 11 Radical scavenging of anilines as a function of their bond-dissociation energy (BDE)

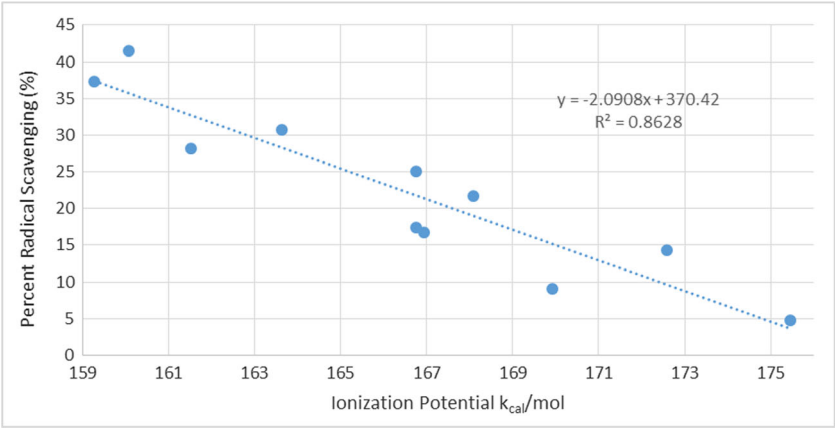


Fig. 12 Effect of ionization potential (IP) of the anilines on their experimental radical scavenging activity

above model compounds suggest it is not necessarily the amount of OH groups, rather their position, that is important. Figure 7 highlights eight of our models with single and multiple hydroxyl groups along with the experimental ABTS scavenging activity.

If the OH content alone was responsible for the activity of the compounds, then compound **1** should have significantly lower activity than compound **4** and compound **7** should have lower activity than compounds **11**–**15**. This is not necessarily the case. Instead, the position of the phenolic –OH groups appears to be important in determining the activity. As the data indicate, the single OH phenol (**1**) possesses 1.4 times higher activity than hydroquinone (**4**). Several benzoic acids were also part of our analysis and the results led to the same conclusion. Most of the benzoic acids that have more than one phenolic –OH group (**11**, **12**, and **14**) have lower activity than 4-hydroxybenzoic acid (**7**). However, compounds **13** and **15**, where the OH groups are in 1,3-positions, respectively, have higher activity than compound **7**. This suggests that the placement of the phenolic –OH groups is of primary importance, and the 1,3-arrangement helps boost the radical scavenging activity. In fact, many of the potent polyphenols commonly investigated in the literature have this 1,3-dihydroxy motif (resveratrol, cyanidin, catechin, quercetin, tannin, etc.) [6, 18, 59].

Anilines and other NH-containing compounds

As the joint analysis of phenols and anilines did not result in coherent SAR, it was decided to analyze the potential relationship between their calculated properties and experimental radical scavenging activity separately. Accordingly, we shift the discussion to primary amino group-containing aromatics (anilines) and other NH-containing compounds. Consisting of compounds **22**–**28** and **31**–**32**, these models showed widespread correlations between the ABTS scavenging data and their physicochemical properties. Anilines (**22**–**27**) had significant correlation between their ABTS scavenging data and dipole moment (Fig. 8).

The dipole moment of a potential radical scavenger may also be connected to its activity [54]. Anilines, unlike substituted phenols, showed a linear trend upon further refining the data (Fig. 8) by removing aniline (**18**) and a few of the

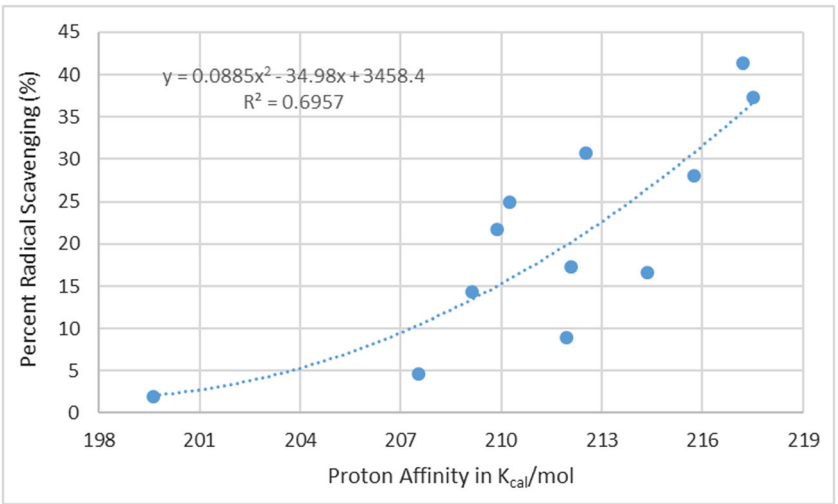


Fig. 13 Effect of proton affinity (PA) of anilines on the ABTS radical scavenging activity

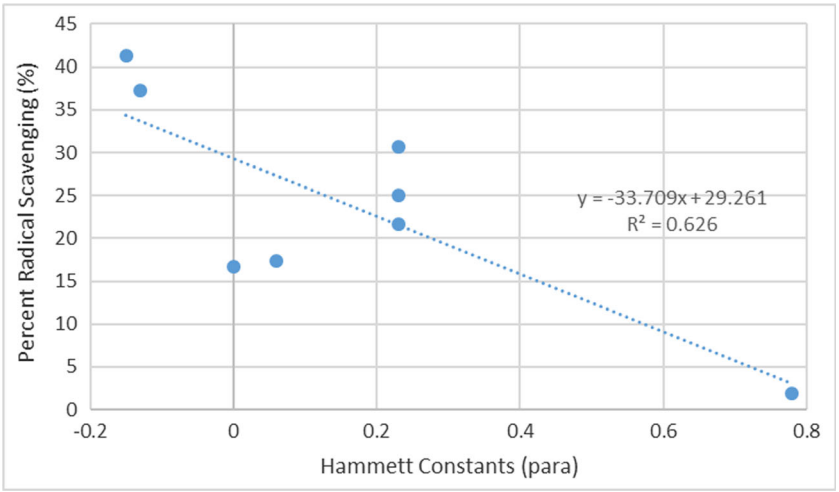


Fig. 14 Radical scavenging of anilines as a function of their Hammett constants (*para*)

compounds with groups that may sterically hinder the interaction of the NH_2 with the bulky radical species (**19**–**21**, **28**, **31**, and **32**). A low dipole moment suggests more pronounced delocalization in the molecule that can contribute to the stabilization of the lone electron left on the spent antioxidant.

Similar to the phenols, it appears that the radical scavenging activity of anilines is also affected by their HOMO energy. In Fig. 9, compounds **18**–**28** and **32** can be seen forming an exponential relationship with activity.

Unlike the phenols, the activity of anilines grew with increasing HOMO energy suggesting that the more likely the anilines were to share the electrons, the better antioxidant they were, which would be expected for compounds reacting through the SET mechanism. Based on previously outlined mechanisms of radical scavenging activity, the ability for anilines to share electrons explains their activity in the ABTS assay. As it was the case with phenols, the LUMO energies for the anilines were also too similar to be able to identify a specific trend in the data (Table 1).

An exponentially growing trend was identified in the HOMO-LUMO gap energy data for anilines **21**–**24**, **26**–**28**, **31**, and **32** (Fig. 10). Much like the HOMO-LUMO gap, energy-activity relationship of the phenol stabilization of the spent antioxidant is very important [55].

The activity vs. BDE plot of the anilines also appears relevant and shows a correlation with the ABTS activity data (Fig. 11). The exponentially declining activity as a function of BDE is in agreement with expectations and with the well-established HAT mechanism, in which a direct H atom transfer from the antioxidant to the radical would ensure the radical scavenging effect.

Ionization potential (IP) is important for the evaluation of antioxidants, as the electron transfer from the scavengers to the radical species is an essential step in the SET mechanism [54]. Anilines show an inverse linear correlation between radical scavenging activity and the ionization potential. As the ionization potential (Fig. 12) of the compound decreases, their activity increases. This is in direct agreement with the literature which suggests that the first step in SET mechanism (the donation of an electron from the antioxidant compound to the radical species) is favorable for these anilines [54, 55].

Activity vs. proton affinity (PA) plots can be used to determine the likelihood of a compound scavenging the radical species through the SPLET mechanism [54, 60]. Higher proton affinity values suggest that the molecule may undergo heterolytic cleavage and release an H^+ to solution (first step of SPLET mechanism) [60]. In Fig. 13, the data show that anilines have proton affinity values higher than 200 kcal/mol and as the proton affinity increases, the radical scavenging activity also increases. The data suggest that anilines do not operate solely under one mechanism; rather, they interact with radicals through a variety of all three pathways.

The correlation of the Hammett constants (σ) was also investigated to identify possible inductive or resonance effects

Table 2 Major properties of phenols that the show considerable fit with the experimental ABTS radical scavenging data

Parameter	Function	Fit (R^2 value)	Note
HOMO energy	$y = 214296x^2 + 97478x + 11.103$	0.902	Monophenols
HOMO energy	$y = 154028x^2 + 63636x + 6591.9$	0.812	Dihydroxy-phenols
HOMO-LUMO gap	$y = 59.255x - 291.33$	0.964	Monophenols
HOMO-LUMO gap	$y = -29.274x^2 - 330.45x - 852.15$	0.861	Dihydroxy-phenols
Bond-dissociation energy (BDE)	$y = -9.5372x + 747.01$	0.737	

Table 3 Major properties of anilines that the show considerable fit with the experimental ABTS radical scavenging data

Parameter	Function	Fit (R^2 value)	Note
Dipole moment	$y = -7.8149x + 44.873$	0.935	
HOMO energy	$y = 26859x^2 + 12248x + 1398.4$	0.744	
HOMO-LUMO gap	$y = -317.9x^2 - 3563.2x - 9941.1$	0.762	
Bond-dissociation energy (BDE)	$y = 2E + 18e^{-0.495x}$	0.730	
Ionization potential (IP)	$y = 2.0908x + 370.42$	0.863	
Proton affinity (PA)	$y = 0.0885x^2 - 34.98x + 3458.4$	0.696	
Hammett constants (σ)	$y = 33.709x + 29.261$	0.626	σ_{para} only

on the activity of the compounds [48]. Using compounds with a similar backbone who have varying substituents allows us to investigate the effects the substituents may have on the overall electron density of the compound and ultimately estimate the stability of the spent antioxidant [54]. The substituents in both the *meta* and *para* positions in the phenols are mostly other –OH groups, so the data collected for phenols does not provide much useful information. The data collected from anilines show a more interesting trend. Figure 14 exhibits a correlation between the radical scavenging potency and the *para*-Hammett constant, suggesting that electron-donating substituents such as ethyl and propyl increase the radical scavenging activity of the anilines. A trend does not develop with the *meta*-Hammett constants. As the Hammett constants include electronic effect of the substituents, the electronic effects of pyramidalization of the NH_2 group can also be considered. In the presence of electron-donating substituents, this pyramidalization is more pronounced, resulting in the move of the N–H bond out of the plane where it can overlap with the aromatic π-system [61]. This overlap weakens the N–H bond and could be, at least partially, responsible for the enhanced radical scavenging effect.

Electron-withdrawing groups such as CF_3 and NO_2 have had a tremendous impact on the activity of the anilines. Interestingly enough, the 2-amino-4-nitrophenol still retained a 43% radical scavenging activity in the ABTS assay. This is most likely due to the presence of the additional OH on the phenyl ring.

Summarizing the above analysis, the factors that appeared to have meaningful effect on the ABTS radical scavenging activity are tabulated in Tables 2 and 3.

It appears that while the phenols and anilines used do not form a unified model set that results in a coherent picture, analyzing them separately, however, yields valuable information. HOMO, HOMO-LUMO gap, and bond-dissociation energy data are of high importance to determine the radical scavenging activity of both groups. Interestingly, these are the only parameters that seem to contribute to the activity of phenols. During the analysis, it was found, that even monophenols and dihydroxy derivatives cannot/should not be handled together. When evaluated separately, these two groups resulted in meaningful fit between the experimental data and calculated HOMO and HOMO-LUMO gap energies. Based on the data obtained, phenols appear to act predominantly via HAT mechanism. The experimental radical scavenging data of aniline derivatives reveal strong radical scavenger properties that are competitive with those of phenols. The theoretical analysis of their properties, however, indicates that while anilines also act via HAT mechanism, their mode of action is somewhat more complicated. Reasonable fits with ionization potential and proton affinity values suggest that these compounds at least partially scavenge radicals via the SET and SPLET mechanisms as well. This complex mode of action could contribute to the versatility of aniline-NH-containing natural and synthetic antioxidants and make them novel candidates in antioxidant-based therapeutic applications.

Conclusions

Analyzing the radical scavenging activity of simple phenol and aniline model compounds as a function of their calculated properties has provided a better understanding of the contributing factors on the radical scavenging capabilities of these compounds. It was observed that phenols, on average, possess higher radical scavenging activity. However, several anilines showed much higher activity than majority of phenols, thus the activity ranges overlap, indicating that anilines are comparable radical scavengers to phenols. Regarding phenols, it has been found that increasing the number of OH groups does not necessarily result in parallel enhancement in radical scavenging activity. It was observed that the position of the multiple OH groups is of particular importance: having them in 1,3-position results in significant, synergistic increase in activity, in contrast to 1,2- or 1,4-positions. The study identified several physical properties that likely govern the radical scavenging activity of phenols and anilines, such as the HOMO energies, HOMO-LUMO gap, and BDE. Anilines appear to act via more complex mechanisms as the ionization potential, dipole moment, and proton affinity all have considerable effect on their radical scavenging activities.

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Abbreviations HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl; ORAC, oxygen radical absorbance capacity; MS, multiple sclerosis; HAT, hydrogen atom transfer; SET, single-electron transfer; SPLET, sequential proton loss electron transfer; DMSO, dimethyl sulfoxide; μ (D), dipole moment; BDE, bond-dissociation energy; IP, ionization potential; PA, proton affinity; σ, Hammett constant

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